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Original article

Within-population variability influences early seedling establishment in four Mediterranean oaks

Victoria González-Rodríguez^a, Isabel C. Barrio^{b,1}, Rafael Villar^{a,*}

^a Área de Ecología, Universidad de Córdoba, Edificio Celestino Mutis, Campus de Rabanales, 14071 Córdoba, Spain

^b Departamento de Zoología, Universidad de Córdoba, Edificio C1, Campus de Rabanales, 14071 Córdoba, Spain

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ABSTRACT

Regeneration of Mediterranean forests is severely limited. Multiple abiotic factors are known to constrain the establishment of woody seedlings at its first phases, such as summer drought or excessive incident radiation, but less attention has been paid to the role of intra-specific variation in seedling performance. In this study we investigate the relative influence of environment (light availability, soil moisture and summer irrigation) and intrinsic factors (seed mass and maternal origin) as determinants of within-population variability in the early establishment of four coexisting Mediterranean oaks (*Quercus ilex*, *Quercus suber*, *Quercus faginea* and *Quercus pyrenaica*), from emergence and early growth to second-year survival in a field experiment. Seed size was a poor predictor of seed and seedling success. Instead, mother identity showed a stronger effect on seedling performance. Time and percentage of emergence, establishment success and morphological traits varied among seedlings from different maternal trees but main drivers for each variable were different for each species. In addition to a direct effect, in many cases mother-related intrinsic traits and seed mass influenced the effects of environmental conditions on seedling performance. The role of intrinsic factors was masked under ameliorated conditions (i.e. summer irrigation), indicating the relevant role of within-population variability to cope with highly heterogeneous and unpredictable Mediterranean environments.

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1. Introduction

A vast proportion of the forested areas in temperate and subtropical regions of the Northern Hemisphere are dominated by species belonging to *Quercus* genus and oak forests are one of the main ecosystem types in Mediterranean areas. However these are shrinking because of changes in land use and climate change with associated habitat loss and very low seedling establishment (Pulido, 2002). Indeed the seedling phase is especially sensitive to environmental conditions (Pulido, 2002; González-Rodríguez et al., 2011b). Summer drought and excessive incident radiation may be major causes of mortality, with several studies demonstrating that moderate shade may be beneficial (Quero et al., 2006; Gómez-Aparicio et al., 2008).

Mediterranean areas experience large intra and inter-annual environmental variation, so plants have to cope with spatial and

* Corresponding author. Tel.: +34 957 218635; fax: +34 957218233.

E-mail addresses: bv2gorov@uco.es (V. González-Rodríguez), b42cabai@uco.es (I.C. Barrio), bv1vimor@uco.es (R. Villar).

¹ Present address: Department of Biological Sciences, University of Alberta, Edmonton AB, T6G 2E9 Canada.

temporal heterogeneity. High phenotypic diversity may enable species to cope with unpredictable and heterogeneous environments (Sánchez-Vilas and Retuerto, 2007). Many studies have found significant among population variation in physiological, structural and growth parameters (Sánchez-Vilas and Retuerto, 2007; Gimeno et al., 2009; Ramírez-Valiente et al., 2009). Within-population genetic variation and variation in maternal genetic or environmental effects on traits like seed mass (Schmitt et al., 1992; González-Rodríguez et al., 2010), leaf traits (Rice et al., 1993; Ramírez-Valiente et al., 2009), seedling growth (Castro et al., 2008) or drought resistance (Leiva and Fernández-Alés, 1998) might also permit a local response to unpredictable and variable environmental conditions (Castro, 1999). Genetic variation may also allow adaptation to local or changing conditions (McKay et al., 2005). Genetic diversity of plant material for restoration is sometimes very reduced, as seed are collected from a few maternal plants. Therefore, from an applied perspective it is important to maintain a high genetic diversity of plant material (for example seed collects from many maternal plants) for future forest restoration actions (Vander Mijnsbrugge et al., 2010).

Seed mass is particularly important for regeneration (Castro, 1999; Merouani et al., 2001). For example, larger seeds germinate

better and the resulting seedlings are larger and survive better (Gómez, 2004a; Quero et al., 2007; González-Rodríguez et al., 2011a). However it is necessary to distinguish between the effect of seed mass and the genetic or environmental quality of the mother. Many studies have evaluated environmental constraints on seedling emergence and survival in Mediterranean oaks (Gómez, 2004b; Quero et al., 2008; Gómez-Aparicio et al., 2008; Tyler et al., 2008; Urbietta et al., 2008; Pérez-Ramos et al., 2010). Some have evaluated the importance of population differentiation (Ramírez-Valiente et al., 2009), but the role of within-population variability in the mechanisms underlying seedling establishment warrants further study. To our knowledge, this is the first field experiment that combines maternal and environmental effects in seedling establishment of various coexisting species at the same time.

The aim of our study was to investigate how seeds of the range of available sizes from different maternal individuals of four late-successional *Quercus* species germinate and how the resulting seedlings establish and survive in response to experimental manipulation of water availability and shading. Specifically, our objectives were: 1) to investigate how seed germination and seedling performance varies with seed size and whether this differs among mother trees, 2) identify critical factors (environmental vs. intrinsic) at each establishment phase (emergence, growth and survival), 3) evaluate the benefits of artificial irrigation simulating a rainy summer in the first year, and its delayed effects on the second year, and 4) compare these effects on four late-successional *Quercus* species that differ in leaf longevity.

2. Methods

2.1. Study area and species

The experiment was carried out on a public property ("La Vegueta del Fresno") at the Natural Park of Sierra de Cardena y Montoro (Córdoba, S Spain) (38° 21' N, 3° 12' W). The climate is continental, with summer droughts, annual rainfall between 570 and 970 mm and mean annual temperature of 15.3 °C. Soils are regosols and consist mainly of sand with granite as bedrock (Quero and Villar, 2009). The experimental site was at 480 m a.s.l., occupied a 2 ha surface and was fenced to exclude large herbivores. It consisted of an open forest of *Quercus ilex* L. subsp. *ballota* (Desf.) with some sparse individuals of *Pinus pinaster* Aiton and *Cistus ladanifer* L. shrubs.

Two evergreen species: *Q. ilex* L. subsp. *ballota* (Desf.) Samp. and *Quercus suber* L., and two deciduous species: *Quercus faginea* Lam. and *Quercus pyrenaica* Willd., were selected for the study. The four species coexist in the Natural Park and have different habitat preferences, but all show regeneration problems. *Q. ilex* is the most common oak in the Iberian Peninsula and occupies mixed woodlands with other *Quercus* species. *Q. suber* is found in shadier areas or in moister soils. *Q. faginea* often occurs close to valley bottoms where there is a temporary flow of water, and therefore, greater water availability. *Q. pyrenaica* is generally found at higher elevations (around 700 m a.s.l.).

2.2. Experimental design

Five mother trees were selected for each species within the same population in the park. Maternal plants were chosen to cover a wide range of seed sizes (Appendix S4); each mother tree was identified by a number according to its mean seed size, with 1 being the lightest and 5 the heaviest (Appendix S4, Electronic Appendix). In autumn 2006, 200 acorns were collected from each mother tree and stored at 2–5 °C. For each species and mother tree a random sub-sample of seeds was used to calculate predictors of dry seed

mass (in all cases $R^2 > 0.90$, $P < 0.05$), a variable widely used in the literature (see Appendix S1 for details). This ensures that differences in water percentage of the seeds do not affect the results. Also, seed dry mass represents the reserves available to seedlings during the first development stages.

In late November 2006, 600 acorns per species with no fungi or predation signs were randomly selected and sowed in the field (120 from each mother tree, 2400 in total). Acorn fresh mass and mother tree were recorded. Twenty-five experimental plots were set up at each of 3 light categories ($n = 75$ experimental plots): 1) open woodlands, 2) beneath cover of *Q. ilex*, and 3) beneath cover of *Q. ilex* and simulated shrub cover. To simulate shrub cover, a green mesh that transmitted 27% of incident radiation was used. Each experimental plot was divided in two replicates (separated 50 cm; 150 replicates in total). Sixteen acorns of randomly drawn mother trees (4 per species) were sown at each replicate, 4 cm deep and 5 cm apart in a square grid of 4×4 acorns. Each replicate was protected by a wire square (50 × 50 cm, 1 cm mesh width), put up and half-buried to avoid predation by rodents (Gómez-Aparicio et al., 2008). Weeds were removed by hand during the first spring to avoid competition.

In each experimental plot, replicates were randomly assigned to an irrigation treatment (irrigated vs. non-irrigated). Water was applied during summer of the first year (70 days, between June and August 2007) at 15–30 days intervals. In total, 51 L m⁻² were added, simulating a rainy summer in this area (data from Consejería de Agricultura, Junta de Andalucía). The distance between replicates (50 cm) was enough to efficiently separate both treatments (soil moisture was in all cases significantly higher in the irrigated replicates; mean values ± sd of volumetric water content in July was $1.53 \pm 1.11\%$ in the irrigated replicates whereas in non-irrigated replicates was $0.46 \pm 0.64\%$; ANOVA $F = 51.2$, $P = 0.000$).

2.3. Data collection

Light availability was explored with hemispherical digital photography. Pictures were taken before nightfall ensuring homogeneous conditions of illumination at the understory and a correct contrast between canopy and sky (Pérez-Ramos et al., 2010). At each plot, a photograph was taken at the seedling level (about 30 cm over the soil) with a horizontally-levelled digital camera aimed at the zenith, using a 180° field of view fish-eye lens. All photographs were taken between April and May 2007. Images were analysed using Hemiview canopy analysis v 2.1, which estimates a global site factor (GSF) by combining the proportion of direct and diffuse radiation.

The volumetric water content of the topsoil (20 cm depth) was monthly measured using a time-domain reflectometer (TDR model 100; Spectrum Technologies Inc., Plainfield, IL, USA). At each plot three measurements were taken per visit from December to May 2007. Measurements were averaged from December to February to account for soil moisture in the pre-emergence period, and from December to May to reflect spring soil water availability.

Experimental plots were visited every two weeks for the first year and once every three months for the second year. We evaluated seedling emergence (i.e. percentage of seeds emerged from sown seeds), time to emergence (number of days after sowing when seedlings emerged to the nearest two weeks), survival (percentage of seedlings surviving from emerged seeds at the end of the first and second year of the experiment) and an overall quantification of seedling establishment success at the end of the experiment (percentage of seedlings surviving from the sown seeds).

Aboveground biomass and aerial leaf mass fraction (LMF_a: leaf mass/aboveground biomass) were estimated at the end of the

growing season (May–July 2007) with non-destructive measurements taken on a randomly-selected sub-sample of the emerged seedlings. Specific leaf area (SLA, leaf area/dry mass) was calculated from an additional fifteen randomly-selected seedlings per mother tree at the end of the growing season (see Appendix S1 for details).

2.4. Data analyses

We tested if time of emergence differed among species using a Linear Mixed Model (LMM), including replicate as a random factor to account for data structure. To investigate the effect of different variables on the phases of establishment we built mixed models for each species, including experimental plot as random factor. For seedling emergence, survival and establishment success Generalized Linear Mixed Models (GLMM) with a binomial distribution of errors and logit link function were used, while for time to emergence LMMs were used. To avoid multicollinearity problems (Graham, 2003), we computed a regression of dry seed mass against maternal identity, and in further analyses we replaced the former variable with the residuals from this regression (we refer them as 'relative seed dry mass'). These residuals represent the relative mass of each seed in relation to the maternal mean, that is, the deviation of each seed with respect to the corresponding maternal mean seed mass. Having removed the variation in seed mass due to maternal identity, these residuals represent the unique contribution of dry seed mass, independent from mother-induced effects (Castro, 1999). Light availability, relative seed dry mass, mother plant and all two-way interactions were taken as fixed effects. In the models for first-year emergence and survival, soil moisture was included (pre-emergence and spring values of soil moisture respectively), and irrigation treatments were included in all post-emergence models. Survival of non-irrigated seedlings was also analysed including light availability, spring soil moisture, relative seed dry mass and maternal plants as fixed effects.

To investigate the influence of mother tree on different growth and morphological traits, i.e. aboveground biomass, aerial leaf mass fraction (LMF_a), and specific leaf area (SLA), we built Linear Mixed Models (LMM) for each species, including experimental plot as a random factor due to the experimental design. Aboveground biomass was square root-transformed to achieve normality. Spring soil moisture, light availability and relative seed dry mass were included as the fixed component. For SLA models we also tested the effect of the interaction between light availability and maternal plant (Rice et al., 1993).

Model selection was based on AIC and Log-likelihood Ratio Tests (LRT) using Maximum Likelihood, and for the binomial GLMMs it followed a backward procedure based on a 'Laplace' approximation of likelihood (Zuur et al., 2009). For GLMMs we report the χ^2 statistics of the likelihood ratio tests between models with and without a certain fixed term (Bolker et al., 2009). The direction of the effect of a variable was based on evaluation of its estimated coefficient. All modelling assumptions were checked (Zuur et al., 2009) and analyses were performed using R 2.10.1 (R Development Core Team, 2009), specifically the packages *lme4* (Bates and Maechler, 2009) and *nlme* (Pinheiro et al., 2009) to build GLMMs and LMMs respectively.

3. Results

3.1. Seedling emergence

Time to emergence differed among the four *Quercus* species studied (ANOVA; $F = 19.06$; $df = 3, 1285$; $P = 0.000$; Table 1). *Q. faginea* had the shortest time to emergence and *Q. ilex* the longest. In all species a percentage of seedlings did not emerge until

Table 1

Mean \pm standard deviation and range (in brackets) of time to emergence, above-ground biomass, aerial leaf mass fraction (LMF_a), specific leaf area (SLA), and percentages of emergence, survival and establishment success in years 1 and 2 for the four *Quercus* species studied. Survival relates to the number of emerged seedlings that are found alive in the following year, while establishment success refers to the number of planted acorns that were recruited at the end of the experiment.

	Evergreens		Deciduous	
	<i>Quercus ilex</i>	<i>Quercus suber</i>	<i>Quercus faginea</i>	<i>Quercus pyrenaica</i>
Time to emergence (days)	170.2 \pm 27.0 [97 – 665]	160.0 \pm 28.6 [97 – 567]	153.8 \pm 31.2 [97 – 791]	155.6 \pm 27.5 [83 – 567]
Emergence (%)	33.6	73.5	54.2	71.0
Survival Y1 (%)	37.9	22.8	19.5	12.4
Survival Y1 non-irrigated (%)	31.1	17.8	14.2	7.21
Survival Y2 (%)	24.0	10.4	10.9	7.7
Establishment Success (%)	8.5	7.8	6.2	5.71
Aboveground biomass (g)	0.54 \pm 0.29 [0.10–1.11]	0.56 \pm 0.27 [0.05–1.29]	0.67 \pm 0.44 [0.11–2.10]	0.67 \pm 0.39 [0.16–2.37]
LMF _a	0.84 \pm 0.06 [0.69 – 0.93]	0.85 \pm 0.06 [0.55 – 0.96]	0.83 \pm 0.06 [0.61 – 0.93]	0.81 \pm 0.06 [0.60 – 0.91]
SLA (m ² kg ⁻¹)	7.3 \pm 1.9 [5.0 – 16.9]	11.5 \pm 2.7 [7.4 – 17.7]	11.3 \pm 2.3 [7.2 – 17.6]	13.7 \pm 2.8 [9.6 – 21.7]

the second year (*Q. ilex*: 4.7%; *Q. suber*: 2.7%; *Q. faginea*: 5.5%; *Q. pyrenaica*: 3.7%). Time to emergence was determined by different variables for each species (Table 2), but in most cases was related to light availability and intrinsic mother-related traits (Fig. 1). No differences were found in mean time to emergence between deciduous species (Tukey contrasts; $z = 0.892$, $P = 0.807$), which were on average lower than for evergreens.

Seedling emergence was highest for *Q. suber* and *Q. pyrenaica* (Table 1), being determined by the individual relative seed dry mass and maternal identity (Table 2). Seed dry mass had a different effect on emergence of seedlings from different mother trees in *Q. faginea* and *Q. ilex* (Table 2; Fig. 2), which in turn showed lower rates of emergence (54.2% and 33.6% respectively; Table 1).

3.2. Seedling survival and establishment success

Summer irrigation increased first-year seedling survival for all four *Quercus* species (Table 2). The greatest effect was detected on *Q. pyrenaica*, for which irrigated seedlings increased their probabilities of survival by 60.9%. These percentages were 45.4, 37.7 and 35.5 for *Q. faginea*, *Q. suber* and *Q. ilex*, respectively. For *Q. suber* other factors were also involved (Table 2). For non-irrigated seedlings, survival after the first summer was mainly determined by mother tree, except for *Q. faginea*, for which none of the studied variables had a significant effect. For *Q. suber*, survival of non-irrigated plants also depended on other factors (Table 2).

Second-year survival was much lower than first year-survival for all species (Table 1). Summer irrigation in the previous year influenced second-year survival for *Q. ilex* and *Q. faginea*, but its effect differed among mother trees (Table 2). In the case of *Q. suber* and *Q. pyrenaica* other factors were involved (Table 2).

Establishment success is the cumulative effect of consecutive processes, from seedling emergence to subsequent survival, and therefore, much lower values were found (between 5.7 and 8.5%; Table 1). Different factors explained establishment success in each species (Table 2). In most cases intrinsic factors were involved; for *Q. ilex* mother origin was the main driver, for *Q. suber* larger seeds had better establishment, and for *Q. pyrenaica* the influence of environmental variables (light availability and irrigation treatment)

Table 2

Linear Mixed Models (LMM) for time to emergence and Generalized Linear Mixed Models (GLMM) for seedling emergence, survival in years 1 and 2, survival in year 1 for non-irrigated seedlings and establishment success of the four *Quercus* species studied. For LMM results of likelihood ratio tests (LRT) are given, while for GLMMs significance (Sig.) is assessed by comparing nested models with a Chi-square statistic. Signs in brackets indicate the direction of the effect of continuous predictors on the response variable. Irrigation effects were always positive. Empty cells indicate no significant effect of any of the predictors included (random effects only). 'GSF': light availability; 'seed mass': relative dry seed mass; 'mother': maternal plant; 'irrigation': presence of summer watering in year 1; 'moisture': soil moisture.

	<i>Quercus ilex</i>			<i>Quercus suber</i>			<i>Quercus faginea</i>			<i>Quercus pyrenaica</i>						
		LRT	Sig.		LRT	Sig.		LRT	Sig.		LRT	Sig.				
Time to emergence	GSF (-)	4.33	0.038	Seed mass (-)	20.60	0.000	GSF*	13.38	0.010	GSF*	10.71	0.030				
				Mother	28.50	0.000	mother Moisture* mother Seed mass (-)	17.34 0.002 9.78 0.002		mother						
		Chisq	df	Sig.	Chisq	df	Sig.	Chisq	df	Sig.	Chisq	df	Sig.			
Emergence	Seed mass*mother	13.34	4	0.009	Seed mass (+)	11.54	1	0.001	Seed mass* mother	29.25	4	0.000	Seed mass (+)	16.48	1	0.000
Survival Y1	Irrigation	3.47	1	0.062	Mother	29.62	4	0.000	Mother	4.68	1	0.031	Mother	18.50	4	0.001
					Irrigation	6.33	1	0.012	Irrigation	4.58	1	0.032	Irrigation	9.93	1	0.002
					GSF*moisture	4.58	1	0.032	GSF*mother	11.46	4	0.022				
Survival Y1 for non-irrigated seedlings	Mother	12.64	4	0.013	Seed mass (+)	5.67	1	0.018	Mother	10.63	4	0.031	Mother	16.91	4	0.002
					Seed mass (+)	5.01	1	0.025	Seed mass (+)	5.01	1	0.025				
					Moisture (+)	7.07	1	0.008	Moisture (+)	7.07	1	0.008				
Survival Y2	Irrigation* mother	10.11	4	0.039	Seed mass (+)	3.64	1	0.056	Irrigation* mother	10.20	1	0.037	GSF*seed mass	7.20	1	0.007
					Seed mass (+)	6.52	1	0.011	Irrigation	5.96	1	0.015	GSF*seed mass	5.18	1	0.023
Establishment success	Mother	9.54	4	0.049									Irrigation* mother	10.65	4	0.031

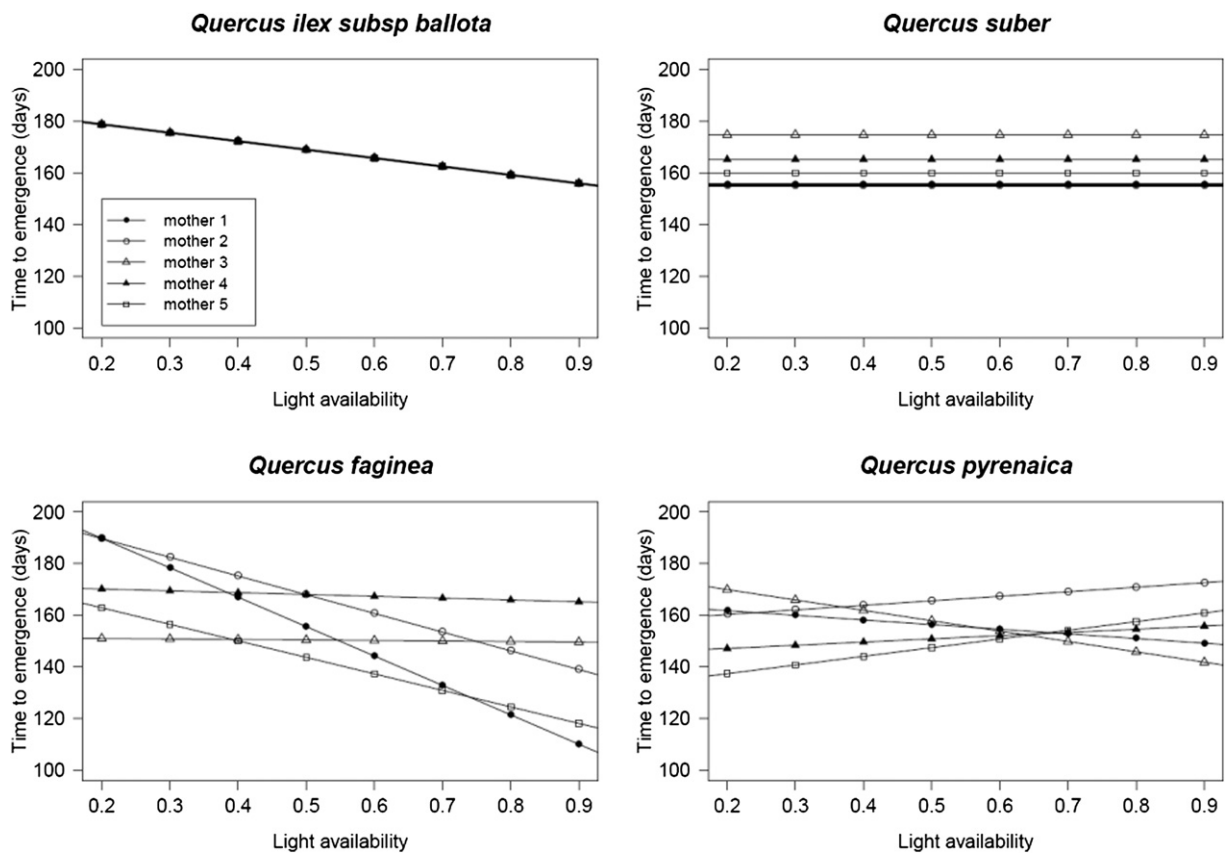


Fig. 1. Predicted values for time to emergence in relation to light availability measured through GSF (Global Site Factor) for the four *Quercus* species studied. Lines indicate predicted values of time to emergence (days) for different mother trees, which are depicted by different symbols. Mother trees were ranked from lightest (1) to heaviest (5) mean dry seed mass. Parallel or overlapping lines (thick lines) indicate non-significant interactions between light availability and mother tree.

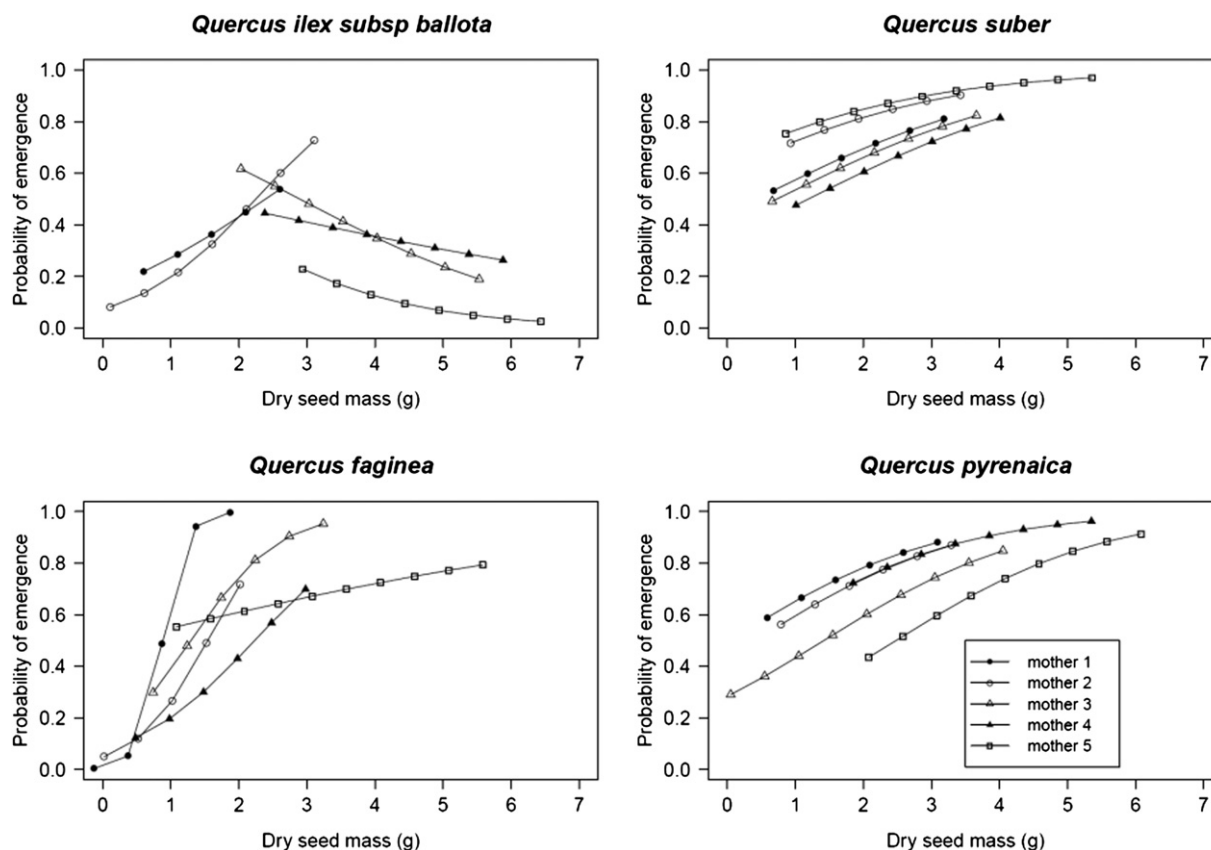


Fig. 2. Predicted probabilities of emergence across values of dry seed mass within the predicted range for each mother tree. Maternal plants are depicted by different symbols. Mother trees were ranked from lightest (1) to heaviest (5) mean dry seed mass. Non-parallel curves indicate a significant interaction between maternal plant and the relative dry seed mass.

was modulated by seed mass and maternal origin. In the case of *Q. faginea* only irrigation treatment played a role.

3.3. Morphological traits and growth parameters

Aboveground biomass at the end of the second growing season for *Q. ilex*, *Q. suber* and *Q. faginea* was only determined by maternal traits (Table 3, Fig. 3). For *Q. pyrenaica* none of the explored factors had a significant effect. No variable influenced aerial leaf mass fraction (LMF_a) for *Q. suber*, but maternal plant had a significant effect for the other species. Additionally for *Q. faginea* light availability had a positive effect on LMF_a (Table 3). For all oak species light availability had a negative impact on specific leaf area. For *Q. suber*, an independent effect of mother tree was also detected,

whereas for *Q. faginea* the response to light availability depended on the mother tree (Table 3).

Appendices S2–S4 show the mean values (±sd) of the different variables measured in the five mother trees of the four species.

4. Discussion

In this study we identified environmental and intrinsic factors that influence early establishment success of four Mediterranean oak species. Individual mother trees varied for how these factors influenced different phases of establishment. Mother trees also varied in how their heavier or lighter seeds performed under different water and light regimes. In contrast to the generalized view, we have also found that seed size is a rather poor predictor of

Table 3
Model results for growth parameters in the four *Quercus* species studied: Linear Models (LM) for estimated aboveground biomass and Linear Mixed Models (LMM) for aerial leaf mass fraction (LMF_a), and specific leaf area. For LM results of F tests are given and degrees of freedom (numerator, denominator) are indicated, while for LMMs log-likelihood ratio tests (LRT) and the corresponding significance levels (Sig.) are shown. Signs in brackets indicate the direction of the effect of continuous predictors on the response variable. Empty cells indicate no significant effect of any of the predictors included (random effects only). 'GSF': light availability; 'seed mass': relative dry seed mass; 'mother': maternal plant.

	<i>Quercus ilex</i>			<i>Quercus suber</i>			<i>Quercus faginea</i>			<i>Quercus pyrenaica</i>					
		F	df	Sig.		F	df	Sig.		F	df	Sig.			
Aboveground biomass	Mother	12.75	4, 36	0.000	Mother	4.59	4, 61	0.001	Mother	13.22	4, 58	0.000	—		
	LRT			Sig.	LRT			Sig.	LRT			Sig.	LRT		
LMF _a	Mother	8.95		0.062					Mother	9.99		0.041	Mother	16.57	0.023
Specific leaf area (SLA)	GSF (–)	15.73		0.000	GSF (–)	12.09		0.000	GSF*	10.21		0.037	GSF (–)	10.17	0.001
					Mother	11.19		0.0245	mother						

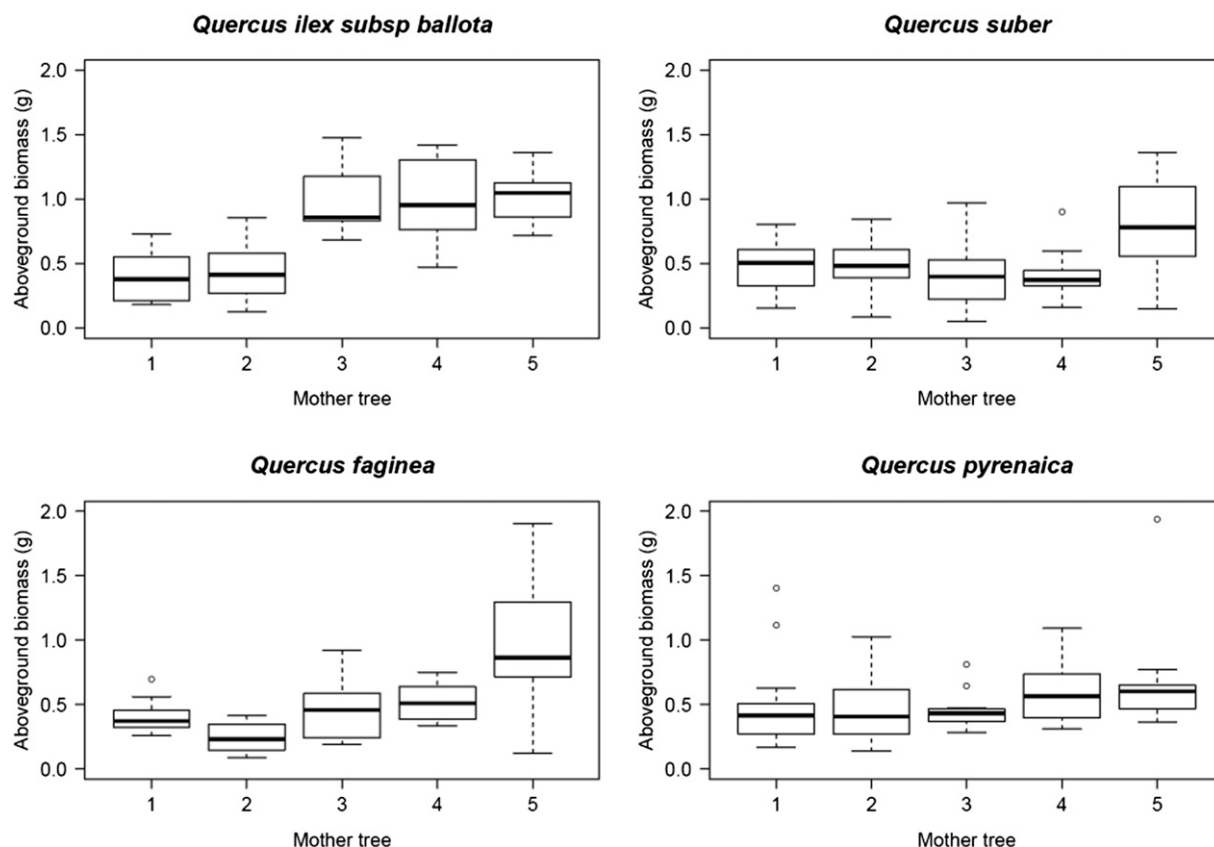


Fig. 3. Box plot for aboveground biomass of one-year-old seedlings conditional on mother tree for the four different oak species studied. Mother trees were ranked from lightest (1) to heaviest (5) mean dry seed mass.

seed and seedling success. Instead, mother identity can have a stronger effect on seedling performance. The strengths of this study are: 1) the comparison of the relative influence of environment vs. intrinsic factors of seeds (seed mass and mother origin) on establishment is explicitly addressed, which has been barely studied in plant ecological research and is an original approach; 2) the use of field conditions instead of a greenhouse experiment is a more realistic approach and 3) the comparison of four oak species that coexist in the study area can give a first impression of how the four species may be affected during the first stages of establishment.

4.1. Emergence

Larger seeds germinated more rapidly and germination was earlier under high light conditions, but these effects were modified by maternal source. Differences between mothers might be related to seed moisture content, which differed among maternal individuals (Appendix S2). Seeds with higher moisture content show more hypocotyl dormancy, which in turn retards emergence of seedlings (Merouani et al., 2001). On the other hand, deciduous species emerged before the evergreen ones, as found by Urbieto et al. (2008). Thus, emergence date appears to be a species-specific character (Laliberté et al., 2008) that can be modified by maternal source.

Another interesting result was the delayed emergence (>1 yr) observed for a proportion of seedlings in all species (between 2.7 and 5.5%), which was also found for *Q. ilex* by Gómez (2004b). This may be explained if some seeds delayed their emergence until summer. Summer drought may then inhibit seedling growth but when environmental conditions for growth improved (high

temperature and water availability), these seeds would reinitiate growth, emerging above ground the following spring. This phenomenon deserves further study under differing environmental conditions.

Surprisingly, water availability did not significantly influence the probability of acorn emergence that varied only with maternal origin and relative seed dry mass. Heavier seeds generally germinate better (Seiwa, 2000), but this relationship may only hold for some maternal plants (Castro, 1999) as we also found for *Q. ilex*. Although other studies have described the effects of environmental factors on emergence, most of them overlooked maternal effects (Tyler et al., 2008; Mendoza et al., 2009). In our case, optimal conditions in spring 2007 may have not limited seedling emergence that was thereby more dependent on seed characteristics.

4.2. Survival

The Mediterranean climate is characterised by large water deficits in which belowground resources are more critical than other environmental factors (Maltez-Mouro et al., 2007). In fact, irrigation was the main factor explaining survival after the first summer for all species, as in Mendoza et al. (2009). This result suggests that the effects of maternal source can be masked under the ameliorated conditions simulated by summer irrigation. In this sense, under severe drought (in our study, just 3 mm of precipitation during summer) maternal traits are the main factors determining survival.

Different effects explained survival after the second summer for each *Quercus* species. Irrigation had a delayed effect in two species

and interestingly, this effect varied across mothers. This delayed effect could be explained because during the second growing season seedlings could develop longer roots and thus reach deeper soil layers. For two species there was a positive effect of relative seed dry mass on second-year survival. Since *Quercus* acorns are recalcitrant (Finch-Savage, 1992) and they decompose soon (<1 year, personal observation), this delayed effect of seed dry mass might be due to a higher investment in belowground belowground biomass, which has been found to increase survival (Lloret et al., 1999).

4.3. Establishment success

In Mediterranean conditions, survival of seedlings stabilizes after two years (Jordano et al., 2008). Overall the factors that limited the different phases were involved in the establishment success of each species. For example, low emergence percentage of *Q. ilex* was offset by higher survival relative to the other oak species. As a result, the percentage of established seedlings after two years was similar for all species (~7%), although it was slightly higher in evergreen than in deciduous oaks, probably related to their differential susceptibility to summer drought (Acherar and Rambal, 1992). Indeed, irrigation had a significant effect for the most drought-sensitive species, *Q. pyrenaica* and *Q. faginea*.

4.4. Growth and morphological traits

Maternal identity explained most variation in growth and morphological traits in the first growing season. Larger seeds generally produce larger seedlings (Quero et al., 2007). In our study, aboveground biomass did not vary with relative seed dry mass probably because of the narrow seed mass variation within mothers. Thus, mother tree may influence seedling biomass through seed mass, but it may also have an influence through other intrinsic traits, such as physiological status or nutrient content (Castro et al., 1999; Merouani et al., 2001).

As expected, SLA was lower under high light conditions (Poorter et al., 2009), and for one species (*Q. faginea*) SLA depended on the interaction of light and maternal identity. Rice et al. (1993) found the same effect in *Quercus douglasii* seedlings. This phenotypic variation in leaf morphology might be genetic or the result of the maternal environment.

4.5. Within-population variability: the role of mother tree

Maternal identity can affect seedling performance through seed size variation (Leiva and Fernandez-Alés, 1998; Castro, 1999; Merouani et al., 2001) but there may also be maternal effects not related to seed size. We have demonstrated that, though in some cases larger seeds germinated better and produced better seedlings than did smaller seeds, seed size alone was a poor predictor of seed and seedling performance. Indeed seeds of similar size from different mothers could differ greatly in success, and small seeds from one mother performed better than large seeds of another, for example, 1.5 g seeds from mother 1 outperformed 5 g seeds from mother 5 in *Q. faginea* (see Fig. 2). Therefore, there is an aspect of seed quality that is influenced by the mothers identity, which may be due to various causes, i.e. genetic or environmental, and, besides that, there is an aspect of seed quality associated with its mass.

We have also found that seeds and seedlings from different mothers responded differently to shading and irrigation treatments. This within-population variability on responses across the resource gradients could be an advantage in highly heterogeneous and unpredictable environments (Castro, 1999; Sánchez-Vilas and

Retuerto, 2007). In order to evaluate this hypothesis, it would be interesting to study these effects on oak populations from less heterogeneous environments. As well, the relative role of intrinsic factors on seedling performance warrants further study in different years with contrasting environmental conditions. Differences found in seedlings from different mothers may be genetic or induced by a different environment of mothers. However, we think most of the variation should be genetic because the mothers of the same species were from the same population and they were very proximate between them. Therefore, the environmental conditions of the different mothers should be very similar, and thus the effect of a different environment of the mothers should be very low.

Woody species have more than 90% of their total genetic diversity within-populations rather than among them (Hamrick, 2004). In the context of rapid climate change predicted for the Mediterranean region (IPCC, 2007), it is crucial to understand whether sufficient genetic variation resides within-populations to allow a successful recruitment and guarantee their persistence (Hamrick, 2004). In this experiment we found that maternal source modifies seedling performance at different stages of establishment, providing a first approach to the understanding of within-population variability patterns in relation to other environmental constraints. Combining this kind of ecological studies with genetic approaches using molecular techniques would provide useful knowledge that completes conservation and restoration efforts (McKay et al., 2005).

5. Conclusions

This field study evaluated maternal influences and their interactions with various factors relevant to Mediterranean oaks establishment. Maternal identity had a dominant role on many of the phases studied. The differences in seed quality among mothers was not completely reflected in the size of the seeds they produce, and large seeds were not always better than small seeds. Indeed, some trees make large seeds that may be worse than smaller seeds produced by other trees. Intrinsic traits influenced emergence, survival, growth and morphological traits, especially under adverse growing conditions (i.e. extreme summer drought). Ameliorating conditions can favour oak survival rendering it less dependent on maternal traits. Environmental effects on seedling performance were also modified by the mother, and this within-population variability could be advantageous in high heterogeneous and unpredictable environments.

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Appendix. Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.actao.2012.04.008.

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