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Masting uncoupling: mast seeding does not follow all mast flowering episodes in a dioecious juniper tree

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Evolutionary selective forces, like predator satiation and pollination efficiency, are acknowledged to be major causes of masting (the variable, periodic and synchronic production of seeds in a population). However, a number of recent studies indicate that resources might also play an important role on shaping masting patterns. Dioecious masting species offer a privileged framework to study the role of resources on masting variation, since male and female plants often experience different reproductive costs and selective pressures. We followed masting and reproductive investment (RI) of the dioecious tree *Juniperus thurifera* in two populations along 10 years and studied the different response of males and females to experimentally increased water and nutrient availability in a third population.

Juniperus thurifera females invested in reproduction three times more resources than males. Such disparity generated different resource-use strategies in male and female trees. Tree-ring growth and water use efficiency (WUE) confirmed that sexes differed in their resource investment temporal pattern, with males using current resources for reproduction and females using resources accumulated during longer periods. Watered and fertilized female trees presented significantly higher flowering reproductive investments than males and experienced an extraordinary mast-flowering event. However, seeding RI and mast seeding were not affected by the treatment. This suggests that although resource availability affects the reproductive output of this species, there are other major forces regulating masting on *J. thurifera*.

During 10 years, *J. thurifera* male and female trees presented high and low flowering years more or less synchronously. However, not all mast flowering episodes resulted in mast seeding, leading to masting uncoupling between flowering and seeding. Since flowering costs represent only 1% of females' total reproductive investments, masting uncoupling could be a beneficial bet-hedging strategy to maximize reproductive output in spite of unpredictable catastrophic events.

> *"Masting is often an adaptive reproductive trait overlaid on the direct influence of weather"* (Kelly and Sork 2002)

Mast seeding (the variable, periodic and synchronic production of seeds in a population) is a phenomenon resulting from the interaction of many functional constraints and evolutionary forces (Janzen 1976, Kelly 1994, Kelly and Sork 2002, Rees et al. 2002). Two of the most well-known evolutionary factors are 1) predator satiation, suggesting that large intermittent seed crops reduce seed-loss due to satiation of predators; and 2) pollination efficiency, proposing that concentrating pollination in some years increases pollination efficiency in wind pollinated species (Kelly and Sork 2002, Rees et al. 2002, Satake and Bjørnstad 2004, Piovesan and Adams 2005).

Nonetheless, masting pulses are also dependent on resource availability and reserves, (Clark and Clark 1987, Norton and Kelly 1988, Sork et al. 1993, Yamauchi 1996, Isagi et al. 1997, Satake and Iwasa 2002). Therefore models benefit greatly from taking into account resource availability (Yamauchi 1996, Isagi et al. 1997, Rees et al. 2002). In a remarkable study Crone et al. (2009) experimentally demonstrated that resource availability was the underlying factor, mediated by pollination efficiency, controlling masting pulses in *Astragalus scaphioides*. If resources play such an important role on masting, the study of dioecious masting species will provide unparalleled opportunities to study the effect of differential male and female sexual investments on masting behavior. Reproduction is expensive and in dioecious plants different sexes often incur in different reproductive costs (Obeso 2002). Males usually invest more resources than females to flowering although overall reproductive investments are usually higher in females due to fructification costs (Delph 1999). Reproductive investments can be compensated for by improving physiological performance, reducing resources allocated to vegetative growth or reducing the frequency of reproduction (Delph 1999, Obeso 2002). However, compensating mechanisms rarely pay off for the totality of resources invested in reproduction by females (Geber et al. 2009), in that case females are expected to:

1) Flower with less intensity than males, but with the same regularity (reviewed by Eckhart 1999).

2) Flower less often (Delph 1999), presenting a mast-flowering behavior (Antos and Allen 1999). Then, males flowering synchronously with females could increase their fitness, which eventually would lead to flowering synchronization between the sexes (Kelly and Sork 2002, Crone et al. 2009).
3) Adopt a bet-hedging behavior in which flowering does not always result in seeding. Plants can benefit from such behavior if female flowering represents a minor cost compared to seeding and environmental variation is unpredictable (Stephenson 1980, 1981, Sutherland 1986, Burd 1994, 1998, Kamoi et al. 2008). In dioecious species males would have no control over the output of this process, but still could benefit from keeping some residual flowering during non mast-flowering years if some male fitness is achieved by residual seeding, or if females' seeding cycle is unpredictable (Morgan 1993, Arnold 1994).

One frequent assumption when studying masting is that male flowering intensity matches female flowering and that subsequent seed set will vary proportionally to flowering intensity in an efficient resource-use process; namely, that flowering and seeding masting are coupled, consequently only seeding is recorded (reviewed by Herrera 1998, Koenig and Knops 2000, Kelly and Sork 2002). Although some studies record both flowering and seeding, they habitually found that flowering and seeding were coupled, and therefore tend to focus on evaluating the importance of pollen limitation (reviewed by Koenig and Ashley 2003). Nevertheless, Sork et al. (1993) and Sork and Bramble (1993) recorded flowering and seeding in masting *Quercus* species and found that many pollinated flowers aborted before reaching maturity due to resource limitation. However, since *Quercus* are monoecious, sexual differences in reproductive tradeoffs could not be accounted for.

The dioecious masting tree *Juniperus thurifera* presents an optimal set of traits to investigate the consequences of differential reproductive investments of male and female trees on masting according to the three possibilities above mentioned, and particularly to test for uncoupling between flowering and seeding. Females produce tiny flowers and costly fleshy cones (throughout the text the terms flower and fruit are used instead of 'reproductive cone' and 'berry like cones' for ease of use). It also inhabits an environment on the limit of its environmental optimal in which very small increases of nutrients and water (only 50 mm year^{-1}) can considerably increase fructification (Montesinos et al. 2010). This should imply that small, unpredictable variations in resource availability (e.g. precipitation) may have a significant effect on reproduction, providing with an optimal framework to test for the advantages of bet-hedging.

In this work we followed flowering from 1999 to 2009 and their correspondent seeding cohorts of male and female trees in two populations and evaluated reproductive investments in order to investigate the role of costs of reproduction and environmental stochasticity on masting behavior. In one of the populations we also studied vegetative tree-ring growth and annual δ^{13}C as an approximation to water use efficiency (WUE hereafter; it evaluates the ability of plants to fix carbon with a minimal loss of water) and related it to reproductive investment patterns of each sex. A manipulative experiment was also performed to disentangle differences between genders in reproductive investment by adding water and nutrient to male and female trees of *J. thurifera* in a third population. With this design we aimed to answer the following specific questions:

1) Do *J. thurifera* male and female trees flower synchronously? 2) Is mast-flowering coupled to mast-seeding? 3) Do females incur in higher reproductive investments than males? 4) Do males and females present the same physiological tradeoffs? 5) How does resource availability affect masting?

Material and methods

Study species

Juniperus thurifera (Cupressaceae) is a dioecious long-lived tree with relict distribution from the Tertiary throughout the western Mediterranean basin (Marcysiak et al. 2007). Together with two closely related vicariant species with similar morphology and biology (*J. excelsa* and *J. procera*) they distribute along the Mediterranean basin, western Asia and eastern Africa. Trees are 4–10 m high and often live for centuries (Bertaudière et al. 1999) and reach sexual maturity at the approximate age of 31 (Pavón-García 2005). Males produce bigger and more numerous flowers than females, and both flower by January. After pollination, females produce berries over a period of 20 months, when seed dispersal occurs (Amaral-Franco 1986, García-Fayos unpubl.). Seeds present an extremely low viability (9.5 ± 8.8%, mean ± SD, Montesinos et al. 2010) and trees' reproductive output varies strongly from year to year. The present distribution in high mountains with unfertile soils constraints population reproductive output (Montesinos et al. 2010) and recruitment (Montesinos et al. 2007).

Study sites

The study was performed in eastern Spain at three different locations:

Population A is located at 1150 m a.s.l. near El Portell de Morella, Castelló. Vegetation is dominated by *J. thurifera* and *Quercus ilex* trees, accompanied by sparse individuals of *J. communis*, *Thymus vulgaris* and *Genista scorpius*. Lichens and herbaceous ephemeral vegetation cover most of the rocky soil surface. The climate is Mediterranean, with harsh winters (the duration of the freezing period is more than 120 days per year), and warm dry summers. Climatological data was obtained from AEMet's Vilafranca del Cid station. Mean annual precipitation is 665 mm with May being the wettest month (81 mm) and July being the driest (35 mm). Annual mean temperature is 11.2°C, with July being the warmest month (mean 20.1°C) and January the coldest (mean 3.8°C).

Population B is located some 100 km from population A, at 1600 m a.s.l. near La Puebla de San Miguel, València. Vegetation is dominated by *J. thurifera* trees with sparse *Thymus godayanus* and *G. scorpius* plants. Lichens and herbaceous ephemeral vegetation cover most of the rocky soil surface. The climate is Mediterranean. Climatological data was obtained from AEMet's Sesga station. Mean annual precipitation is 536 mm with May being the wettest month (66 mm) and July the driest (27 mm). Annual mean temperature is 12.6°C, with July being the warmest month (mean 20.9°C) and January the coldest (mean 4.2°C).

Population C is located at only 2 km from population B, at 1500 m a.s.l. near La Puebla de San Miguel, València. Climate and vegetation are similar to population B.

Reproductive investment

In 1999 we selected a set of trees of similar age distribution on populations A and B. On each population a set of 20 male trees and 20 female trees were marked and several reproductive and vegetative variables were measured from that year to 2009. The same procedure was followed from 2003 to 2009 for population C. From April 2003 to March 2005, half of the male and female trees from population C were watered and fertilized (fertirrigated) monthly, randomly assigning 10 male and 10 female trees to each of the watering treatments (total of 40 trees). Trees were fertirrigated fortnightly in June and July, the driest months, and fertirrigation did not occur between November and February, when temperature was extremely low and precipitation was high. On each fertirrigation date every tree was supplied with 100 l of fertilization solution (2 g l^{-1} of 18N:11P:18K plus oligoelements). This resulted in a net yearly dose of 1000 l of water and an effective dose of 360 g of N; 220 g of P and 360 g of K per year. Water was distributed on 20 m^2 around trunks resulting in an approximate increase in precipitation of 50 mm per year. This meant an approximate increment of 9.5% over the accumulated rain on population C along the two experimental years (1059 mm over the two years) and an increment of 21.7% over the accumulated summer precipitation (138 mm) (Fig. 1).

Carbon tends to reflect the allocation patterns of the most limiting resources (Reekie and Bazzaz 1987, de Jong and Klinkhamer 2005), since allocation to biomass generally integrates allocation of other resources (Delph 1999, Bazzaz et al. 2000), especially when comparing individuals from the same species (Mendez and Karlsson 2007). Consequently reproductive investment (RI) is quantified in terms of dry biomass in this study. To study RI accounting for intra-individual variability we counted the total number of male and female flowers every January (Fw), and ripe female fruits every October (Fr2, 22 months old) in 10 branches randomly sampled all around each tree along ten consecutive years in populations A and B, and six years in population C. Flowers measured in January were accounted for as reproductive investment from the previous year because they were fully preformed in the previous summer (Montesinos unpubl.). Branch sampling was re-randomized each year. In 2003 and 2004 we collected and weighed ten flowers (Fw), ten unripe female fruits (Fr1, 12 months old) and ten ripe female fruits (Fr2, 22 months old) from each of 10 male and 10 female trees from population B. Flowers and female fruits were dried at 50°C for 48 h before weighting. For female fruits, we calculated the weight of 12 months old fruits relative to the mature female fruits weight. We determined that only 36% of the weight of a ripe female fruit (Fr2) was produced during the last year, and 64% during the first year (Fr1). As a result, mast-seeding years are preceded by a peak in RI. This proportion between Fr1 and Fr2 did not significantly differ between years (F = 2.52, DF = 1, p = 0.129). Consequently, subsequent yearly reproductive investment (RI) per branch was calculated as:

$$RI_t = (W_{Fw} \times N_{Fw(t)}) + (0.36 \times W_{Fr2} \times N_{Fr2(t)}) \\ + (0.64 \times W_{Fr2} \times N_{Fr2(t+1)}) - (W_{Fw} \times N_{Fr2(t+1)})$$

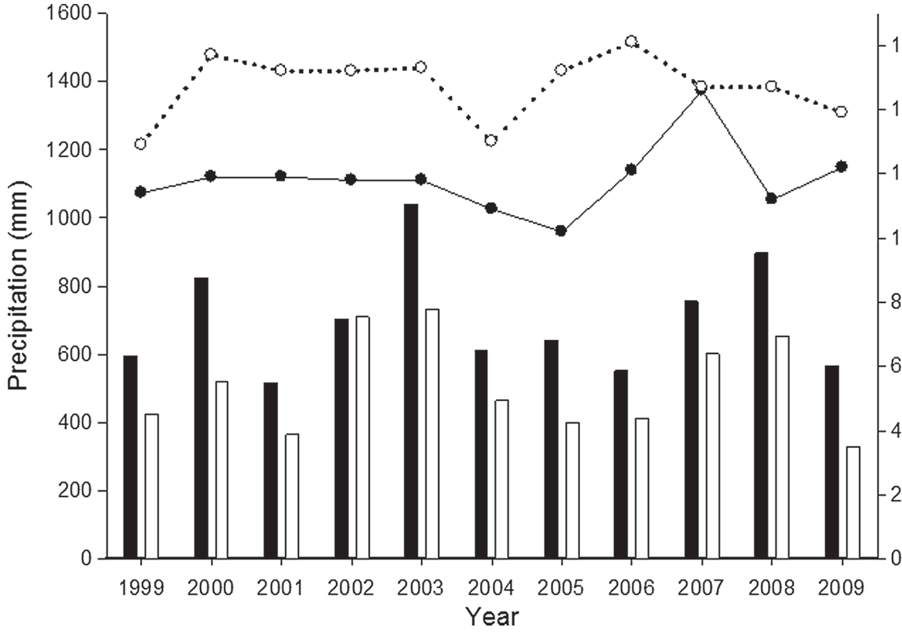

Figure 1. Annual mean precipitation (mm) and annual mean temperature (°C) for the weather stations of Vilafranca del Cid (solid bars and line; population A) and Sesga (white bars and dotted line; populations B and C). Data provided by AEMet.

in which W refers to dry weight of each flower or fruit in grams; N, number of flowers or fruits; t, current year and t + 1, following year.

Using the allometric methodology of Montès et al. (2000) to estimate aboveground tree weight based on measures of tree height and canopy cover, we concluded that our selected trees from all three populations did not differ in their biomass or size distribution (mean ± SD = 577 ± 114 kg; Kolmogorov–Smirnov $Z = 0.41$, $p = 0.996$). *Juniperus thurifera* branches are defoliated near the trunk and present a tuft of terminal leaves. Males (length × width = 1095 ± 419 mm^2, $n = 50$) and females (1074 ± 382 mm^2, $n = 50$) presented similar branch-tuft size and differences were statistically not significant (Kolmogorov–Smirnov $Z = 1.00$, $p = 0.270$). Therefore, mean RI per branch gives a convenient, proportional and size independent approximation to global RI since bigger trees would have more branches than smaller ones.

To estimate annual vegetative growth and physiological costs, in July 2005 we extracted two dendrochronological wood cores from each marked individual from population B and ring growth from 2000 to 2005 was measured using standard dendrochronological techniques.

Masting

Since there is no clear boundary between masting and non-masting species (Herrera 1998) a quantitative approach to how much masting a determinate species is (i.e. variable, synchronic and periodic) can be useful from an evolutionary point of view (Koenig et al. 2003). To give a quantitative approach to our study we calculated the indexes proposed by Herrera (1998) to quantify masting (CVp and CVi) and the mean Pearson's pairwise correlation coefficient (r) to study the degree of supra-annual synchrony among trees (Buonaccorsi et al. 2003). Coefficients of variation (CV = SD/mean) for male and female flowering RI and for total female RI were calculated independently for each population. CVp was calculated as the population coefficient of variation for RI among years. CVi was calculated as the mean of within-plant coefficients of variation among years in RI.

To differentiate between masting and non masting years we used the most restrictive and conservative method according to a recent review of 36 datasets (LaMontagne and Boutin 2007, 2009). Annual RI is expressed as a standardize deviate based on long-term average RI and variability of the dataset (10 years in our study) according to the following expression (LaMontagne and Boutin 2009):

> *"Annual deviate from the long-term mean$_i$ = (mean RI$_i$ in year 't' − long-term mean RI$_i$)/standard deviation calculated over all years$_i$"*

RI of years below the long-term mean will present negative standard deviates while mast years will present relatively high, positive, standard deviates. We considered that a particular year was a mast year if the annual deviate value was on or above the absolute value of the lowest standardized deviate for each particular population.

Physiological costs ($\delta^{13}C$)

As a surrogate of water use efficiency (WUE) we analyzed $\delta^{13}C$ isotopic discrimination by extracting a different set of cores from marked trees at population B in July 2005. Tree rings from year 2000 (previous to a mast-seeding year), 2001 (mast-seeding year) and 2004 (one of the lowest reproductive investments for both genders) were carefully separated. Cellulose was extracted following the protocol proposed by Freyer (1979). Samples were milled to dust and the cellulose extracted from each ring was analyzed at the Laboratorio de Isótopos Estables (Univ. Autónoma de Madrid, Spain) to determine the C isotope composition. After combustion of the samples in an elemental analyzer the $^{13}C/^{12}C$ ratios were determined with a continuous flow isotope mass ratio spectrometer with a measurement error of 0.1‰. The isotopic values are expressed in delta notation and calculated as:

$$\delta^{13}C(‰) = (R_{sample}/R_{standard} - 1) \times 1000$$

where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ ratios of the sample and the Pee Dee Belemnite carbonate standard, respectively.

Statistical analyses

Linear mixed models (LMM) analysis permits the study of time-series accounting for temporal autocorrelations of repeated measurements taken among years within each of the individual subjects studied (Bolker et al. 2009). LMM tests with autoregressive covariance structure of order one were run to test for differences between sexes and years and for correlations between variables. Year was used as a repeated effect variable and each of the individual trees as subject variable. Together with the F-value and degrees of freedom and significance, an estimate provides with an estimate of fixed effects of the model (SPSS 19.00). Aboveground tree biomass was used as covariate factor to correct for possible differences related to tree size. Statistical analyses were performed by using SPSS 19.00 and R 2.11.1. Unless otherwise indicated all values are shown as mean ± SD.

Results

Reproductive investment and masting

According to the indexes proposed by Herrera (1998) to quantify masting (CVp, CVi), and to the pairwise correlation coefficient (r) (Buonaccorsi et al. 2003), variation in reproductive investment (RI) of populations A and B presented a moderately high degree of inter-annual variability and population synchrony (Table 1). On population C experimental addition of water and nutrients had small or no impact on the indexes. Although population C control trees showed an abnormally low female flowering synchrony the value for watered individuals was similar to that of controls from the other two populations.

Populations A and B presented a similar pattern of reproductive variation. Male and female trees flowered synchronically (Pearson correlation population A: $r = 0.703$,

Table 1. Components of synchrony for the reproductive investment (RI) of male and female flowering (Fw); total intersexual flowering investment (total Fw); total fruiting investment (total Fr) and total reproductive investment of females (total Female). CVp refers to the population coefficient of variation (SD/mean) for RI among years. CVi refers to the mean of within-plant coefficients of variation among years in RI. These indexes have been shown to range between 0.71 and 1.67 for several *Juniperus* species and from 0.19 to 2.85 for a wide range of species (Herrera 1998). *r* refers to the degree of supra-annual synchrony among trees expressed by the mean Pearson's pairwise correlation coefficient and its SE, this coefficient ranks from −1 (complete discordance) to 1 (complete synchrony) (Buonaccorsi et al. 2003).

	Male Fw	Female Fw	Total Fw	Total Fr	Total Female
Population A					
CVp	0.90	1.00	0.88	1.87	1.63
CVi	1.35	1.86	1.61	1.83	1.61
r (± SE)	0.50 ± 0.02	0.38 ± 0.03	0.38 ± 0.03	0.92 ± 0.01	0.79 ± 0.02
Population B					
CVp	0.90	1.36	0.91	0.85	0.84
CVi	1.69	1.98	1.85	1.25	1.24
r (± SE)	0.47 ± 0.03	0.58 ± 0.03	0.52 ± 0.01	0.30 ± 0.02	0.30 ± 0.03
Population C – control					
CVp	0.58	1.23	0.58	1.42	1.41
CVi	1.04	1.82	1.43	1.41	1.36
r (± SE)	0.30 ± 0.06	0.08 ± 0.06	0.15 ± 0.04	0.97 ± 0.01	0.91 ± 0.02
Population C – watering					
CVp	0.54	1.35	0.55	1.49	1.49
CVi	1.13	1.62	1.37	1.32	1.32
r (± SE)	0.32 ± 0.07	0.62 ± 0.08	0.37 ± 0.04	0.72 ± 0.07	0.72 ± 0.07

$p = 0.016$; population B: $r = 0.716$, $p = 0.013$; Fig. 2) with three clear and consistent mast-flowering peaks on 1999, 2001 and 2006 (females of population A also on 2009; black bars on SD figures indicate masting years). Such mast-flowering peaks should have resulted in three mast-seeding peaks on 2001, 2003 and 2008 respectively. However, clear mast-seeding events where recorded only on one of those years for population A (2001); and on two years for population B (2001 and 2008) (Fig. 3). Investment in female flowers (Fw) was very low compared to fruits. Female fruits gain 64% of their weight one year before ripening (see methods). This is due to the strong reproductive investment needed to increase the size of female unripe fruits (Fr1, 12 months old). Contrarily, ripening of female fruits (Fr2, 22 months old) demands a lower weight investment (36%). Notice that since we are evaluating reproductive investments and not just flower or fruit counts, the plots show a higher RI in the year previous to a mast-seeding year than in the mast-seeding year itself, due to higher RI on growing Fr1 than on ripening Fr2 (Fig. 3).

On populations A and B, mean reproductive investment of females among years (1.27 ± 1.75 g) was more than three times higher than that of males (0.42 ± 0.37 g). Females invested in flowers less than 1% of the resources devoted to reproduction (0.01 ± 0.01 g). Reproductive investment was highly variable among years for both sexes (Fig. 3) and during some particular years male trees eventually invested a similar or even higher amount of resources to reproduction than female trees, resulting in a significant interaction (Table 2).

Watering and fertilization did increase female flowering RI significantly ($F = 5.04$, $DF = 1,40$, $p = 0.030$) but did not significantly increase male flowering RI ($F = 2.75$, $DF = 1,41$, $p = 0.105$) nor female seeding RI ($F = 0.44$, $DF = 1,43$, $p = 0.512$) (Fig. 2C, 3C). However, watering and fertilization had an interesting effect on the standardized deviates used to define mast-flowering years. According to this convention, control females presented only one mast-flowering event on 2008 while watered individuals presented one mast-flowering year on 2004 but not on 2008. We believe that the lack of the 2008 mast-flowering event is an artifact forced by the high RI values obtained from watered individuals on 2004, which are forcing the mean so high that the RI of 2008 (or even 2006 for watered females) is comparatively too low as compared to 2004. Therefore, watering could have triggered at least one mast-flowering event (2004). We could not detect any mast-flowering event during 2006 which should be preceding the detected mast-seeding event of 2008 on population C.

Tree-ring growth followed a similar pattern for individuals of both sexes. Mean tree-ring growth for male trees (0.98 ± 0.59 mm) was similar to that of female trees (0.94 ± 0.53 mm) and temporal variations were similar (Table 2). Male trees presented a negative correlation between reproductive investment and tree-ring growth (estimate $= -0.34 \pm 0.12$, $F = 8.41$, $DF = 1,52$, $p = 0.005$). In contrast, female trees did not show any relationship between vegetative and reproductive investment in the short term neither for total RI (estimate $= -0.01 \pm 0.03$, $F = 0.01$, $DF = 1,74$, $p = 0.990$) nor for flowering RI (estimate $= -3.00 \pm 3.42$, $F = 0.77$, $DF = 1,74$, $p = 0.384$), but see Montesinos et al. (2006) for a long term relationship for females.

Physiological costs (δ^{13}C)

Male (−24.99 ± 1.34‰) and female trees (−24.54 ± 1.26‰) from population B showed no differences in δ^{13}C between sexes or years (Table 2). Interaction was not significant. Moreover δ^{13}C did not correlate with reproductive investment (estimate $= 0.03 \pm 0.07$, $F = 0.25$, $DF = 1,67$, $p = 0.193$) or tree-ring growth (estimate $= -0.39 \pm 0.30$, $F = 1.73$, $DF = 1,67$, $p = 0.193$).

Interestingly, males presented a positive relationship between tree size and annual δ^{13}C (estimate $= 0.0011 \pm 0.0004$, $F = 6.15$, $DF = 1,16$, $p = 0.025$), indicating a

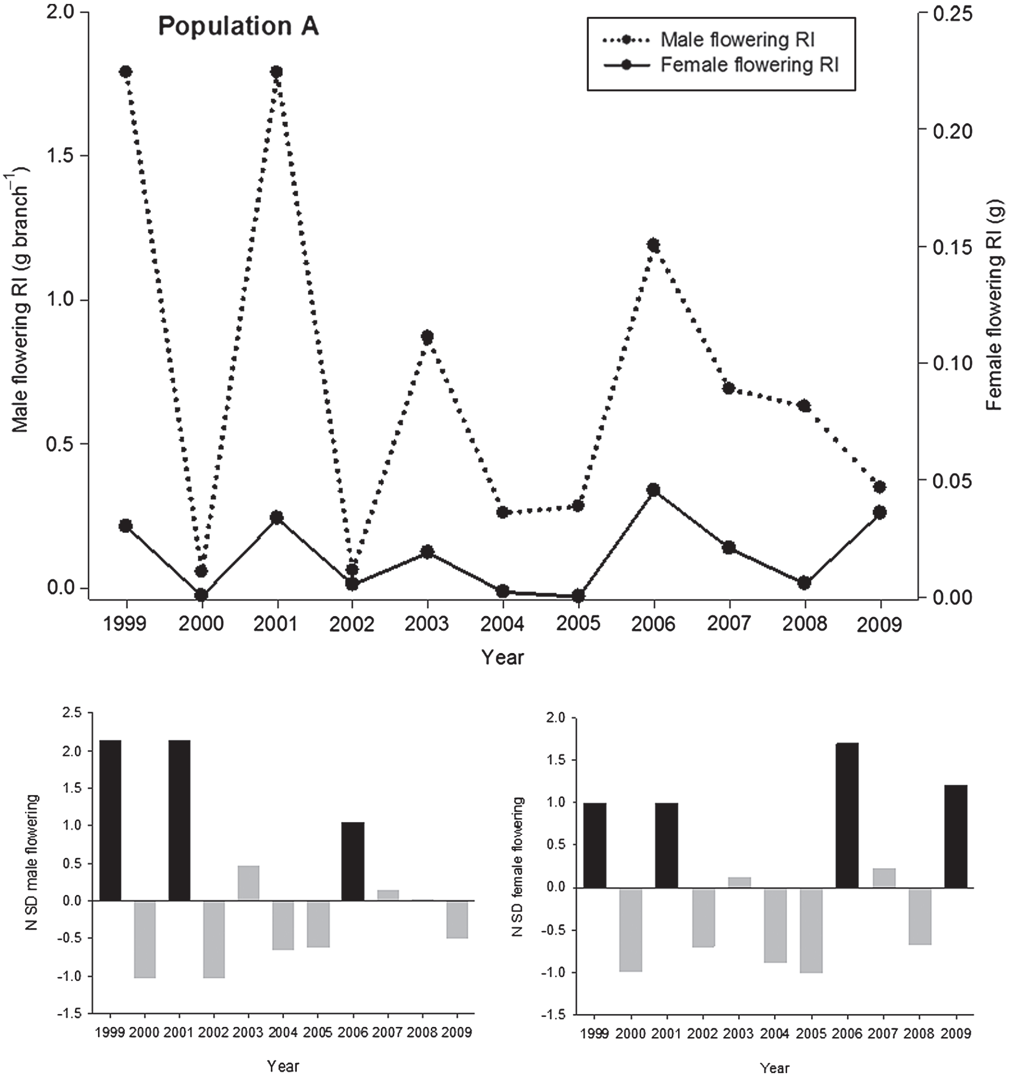

Figure 2. Mean flowering reproductive investment per branch (top plot) and number of standard deviations (bottom plots) of annual flowering RI from the long-term inter-annual mean. Black bars indicate years in which N SD value was on or above the absolute value of the lowest standardized deviate of each population. Compare with total RI on Fig. 3. Note that different graphs have different y-axes scales.

slightly better water use efficiency and a less stressed condition for larger males. Females did not present such relationship (estimate = 0.0004 ± 0.0005, F = 0.63, DF = 1,20, p = 0.435) showing again a physiology less dependent of within-annual conditions.

Discussion

Our study shows five unequivocal conclusions: 1) *Juniperus thurifera* male and female trees flower synchronously; 2) females experienced less mast-seeding than mast-flowering episodes, leading to uncoupling between mast-flowering and mast-seeding; 3) females incur in markedly higher reproductive investment than males, even though their flowering costs are less than 1% of their total investment; 4) reproduction and growth present different negative relationships in males and females: males present a negative relationship on the short term (present study) while females did it on the long term (Fig. 3, Montesinos et al. 2006); furthermore, males present a positive relationship between tree size and water use efficiency (WUE) on an annual basis while females do not; and 5) increased resource availability increased female flowering RI and prompted a mast-flowering event.

Our data indicate that males and females present differences in their resource use patterns, with males presenting short term responses and negative correlation between tree ring-growth and reproductive investment and females probably storing resources which are not used immediately for future reproductive events thus preventing any correlation to occur in the short time. Tradeoffs between vegetative and reproductive functions are common among plants and particularly in coniferous trees (Koenig and Knops 1998). Although the presence of negative relationships does not necessarily indicate an actual tradeoff (Knops et al. 2007), many studies confidently document cases in which reproductive female plants present lower leaf and tree-ring growth and lower water use efficiency than males, especially under stressful conditions (Dawson and Bliss 1989, Obeso et al.

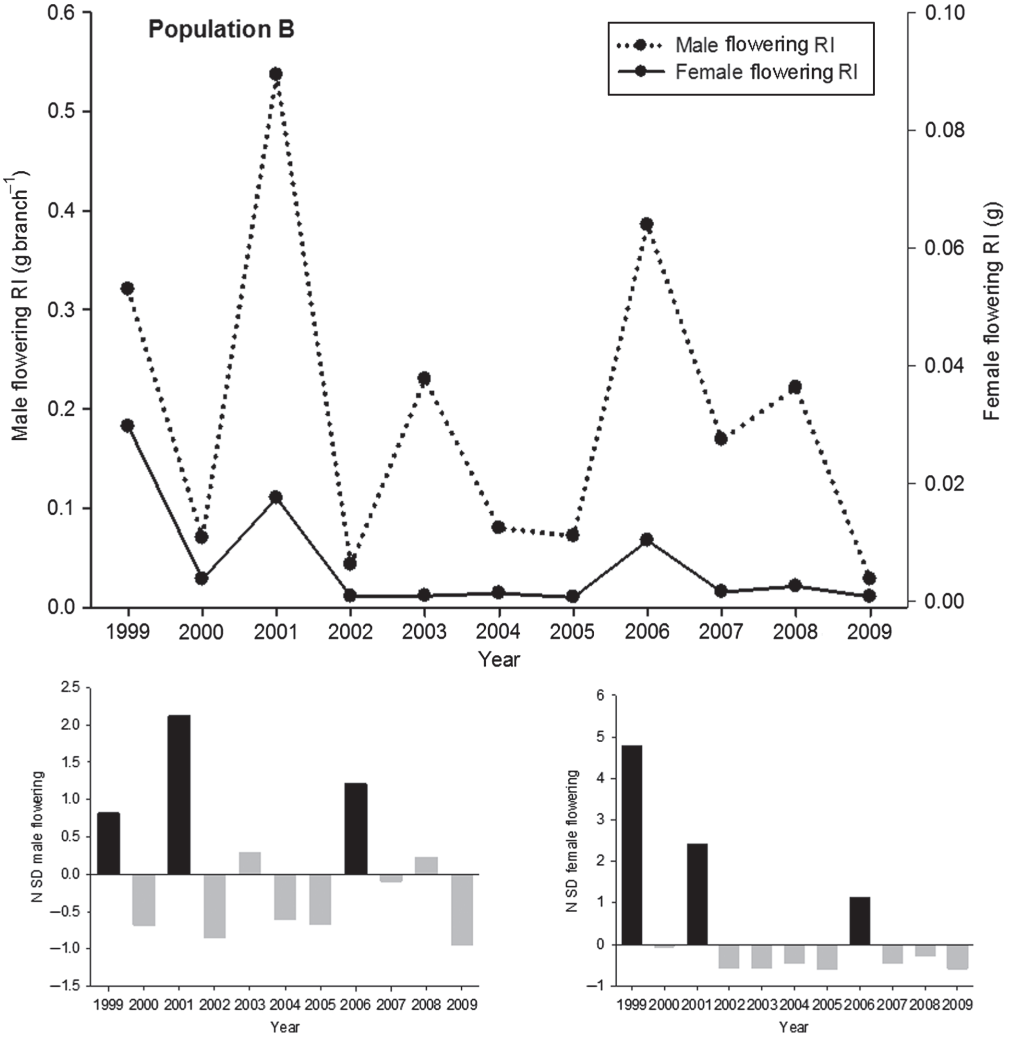

Figure 2. (Continued)

1998, Dawson et al. 2004, Montesinos et al. 2006, 2012). This is coherent with *J. thurifera* water use efficiency data: if females are distributing costs among years it is unlikely to detect differences in physiological costs on an annual basis; however small males, more dependent on current resources income, do experience lower water use efficiency (WUE) on an annual basis. In terms of total reproductive investment (RI) male and female trees differed in their relationship between vegetative growth and reproduction as well. Male trees flowered with an intensity pattern which was negatively correlated with tree-ring growth. A negative correlation between growth and reproduction is consistent with the resource switching hypothesis (Kelly and Sork 2002, Monks and Kelly 2006) according to which plants allocate resources successively into, and then away, from reproduction (Koenig and Knops 2000, Monks and Kelly 2006). However, females of *J. thurifera* concentrated most of their reproductive investment on scarce particular years, reducing total reproductive investment for many years after a mast-seeding episode, as is common in many masting species (Kelly 1994, Kelly and Sork 2002). In coniferous species maturation cycles of more than one year are common (Castroviejo et al. 1986, McDowell et al. 2000, Wesche et al. 2005), and plants often distribute their reproductive costs among years, resulting in long masting cycles (Norton and Kelly 1988, Herrera 1998, Koenig et al. 2003). Plants can store resources in different parts of the plant, and carbohydrates stored during years with low reproduction can be invested during highly reproductive years (Kelly 1994, Antos and Allen 1999, Henery and Westoby 2001, Miyazaki et al. 2002, Sala 2006, Crone et al. 2009). This often results in undetectable tradeoffs between growth and reproduction (Obeso 2002). In a study in which females of the dioecious shrub *Rhamnus alpinus* had higher RI and lower tree-ring growth in the long term (Bañuelos and Obeso 2004), both sexes presented the same short-term tree-ring pattern and there was no short-term correlation between tree-ring growth and RI. In our study only males showed a short-term relationship between growth and reproduction, while a negative relationship on females was detectable only in the long term (Fig. 4). The pattern of resource use on male and female trees suggests that males are using for reproduction resources that they obtained during the current year while females depend on resources stored during a longer period. Using

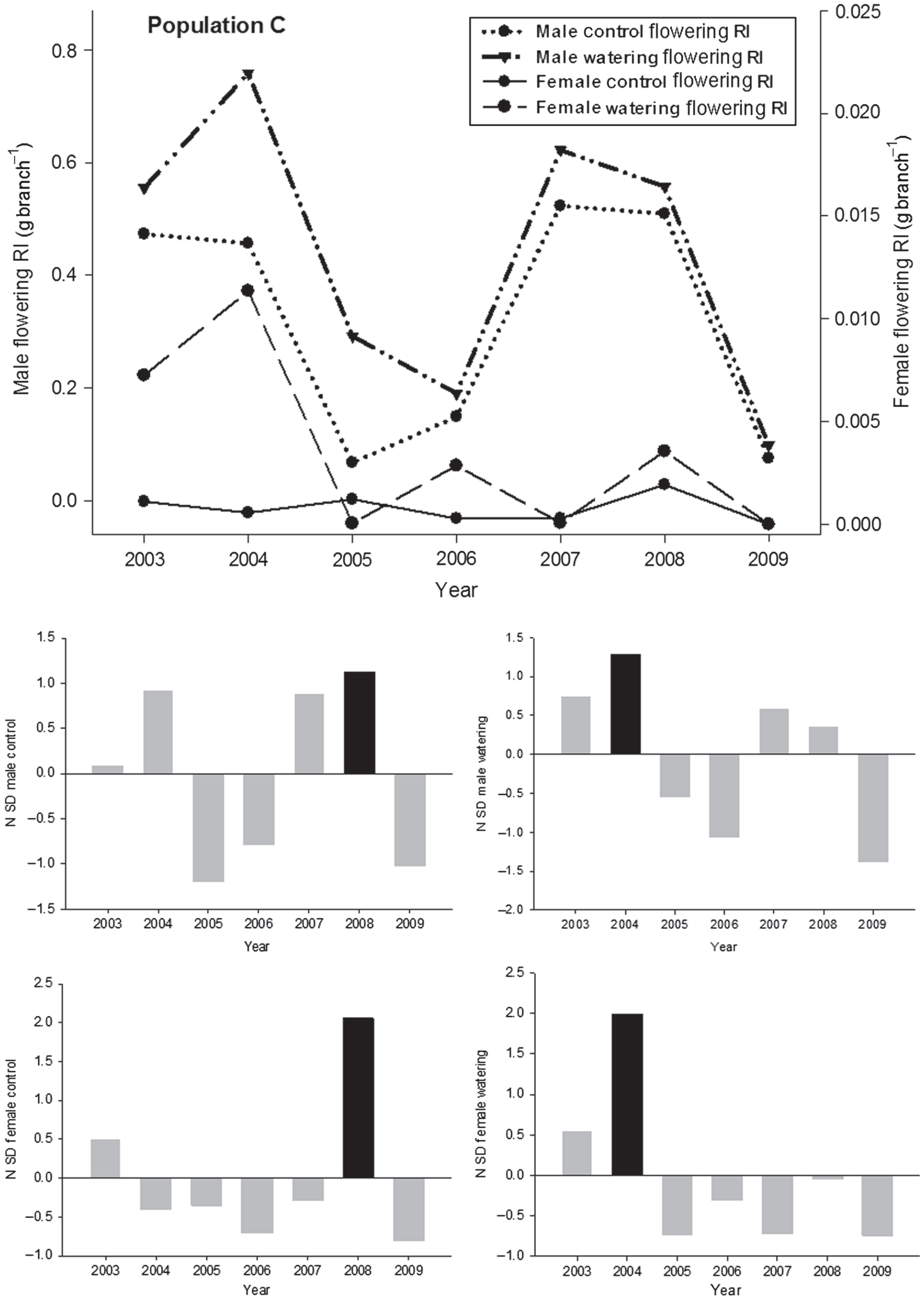

Figure 2. (Continued)

a nomenclature common in the study of animal resource use, males would act as 'income breeders' and females as 'capital breeders' (Stearns 1992, Beck et al. 2003). Coherently, our data also suggests that *J. thurifera* males and females are using their resources in a different temporal frame, with males using current resources for reproduction and females using resources accumulated during longer periods, making costs of reproduction undetectable on the short term and optimizing bet-hedging strategies in female trees thanks to low flowering costs. This implies that resource allocation patterns and tradeoffs might need to be studied in different temporal windows in order to be detected.

Experimental increase of resource availability did increase female flowering and did trigger a mast-flowering event (2004). Such mast-flowering did not result in a mast-seeding event though. This suggests that although the ultimate causes of masting might be adaptative (Kelly and Sork 2002,

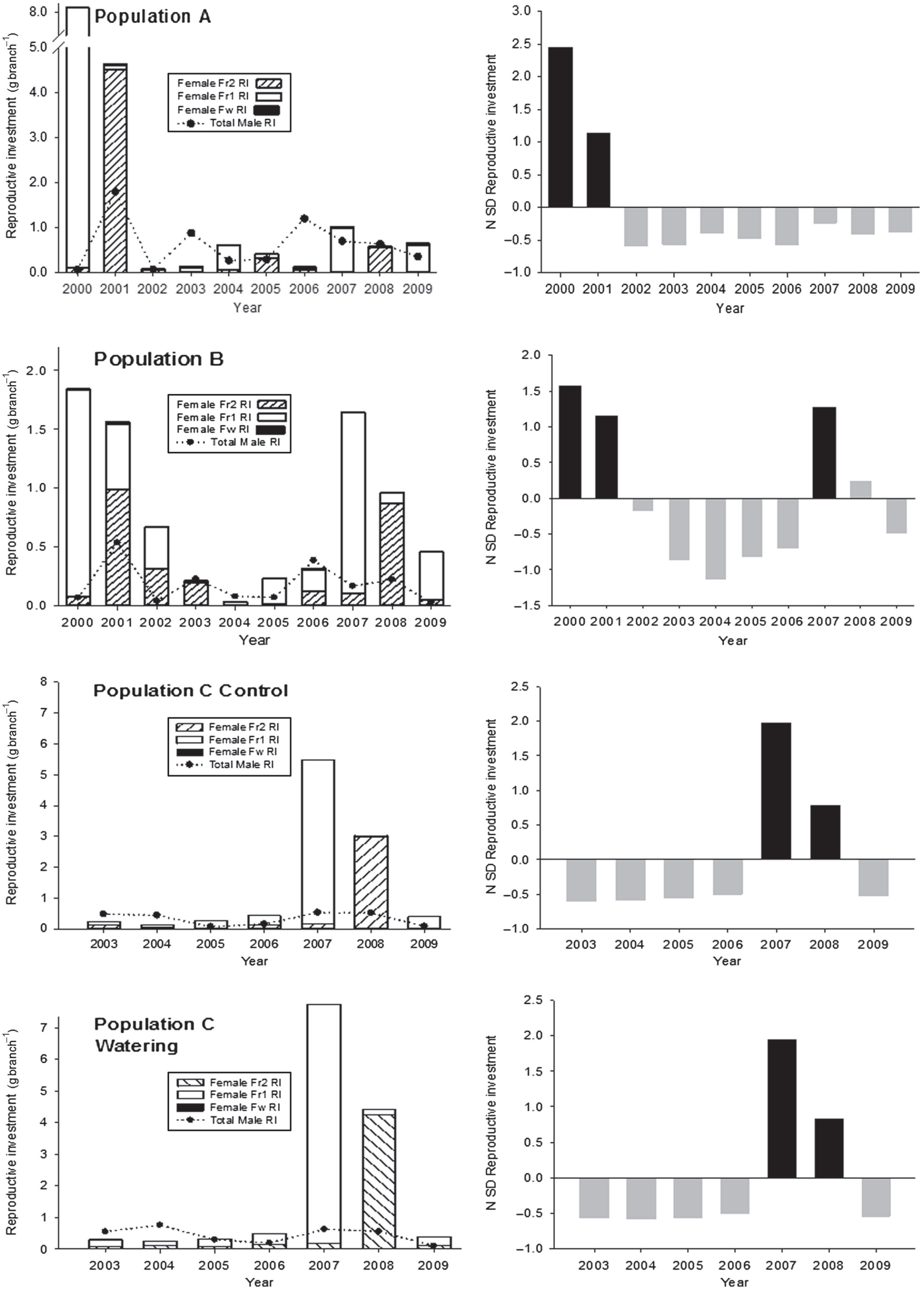

Figure 3. Total mean reproductive investment per branch in g (left). Females' RI is divided into flowering (Fw), 12 months green fruits (Fr1), and 22 months ripe fruits (Fr2). Number of standard deviations of annual seeding RI from the long-term inter-annual mean (right). Black bars represent years in which N SD value was on or above the absolute value of the lowest standardized deviate of each population. Due to *J. thurifera* fruit development particularities (see Methods), a masting year will typically occur a year after an RI peak (Discussion).

Table 2. Effect of sex and year on reproductive investment (RI) for all the studied populations and on tree-ring growth and $\delta^{13}C$ (population B). F-values and their significance levels were calculated by linear mixed models accounting for temporal autocorrelation among years. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

	Sex	Year	Sex × Year
RI population A	20.66***	18.99***	14.87***
RI population B	14.68***	6.77***	6.75***
RI population C - control	15.49***	7.20***	7.25***
RI population C - watering	12.13**	5.10**	4.60**
Tree-ring growth (population B)	0.07	25.07***	0.33
$\delta^{13}C$ (population B)	1.58	1.29	0.13

Rees et al. 2002, Satake and Bjørnstad 2004, Piovesan and Adams 2005) resource availability does play an important role in defining mast-flowering at least for females, the gender with higher reproductive investments. Interestingly, the mast-seeding event detected in most populations on 2008 was not preceded by a mast-flowering event on population C during 2006. This suggests that *J. thurifera* is able to produce high seed crops even from initially small flower-sets, supporting masting uncoupling in yet another, unexpected way.

Anyhow the detected uncoupling between flowering and seeding implies a waste of flowering investments and reduces the number of significant reproductive events from three or four to only one or two every ten years. Can this be adaptative? In the introduction we enumerated a number of conditions for plants to benefit from bet-hedging. Trees would benefit from producing flowers more often than they are capable to produce seeds if bad years are unpredictable and if flowering costs are low. Under such circumstances at least some of the mast-flowering episodes would be able to produce seeds, while investing all resources during fewer mast-flowering events could be risky if resources become unpredictably low or other catastrophic event takes place. *J. thurifera* female flowering represented less than 1% of the total inter-annual females' reproductive investment and only 2% of that of males (see Fig. 2 and note that males and females are drawn in different scales). Therefore making 'mast-flowering bets' would be cheap in terms of resources, particularly for female trees, which are investing overall three-fold more resources to reproduction than males. On the other hand resource scarcity is limiting reproduction in *J. thurifera*, and year to year small differences in nutrients and water availability can have a significant impact on seed set (Montesinos et al. 2010). Under such circumstances, small unpredictable variations in annual precipitation or nutrient availability can have a significant effect on reproduction, synergistically benefiting bet-hedging.

A major environmental factor potentially causing masting uncoupling could be extraordinary drought events or extraordinary high or low temperature events. We explored the relationship between reproduction variables and climatic data during the studied period for populations A and B (precipitation, summer precipitation, minimum and maximum mean temperatures and absolute minimum and maximum temperatures) and did not find any significant relationship between precipitation shortage or extreme temperature events and the failed mast-seeding events or to RI (data not shown). However, our data set is relatively short, accounting with only one or two mast-seeding failures after only three mast-flowering episodes, and therefore we cannot discard these or other environmental factors as the causes for massive reproductive losses. Anyhow, these catastrophic or climatic events could play a role in population synchrony (Schauber et al. 2002, Kelly and Sork 2002, Kelly et al. 2008), although a longer temporal series would be needed to study it.

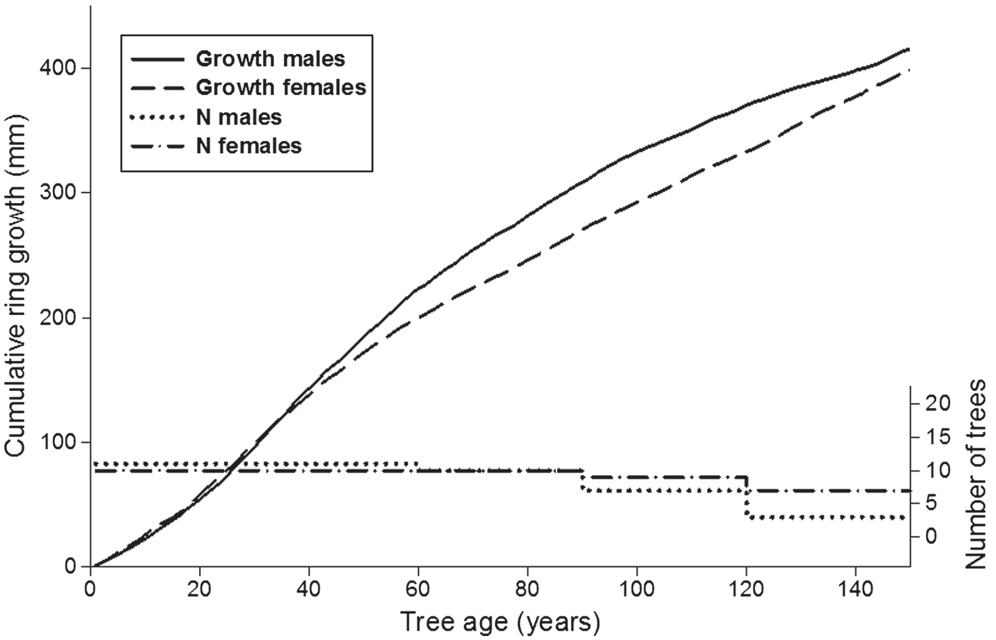

Figure 4. Relationship between cumulative ring growth and tree age. *J. thurifera* male and female trees grow similarly until sexual maturity (approx. 30 years), after which male trees grow significantly more than female trees. Sample sizes are indicated. From Montesinos et al. (2006) by permission of Annals of Botany and Oxford Univ. Press.

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