

# Seedling establishment in *Olea europaea*: Seed size and microhabitat affect growth and survival<sup>1</sup>

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**Abstract:** We investigated the effects of seed size and microhabitat on the dynamics of seedling establishment in *Olea europaea*. We tested the hypothesis that the type of microhabitat suitable for *O. europaea* recruitment is influenced by microhabitat-specific relationships between seed size and seedling growth rate. To test this hypothesis we conducted field experiments evaluating seedling growth and survival as a function of seed size, microhabitat (determined by presence/absence of scrub cover), and competition (established by means of seedling density gradients). Our results show that, first, seed size was related to seedling size and to differential growth rate. Second, seedling growth rate was negatively correlated with initial seedling size, although this correlation varied among microhabitats and temporally. Third, seedling survival differed between microhabitats and was positively affected by initial seedling size until the end of the summer drought. Finally, the effect of seedling size on survival was partly mediated by its negative relationship with growth rate. However, the combined effect of size and growth on seedling survival varied between microhabitats. We conclude that establishment depended on microhabitats through facilitation and inhibition processes, and that seed size-related growth rate strongly affected seedling fate. It is noteworthy that the effectiveness of the size-correlated growth depended on microhabitat.

**Keywords:** seed size, seedling growth rate, seedling size, seedling survival, water stress, wild olive.

**Résumé :** Nous avons étudié les effets de la taille des graines et du microhabitat sur la dynamique d'établissement des plantules chez l'olivier (*Olea europaea*). Nous avons vérifié l'hypothèse selon laquelle le type de microhabitat favorable au recrutement de *O. europaea* est influencé par des relations existant entre la taille des graines et le taux de croissance des plantules qui sont spécifiques au microhabitat. Nous avons mené des expériences sur le terrain visant à évaluer la croissance et la survie des plantules en fonction de la taille des graines, du microhabitat (déterminé par la présence ou l'absence de couvert arbustif) et de la compétition (établie à l'aide de gradients de densité de plantules). La taille des graines est reliée à la taille des plantules et au taux de croissance différentiel. Le taux de croissance des plantules est corrélé de façon négative à la taille initiale des plantules. Cette corrélation varie toutefois dans le temps selon les microhabitats. La survie des plantules diffère entre les microhabitats et est influencée de façon positive par leur taille initiale jusqu'à la fin de la sécheresse estivale. Enfin, l'effet de la taille des plantules sur leur survie est partiellement atténué par la relation négative qui existe entre la taille et le taux de croissance. Les effets combinés de la taille et de la croissance sur la survie des plantules varient toutefois selon les microhabitats. En conclusion, l'établissement des jeunes oliviers dépend des processus de facilitation et d'inhibition liés aux microhabitats. Le taux de croissance, relié à la taille des graines, a une forte influence sur le destin des plantules. La relation qui existe entre la taille et la croissance est toutefois fortement influencée par le microhabitat.

**Mots-clés :** stress hydrique, survie des plantules, taille des graines, taille des plantules, taux de croissance des plantules, olivier sauvage.

**Nomenclature:** Please give reference of nomenclature books for latin binomials mentioned in the text.

## Introduction

Seed and seedling stage are key elements during recruitment of most plant species. Environmental factors affecting seedling development and survival (e.g., light, water, nutrients, temperature, other plants, or enemies) can severely limit the recruitment dynamics of plant populations. These factors can covary in complex ways at small spatial scales within a habitat, forming so-called

microhabitats or micro-environments (Belsky *et al.*, 1989; Sipe & Bazzaz, 1995; Kolb & Robberecht, 1996) and making recruitment highly variable. To understand plant population recruitment it is thus necessary to analyze how seedlings respond to microhabitat variability. This response may become apparent as changes in growth rate, leaf shape, or biomass allocation (Gross, 1984; Tripathi & Khan, 1990; Saverimuttu & Westoby, 1996; Broncano, Riba & Retana, 1998; Huante, Rincón & Chapin, 1998), and it may be highly influenced by plant traits with limited plasticity, like seed or seedling size, physiology, and

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growth form (Fenner, 1983; Gleeson & Tilman, 1994; Cornelissen, Castro-Diez & Hunt, 1996; Bonfil, 1998; Lloret, Casanovas & Peñuelas, 1999).

Seedling responses to environmental variability can be analyzed by spatial modeling of population dynamics (where the environment determines seedling survival) or by modeling of plant strategies (where plant attributes determine seedling survival in different environments). Studies focusing on population dynamics have frequently addressed the final outcome of the process in terms of number of seedlings recruited and thus have disregarded the relationships among growth, survival, and plant attributes and strategies (but see Chapin & Bliss, 1989; George & Bazzaz, 1999). Most studies focusing on plant strategies have relied heavily on eco-physiological traits (but see Grime, 2001). They have pointed out the importance of functional traits, such as growth rate or biomass allocation, under certain environmental conditions, like early successional stages (Gleeson & Tilman, 1994; Berkowitz, Canham & Kelly, 1995), shade (Walters & Reich, 1996; Saverimuttu & Westoby, 1996; Reich, 2000), arid environments, and nutrient-poor soils (Jurado & Westoby, 1992; Lloret, Casanovas & Peñuelas, 1999). These two perspectives illustrate a dual nature (ecophysiological and demographic) of the population and community dynamics, but must be integrated to understand final recruitment patterns (Bazzaz, 1996). This integrated view has been very fruitful in unraveling the dynamics of old-field succession in temperate forests (e.g., Bazzaz, 1996 and references therein) and gaps in tropical forests (Dalling & Hubbell, 2002; Wright, 2002).

One hypothesis rising from such integration is that plant traits like seed size or specific leaf area (SLA) can constrain the set of environments adequate for recruitment. This hypothesis is mostly at the core of studies that analyze the relationships between seed size, growth rate (or related parameters), and habitat conditions through multispecific comparisons (Marañón & Grubb, 1993; Saverimuttu & Westoby, 1996; Walters & Reich, 2000). Here we test this hypothesis at the within-species level with *Olea europaea* (hereafter *Olea*), a Mediterranean tree. Previous work with this species has demonstrated the importance of seed size as a key life-history trait (Rey *et al.*, 1997; Alcántara *et al.*, 2000a,b; Alcántara & Rey, 2003). Moreover, Rey and Alcántara (2000) have demonstrated that the recruitment of *Olea* is confined to the shade of heterospecific shrubs, being inhibited by conspecific plants and in full sun.

Here we explore the relationships between seed size and the observed pattern of recruitment among different microhabitats. In this context, the above hypothesis specifically states that the type of microhabitat suitable for *Olea* recruitment is determined by microhabitat-specific relationships between seed size and seedling growth rate. Testing this hypothesis requires two steps: 1) demonstrating that seed and/or seedling size correlate with seedling growth rate, and that such correlation varies among microhabitats; and 2) demonstrating the link of seedling size with seedling survival through its effect on the growth rate in different microhabitats.

## Methods

### STUDY SPECIES AND AREA

The wild olive tree (*Olea europaea* var. *sylvestris*, Oleaceae) occurs along the northern Mediterranean basin. Fruits (drupes) ripen during autumn and winter and contain a single seed (embryo plus endosperm) wrapped in a hard endocarp. Propagules (seed plus endocarp; seed hereafter) average  $10.6 \pm 1.8$  mm in length,  $6.2 \pm 0.8$  mm in width, and  $226.0 \pm 75.0$  mg in fresh mass (mean  $\pm$  SD). Mean seed size, as estimated by seed mass, differs considerably between trees in the same year, ranging from 22.8 to 325.6 mg (Alcántara *et al.*, 2000b). Small- to medium-sized frugivorous birds, mainly in the genera *Turdus* and *Sylvia*, disperse the seeds.

The study was conducted in Sierra Sur de Jaén ( $37^{\circ} 40' N$ ,  $3^{\circ} 45' W$ ; Jaén province, southern Spain) in a dense scrubland dominated by wild olive trees ( $17 \text{ trees} \cdot \text{ha}^{-1}$ ), *Quercus coccifera*, *Pistacia terebinthus*, and *Phillyrea latifolia*. Scrubland patches of a few hectares are scattered among olive orchards, pine forests, and old fields. This landscape is common in the study region. The climate is of Mediterranean type, with a mean annual temperature of  $16^{\circ} C$  and mean annual rainfall of 539 mm. The dry period typically extends from June to October, when the balance between soil water reserves, evapotranspiration, and rainfall determines a period of water deficit. During the dry season, the mean monthly temperature ranges from  $23.0^{\circ} C$  in June to  $27.4^{\circ} C$  in July, whereas rainfall is below  $10 \text{ mm} \cdot \text{month}^{-1}$  in July and August (Junta de Andalucía, 1996).

### SOWING EXPERIMENT: RELATIONSHIP BETWEEN SEED SIZE AND SEEDLING GROWTH

In November 1994, we sowed a total of 2,400 seeds distributed in 72 sites ( $25\text{-cm} \times 25\text{-cm}$  square plots) according to a three-factor design: cover (scrub cover *versus* open interspaces), density (three sowing densities: 16, 25, or 64 seeds per site), and seed size (small [ $< 0.1$  g], medium [ $0.1\text{-}0.2$  g], and large [ $> 0.2$  g]). These classes represent the sizes dispersed by three different sets of avian frugivores in the study area (Rey *et al.*, 1997). Information about the frequencies of different seed sizes can be found in Alcántara *et al.* (2000b). The sowing sites were randomly distributed within each cover type. Sowing sites were protected from rodents with a wire mesh. Six of these sites were severely disturbed by animals and were not considered in the analyses. Germination was monitored every 10 d following the first seedling emergence (December 1995), until May 1996. Within 10 d of emergence, we measured width and length of one cotyledon from each seedling and estimated its area as the surface of an ellipse. We further estimated leaf expansion until May (130 d after the first seedling emerged) by periodically recording length and width of each new leaf and assuming an oval leaf surface. With this information, we estimated leaf area growth rate (LAGR) as

$$\text{LAGR} = \log(\text{LA})/\text{number of days after emergence} \quad [1]$$

I have interchanged Appendix I and II because Appendix II was cited before Appendix I. OK?

where LA is total leaf area (including cotyledons) at the end of the study. Using LA to estimate plant growth might be misleading if the plant increases its aboveground biomass with no increment in LA (*i.e.*, reducing its SLA). However, changes in total biomass and relative growth rate are more clearly related to total leaf area than to SLA in *Olea* seedlings under different light treatments (Fetene & Feleke, 2001). Moreover, Pritsa *et al.* (2003) show that both mean and total leaf area are similarly and positively correlated with SLA in seedlings of different *Olea* cultivars. Therefore, we can assume that LAGR is a good proxy for aboveground biomass growth in *Olea* seedlings.

#### TRANSPLANT EXPERIMENT: EFFECTS OF SIZE, DENSITY AND MICROHABITAT ON SEEDLING GROWTH AND SURVIVAL

In March 1996 we collected recently emerged seedlings from places across the study area with a deep litter layer, where roots were loosely attached to the soil. Seedlings were transferred to the laboratory, where we randomly sampled 100 individuals to characterize shoot length, root length, cotyledon width and length, and total fresh and dry mass (see [Appendix I](#)). This information allowed us, first, to identify some basic anatomical parameters that could be used as estimators of seedling size in the field. We found that fresh mass correlated better (through partial correlation analysis) with cotyledon area ( $r_{\text{partial}}: 0.80, P < 0.01, n = 100$ ) than with shoot height ( $r_{\text{partial}}: 0.43, P < 0.01, n = 100$ ) or root length ( $r_{\text{partial}}: 0.38, P < 0.01, n = 100$ ). Furthermore, cotyledon area was also highly correlated with seedling dry mass ( $r = 0.82, P < 0.01, n = 20$ ). Therefore, cotyledon area was a good predictor of seedling size. Second, it allowed us to analyze the allocation among root, shoot, and cotyledons at an early stage of seedling development, therefore assessing whether leaf expansion could be a good proxy for growth in biomass. Partial correlations indicated that cotyledon area and shoot height were significantly correlated ( $r_{\text{partial}}: 0.52, P < 0.01, n = 100$ ). However, root length was independent of both shoot length ( $r_{\text{partial}}: -0.15, P > 0.1, n = 100$ ) and cotyledon area ( $r_{\text{partial}}: 0.04, P > 0.1, n = 100$ ). Therefore, at least during the early development of the seedlings, the allocation to aerial parts seems to be independent of the allocation to roots. Furthermore, we found an allometric relationship among seedling mass and LA ( $b = 0.84 \pm 0.05, r^2 = 0.70, F_{1,99} = 235.3, P < 0.001$ , regression with log transformed data; (otherwise stated, parameter values are indicated SE hereafter), which illustrates that growth measured in terms of leaf area is a good surrogate for growth measured in terms of biomass.

The remaining collected seedlings were individually transplanted into 350-cm<sup>3</sup> root-trainers filled with soil from the study site, watered daily, and kept at room temperature, light, and humidity. After 2 weeks, healthy seedlings were individually tagged and transplanted back, with a soil core, into the field according to the following design. Twelve transplant sites, with 16 transplanted seedlings each, were randomly established under the canopy of the three dominant scrub species (microhabitats) in the study area (*O. europaea*, *P. latifolia*, and *Q. coccifera*) and in open interspaces. Different types of

scrub cover were considered to represent distinct micro-environments for seedling establishment. In August 1996, we measured some soil properties (texture, fertility, and water retention) and in March 1997, we measured photosynthetically active radiation (PAR hereafter) in each transplant site. Appendix II shows that the four microhabitats effectively represented distinct micro-environments differing mainly in potassium concentration, water retention capacity, texture, and PAR.

We created three classes of seedling density by varying the distance between plants: high, medium, and low density (seedlings separated by 3, 7, and 16 cm, respectively). The high-density category promoted direct competition for light from the beginning of the experiment, since the mean cotyledon length was *ca* 1.5 cm. All three densities were naturally found in the field (Rey & Alcántara, 2000). Each microhabitat  $\times$  density combination was replicated four times in a fully crossed two-factor design with 48 transplant sites. We measured cotyledon area (LA<sub>0</sub>) of each seedling when transplanted.

Seedlings were watered (0.5 mm  $\cdot$  m<sup>-2</sup> every 3 d) during the first 2 weeks to minimize death due to transfer shock. Seedlings dying within the first week after transplanting were replaced. We started recording seedling growth and survival after 4 weeks. Seedling survival was checked every 10 d until July 1996 and then monthly until October 1997. Mortality causes were classified as herbivory (shoots eaten, including cotyledons, leaves, and buds; or seedlings found in previous checks to have lost more than 50% of their leaf area); fungal attack (rotting; seedlings showed stem necrosis in previous checks); physical damage (seedlings chopped off by fallen twigs, stones, or trampling); water stress (dry seedlings without evident damage in previous checks); other causes, including missing seedlings and dead ones not included in any of the former categories (*e.g.*, irregular growth or discoloured leaves).

We estimated growth by periodically recording the number of expanded leaves of each seedling. In addition, in April, May, and October 1996 and in May 1997, we estimated total leaf area (LA, including cotyledons and true leaves) and calculated LAGR for early growth (April-May 1996, mainly dependent on cotyledon reserves); growth during the dry period (June-October 1996, with seedlings under water stress, see above); and growth during the period of favourable weather (November 1996 to May 1997). We estimated LAGR for each period as

$$\text{LAGR} = \frac{[\log(\text{LA}_t) - \log(\text{LA}_{t-1})]}{\text{number of days between measures}} \quad [2]$$

where LA<sub>t</sub> is total leaf area at the end of period t and LA<sub>t-1</sub> is total leaf area at the end of the previous period.

#### STATISTICAL ANALYSES

##### SEEDLING GROWTH

Testing the hypothesis formulated in the introduction first requires us to confirm that seed and/or seedling size correlates with seedling growth rate, and that such correlation varies among micro-environments (*i.e.*, microhabi-

tat and density). We performed statistical tests that appropriately consider simple and interaction effects implicit in this prediction. Specifically, the sowing experiment was analyzed using a General Linear Model with LAGR as dependent variable and emergence date as a covariate (GLM procedure, SAS Institute, 1996a,b). Data from the transplant experiment were analyzed with a mixed model ANCOVA with LAGR as the dependent variable, using the procedure MIXED (SAS Institute, 1996a,b), which allows both fixed (microhabitat and density) and random factors (transplant site, nested in both main effects) in the analysis. Given that separate analyses were done for each growth period, we incorporated seedling size at the beginning of each period ( $LA_{t-1}$ ) as a covariate. Finally, the interaction of  $LA_{t-1}$  with microhabitat and seedling density was added to test whether the effect of seedling size on growth differed in different environments. The model was fitted using “restricted maximum likelihood” (REML; SAS Institute, 1996a,b). We conducted separate analyses for each of the growth periods described above. These periods are characterized by very different water availability and in fact reflect the difference between a first growth stage, when the seedlings still depend on seed reserves, and later stages, when seedlings depend on external resources.

#### SEEDLING SURVIVAL

The second step needed to test the hypothesis is to assess whether the relationship between seedling size and growth results in different probabilities of seedling establishment under different microhabitats and densities. Thus, from the transplant experiment we analyzed *i*) the spatial heterogeneity of the probability of establishment; *ii*) the overall relationship between survival, growth, and seedling size; and *iii*) dependence of this relationship on microhabitat.

To analyze the spatial variation in seedling establishment, we used a Generalized Linear Model with binomial distribution and a logit link function (GENMOD procedure, SAS Institute, 1996a). The dependent variable was the proportion of seedlings surviving in each transplant at the end of the experiment, and the main fixed effects were microhabitat and seedling density.

We conducted a binomial regression (with logit link function) to examine the overall relationship between seedling survival, growth, and size. Specifically, we tested whether seedling survival at the end of a given period was related to seedling size at the beginning of that period and to seedling growth during the previous one. The effect of microhabitat type on such relationships was further explored by conducting a proportional hazard regression (PHREG procedure, SAS Institute, 1995) where seedling lifespan was the dependent variable and the life-time predictors were the initial seedling size at the time of the transplant ( $LA_0$ ) and LAGR during the initial growth. Microhabitat was considered as a stratification factor in the model.

## Results

#### SEEDLING GROWTH IN RELATION TO SEED SIZE

No seed germinated the first year, but 472 (19.6%) germinated during the second one. Seed size positively

influenced germination (ANOVA,  $F_{2,60} = 20.7$ ,  $P < 0.001$ ), the percentage of small, medium-sized, and large seeds germinating being  $16.3 \pm 2.8\%$ ,  $26.7 \pm 2.8\%$ , and  $47.9 \pm 3.2\%$ , respectively. Germination also depended on microhabitat ( $F_{1,60} = 44.9$ ,  $P < 0.001$ ), being considerably higher under scrub cover ( $41.4 \pm 2.3\%$ ) than in open interspaces ( $17.8 \pm 2.5\%$ ). The effect of seed size was consistent under scrub cover and open interspaces, as suggested by the non-significant interaction effect ( $F_{2,60} = 1.38$ ,  $P = 0.26$ ). Similarly, when testing the effects of seed size, microhabitat, and sowing density (and their interactions) on initial cotyledon area, we only found a positive effect of seed size on cotyledon area ( $F_{2,37} = 24.4$ ,  $P < 0.001$ , considering only sites where at least two seeds germinated):  $45.0 \pm 3.2 \text{ mm}^2$ ,  $71.8 \pm 5.5 \text{ mm}^2$ , and  $89.3 \pm 6.0 \text{ mm}^2$  in seedlings from small, medium-sized, and large seeds, respectively. This positive effect was consistent across microhabitats and densities (*i.e.*, interactions with microhabitat or density were not significant). Growth was affected by seed size, but its effect depended on the microhabitat (Table I). Specifically, LAGR was negatively related to seed size in open interspaces but was independent of size under scrub cover (Figure 1). Emergence date had a positive effect on LAGR, such that late-emerging seedlings had a higher growth rate.

#### SEEDLING GROWTH IN THE TRANSPLANT EXPERIMENT

The effects of seedling size, density, and microhabitat on seedling growth are summarized in Table II. Microhabitat type affected growth only during the initial period. Seedlings in open interspaces had the lowest LAGR ( $5.12 \times 10^{-3} \pm 0.87 \times 10^{-3} \text{ mm}^2 \cdot \text{d}^{-1}$ ), followed by those beneath the close coppice of *Q. coccifera* ( $6.48 \times 10^{-3} \pm 0.37 \times 10^{-3} \text{ mm}^2 \cdot \text{d}^{-1}$ ), while seedlings under *O. europaea* and *P. latifolia* had higher and similar LAGR ( $7.73 \times 10^{-3} \pm 0.38 \times 10^{-3} \text{ mm}^2 \cdot \text{d}^{-1}$  and  $7.84 \times 10^{-3} \pm 0.37 \times 10^{-3} \text{ mm}^2 \cdot \text{d}^{-1}$ , respectively). Seedling density had a negative effect on growth only during the dry period, when the LAGR of seedlings transplanted at high density was  $0.36 \times 10^{-3} \pm 0.16 \times 10^{-3} \text{ mm}^2 \cdot \text{d}^{-1}$ , while the LAGR of seedlings at medium and low density scored  $1.02 \times 10^{-3} \pm 0.10 \times 10^{-3} \text{ mm}^2 \cdot \text{d}^{-1}$  and  $1.36 \times 10^{-3} \pm 0.09 \times 10^{-3} \text{ mm}^2 \cdot \text{d}^{-1}$ , respectively. Seedling size, estimated as  $LA_{t-1}$ , had a significant effect on growth only during the initial and dry periods. Interestingly, the size effect varied significantly between microhabitats (note the different slopes in Figure 2), as evidenced by the significant interaction between microhabitat and  $LA_{t-1}$  (Table II). In any case, the coefficient of the “size effect” was negative and differed from 0 in all microhabitats ( $P < 0.05$  in all cases except *Q. coccifera* during the dry period [ $P < 0.1$ ], see Figure 2). Thus, smaller seedlings grew faster during the first months but not after the dry period. Different LAGRs in the first months did not compensate for the initial differences in seedling size, since there was a positive correlation between cotyledon area and the final LA ( $r = 0.39$ ,  $P < 0.001$ ,  $n = 321$ , considering only seedlings alive at the end of the study).

Only transplant site accounted for variation in mean LAGR during the period of favourable weather. In fact,

TABLE I. Results of General Linear Model analysis testing the effect of microhabitat, seed size, sowing density, and emergence date (included as a covariate) on seedling growth rate (LAGR, see text) estimated from the sowing experiment. A previous step in the analysis (not shown) tested the microhabitat  $\times$  emergence date and seed size  $\times$  emergence date interactions (*i.e.*, a heterogeneity of slopes ANCOVA model), but given that no significant interaction effect was found, we proceeded with the homogeneity of slopes model.

Effect	df	F	P
Microhabitat (M)	1	0.82	0.36
Seed size (SS)	2	2.31	0.10
Sowing density (SD)	2	0.30	0.74
M $\times$ SS	2	3.86	0.02
M $\times$ SD	2	0.54	0.58
SS $\times$ SD	4	0.63	0.64
Emergence date	1	2,383.5	< 0.001

the effect of transplant site was significant in all three periods, suggesting that growth conditions varied at a small scale within microhabitats.

#### SEEDLING SURVIVAL

Two years after transplanting, 37.9% of the seedlings were still alive. Water stress was responsible for 76.2% of the deaths, physical damage for 7.6%, and rotting for 6.0%. Pests (3.2%) and herbivores (2.7%) caused minor losses. The proportion of seedlings surviving at the end of the study was significantly affected by microhabitat, but not by seedling density or the interaction between the two factors (Table III). Seedlings in open interspaces had the highest mortality rate, followed by those under wild olive trees. Mortality was much lower under *P. latifolia* and *Q. coccifera*.

Concerning the influence of LAGR and  $LA_{t-1}$  on seedling survival, we found that during the dry period, higher previous LAGR had a negative effect on seedling survival (binomial regression,  $b = -0.06 \pm 0.02$ ;  $\chi^2 = 6.83$ ,  $P < 0.01$ ). In contrast, a higher initial seedling size was advantageous for survival during this stressful period ( $b = 0.006 \pm 0.001$ ;  $\chi^2 = 62.7$ ,  $P < 0.001$ ). During the next period, with milder climatic conditions, neither the previous LAGR nor seedling size significantly affected subsequent survival ( $P > 0.05$  in both cases). Consequently, seedlings that allocated more resources to growth during their first months jeopardized their survival during the summer. However, after the dry period, LAGR became independent of seedling size (see above) and did not affect survival. A proportional hazard regression model (Table IV) further showed a decrease in mortality with seedling size and an increase with the initial LAGR. However, this pattern differed between microhabitats (stratification effect: Wald  $\chi^2 = 21.2$ ,  $P < 0.001$ ). Larger cotyledon area decreased mortality (although it was not significant under *Q. coccifera*), whereas growth rate significantly increased it only under *Q. coccifera* and open interspaces.

### Discussion

Seed size was significantly related to seedling mass, a result that has been found for many other plants (Kang,

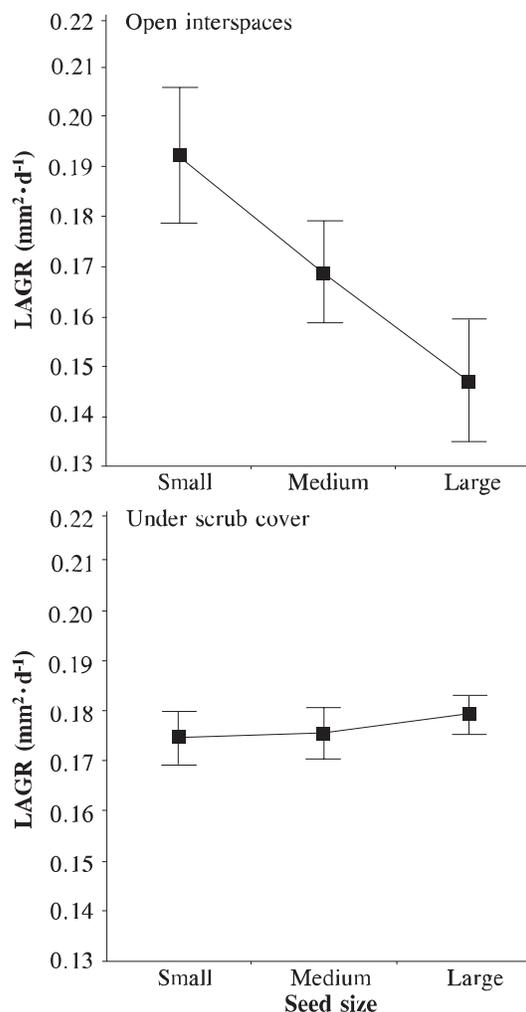


FIGURE 1. Growth rate (LAGR) for seedlings emerged from seeds of different size (small [ $< 0.1$  g], medium [ $0.1-0.2$  g], and large [ $> 0.2$  g]) and in different microhabitats. Least square mean LAGR ( $\pm 1$  SE) derived from the analysis shown in Table I is indicated for each combination of seed size and microhabitat.

Jaschek & Bawa, 1992; Moegenburg, 1996; Bonfil, 1998). The high variability in wild olive seed size (Alcántara, 1998) creates a large range in initial seedling size, with the potential to determine differences in growth and survival.

#### FACTORS AFFECTING SEEDLING GROWTH

The importance of the factors affecting growth varied according to the stage of seedling development. During the first months of life, growth rate was negatively related to cotyledon size and hence to seedling size. This has been observed for several species (Zimmerman & Weis, 1983; Gross, 1984; Zhang & Maun, 1990) although it is not a general pattern (Choe *et al.*, 1988; Meerts & Garnier, 1996; Bonfil, 1998). Growth rate further depended on microhabitat during this period, probably reflecting different abiotic conditions.

Seedling growth rate decreased considerably during the dry period, probably as a consequence of the synergic action of water deficit and excessive PAR, which may promote photoinhibition (see Demming-Adams, 1992 for

TABLE II. Mixed model ANOVA analyzing seedling growth (LAGR) in the transplant experiment during three periods: April to May (initial growth), June to October (period of water deficit), and November to May (favourable weather).  $LA_{t-1}$  had a significant effect on LAGR during the first and second periods, but LAGR *versus*  $LA_{t-1}$  plots (see Figure 2) showed that the slope was different for each microhabitat and density. Thus, we proceeded with a separate-slopes model analysis.

Periods	April-May			June-October			November-May		
	$\chi^2$	df	P	$\chi^2$	df	P	$\chi^2$	df	P
<b>FIXED EFFECTS<sup>1</sup></b>									
Microhabitat	15.76	3,36	< 0.001	5.26	3,29	0.415	2.25	3,28	0.52
Density	0.52	2,36	0.80	11.25	2,29	< 0.01	0.58	2,28	0.75
Microhabitat $\times$ density	3.90	6,36	0.69	11.40	6,29	0.077	4.64	6,28	0.59
<b>COVARIATES</b>									
$LA_{t-1} \times$ microhabitat	20.70	3,522	< 0.001	13.04	3,29	< 0.01	3.89	3,27	0.27
$LA_{t-1} \times$ density	1.99	2,522	0.37	1.96	2,29	0.38	0.37	2,27	0.83
<b>RANDOM EFFECTS</b>									
Transplant site	Wald Z	P		Wald Z	P		Wald Z	P	
Residuals	2.97	< 0.01		2.43	0.01		2.45	0.01	
	13.88	< 0.001		11.92	< 0.001		11.65	< 0.001	

<sup>1</sup> Test: Wald  $\chi^2$  type III

a review; Angelopoulos, Dichio & Xiloyannis, 1996 for photoinhibition in *Olea*). During this period competition among seedlings occurred, since growth was negatively affected by density in the transplant sites. Furthermore, growth rate was still negatively affected by seedling size. The negative relationship between size and growth rate during the initial and dry periods was significant in all microhabitats, although its magnitude differed, being especially dramatic in open interspaces (Figure 2).

Compared to the initial period, growth resumed at a lower rate after summer. Moreover, at this time neither the extrinsic factors evaluated here nor seedling size influenced growth rate, suggesting that seedlings had depleted the seed reserves (De Steven & Putz, 1984) and that only fine-scale environmental variation occurring among transplant sites was important for seedling growth at this point.

These results corroborate the existence of a size-mediated ecophysiological response of seedlings to environmental variation, which is set at the earliest stages of the seedling development.

#### CAUSES OF MORTALITY AND ENVIRONMENTAL FACTORS AFFECTING SEEDLING SURVIVAL

Water stress was the major mortality cause for *Olea* seedlings. This was also found when studying natural recruitment of wild olives at the same study area (Rey & Alcántara, 2000) and has been reported for many woody plant species in southern Spain (*Phillyrea latifolia*, Herrera *et al.*, 1994; *Pistacia lentiscus*, García-Fayos & Verdú, 1998; *Juniperus communis*, García, 2001; *Pinus sylvestris*, Castro, Zamora & Hodar, 2002; Castro *et al.*, 2002).

Seedling transplants show that mortality was highly influenced by microhabitat but not by density. In contrast to our findings concerning seedling growth, we detected no intra-specific competitive effects on survival. This demonstrates the important contribution of micro-environmental abiotic characteristics (here summarized as microhabitat effects) to recruitment during the early seedling stages. The influence of microhabitat on seedling survivorship is probably related to facilitation by heterospecific scrubs and to inhibition by conspecific adults. Although the mechanisms of such processes are not disclosed here, some microenvironmental characteristics render useful

insights. Open interspaces were clearly adverse for transplanted seedlings, which is consistent with the lack of natural recruitment found in such sites (Rey & Alcántara, 2000). This is likely related to the stressful combination of low soil water retention capacity and very high incident radiation occurring in this microhabitat. Moreover, incident radiation is probably related to high temperatures, and subsequently to very low soil moisture, registered in open interspaces during the summer in the Mediterranean region (Retana *et al.*, 1999; Gómez, in press). In contrast, as shown by this study, scrub cover ameliorates such stressful conditions, ultimately enhancing seedling survival (see similar results with other Mediterranean scrubs in Retana *et al.*, 1999). Regarding the inhibition under conspecifics, our results show that it is not mediated by seedling density. There is no reason to presume worse hydraulic capacity under wild olive trees than under other shrubs (see Appendix II). Conspecific allelopathy (Nilsson, 1994; Jäderlund, Zackrisson & Nilsson, 1996) and/or competition for soil resources with adults are possible explanations that cannot be ruled out.

#### RELATIONSHIPS AMONG SEEDLING SIZE, GROWTH, AND SURVIVAL

Growing evidence suggests advantages of large seeds under a wide variety of hazards (Westoby, Leishman & Lord, 1996), including water stress. Thus, if size were functional in relation to this factor, we should find a positive relationship between survival and seed size in microhabitats prone to water deficit (*e.g.*, open interspaces) and a minor advantage of size in microhabitats prone to water retention (*i.e.*, under scrubs; Joffre & Rambal, 1993; Verdú & García-Fayos, 1996; Pugnaire, Haase & Puigdefábregas, 1996). In our study, seed size was positively related to seedling size, and the latter was positively related to the short-term probability of seedling survival (Weller, 1985; Tripathi & Khan, 1990; Walters & Reich, 1996). The influence of size on seedling survival varied among microhabitats. Specifically, it was not significant under *Quercus*, where water retention is usually high (Joffre & Rambal, 1993).

Several mechanisms have been proposed to explain the general benefits of seed size for survival (Westoby, Jurado & Leishman, 1992; Westoby, Leishman & Lord,

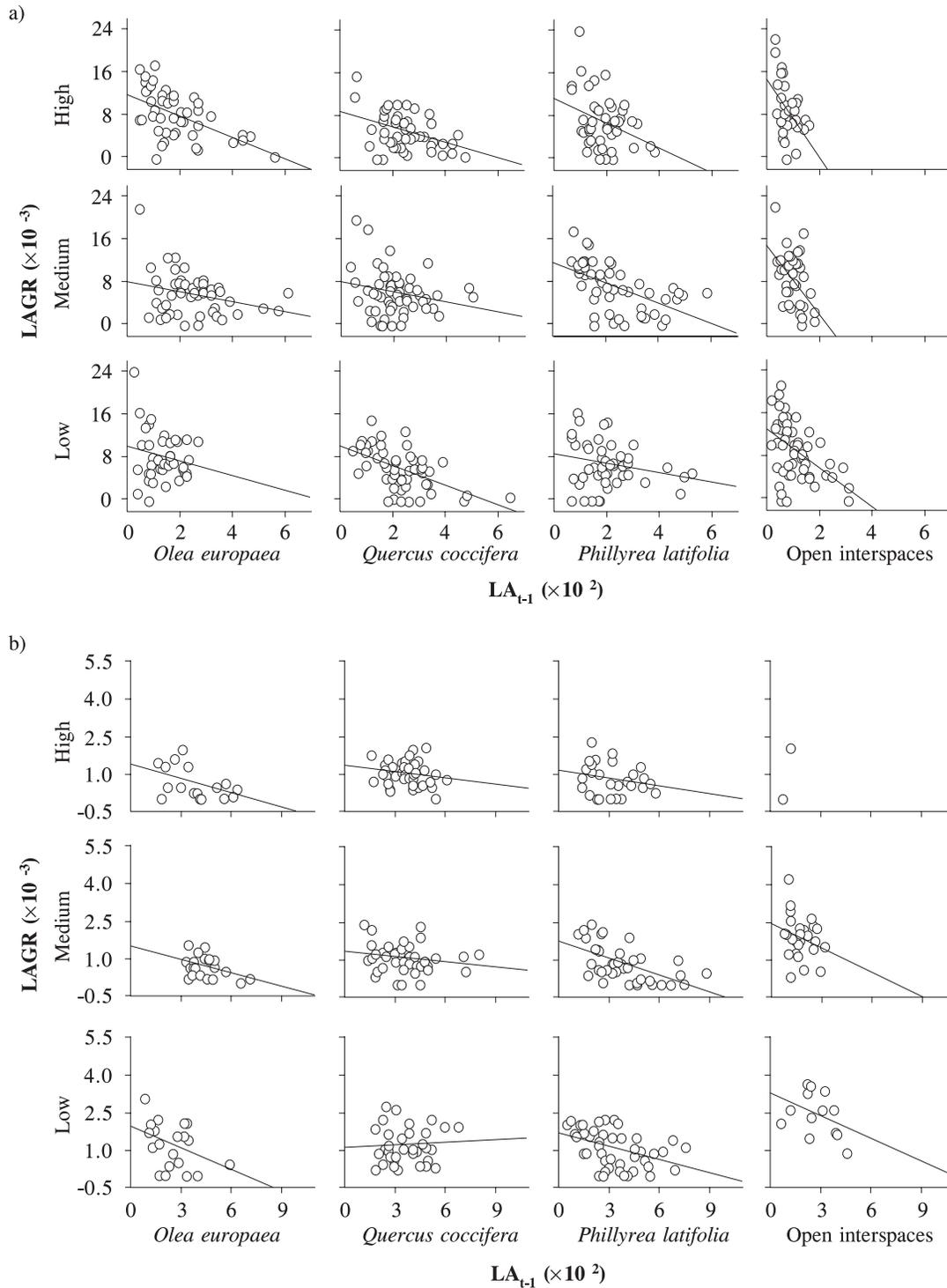


FIGURE 2. Relative growth rate as a function of leaf area under different combinations of microhabitat (columns) and density (rows). Leaf area ( $LA_{t-1}$ ) is measured in  $mm^2$ , whereas leaf area growth rate (LAGR) is measured in  $mm^2 \cdot d^{-1}$ . a) Data for initial growth period, b) data for the period of water stress.

1996). Among them, a seed-size-correlated metabolic effect seems particularly relevant for this study, since it relates size to the variable we used to evaluate seedling response to micro-environmental variation. Growth rate during the first months was negatively related to size, suggesting that larger seedlings consumed their reserves at a lower rate, with the associated benefits of reduced

respiratory costs and delayed dependence on external resources. Consequently, these seedlings were less susceptible to negative carbon balance during the long Mediterranean summer drought. This probably contributes to a positive effect of size on survival mediated by the amount of reserves. Larger wild olive seeds contain higher net and relative amounts of endosperm (Alcántara,

1998), which would allow them to allocate more resources to early growth or to self-maintenance during critical periods. In any case, as expected from different micro-environmental conditions, these seed-size-correlated effects varied among microhabitats, being less intense under less stressful environments.

Thus, our results support the second part of the hypothesis addressed in this study, namely that the relationship between seedling traits and growth extended to a differential ability of seedlings to establish in different micro-environments within the same habitat.

Summarizing, this study experimentally demonstrates the importance for seedling establishment of some of the mechanisms suggested from observational studies of the recruitment in *Olea*: the occurrence of facilitation by heterospecific scrubs and inhibition under conspecifics and in open interspaces (Rey & Alcántara, 2000). In addition, we demonstrate that a trait-mediated ecophysiological response of seedlings to their microenvironment can partly explain the observed spatial pattern of seedling establishment: the higher growth rate of seedlings from small seeds was especially detrimental in open interspaces, where the frequency distribution of seed size was largely biased towards the smallest seeds (Alcántara *et al.*, 2000b). The low probability of survival of seedlings from small seeds in open interspaces thus accentuates the differences in recruitment among open interspaces and scrub-covered microsites, where medium and large seeds were more abundant.

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TABLE III. Two-way ANOVA of the proportion of seedling survival at the end of the transplant experiment (means ± SD). Data are analyzed following a binomial distribution with a logit link function and type III sum of squares. Both microhabitat and density have been entered as fixed effects. Significant differences between microhabitats are shown with different superscripts. The interaction effect was not significant ( $\chi^2_{(3,36)} = 4.31$ ;  $P = 0.64$ ).

Microhabitat	Mean ± SD	Density	Mean ± SD
$\chi^2_{(3,36)} = 13.97$ ; $P = 0.0030$		$\chi^2_{(2,36)} = 3.57$ ; $P = 0.17$	
<i>Olea europaea</i>	0.30 ± 0.07	High	0.33 ± 0.08
<i>Phillyrea latifolia</i> <sup>b</sup>	0.57 ± 0.07	Medium	0.50 ± 0.08
<i>Quercus coccifera</i> <sup>b</sup>	0.60 ± 0.07	Low	0.42 ± 0.07
Open interspaces <sup>a</sup>	0.19 ± 0.08		

TABLE IV. Proportional Hazard “Cox” regression testing the effect of microhabitat, LAGR, and LA<sub>0</sub> on seedling mortality. Results for the whole data set and by microhabitat are shown (after assessing statistical significance of microhabitat as stratification variable). Significance levels are indicated as <sup>ns</sup> $P > 0.05$ , \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

Microhabitat	Model	LAGR		LA <sub>0</sub>	
	Wald $\chi^2$	Coefficient ± SE	Wald $\chi^2$	Coefficient ± SE	Wald $\chi^2$
All	27.93***	0.006 ± 0.013	17.11***	-0.003 ± 0.001	12.77***
<i>Olea europaea</i>	8.79*	-0.021 ± 0.030	0.48 <sup>ns</sup>	-0.005 ± 0.002	7.88**
<i>Phillyrea latifolia</i>	8.71**	-0.028 ± 0.032	0.76 <sup>ns</sup>	-0.005 ± 0.002	6.95**
<i>Quercus coccifera</i>	6.97*	0.085 ± 0.035	5.83*	-0.002 ± 0.002	1.39 <sup>ns</sup>
Open interspaces	8.52**	0.050 ± 0.025	3.98*	-0.003 ± 0.002	4.03*

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APPENDIX I. Main anatomical parameters of *Olea europaea* var. *sylvestris* seedlings used in this study.

	Mean ± SD	n
Fresh mass (g)	0.1251 ± 0.0459	100
Dry mass (g)	0.0253 ± 0.0087	20
Cotyledon area (mm <sup>2</sup> )	121.15 ± 32.21	100
Root length (mm)	39.32 ± 8.07	100
Shoot length (mm)	38.25 ± 10.05	100
% Water*	84.74 ± 3.68	20

\* (Fresh mass - dry mass) × 100 / fresh mass

APPENDIX II. Summary of the environmental conditions (means ± 1 SE) of the microhabitats considered in this study. Photosynthetically active radiation measurements (PAR) were obtained between 0800 and 1800 on two cloudless days (March 11<sup>th</sup> and 14<sup>th</sup>) using a Quantum Sensor LI-190SA coupled with a Datalogger LI-1000 (LI-COR, Lincoln, Nebraska, USA). Soil properties were determined in the Instituto Agroalimentario de Atarfe (Junta de Andalucía) according to conventional procedures (n = 12, df = 3,44, \*P < 0.1, \*P < 0.05, \*\*\*P < 0.001).

Abiotic conditions	<i>Olea europaea</i>	<i>Phillyrea latifolia</i>	Microhabitats <i>Quercus coccifera</i>	open interspaces	<b>Title needed</b>
PAR (μmol m <sup>-2</sup> s <sup>-1</sup> )	120.23 ± 34.07	75.74 ± 20.66	80.24 ± 19.31	332.01 ± 49.91	13.29***
SOIL PROPERTIES					
Phosphorus (ppm)	39.42 ± 7.32	25.08 ± 2.85	24.83 ± 4.83	26.25 ± 4.05	1.96
Nitrogen (%)	0.76 ± 0.12	0.82 ± 0.10	0.68 ± 0.07	0.60 ± 0.07	1.07
Potassium (ppm)	1,022.92 ± 144.35	833.75 ± 62.08	829.17 ± 47.48	350.83 ± 38.15	11.58***
Organic carbon (%)	13.28 ± 2.23	14.88 ± 2.03	12.21 ± 1.36	9.63 ± 1.23	1.57
pH	7.97 ± 0.07	7.93 ± 0.05	7.95 ± 0.04	8.03 ± 0.03	0.81
pF 1/3 atm (%) <sup>a</sup>	43.15 ± 3.18	52.34 ± 3.91	46.10 ± 2.56	40.46 ± 2.13	2.87*
pF 15 atm (%) <sup>b</sup>	32.98 ± 3.86	40.09 ± 4.59	34.58 ± 2.83	26.32 ± 2.04	2.67*
Clay (%)	26.35 ± 3.54	28.98 ± 4.05	34.88 ± 1.61	21.15 ± 3.24	3.32*
Silt (%)	55.55 ± 5.01	56.24 ± 4.22	51.22 ± 1.98	62.01 ± 3.78	1.30
Sand (%)	18.10 ± 3.40	14.78 ± 2.43	14.09 ± 1.17	17.50 ± 1.66	0.56

<sup>a</sup>Soil moisture at the water holding capacity; <sup>b</sup>soil moisture at the permanent wilting point.

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- Les tirés à part sont tous brochés à plat; ils sont fournis dans le même format que celui de la revue ; ils ne comprennent pas de couverture.
- Ces prix sont sujets à changements si la commande est reçue après la date limite indiquée au verso.
- Les frais de poste et les taxes applicables s'ajoutent aux prix mentionnés.
- Le délai accordé pour le paiement est de 30 jours.
- Les prix sont indiqués en dollars canadiens.
- Reprints are all side-wire stappling; they are in the same size and format than the journal; all are without cover.
- Prices are subject to changes when order is received after the deadline indicated on reverse side.
- Postage charges and applicable taxes will be added to prices quoted above.
- Payment term is 30 days.
- Prices are given in Canadian dollars.