Increased drought reduces acorn production in *Quercus ilex* coppices: thinning mitigates this effect but only in the short term

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Summary

In order to explore the effects of climate change on Mediterranean regenerating forests, we experimentally assessed the effects of increased drought on the reproductive attributes of *Quercus ilex* over a 4-year period (2005–2008). We also investigated whether traditional thinning (selection of one to a few stems per stump) could mitigate the consequences of increased drought in oak coppices. Increased drought reduced the number of reproductive trees, mean number of female flowers produced and acorn crop size, although most of these effects appeared only in the last 2 years of the experiment. In a different way, thinning enhanced all reproductive attributes, but its main effects were transient and covered only 1 or 2 years after the application of the treatments. Our results indicate that a moderate reduction in rainfall (15 per cent) reduces the reproductive ability of *Q. ilex*. This may have long-term negative consequences for recruitment as well as for the fauna feeding on acorns. Although traditional thinning may mitigate the consequences of increased drought, it has a remarkably short-term effect. This highlights the need to re-examine traditional forestry practices as potential adaptive strategies for coping with climate change in Mediterranean regenerating forests.

Introduction

Climate change means new challenges for forest conservation (Chapin *et al.*, 1990). The increase in temperature by ~ 0.8°C and the decrease in precipitation by ~5 per cent in the Mediterranean Basin during the last century (Begni *et al.*, 2001) have led to increased evapotranspiration and aridity (Piñol *et al.* 1998; De Luis *et al.*, 2001). Summer drought is already one of the main factors limiting plant distribution and growth in Mediterranean-type climates (Mooney, 1983; Terradas and Savé, 1992); if climatic conditions become even drier, Mediterranean ecosystems will be particularly affected (Ogaya *et al.*, 2003, Limousin *et al.*, 2010, Ozturk *et al.*, 2010). Whether forests will be able to adapt to such conditions is a matter of concern (Scarascia-Mugnozza *et al.*, 2000; Sabaté *et al.*, 2002). In this context, it becomes crucial to find appropriate forest management strategies that mitigate the impacts of climate change on forests (Lindner, 2000; Resco *et al.*, 2007; Cotillas *et al.*, 2009).

The negative consequences of increasing drought have been thoroughly documented in Mediterranean-type forests, such as the recurrence of tree mortality episodes (Martínez-Vilalta and Piñol, 2002; Lloret *et al.*, 2004) and reduction of primary and secondary growth (Corcuera *et al.*, 2004a, b; Cotillas *et al.*, 2009). In comparison, the potential effect of drought on tree reproduction has been much less explored. For example, a shortage in water availability may constrain seed production by reducing flowering (Ogaya and Peñuelas, 2007a) and increasing seed abortion (Espelta *et al.*, 2008). Drought may affect not only the magnitude of the final seed crop size produced, but many other parameters related with reproduction as well, such as: (1) delaying the reproductive onset of trees (Moya *et al.*, 2008), (2) altering the phenology of seed production (Peñuelas *et al.*, 2002), (3) decreasing seed size (Long and Jones, 1996) and (4) increasing interannual variability in seed crop size (Kelly and Sork, 2002; Espelta *et al.*, 2008). These changes may trigger negative effects on tree recruitment

either reducing seed supply (Espelta *et al.*, 2008; Espelta *et al.*, 2009b) or limiting seedling establishment (Lloret *et al.*, 2004). This can be especially dramatic in young forests regenerating after disturbances by constraining the successional dynamics and the colonization of new sites.

The evergreen *Quercus ilex* L. (holm oak) is one of the most widespread tree species in the Mediterranean Basin, present over a large area extending 6000 × 1500 km, from Portugal to Syria and from Morocco and Algeria to France (Quezel and Medail, 2003). Due to a long history of repeated disturbances (e.g. wildfires, clear-cutting, livestock grazing), most Mediterranean *Q. ilex* forests are 'coppices' of multi-stemmed stumps with slow growth rates (Terradas, 1999). Acorn production and seedling establishment in regenerating oak populations are crucial for long-term maintenance (Ducrey and Toth, 1992; Retana *et al.*, 1999) because (1) sexual reproduction is the only mechanism that confers genetic variability and (2) new areas can be colonized when animals disperse the acorns. Because the re-sprouting oaks produce low acorn crops during the first years after the mentioned disturbances, the few acorns that are produced are especially important for oak regeneration. However, difficulties in the sexual regeneration of Mediterranean oak coppices have been thoroughly documented (Espelta *et al.*, 1995; Gracia *et al.*, 2001; Cañellas *et al.*, 2004; Acacio *et al.*, 2007). This situation may become even worse if increasing drought conditions lead to a decrease of acorn production.

One practice traditionally employed to improve the performance of oak coppices is the conversion of those coppices to high forests by cutting the weakest stems, leaving one or two of the best stems per stump (Serrada *et al.*, 1996). This thinning may reduce the competition for resources, such as water and nutrients among the remaining stems (Cañellas *et al.*, 2004; Moreno *et al.*, 2007), and thus result in an increase in tree growth and survival (Retana *et al.*, 1992; Espelta *et al.*, 2003; Rodríguez-Calcerrada *et al.*, 2008).

Despite the importance of seed supply for long-term maintenance of plant populations, the consequences of increased drought on the reproductive patterns of holm oak forests have seldom been explored (but see Ogaya and Peñuelas, 2007a). Moreover, whether or not the traditional thinning can mitigate these potential negative effects remains elusive. Therefore, the main aims of this study were (1) to analyse the effect of increased drought on several reproductive traits of *Q. ilex* (onset of reproduction, flowering intensity, seed crop size and size of acorns) in coppices regenerating after fire and (ii) to assess whether traditional thinning conducted in these oak coppices can reduce the potential negative effects of increased drought. Furthermore, we examine whether the effects of drought and thinning may vary with time, as very few experiments study their influence for several years after the treatment (for different *Quercus* sp. see Hanson *et al.*, 2001; Ogaya *et al.*, 2003; Limousin *et al.*, 2009).

To achieve these objectives, we performed a 4-year experiment (2005–2008) in a *Q. ilex* coppice, in which we factorially combined two experimental treatments: drought (increased versus natural drought) and thinning (thinning versus no thinning). We expect that our results will help foresters understand the reproductive performance of this Mediterranean oaks under increased drought and will aid in the development of thinning guidelines for these forests to promote forest regeneration and conservation in light of climate change.

Materials and methods

Study site and species

The study site is located in the region of Bages, Catalonia, NE of Spain (41° 44′ N, 1° 39′ E), at a mean elevation of 800 m above the sea level. The mean annual temperature is 12°C and annual precipitation is 600 ± 135 mm. Climate in the area is dry-subhumid Mediterranean according to the Thornwaite index (Thornwaite, 1948). The forest is an oak coppice dominated by multi-stemmed stools, regenerating after a wildfire in 1998 (Espelta *et al.*, 2002). For further details about the study site, see Cotillas *et al.* (2009). *Q. ilex* is the dominant tree species accompanied by the winter-deciduous *Q. cerrioides* Willk. & Costa and scattered Aleppo pine (*Pinus halepensis* Mill.). *Q. ilex* is a Mediterranean evergreen oak that flowers from April to May; acorns mature during the summer and are dispersed from September to December of the same year (Espelta *et al.*, 2009a).

Experimental design and sampling protocol

The experimental study was conducted from January 2005 to November 2008. In order to test for the effects of drought and thinning on the reproductive patterns of *Q. ilex* trees, we factorially combined two experimental treatments: drought (natural drought versus increased drought) and thinning (thinning versus no thinning), with three replicates (plots) per combination. The 12 plots (15 × 15 m) were randomly distributed in the sampling area. Increased drought was simulated by means of drainage channels installed across the experimental plots to intercept precipitation (Figure 1). The drainage channels were 15 cm wide and were installed at 50 cm above the ground, every 0.5–1 m across the plot, resulting in approximately a 15 per cent reduction in precipitation. Thinning was conducted from below in a manner traditionally done in these coppice stands (Espelta *et al.*, 2002). The treatment consisted of removing all the smallest stems per stump, leaving from one to three of the largest stems per stump (Figure 1). On average, this resulted in a reduction in 20–30 per cent of total stump basal area per plot and an increase in growth rate of the residual stems (see Cotillas *et al.*, 2009). Soil moisture was checked regularly by means of Time Domain Reflectometers (Tekronix 1502C; Tektronix, Beaverton, OR, USA) connected to the ends of three 25-cm-long stainless steel cylindrical rods driven into the soil. Seasonal moisture averages were calculated. For the purposes of the present study, we only retained and used the mean soil moisture

values in spring and summer, as we assumed that the environmental conditions during these two seasons are the most important in determining reproductive success in Mediterranean oaks: spring conditions relate to flowering and summer water stress relates to acorn maturation (Espelta *et al.*, 2008; B. Sánchez-Humanes, unpublished results).

After the experimental treatments were applied, we inventoried and tagged all *Q. ilex* individuals (multi-stemmed stumps) present in the plots. We measured the density of trees per plot and basal diameter of all stems per stump every year in February, before the onset of vegetative growth. The sum of the basal area of all stems per stump (stump basal area) was calculated as an overall measure of tree size (Cotillas *et al.*, 2009). The main structural characteristics of the experimental plots assigned to the different

drought and thinning levels at the onset of the experiment are shown in Table 1 (see also Cotillas *et al.*, 2009).

The reproductive response of *Q. ilex* trees to the experimental treatments was assessed by measuring (1) production of female flowers, (2) production of fertilized acorns (number of acorns in the middle of summer, after non-fertilized flowers have dropped) and (3) production of mature acorns (number of fully mature acorns, before acorn dispersal) every year in all oaks per plot. Because of the small size of the oaks, we were able to make absolute counts of the abovementioned variables for every oak. To test the effects of the experimental treatments on the size of acorns, up to 30 sound mature acorns were randomly collected every year from each plot. The acorns' length and width were measured with the aid of a digital calliper, and their volume was calculated as the volume of a spheroid. The
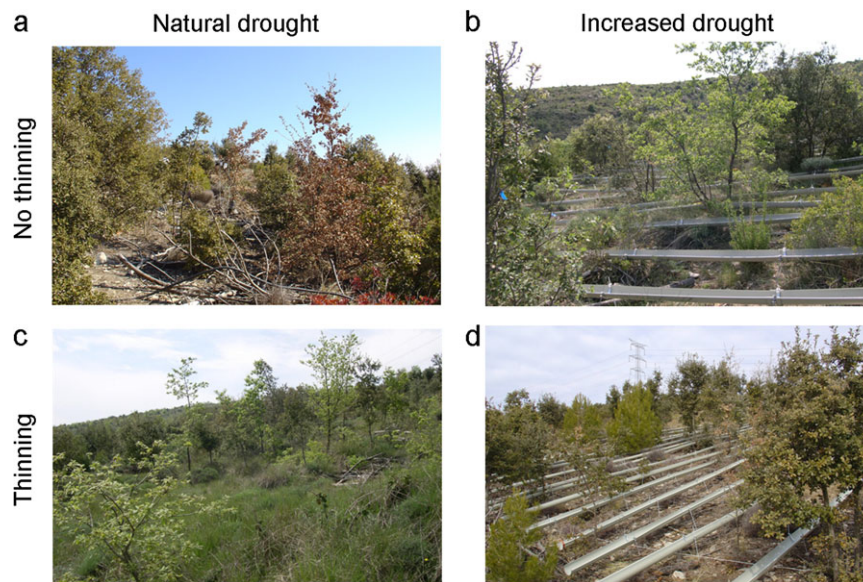

Figure 1. Experimental treatments. Picture of the experimental plots, showing the four combinations of the applied treatments: (a) no thinning and natural drought, (b) no thinning and increased drought, (c) thinning and natural drought and (d) thinning and increased drought.

Table 1: Main structural characteristics of *Quercus ilex* in the experimental plots assigned to the two drought and thinning treatments at the onset of the experiment

Plot	Drought	Forest thinning	Stem density (i ha^{-1})	Stump density (i ha^{-1})	Mean stump basal area (cm^2)	Mean stem height (cm)
1	Natural drought	No thinning	6630	2127	18.9	108
2	Natural drought	No thinning	4307	1335	32.5	138
3	Natural drought	No thinning	5661	1506	41.1	156
4	Natural drought	Thinning	3176	2175	23.3	164
5	Natural drought	Thinning	3746	2087	31.2	182
6	Natural drought	Thinning	2622	1766	19.5	141
7	Increased drought	No thinning	7597	2377	34.2	138
8	Increased drought	No thinning	5383	1630	26.2	125
9	Increased drought	No thinning	3188	1204	23.6	111
10	Increased drought	Thinning	2499	1781	18.9	139
11	Increased drought	Thinning	4197	2912	15.8	151
12	Increased drought	Thinning	2436	1638	22.4	160

acorns were dried (70°C, 96 h) and weighed to the nearest 0.001 g. Due to the low acorn production in some of the experimental treatments and the occurrence of poor seeding years (see the Results section), mean acorn volume and dry weight were calculated on a per plot basis.

Data analysis

The effects of the drought and thinning treatments on spring and summer soil moisture were analysed through two separate repeated measures analysis of variance (ANOVA) models. In these models, thinning and drought treatments were the independent variables, plot was the subject variable and year was the intra-subject variable. We conducted two separate analyses for spring and summer seasons, respectively, because the moderate number of replicates (three plots) for each combination of drought and thinning levels did not allow us to conduct a more detailed analysis including both season (spring and summer) and year (2004–2007) as intra-subject (two repeated) variables.

The effect of the experimental treatments on the yearly proportion of flowering trees (i.e. trees that flowered, independently of whether female flowers became mature acorns or not) and fruiting trees (i.e. trees that produced mature acorns) was analysed by means of a repeated measures Generalized Linear Model with a binomial error and a logit link function. In these analyses, plot was the subject variable and year the intra-subject variable. We used tree density as a covariate to control for differences among the experimental plots (Table 1, see also Cotillas *et al.*, 2009). In a similar way, the effects of the experimental treatments on the number of female flowers and mature acorns produced per tree were analysed by means of a repeated measures Generalized Linear Mixed Model with a Poisson error and a log link function, where tree was the subject variable and year was the intra-subject variable. In these analyses, we included plot as a random factor and basal area of each individual as a covariate to control for differences in tree size. The year 2006 was dropped from the analysis of the number of female flowers because we lacked quantitative data for that year.

To assess whether the effects of drought and thinning might have a different influence in the process of pollination and fertilization (i.e. the proportion of female flowers that became fertilized acorns) and acorn development (the proportion of fertilized acorns that reached maturity), we conducted a repeated measures Generalized Linear Mixed Model analysis with a Poisson error and a log link function. In this analysis, the dependent variable was the number of acorns in a precise stage of development (fertilized or mature acorns per tree) and the offset variable was the number of acorns in the previous stage (flowers or fertilized acorns, respectively), both transformed to their logarithmic form. The independent variables included in the analysis were thinning, drought, year and the stage of acorn development (fertilized acorns versus mature acorns). Stage was the intra-subject variable, which is repeated for every combination of tree × year (subject variable). As in the previous analysis, plot was introduced as a random factor and basal area as a covariate.

The influence of the experimental factors and year in mean acorn size per plot was analysed by means of a repeated measures ANOVA.

To evaluate whether the effects of increased drought and thinning on a per tree basis resulted in general differences among forest plots, we carried out a Generalized Linear Model of the effects of the experimental factors and year on the total number of mature acorns produced per plot (acorns ha−1). To control for differences in forest structure among plots, we included oak density as a covariate in this analysis.

All the mentioned Generalized Linear Models were performed with SPSS statistical software ver. 15.0 by using the GENLIN procedure. Quasi-likelihood models were used to deal with overdispersion.

Results

Soil moisture in spring and summer showed a high interannual variability (factor year: $F = 95.5$, $P < 0.001$ and $F = 25.5$, $P < 0.001$, respectively), as expected in a Mediterranean-type climate: soil moisture values in spring were lower in 2005 (10.2 ± 0.3 per cent) and 2006 (9.7 ± 0.3 per cent) than in 2007 (17.9 ± 0.5 per cent) and 2008 (16.4 ± 0.7), while values in summer were higher in 2005 (11.1 ± 0.3 per cent) in comparison to the rest of the years (9.0 ± 0.4 in 2006, 8.9 ± 0.5 in 2007 and 8.5 ± 0.2 in 2008). Throughout the 4 years monitored, the mean soil moisture in spring was higher in natural- than in increased-drought plots (respectively, 14.3 ± 0.4 per cent versus 12.8 ± 0.4 per cent; $F = 6.2$, $P = 0.038$), while the interaction thinning × year ($F = 3.5$, $P = 0.030$) revealed higher values in thinned than in non-thinned plots only in the first 2 years after the experimental treatment was applied (2005 and 2006 in Figure 2A). Soil moisture values in summer were not influenced by thinning ($F = 0.2$, $P = 0.694$) but they changed according to the interaction drought × year ($F = 4.1$, $P = 0.017$): no differences were found among natural- and induced-drought plots in 2005 and 2006, but lower values of soil moisture were observed in plots subjected to increased drought in 2007 and 2008 (Figure 2B).

Only 31 per cent of oaks flowered at least one of the 4 years of study, while this percentage decreased to 23 per cent with regard to fruiting trees. The mean size (basal area) of flowering and fruiting trees was higher than for non-reproductive ones (respectively, 39.15 ± 2.16 cm^2 versus 10.21 ± 0.93 cm^2 and 43.08 ± 2.69 cm^2 versus 14.18 ± 1.08 cm^2). Concerning the two experimental factors, most reproductive characteristics of oaks significantly differed according to the drought and thinning treatments applied (Tables 2 and 3), although the sign and the strength of these differences varied with time (interactions with the Year effect in Tables 2 and 3). Interestingly, an interaction between drought, thinning and year was observed for the proportion of flowering trees and the mean number of female flowers produced per tree, yet neither for the proportion of fruiting trees nor for their mean acorn crop size. As shown in Figure 3A, the combined effects of thinning and drought on the proportion of flowering trees differed
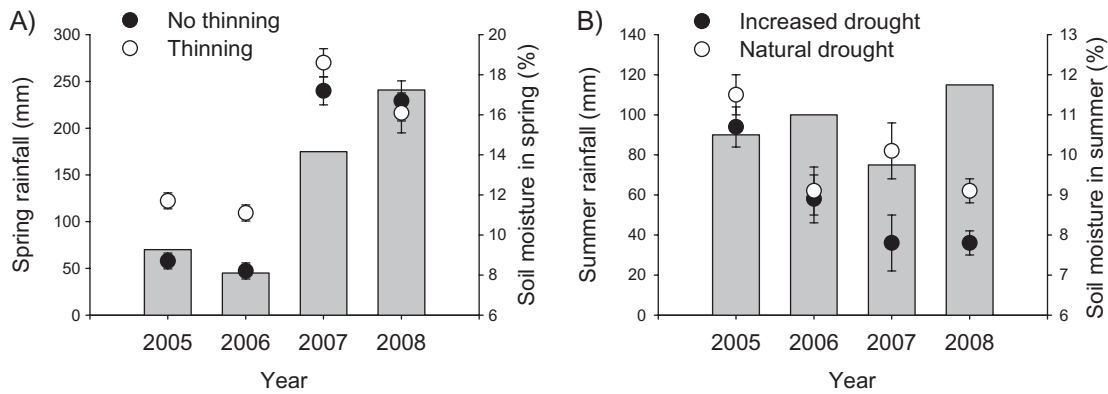
Figure 2. Rainfall and soil moisture in thinning and drought treatments. Annual rainfall (in grey bars) and mean ± SE soil moisture values in spring in thinned versus no thinned plots (A) and in summer in natural versus increased drought (B) during the 4 years monitored in this experiment (2005, 2006, 2007 and 2008). *N* = 48.

Table 2: Results of the repeated measures Generalized Linear Model for the effects of drought (natural versus increased), forest thinning (thinning versus no thinning), year (2005, 2006, 2007 and 2008) and tree density on the proportion of flowering and fruiting trees per plot

		Flowering trees		Fruiting trees	
Effects	d.f.	Chi-square test	*P*	Chi-square test	*P*
Drought (D)	1	0.5	0.482	0.1	0.796
Thinning (T)	1	**4.5**	**0.033**	**5.5**	**0.019**
D × T	1	0.1	0.898	0.1	0.742
Year (Y)	3	**259.2**	**<0.001**	7.0	0.073
Y × D	3	**28.0**	**<0.001**	**12.5**	**0.006**
Y × T	3	**11.7**	**0.009**	**51.7**	**<0.001**
Y × D × T	3	**60.4**	**<0.001**	2.8	0.417
Tree density	1	0.7	0.389	1.5	0.228

Significant coefficients at α = 0.05 are indicated in bold. *N* = 48.

Table 3: Results of the repeated measures Generalized Linear Mixed Model for the effects of drought (natural versus increased), forest thinning (thinning versus no thinning), year (2005, 2006, 2007 and 2008), plot and tree size (basal area) on the number of female flowers and mature acorns produced per tree

		Female flowers			Mature acorns	
Effects	d.f.	Chi-square test	*P*	d.f.	Chi-square test	*P*
Drought (D)	1	**10.7**	**0.001**	1	**7.6**	**0.006**
Thinning (T)	1	**14.7**	**<0.001**	1	**9.3**	**0.002**
D x T	1	1.2	0.267	1	0.1	0.724
Year (Y)	2	**50.5**	**<0.001**	3	**92.0**	**<0.001**
Y x D	2	2.6	0.274	3	**11.0**	**0.012**
Y x T	2	**17.9**	**<0.001**	3	**43.4**	**<0.001**
Y x D x T	2	**8.4**	**0.015**	3	4.4	0.225
Plot	8	14.5	0.069	8	**33.7**	**<0.001**
Tree size	1	**75.5**	**<0.001**	1	**14.8**	**<0.001**

For female flowers, the analysis was restricted to 2005, 2007 and 2008. Significant coefficients at α = 0.05 are indicated in bold. *N* = 336 and 244, respectively.
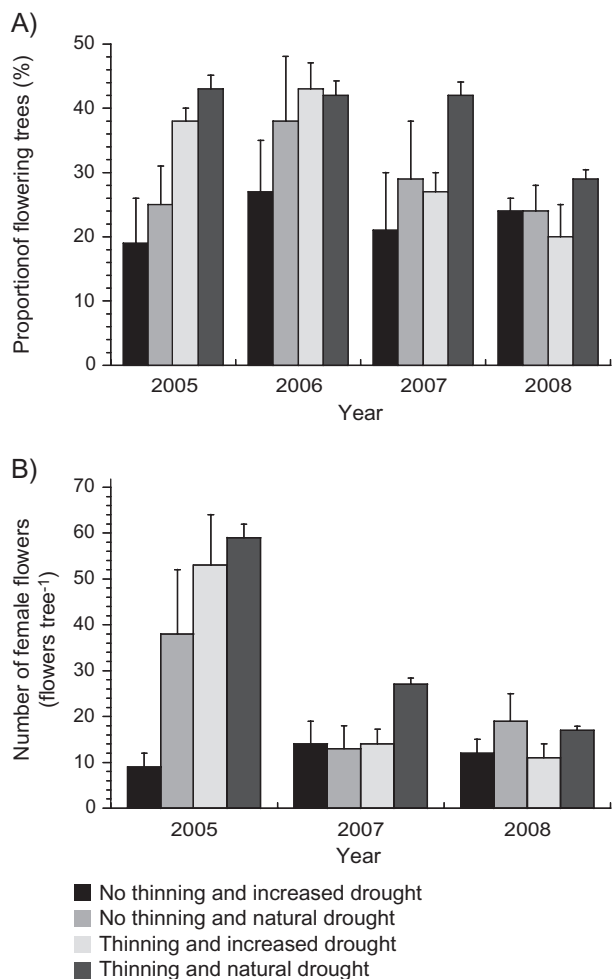

Figure 3. Flowering trees and number of female flowers under thinning and drought treatments. (A) Mean ± SE proportion of flowering trees (*N* = 48) and (B) number of female flowers produced per tree (*N* = 336) in thinned and no thinned plots subjected to natural or increased drought during the 4 years experiment.

over the 4 years of study, with three remarkable results: (1) no differences throughout the experiment were observed among non-thinned plots subjected either to natural or to increased drought, (2) thinning enhanced the proportion of flowering trees in comparison to non-thinned plots, only the first year after the experiment onset (2005), and (3) significant differences among the two most negative and positive situations in the hypothetical gradient of resource availability (non-thinned plots subjected to increased drought versus thinned plots subjected to natural drought) were observed in 3 of the 4 years (2005, 2006 and 2007). A similar effect of the interaction between drought and thinning was observed for the mean number of female flowers produced per tree (Figure 3B): i.e. (1) thinning enhanced the mean number of flowers produced per tree (both in natural- and increased-drought conditions) only the first year after the experiment onset (2005) and (2) the positive effects of thinning decreased with time, especially in thinned plots subjected to increased drought.

In addition to the reported effects on flowering, thinning and drought also significantly influenced the proportion of fruiting trees as well as the number of acorns produced, although the two experimental factors did not interact (Tables 2 and 3). As previously shown for flowering (see above), thinning enhanced the proportion of fruiting trees and their mean acorn crop size but with a transient and short-term effect: i.e. according to the interaction thinning × year (Tables 2 and 3) differences between thinned and non-thinned plots were only observed in 2005 (Figure 4A and B). Whereas differences due to thinning were only observed at the beginning of the experiment (2005), differences between the two drought levels appeared mostly in 2007 and 2008 (interaction drought × year in Tables 1 and 2): i.e. in plots subjected to increased drought, the proportion of fruiting trees was lower both in 2007 and 2008 (Figure 5A) and so was their mean acorn crop size (Figure 5B).

The results of the detailed analysis of drought and thinning effects on the likelihood of success during the two stages involved in acorn development (from female flowers to fertilized acorns and from fertilized to mature acorns) are shown in Table 4. The developmental stage had a significant influence on the overall success of acorn production: 25 ± 2 female flowers per tree were produced. Roughly, 66.7 ± 0.2 per cent of the female flowers became fertilized acorns, yet only 30.1 ± 0.2 per cent of fertilized acorns reached maturity. However, differences in the relevance of these two processes were found between years (interaction year × stage in Table 4): differences were much larger in 2007 in comparison to 2005 and 2008 (89 ± 2 per cent and 25 ± 2 per cent of acorn fertilization and maturation in 2007 versus 57 ± 3 and 34 ± 3 per cent in 2005 and 2008). On the contrary, the effects of drought and thinning did not vary over the 4 years. The interaction thinning × stage indicates that the effect of thinning on increasing mature acorn crop size was due not to an effect on the number of flowers fertilized (68 ± 2 per cent in thinned versus 65 ± 3 per cent in non-thinned plots), but mostly to an increase in the number of fertilized acorns that reached maturity (33 ± 2 per cent in thinned plots versus 25 ± 3 per cent in non-thinned plots). In a different way, the significance of the drought effect, yet the lack of any interaction with the factor stage (Table 4), indicates that increased drought reduced the size of the mature acorn crop due both to a decrease in the proportion of fertilized flowers and the proportion of acorns that reached maturity (accounting for both processes together, 20 ± 2 per cent of flowers became mature acorns in plots under natural-drought conditions versus 19 ± 2 per cent in plots subjected to increased drought).

Differences on a per tree basis resulted in differences among plots on the total number of acorns produced: i.e. acorn production decreased with drought and increased with thinning, although there was a high year to year variability (respectively, $\chi^2 = 180.2$, $P < 0.001$ and $\chi^2 = 17.4$, P <0.001). Acorn crop size was higher in 2005 and 2007 in thinned plots (5169 ± 1062 versus 582 ± 189 and $5030 \pm$
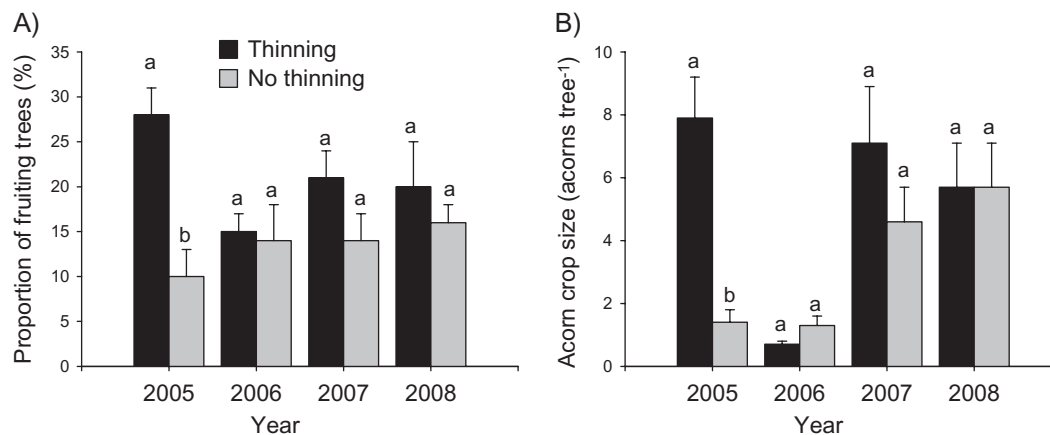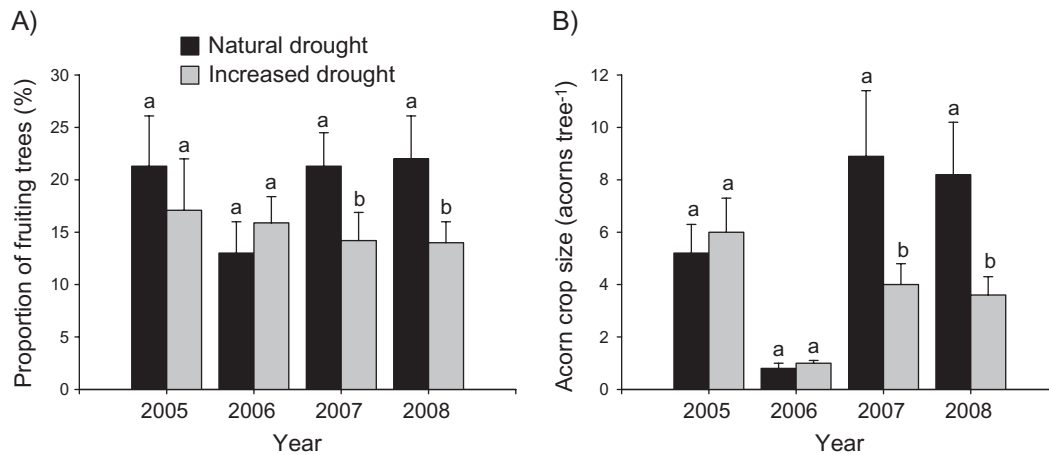

Figure 4. Fruiting trees and number of acorns in thinned versus no thinned plots. (A) Mean ± SE proportion of fruiting trees ($N = 48$) and (B) number of mature acorns produced per tree ($N = 244$) in thinned and no thinned plots during the 4 years experiment. Different letters indicate significant differences among the two thinning levels per year according to the least squares means test.

Figure 5. Fruiting trees and number of acorns in natural- versus increased-drought plots. (A) Mean ± SE proportion of fruiting trees ($N = 48$) and (B) number of acorns produced per tree ($N = 244$) in plots subjected to natural or increased drought during the 4 years experiment. Different letters indicate significant differences among the two drought levels per year according to the least squares means test.

Table 4: Results of the repeated measures Generalized Linear Mixed Model for the effects of drought (natural versus increased), forest thinning (thinning versus no thinning), year (2005, 2007 and 2008), stage of acorn development (fertilized, mature) plot and tree size (basal area) on the likelihood of survival during acorn development (from female flowers to fertilized acorns and from this stage to mature acorns)

Effects	d.f.	Survival during acorn development	
		Chi-square test	*P*
Drought (D)	1	**13.4**	**<0.001**
Thinning (T)	1	**15.7**	**<0.001**
Stage (S)	1	**251.2**	**<0.001**
D × T	1	0.3	0.603
D × S	1	0.1	0.770
M × S	1	**4.7**	**0.030**
D × T × S	1	**3.9**	**0.048**
Year (Y)	2	5.2	0.074
Y × D	2	3.9	0.143
Y × T	2	3.7	0.154
Y × S	2	**12.6**	**0.002**
Y × D × T	2	3.16	0.206
Y × D × S	2	2.20	0.332
Y × T × S	2	1.86	0.394
Plot	8	**31.8**	**<0.001**
Tree size	1	**11.0**	**0.001**

Significant coefficients at $\alpha = 0.05$ are indicated in bold. N= 336.

1977 versus 1831 ± 580 acorns ha^{-1}), while acorn production was higher in natural- than in increased-drought plots in 2007 and 2008 (4789 ± 2082 versus 2073 ± 442 and 4636 ± 949 versus 1743 ± 579 acorns ha^{-1}).

Acorn size was barely influenced by any of the experimental treatments applied, and the only significant differences were found between years (repeated measures ANOVA, d.f. = 3, $F = 36.2$, $P < 0.0001$): mean acorn size was larger in 2005 (1.9 ± 0.2 cm^3) than in 2006, 2007 and 2008 (respectively, 0.8 ± 0.2 cm^3, 0.5 ± 0.1 cm^3, 0.8 ± 0.3 cm^3).

Discussion

This study presents two main contributions to understanding the effects of a new climatic scenario (increased drought) on *Q. ilex* coppices in the Mediterranean Basin. First, increased drought, mediated by a small reduction in rainfall (15 per cent), may not cause immediate changes in mortality or growth (see the results of Cotillas *et al.*, 2009 for the same study area) yet it may reduce the reproductive ability of *Q. ilex*. This may have long-term negative consequences for the regeneration and maintenance of these oak forests as well as for the fauna feeding on acorns. Second, traditional thinning methods enhance reproduction ability and it may mitigate some of the negative consequences of increased drought, yet it has a remarkable short-term effect. This highlights the need to re-examine the consideration of traditional forest thinning practices as potential strategies for coping with the effect of climate change on the reproductive success of this stands.

Although we could well simulate a decrease in soil moisture mediated by a reduction in rainfall, the negative consequences of increased drought observed in this study may be partly underestimated when compared with those occurring under the forecasted climate change scenario in the Mediterranean Basin. In fact, our design incorporated neither the raising of temperatures predicted nor the occurrence of extreme drought episodes in summer (IPCC, 2007). The inclusion of these factors could make the positive impact of thinning on increasing reproductive ability even more transient.

It is a well-known fact that plants need to achieve a certain size before they start reproducing because previous accumulation of resources is necessary (for oaks, see Goodrum *et al.*, 1971; Abrahamson and Layne, 2002a, b). Interestingly, although traditionally viewed as a species

that bases its resilience to fire on a successful re-sprouting strategy, in our study, *Q. ilex* began to produce acorns at least at a post-fire regeneration age of 7 years (similar to the results of Abrahamson and Layne, 2002a for oak species in xeric and fire-prone environments in Florida). Clearly, the mean acorn crop size in these young trees is extremely low in comparison to the values reported for more mature oak woodlands or forests, but the high density of trees in these coppices (c.a. 1500 stumps ha^{-1} in our study area) may render a large crop size on a population basis. Such a reproductive precocity in *Q. ilex* could grant this species the chance to expand its distribution soon after the fire event (Ostertag and Menges, 1994), at least in contrast to heterospecifics with a later reproductive onset (e.g. *Q. cerrioides* in our study area). Clearly, future research should be aimed at assessing whether these early acorn crops may encounter suitable micro sites for germination and establishment and the potential role played by acorn dispersers (e.g *Apodemus sylvaticus*, *Mus spretus*, *Garrulus glandarius* in our study area) in the likelihood of colonization of surrounding areas.

Increased drought reduced the number of flowering and fruiting trees and the production of female flowers and mature acorns per tree. Although *Q. ilex* is recognized as a drought-tolerant species (Terradas, 1999), seeding may be highly dependent on water availability (Siscart *et al.*, 1999; Ogaya and Peñuelas, 2007a). Interestingly, our results indicate that drought not only during summer but also during spring diminished the acorn crop size, by decreasing the production of female flowers and the proportion of those that were fertilized. This result partly contrasts with previous studies that have ascribed to the process of acorn maturation a pre-eminent role in determining acorn crop size, by considering flowering and fertilization to be two less resource-demanding processes and thus less affected by resource shortage (see Sork, 1993; Espelta *et al.*, 2008). Although soil moisture during summer was in a similar range of values during this 4-year experiment (see Figure 2), the negative consequences of increased drought for acorn production became more evident over the last 2 years (Figure 5). This suggests the potential existence of cumulative negative effects of sustained drought, either in the soil compartment (e.g. a progressive lowering of moisture in the entire soil profile) or at the plant level (e.g. a progressive exhaustion of resources needed for seed production). Unfortunately, up to now, there have been very few studies including long-term experiments to test the effect of sustained drought (but see Ogaya and Peñuelas, 2007a, b; Limousin *et al.* 2009, 2010), and they have not yet identified the ultimate cause of these potentially accumulative effects.

Thinning to remove the weakest stems per stump had a positive effect on the reproductive ability of *Q. ilex* trees. Thinned plots had a proportion of flowering and fruiting trees 1.3 and 1.4 times higher than non-thinned ones, and both flowering and acorn production increased in trees in the former plots. Numerous studies conducted in Mediterranean oak coppices have stressed the benefits of thinning to reduce intra-individual competition between stems and thus increase resource availability (light, water and nutrients) for the remaining stems (Espelta *et al.*, 2003 and references therein). From a functional point of view, thinning involves the sudden rupture in the established partition of biomass among above- and below-ground compartments, thus leading to an extraordinary increase in the root-to-shoot ratio (Canadell and López Soria, 1998; Riba, 1998). Moreover, this practice has a beneficial effect on ventilation and irradiation due to the overture of the canopy layer (Mayor and Roda, 1993), which may indirectly enhance seed production by facilitating pollination (see for the effects of thinning in pine forests Barnett and Haugen, 1995; Grayson *et al.*, 2004; Verkaik and Espelta, 2006). Therefore, the benefits of thinning in oak coppices have been extensively reported for tree growth (Ducrey and Toth, 1992; Retana *et al.*, 1992; Gracia *et al.*, 1999), and to a lesser extent for tree reproduction (Zulueta and Montero, 1982). However, very often, several studies have also highlighted the short and transient effects of this type of thinning (Cutter *et al.*, 1991; Mayor and Roda, 1993; Healy *et al.*, 1999; Espelta *et al.*, 2003). In our study, the effect of thinning on reproduction mostly disappeared only 1–2 years after the treatment was applied (Figures 4 and 5). Two main processes could be responsible for making the increase in resource availability quickly disappear and return to the previous situation of above- and below-ground biomass partitioning: a quick expansion of the canopy in the retained stems (Gracia *et al.*, 1999; M. Cotillas, unpublished results) and, primarily, the vigorous production of a new wave of re-sprouts (new stems) from the stump (Espelta *et al.*, 2003; Cotillas *et al.*, 2009). In fact, among thinned plots, the benefits of thinning for acorn production disappeared earlier in plots subjected to increased drought (see Figure 3B), where the production of new re-sprouts was more intense (Cotillas *et al.*, 2009).

Our results draw attention to the importance of drought and thinning levels for *Q. ilex* reproduction. Intriguingly, in spite of their relevance, the two experimental factors applied did not interact or interacted weakly (except for the proportion of flowering trees). Is this a consequence of the different timing they have in their effects? As our results suggest, stronger and immediate effects of thinning suppress those of increased drought but once this transient effect has disappeared, differences mediated by drought may arise. Clearly, a longer series of data, as well as new experiments combining a wider range of drought and thinning levels, would help to answer this question and to fine-tune appropriate forestry practices to help Mediterranean forests cope with climate change. In addition, further research should be conducted on the demographic consequences of a lower acorn production (recruitment and establishment of *Q. ilex* seedlings) as well as its potential consequences for the trophic cascades based on this resource.

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