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Maternal influences on seed mass effect and initial seedling growth in four *Quercus* species

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ABSTRACT

Seed mass represents the reserves available for growth in the first stages of plant establishment. Variation in seed mass is an important trait which may have consequences for growth and survival of seedlings. Three mechanisms have been proposed to explain how seed mass influences seedling development: the reserve use effect, the metabolic effect and the seedling-size effect. Few studies have evaluated at the same time the three hypotheses within species and none have evaluated the effect of the mother trees. We studied four *Quercus* species by selecting five mother trees per species. Seeds were sown in a glasshouse and the use of seed reserves, seedling growth and morphology were measured. Considering all mothers of the same species together, we did not find the reserve effect for any species, the metabolic effect was observed in all species except for *Quercus suber*, and the seedling-size effect was matched for all the species. Within species, maternal origin modified the studied relationships and thus the studied mechanisms as we did not observe seed mass effects on all mothers from each species. Moreover, the metabolic effect was not found in any mother of *Quercus ilex* and *Quercus faginea*. We concluded that a maternal effect can change seed mass relationships with traits related to seedling establishment. The conservation of this high intra-specific variability must be considered to guarantee species performance in heterogeneous environments and in particular in the current context of climate change.

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1. Introduction

Natural oak regeneration is constrained by different factors such as acorn predation, summer drought, soil impoverishment or herbivores (Pulido and Diaz, 2005; Pérez-Ramos and Marañón, 2008; Gómez-Aparicio et al., 2008). Many studies have focused on the factors driving seedling recruitment of *Quercus* at different levels (Castro et al., 2006; Pausas et al., 2006). One important trait is seed mass as it represents the amount of reserves available for the growth of an embryo in the first stages of its life, thus having important consequences on plant early establishment (Navarro et al., 2006; Pérez-Ramos et al., 2010). Larger-seeded plants could have advantages such as higher germination or emergence rates (Vázquez, 1998; Gómez, 2004; Urbietta et al., 2008) and a greater probability of survival (Gómez, 2004; Moles and Westoby, 2004; Baraloto et al., 2005), especially in adverse environments like deep shady areas (Leishman and Westoby, 1994; Saverimuttu and

Westoby, 1996) or in nutrient-poor soils (Milberg and Lamont, 1997). Moreover, seed mass seems to be a good predictor of other morphological traits describing performance of species in their environment such as specific leaf area (SLA) or leaf mass fraction (LMF) in different habitats (Poorter and Rose, 2005; Quero et al., 2008a).

Westoby et al. (1996) proposed three hypotheses to explain the mechanisms leading to a more successful establishment of larger-seeded species. Firstly, the “reserve effect” postulates that the larger-seeded seeds retain a larger amount of reserves, thus leaving a higher proportion of the reserves available to stand up to subsequent stress episodes (Westoby et al., 1996; Bonfil, 1998), and would increase survival possibilities under adverse environment conditions. The second hypothesis is the so-called “metabolic effect”, in which it is predicted that species with larger seeds have a slower relative growth rate (RGR) (Marañón and Grubb, 1993; Baraloto et al., 2005). This effect has been observed specially during the early growth of species from different ecosystems (Poorter and Rose, 2005; Pérez-Ramos et al., 2010).

Finally, the “seedling-size effect” suggests that larger seeds produce larger seedlings (Hendrix et al., 1991; Chacón and

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Bustamante, 2001; Green and Juniper, 2004; Castro et al., 2008). These seedlings can develop longer roots thus reaching deeper layers of the soil, in which there are more water resources available (Milberg and Lamont, 1997). In addition, they have a greater aboveground growth, which permits them to develop a larger photosynthetic surface.

Quero et al. (2007) have suggested that the three hypotheses are connected. Therefore, the relationship between the seed and the seedling biomass is mediated by two effects: the use of the reserves and the metabolic effect. Increasing the use of reserves has a positive effect on seedling biomass, but a strong metabolic effect (a negative relationship between RGR and seed mass) could lead to a lack of any relationship between seed mass and seedling biomass. Most studies on the implications of the variation in seed size have been carried out by comparing different species. Differences in mean seed size between species have been interpreted as being differential adaptations to a wide spectrum of ecological niches (Westoby et al., 1996). However, studies at an intra-specific level are also of interest, since they can provide a better understanding of the effect of variation in seed size on seedling establishment (Bonfil, 1998). Many studies have detected intra-specific variations in mean seed size within and between populations (Michaels et al., 1988; Ågren and Gren, 1989; Susko and Lovett-Doust, 2000; Castro et al., 2008; Rámirez-Valiente et al., 2009). This variability is also reflected in other characters like germination or aerial biomass (Sills and Nienhuis, 1995; Castro et al., 2008). This between-plant variability seems to be due to genetic differences between mother plants as well as environment conditions present at the moment of the seed's development (Wulff, 1986; Ågren and Gren, 1989; Baskin and Baskin, 2001; Castro et al., 2008; Souza et al., 2010).

Quercus genus has high genetic diversity (Michaud et al., 1992), and phenotypic plasticity (Quero et al., 2008b). The existence of inter-individual variation in acorn yields has been studied for different species (Ramírez and Gómez, 1982; Ducouso et al., 1993; Gómez, 2004). In fact, acorn size is a factor traditionally used for the selection of trees in open forest areas (*dehesas*) (Vazquez, 1998). Tilki and Alptekin (2005) found differences in germination rates among *Quercus aucheri* seeds from different provenances. Fernández-Rebollo et al. (2008) have found differences in acorn moisture percentage and chemical composition within populations of *Quercus ilex*. This high variability among and within populations is also reflected in leaf traits (Bruschi et al., 2003; González-Rodríguez and Oyama, 2005; López de Heredia and Gil, 2006) and ecophysiological characteristics (Leiva and Fernández-Alés, 1998; Himrane et al., 2004; Sánchez-Vilas and Retuerto, 2007). Therefore, the mother provenance may determine seed mass, and also other traits associated with the seedling establishment (Castro et al., 2008). Since Leiva and Fernández-Alés (1998), no specific studies in order to assess maternal effects in *Quercus* species on seed-seedling relationships have been developed.

The aims of this study were: (1) to test the three seed mass effect hypotheses (Westoby et al., 1996) at the same time for seeds collected from different mother trees, and made comparisons within and across four Mediterranean oak species and (2) to study the effect of the mother tree on seedling morphological attributes. *Quercus* acorns, as mentioned above, have a great inter-individual variability in seed mass. They also have two cotyledons, which are not photosynthetic but serve as resource storage (Bonner, 2003), making them suited for study of seed reserves. Furthermore, the study of traits related to oak establishment is of great interest due to their limited regeneration in the Mediterranean Basin (Marañón et al., 2004). To our knowledge, this is the first work that combines an inter-specific and intra-specific approach in the study of seed mass effects on establishment.

2. Material and methods

2.1. Selection of species and study area

Two evergreen species: *Q. ilex* L. subsp *ballota* (Desf.) Samp. and *Quercus suber* L., and two deciduous: *Quercus faginea* Lam. and *Quercus pyrenaica* Willd. were selected for the study in the Natural Park of Sierra de Cardeña and Montoro (Sierra Morena, Córdoba, S Spain) (38° 21' N, 3° 12' W). The Natural Park "Sierra de Cardeña y Montoro" (S Spain) has a smooth topography with slopes between 10% and 25%, and altitudes from 200 to 800 m. The climate is Mediterranean semi-humid and Mediterranean humid, and the average annual rainfall ranges from 570 to 970 mm. Temperatures are characterized by typically continental variations, with a mean temperature of 15.3 °C. Soils are mainly composed of sandy and acid granitic soils (Quero, 2007). The four species coexist in the Natural Park and show regeneration problems with a very low proportion of seedlings and saplings due mainly to herbivory and drought. Although the four species can coexist, they have different habitat preferences. *Q. ilex* is the species most represented in the Iberian Peninsula, occupying woodland formations with other *Quercus* species (*Q. suber*, *Q. faginea* and *Q. pyrenaica*) (Blanco et al., 1998). *Q. suber* is found in shady areas or with more water available in the soil than where *Q. ilex* is found. *Q. faginea* is often observed close to valley bottoms where there is a temporary flow of water, and, therefore, there is higher water availability. *Q. pyrenaica* is generally found at a higher altitude than the other *Quercus* species.

2.2. Seed collection

Five mother trees were selected for each of the species studied. The trees of each species were located in the same population and were chosen to cover a wide range of seed sizes (dry seed mass range: *Q. ilex* 0.69–6.50 g; *Q. suber* 1.02–5.12 g; *Q. faginea* 0.21–4.68 g; *Q. pyrenaica* 0.72–5.86 g). From October to December 2006, at least 200 acorns without any apparent damage were collected from each tree. Acorns were stored in a cold chamber (2–5 °C) until December, 2006. In a subsample of ten acorns from each mother tree, fresh weight was determined and then oven-dried at 70 °C for at least 48 h. For each acorn, the pericarp was removed from the cotyledons and the dry mass of each of the parts obtained. Linear regression equations between acorn fresh mass and seed dry mass were developed for each mother tree allowing to estimate the seed dry mass from the acorn fresh mass (R^2 ranging between 0.88 and 0.99) (Appendix S1, see Supplemental Data with the online version of this article).

2.3. Plant cultivation

In December 2006, 10 acorns from each mother tree were selected from the stored ones, giving a total of 200 seeds (10 acorns × 5 mothers × 4 species). These acorns did not have any fungi or signs of predation, and were selected by flotation in order to eliminate any damaged ones. After weighing each fresh acorn, they were individually sown in pots in a glasshouse at Córdoba University, Spain (37° 51'N, 4° 48'W, at 100 m above sea level). See fresh and dry seed mass mean values ± SD in Appendix S2.

The arrangement of the replicates in the glasshouse was completely random. Pots were made with PVC tubes of 50 cm in height and 10.5 cm in diameter to avoid as much as possible root interference with pots. The substrate was a mixture of peat, sand and perlite at a 2:1:1 volume. Plants were watered until saturation by a daily drip irrigation system to ensure that water was not a limiting factor in their growth. The mean temperature was 16.4 ± 7.4 °C during the three months of the experiment. The

mean \pm SD of the photosynthetic active radiation measured (with EMS7, canopy transmission meter, PPsystem, UK) during a clear day (February 2th, 2007) was $574 \pm 191 \mu\text{mol photon m}^{-2} \text{s}^{-1}$, and the total daily mean radiation throughout the experiment was $11.9 \text{ mol m}^{-2} \text{day}^{-1}$. Previous studies have not shown any effect of the glasshouse structure on light quality (red: far red ratio = 1) (Quero et al., 2007). Every two days, individual emergence and number of leaves was recorded.

2.4. Seedling harvest

During February and March 2007 (67 ± 12 days after sowing), ten seedlings for each mother tree were randomly selected and harvested when its first group of leaves was completely unfurled (following Green and Juniper, 2004). At that moment, seedlings were considered independent from the seed reserves. After carefully taking the roots out of the substrate, each plant was divided up into leaves, stem, roots and remaining cotyledons and the fresh mass of each part was obtained. Leaves of each seedling were placed in individual bags with moistened filter paper and kept in a portable icebox until they were taken to the laboratory. The rest of the samples (stems and roots) were placed in paper envelopes. Once in the laboratory, all the leaves were scanned (HP Scan-jet 6300c), placed in envelopes and oven-dried at 70°C for a minimum of 48 h, and subsequently weighed to obtain their dry mass. From the leaf images, the total leaf area of each harvested seedling was calculated using image analysis software (Image Pro-plus 4.5; Media Cybernetics, Inc. Bethesda, MD, USA).

The used seed reserves (*USR*) were calculated as $USR = M_i - M_r$, where M_i is the initial seed dry mass (determined from the regressions in Appendix S1, see Supplemental Data with the online version of this article) and M_r the dry mass of the remaining seed (cotyledon mass was only considered because this is where the seed reserves are localized). The relative growth rate (*RGR*) was calculated according to Steege et al. (1994) and Quero et al. (2007) as $RGR (\text{mg g}^{-1}\text{day}^{-1}) = (\log S - \log USR)/\text{time}$, where S is the dry mass of the seedling with no cotyledons. The efficiency in the use of the reserves was calculated as Efficiency of reserve use (%) = $S \times 100/USR$. Leaf area ratio (*LAR*) was calculated as the total area of leaves divided by the total seedling dry mass. Seedling biomass allocation—root mass fraction (*RMF*), stem mass fraction (*SMF*), and leaf mass fraction (*LMF*)—was calculated as the dry mass of root, stem, and leaves, respectively, divided by the total seedling dry mass (Hunt, 1990). Time of emergence was the time between sowing and stem emergence.

Turnbull et al. (2008) have found that because *RGR* declines as individual plants grow, it could be heavily biased by initial size. In our study, we harvested all plants at the same time (about 67 days after sowing) when the development stage was similar (when its first group of leaves was completely unfurled) to avoid this confounding factor.

2.5. Statistical analysis

We studied the difference in seed mass between mother trees for each species by an analysis of variance where seed mass was the dependent variable and mother tree the random factor.

To evaluate the three hypotheses on seed mass effect, bivariate trait relationships were analysed by fitting Standardised Major Axis (SMA) lines to log scaled variables. SMA techniques provide a better estimate of the line summarizing the relationship between two variables (i.e., the main axis along which two variables are correlated) to that of ordinary linear regression, because the residual variance is minimized in both “x” and “y” dimensions, rather than the y dimension only (McArdle, 1988; Sokal and Rohlf, 1995). The

analysis also determined the differences between the slopes obtained for each species or for each mother tree, so that a significant *P* indicated differences between the slopes of the groups studied. The free software statistics package SMART (Warton et al., 2006) was used.

For the *reserve effect* hypotheses to be supported two conditions must be fulfilled: 1) the slope of the relationship between the seed mass and the used seed reserve should be lower than 1, and, 2) the slope (*S*) of the seed mass–seedling biomass relationship should be significantly greater than the slope of the seed mass–reserves used relationship (Green and Juniper, 2004). If the slopes of these scaling relationships were the same, the ratio of reserve mass to seedling biomass would be the same across species or mothers differing in seed mass. This would be inconsistent with the idea that seedlings from larger-seeded species are better provisioned to deal with hazards than those of smaller seeded species (Green and Juniper, 2004). For the *metabolic effect* to be supported, the relationship between the seed mass and *RGR* should be significant and negative. For the *seedling-size effect* to be supported there should be a positive relationship between the seed mass and the seedling biomass. Firstly, these relationships were evaluated for each of the four species, and secondly within the species for each of the mother trees.

We set up a mixed model ANCOVA to study the effects of mother tree (random factor) and seed mass (covariable) on different variables (time of emergence, total leaf area, efficiency of use of reserves, *RMF*, *SMF*, *LMF*, *LAR*). When necessary, a logarithmic transformation of the data was made to fulfill the requirements of normality and variance homogeneity (Zar, 1984). The statistical analyses were done using STATISTICA (version 7.1, Statsoft Inc.).

Significance was fixed at the 0.05 level throughout the study. In order to control the inflation of type I error derived from repeated testing, the false discovery rate (FDR, the expected proportion of tests erroneously declared as significant) criterion was applied to repeated test tables throughout the paper. The FDR was controlled at the 5% level using a standard step-up procedure (see García, 2004).

3. Results

3.1. Inter-specific level

3.1.1. Testing the three hypotheses

The results of the SMA analysis for testing the three hypotheses are shown in Fig. 1 (analysis per species, considering together the different mother trees). For the *reserve effect hypothesis*, a significant and positive relation between seed mass and the reserves used was observed for all the species (with very high correlation coefficients ranging from 0.83 to 0.95, $P < 0.001$; Fig. 1A). The SMA slopes of *Q. ilex* ($S = 0.87$) and *Q. faginea* ($S = 0.91$) were significantly lower than 1 ($P < 0.05$) and for *Q. pyrenaica* the slope ($S = 0.87$) was near significant ($0.1 > P > 0.05$). This indicates that, in these species, an increase in the seed mass produces a proportionally lower increase in the consumption of the reserves, therefore fulfilling the first condition of the reserve effect. However, the second condition (the slope of the seed mass–seedling biomass relationship should be significantly greater than the slope of the seed mass–reserves used relationship) was not fulfilled, so that the reserve effect was not matched for any species.

For the *metabolic effect hypothesis*, there were different trends depending on the species. In *Q. ilex*, *Q. faginea* and *Q. pyrenaica*, the larger seeds had slower *RGR* (with correlation coefficients ranging from 0.38 to 0.55, $P < 0.01$, Fig. 1B). However, for *Q. suber* no relationship between the seed mass and *RGR* was observed ($P > 0.01$).

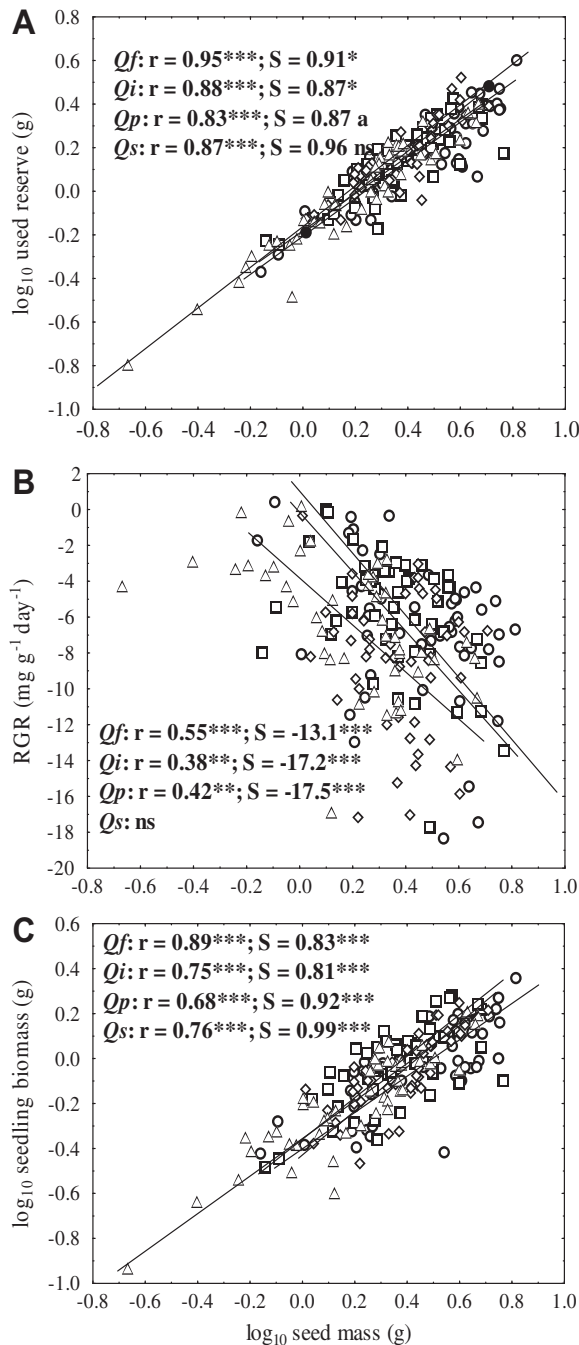


Fig. 1. Relationships of initial seed dry mass versus (A) used seed reserve, (B) relative growth rate (RGR), and (C) seedling biomass after ca. 67 days of growth in four *Quercus* species. Pearson correlation (r) and significance are indicated as: ns, not significant; a, $0.05 < P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. The standardized major axis regression (SMA) lines are given when they are significant or marginally significant ($0.1 > P > 0.05$). All values remained significant after controlling the false discovery rate. The slope of each SMA regression (S) and their significance against the null model ($S = 1$ for the reserve effect, and $S = 0$ for the metabolic effect and the seedling-size effect) is given. Δ : *Quercus faginea* (Qf); \circ : *Quercus ilex* (Qi); \square : *Quercus pyrenaica* (Qp); \diamond : *Quercus suber* (Qs).

Therefore, for the three species (*Q. ilex*, *Q. faginea* and *Q. pyrenaica*) the metabolic effect was fulfilled.

All the species showed an increase in seedling biomass related to an increase in seed mass (with correlation coefficients of between 0.68 and 0.89, $P < 0.001$) and, therefore, the seedling-size effect hypothesis was fulfilled (Fig. 1C).

3.2. Intra-specific level

3.2.1. Seed mass variability

As expected, seed mass varied across maternal trees (Fig. 2). However, some mother trees had seeds with similar sizes; for instance, no differences were found between the seeds of mothers 4 and 5 in *Q. ilex* (Tuckey HSD post-hoc test, Fig. 2). A similar situation was found for the rest of the species, since differences were found between some mother trees but not between others. All the mother trees had coefficients of variation in seed mass between 16% and 42% (Fig. 2), so there was also a variation within the seeds of one mother tree.

3.2.2. Testing the three hypotheses

For the reserve effect hypothesis, the relationship between the seed mass and the use of the reserves was positive and significant for all the mother trees of *Q. faginea* (correlations coefficients ranging from 0.74 to 0.99, $P < 0.05$, Fig. 3A). Mother trees M_1 , M_2 , M_3 and M_5 had a slope (S) non-significantly different from 1 (slopes from 0.93 to 1.15, $P > 0.05$, Fig. 3A), but mother tree M_4 showed a slope steeper than 1 ($S = 1.84$, $P < 0.001$, Fig. 3A). This indicates that an increase in seed mass produced a proportionally higher consumption of seed reserves; the opposite to the reserve effect. Seeds produced by mother tree M_4 were different in size from those of mother trees M_1 and M_2 , but no significant differences were found with mother trees M_3 and M_5 (Fig. 2). Thus, the different response was not caused by differences in seed mass. *Q. ilex* showed a significant and positive relationship between seed mass and the reserves used for mother trees M_1 , M_2 and M_5 (correlation coefficients ranging from 0.78 to 0.88, $P < 0.05$, Fig. 3A). The SMA slope for mother M_1 and M_2 was not different from 1 but that of mother tree M_5 ($S = 1.81$) was higher than 1 ($P < 0.01$) (Fig. 3A), which was contrary to the reserve effect hypothesis. Two mother trees, M_1 and M_3 of *Q. pyrenaica*, showed a significant increase in the use of seed reserves with the increase in seed mass. Finally, all the mother trees of *Q. suber* showed a significant and positive relationship between seed mass and the use of its reserves (correlation coefficients from 0.69 to 0.88; $P < 0.05$, Fig. 3A). In all the mother trees, the slope was close to 1, although for mother M_3 there was a pattern ($0.1 > P > 0.05$) of a slope lower than 1 ($S = 0.72$).

Concerning the metabolic effect hypothesis, the relationship between seed mass and RGR showed differences among mother trees. Mother trees of *Q. ilex* and *Q. faginea* did not show a significant relationship between seed mass and RGR (Fig. 3B). One mother tree (M_5) of *Q. pyrenaica* showed a significant and negative relationship between seed mass and RGR and for mother tree M_4 there was also a negative pattern ($0.1 > P > 0.05$), where RGR decreased as the seed mass increased. In the case of *Q. suber*, only M_1 showed a significant and negative relationship between seed mass and RGR ($r = 0.81$, $P < 0.01$). No metabolic effect was observed in the rest of the mother trees.

For the seedling-size effect within each species, differences between mother trees were also found (Fig. 3C). For *Q. ilex*, mother trees M_1 and M_5 showed a significant and positive relationship between seed mass and seedling biomass; for mother tree M_3 , the relationship was nearly significant ($r = 0.56$, $0.1 > P > 0.05$). *Q. suber* presented a positive and significant relationship between seed mass and seedling biomass for all mothers (except M_1), thus confirming the seedling-size effect (Fig. 3C). All mother trees of *Q. faginea* (except mother tree M_2), showed a significant and positive relationship between seed mass and seedling biomass (correlation coefficients ranging from 0.73 to 0.95; $P < 0.05$, Fig. 3C). The slopes were significantly different from zero for all the mother trees, with mother M_3 and M_4 being the highest ($M_3: S = 2.62$, $M_4: S = 1.73$).

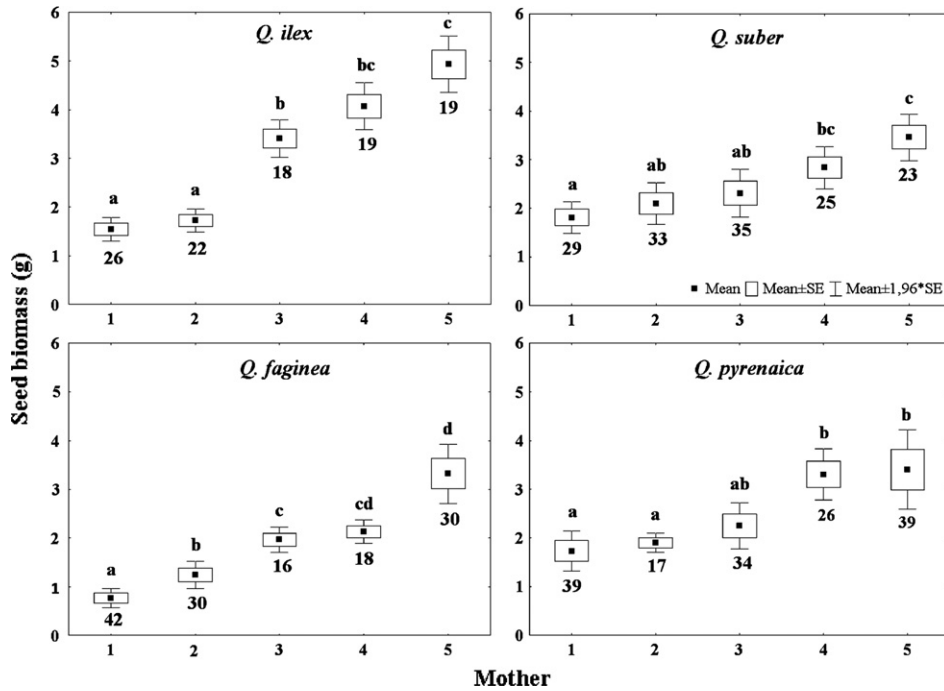


Fig. 2. Mean seed mass of the different mothers for each species. Different letters represent statistically different groups according to post-hoc Tukey test ($P < 0.05$). The coefficient of variation is also shown below each box.

Thus, confirming the seedling-size effect for all mothers (except M_2). Finally, for *Q. pyrenaica*, only two mother trees (M_1 and M_3) displayed a significant and positive relationship between seed mass and seedling biomass.

3.2.3. Maternal influences on morphological variables

The effect of the mother tree was significant for different variables depending on the species (Table 1). The mother effect was observed in the total leaf area of *Q. ilex* seedlings. There was a significant

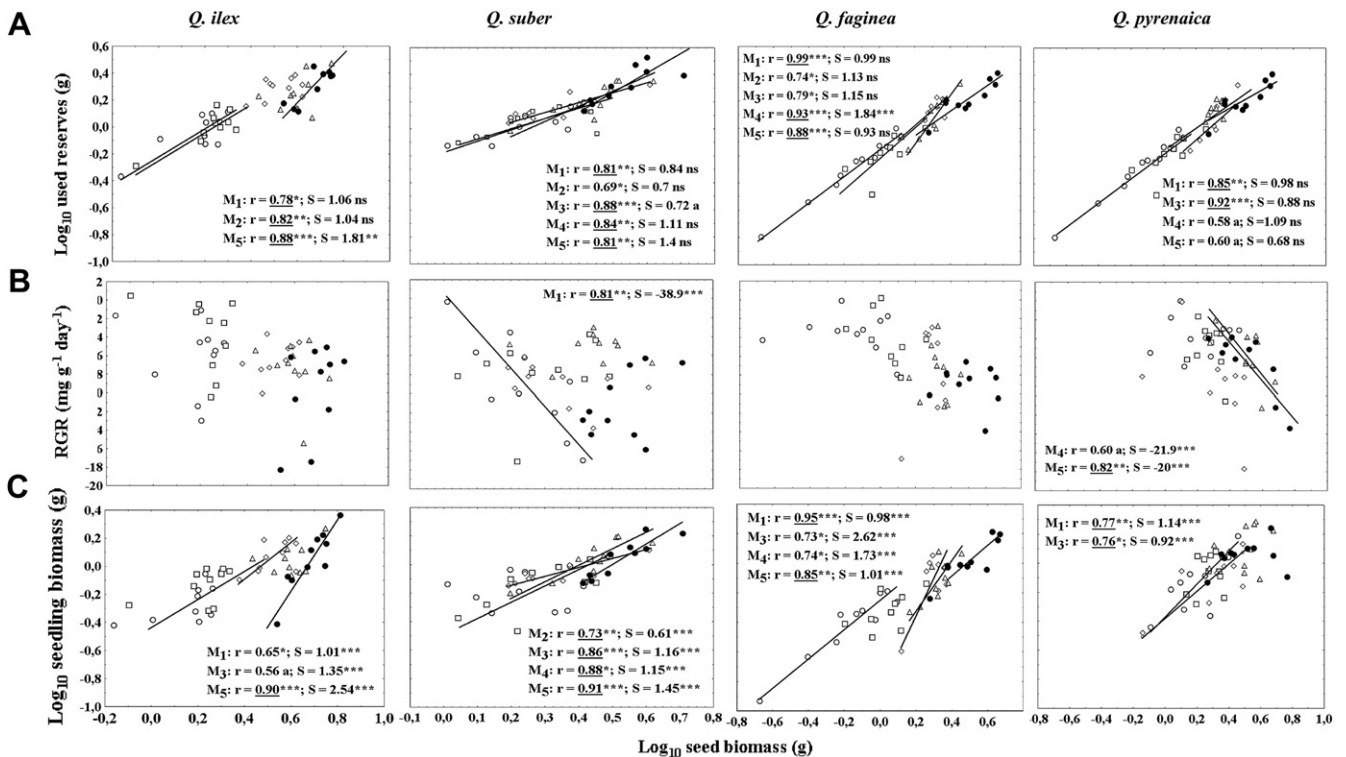


Fig. 3. Relationships of initial seed dry mass versus (A) used seed reserve, (B) relative growth rate (RGR), and (C) seedling biomass after ca. 67 days of growth within five progenitors of the four *Quercus* species. Pearson correlation (r) and significance are indicated as: ns, not significant; a, $0.05 < P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. r values remaining significant after controlling the false discovery rate are underlined. The standardized major axis regression (SMA) lines are given when they are significant or marginally significant ($0.1 > P > 0.05$). The slope of each SMA regression (S) and their significance against the null model ($S = 1$ for the reserve effect, and $S = 0$ for the metabolic effect and the seedling-size effect) is given. Mother trees: ○ M_1 ; □ M_2 ; ◇ M_3 ; △ M_4 ; ● M_5 .

Table 1
Results of mixed model ANCOVA to study the effects of mother tree (M, random factor), seed mass (covariable) and the interaction between both factors (M × Seed) on different variables (time of emergence, total leaf area, efficiency of use of reserves, RMF, SMF, LMF and LAR). Numbers indicate the % of variance explained by each factor calculated as SS_x/SS_{total} , where x is the factor. R^2 (x 100) is the variance explained by the model. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Values of the variance explained by each factor remaining significant after controlling the false discovery rate are underlined. Efficiency use is the calculated as: seedling biomass with no cotyledons × 100/reserves used; RMF (root mass fraction; root/plant biomass), SMF (stem mass fraction; stem/plant biomass), LMF (leaf mass fraction; leaf/plant biomass), LAR (leaf area ratio; total leaf area/plant biomass). All biomass was expressed on dry mass basis. Significant values ($P < 0.05$) are written in bold letters.

Species	Effect	Time emergence	Total leaf area	Efficiency use	RMF	SMF	LMF	LAR
<i>Q. ilex</i>	Mother (M)	8.39	15.83**	12.35	7.37	3.53	11.83	12.32
	Seed	13.76	28.35**	0.18	0.90	3.97	0.01	1.24
	M × Seed	17.20*	11.52	7.89	6.22	5.21	8.12	6.60
	R^2	45.61**	74.30**	28.01	15.50	15.51	22.21	34.60*
<i>Q. suber</i>	Mother (M)	17.20*	14.77	23.23*	5.22	7.51	4.26	13.37
	Seed	1.51	5.45	1.37	13.04*	0.51	17.0**	4.09
	M × Seed	17.39*	9.46	22.74*	6.76	13.63	3.25	12.10
	R^2	49.98***	30.01	39.07**	53.6***	34.83*	50.4***	30.10
<i>Q. faginea</i>	Mother (M)	7.58	14.27	12.78	5.31	6.34	5.25	8.59
	Seed	0.86	6.67	1.33	12.71	0.70	15.99*	4.13
	M × Seed	13.70	10.94	10.09	10.30	7.67	7.77	13.64
	R^2	39.97*	59.61***	35.21*	36.72*	12.64	40.34*	38.76*
<i>Q. pyrenaica</i>	Mother (M)	17.16	8.11	2.47	19.6**	22.41**	13.72	19.4**
	Seed	0.06	19.84**	3.19	4.31	0.92	5.34	9.01
	M × Seed	10.36	10.35	3.64	18.24*	15.12	14.33	19.8**
	R^2	31.55	43.80**	24.71	29.79	45.22**	23.27	36.99*

interaction of mother and seed mass on time of emergence which means that time of emergence was affected by seed mass but the effect was different across mothers. For *Q. suber*, seeds of different mother trees differed in time of emergence and efficiency use of seed reserves. Moreover, maternal source modified seed mass effects on these traits. For *Q. faginea* no mother effects were observed for any of the variables studied. Seedlings of *Q. pyrenaica* differed among mother trees in the root and stem ratios (RMF, SMF) and the leaf area ratio (LAR), and there was a significant interaction between seed mass and mother in root mass ratio and LAR.

4. Discussion

We analyzed the influence of the seed mass on the seedling-size by means of the use of its reserves and of its growth rate, both within and across four oak Mediterranean species. We found significant among-trees differences in these relationships, thus showing that maternal origin may modify seed mass effects.

4.1. Seed mass effect

As described above, the seed mass effect can be explained by three hypotheses: *reserve effect*, *metabolic effect* and *seedling-size effect*. Two conditions are necessary in order for the *reserve effect* to be accomplished (Green and Juniper, 2004). First, the slope of the relationship between the initial seed mass and the use of reserves should be less than one. Second, the slope of the seedling-size effect should be greater than that of the reserve effect. In our experiment, the first condition was met for *Q. ilex* and *Q. faginea*, but the second condition was not fulfilled, so that the reserve effect was not supported, contradicting results found by Kidson and Westoby (2000). This effect has not been clearly shown for the genus *Quercus* (Quero et al., 2007) and nor in other species (Green and Juniper, 2004) so it seems that the reserve effect is not generalized. It is known that *Quercus* acorns are recalcitrant so their viability is very short as they are desiccation-sensitive, and, moreover, they decompose soon because of their high moisture content (Roberts, 1973; Finch-Savage, 1992). Therefore, for this type of seed, there is no a clear advantage in retaining their reserves as they will not be useful later. Most of the studies on reserve effect are on total reserves (mainly

carbohydrates), but other resources may also be important, as for example nutrients. In this way, a recently study found that *Q. ilex* seedlings depend on remobilization of acorn N reserves to roots during the first stages of life (Villar-Salvador et al., 2009).

Interestingly, different trends have been found when analyzing the seed mass hypothesis between mother trees within species. In the case of the reserve effect, differences between mother trees cannot be explained by mean seed mass differences, so it appears to be a factor associated with the mother tree, which has an influence on the use of the seed reserves. We have found a contrary effect to the reserve effect for some mothers (i.e., a higher increase of the reserves used with an increase in seed mass) (M_5 in *Q. ilex*, M_4 in *Q. faginea*). This result could be due to the recalcitrant characteristics of *Quercus* acorns as explained above, where a fast mobilization of the seed reserves could be another successful strategy.

The *metabolic effect* hypotheses assumes that seedlings from larger seeds have a slower RGR. This hypothesis was confirmed for all the species except for *Q. suber*, corroborating results of Quero et al. (2007). This effect could be due either to lower respiration rates, a slower consumption of the seed resources or a lower efficiency in the conversion of seed reserves to seedling biomass. Turnbull et al. (2008) found that calculation of RGR could be heavily biased by initial size. In our study, because we harvest all plants at the same time and with similar development stage we think this problem does not occur.

The metabolic effect changes dramatically within species considering the mother trees. For example, although there was a significant and negative relationship between seed mass and RGR for *Q. ilex* and *Q. faginea*, we did not find any metabolic effect in any mother of the two species. This could be due to each mother tree occupying a small range of seed mass (Fig. 2), so it is possible that there was not enough variability in the seed size within mother trees to show this effect. This would also indicate that RGR is not strongly associated with seed mass as the reserves used or seedling-size are, whose effects appear even for small ranges of seed mass (Fig. 3). Similar results were found by Castro et al. (2008) in Scots pine seedlings from different maternal plants. In that study RGR was weakly correlated to seed mass, suggesting that the relationship between RGR and seed mass is not causal, but reflects an evolutionary covariation in these traits.

According to the *seedling-size effect* hypotheses, a larger seed mass is related to a larger seedling biomass, which would confer a series of advantages on the seedling in its establishment (Hendrix et al., 1991; Eriksson, 1999; Chacón and Bustamante, 2001; Khan, 2004; Castro et al., 2008). In this study, this hypothesis has been supported by all species. Quero et al. (2007) only observed the seedling-size effect in two out of four *Quercus* species under similar light availability. However, they studied seedlings in a more advanced ontogenetic phase, in which seed mass effects might be masked with photosynthetic gains. Light conditions may influence the seedling-size effect, as this effect was mainly observed under moderate or dense shade conditions by Quero et al. (2007) and Leishman and Westoby (1994). However, Chacón and Bustamante (2001) and Quero et al. (2008a), found the *seedling-size effect* under different environment conditions: both in drought and irrigation regimes, the latter being comparable to the conditions in our study.

Considering the mother tree, at least for one mother tree per species, the seedling-size effect was not found. It seems that, regardless of the seed size, there are other factors related to maternal influence. Different causes could be responsible for this, such as a different chemical composition of the seeds, efficiency of reserve use or biomass allocation (Leiva and Fernández-Alés, 1998; Rodríguez-Estévez et al., 2008).

To summarize, an intensive use of the reserves determined larger seedlings in the first development stages, as in the case of M_4 of *Q. faginea*, or M_5 of *Q. ilex* (Fig. 3). These results encourage the idea that the production of larger seedlings from larger seeds is more related to the amount of reserves stored in the cotyledons and their use rather than to the initial growth rates (Baskin and Baskin, 2001; Castro et al., 2008). However, for some mother trees, the metabolic effect showed up (M_4 and M_5 of *Q. pyrenaica* or M_1 of *Q. suber*) and this was the cause of the lack of relationship between seed mass and seedling biomass. This was predicted by the model of Quero et al. (2007). For other mother trees (M_2 of *Q. faginea*) neither the metabolic effect nor the seedling effect were found. For some reason, these plants were less efficient in the use of their reserves. The chemical composition and proportion of carbohydrates in the seeds or the respiration rates during germination could offer some explanation.

4.2. Assessing maternal influences

As described above, we found different trends between mother trees when evaluating seed mass effects, and maternal origin seems to determine other traits acting independently from the seed size, such as acorn genetic variability or physiological status (Merouani et al., 2001; Goodman et al., 2005). This idea is corroborated by other studies in which a mother effect in *Q. ilex* on characteristics associated with establishment and survival was found (Leiva and Fernández-Alés, 1998).

The mother effect could also introduce differences into certain morphological characteristics of the plants. For instance, in the case of *Q. pyrenaica*, the investment in their roots and stems was different depending on the mother, indicating diverse strategies. Some plants invest more in their roots so that they can capture more nutrients and reach deeper layers of the soil, whereas the strategy of others is to develop more photosynthetic tissue and support organs to grow faster (Villar et al., 2008).

It has been proposed that maintaining high phenotypic diversity may be crucial for species inhabiting in heterogeneous environments (Sánchez-Vilas and Retuerto, 2007). In this way, intra-species and interspecies variability needs to be considered for an applied perspective. One widely employed practice in seed companies and restoration programs is to select mother trees with

big acorns, from which more robust plants could be obtained (Vázquez, 1998). Sometimes collection of seeds is done in a limited number of mother trees, which lead to plantations or restoration programs, where genetic diversity is significantly reduced (Rajora, 1999; Burgarella et al., 2007). Moreover, as Leiva and Fernández-Alés (1998) and this study have found, seedlings from different mothers showed a different biomass allocation pattern, which could confer a different drought resistance. Therefore, if we consider that retention of high variability and genetic resources is vital for species to survive in unpredictable and heterogeneous environments and face climate change, these practices should be revised.

5. Conclusions

This experiment was aimed to address the three hypotheses of seed mass effect (Westoby et al., 1996) for four species of *Quercus* and among different mother trees of each species. At species level, the reserve effect was not observed for any species, probably because of the recalcitrant character of *Quercus* seeds. The metabolic effect was observed in three species, and the seedling-size effect was matched for all the species. Interestingly, within species, the mother trees introduced differences in the three hypotheses, as seed effects were found just for some mothers. In general, it seems that the production of larger seedlings from larger seeds is more related to the amount of reserves stored in the cotyledons and its use rather than to the initial growth rates. Further research is needed on the mechanisms regulating these relationships (such as the composition of the reserves, the respiration rates and the mobilization of carbohydrates). This study supports the idea that the high variability in mean seed mass within populations in *Quercus* species could affect other traits associated with establishment. Therefore, it is important to conserve this diversity as it provides the potential for surviving in heterogeneous environments and in the current context of climate change.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at [doi:10.1016/j.actao.2010.10.006](https://doi.org/10.1016/j.actao.2010.10.006).

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