ORIGINAL PAPER

Regional and local variation in seedling emergence, mortality and recruitment of a perennial herb in Mediterranean mountain habitats

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Abstract Spatial patterns of seedling regeneration of woody Mediterranean plants are widely documented, with seedlings and juveniles growing almost exclusively under shrub and tree canopy. Neighbourhood habitat amelioration and consequent facilitative effects on recruitment have been extensively suggested as the major determinant of such spatial pattern for Mediterranean vegetation. Much less is known on recruitment patterns of perennial herbs. As herbs differ from woody plants in many relevant ecological aspects and in their life cycles, we would expect particularities in their recruitment dynamics. We analysed the spatial (regional, local and fine-scale) variation in environment (herb cover, litter depth, air temperature and irradiance) and its relationship with seedling emergence, mortality, and recruitment of the perennial herb *Helleborus foetidus* (Ranunculaceae) during 2 years. We conducted the study in three distant localities in south-eastern Iberian Peninsula (defining the regional scale). Local scale was defined in terms of microhabitat type based on three categories of vegetation cover (under evergreen shrubs or trees, under deciduous spiny-shrubs, and in open interspaces). Finally, fine-scale variation was defined in terms of 1 m^2 sampling plots. Our results showed regional, local and fine-scale variations in recruitment, with the major source of variation changing across scales. Seed input was important in determining differences in recruitment both at the largest (regional) and the finest scale. Environment had minor importance in shaping differences in recruitment at these two scales. At the local scale, variation between microhabitats was mainly related to differences in seedling survival through facilitative effects (alleviation of water and irradiance stresses) of shrub and tree cover, although such facilitative effect was not consistent in all localities or years. Our study thus points to a similarity in the spatial patterns of recruitment (and in the environmental factors shaping such patterns) between Mediterranean woody plants and perennial herbs, but much more effort is needed to assess the generality of such similarity.

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Introduction

The seedling stage is a critical step in demographical, ecological and evolutionary plant dynamics (Fenner 1987; Kitajima and Fenner 2000). It is the last stage in the plant regeneration cycle where dramatic reductions of recruitment potential appear (Harper 1977; Kitajima and Fenner 2000). Many biotic and abiotic factors affect seedling establishment. Biotic factors include herbivory (Howe 1990; Ida and Nakagoshi 1996), pathogen infections (Augspurger and Kelly 1984; Shibata and Nakashizuka 1995), seed dispersal (Rey and Alcántara 2000; Russo and Augspurger 2004), litter (Facelli and Pickett 1991; Eriksson 1995) and competition with herbs (DeSteven 1991). Influential abiotic factors include light (Walters and Reich 1996 and references therein), soil physical and chemical properties (Herrera 2002; Rey et al. 2004), and water stress during drought periods (Herrera et al. 1994; Rey and Alcántara 2000). All these factors are highly variable in space and time (see Gómez-Aparicio et al. 2005a and references therein). Understanding recruitment dynamics requires analysing plant responses to these variable factors. Thus, many studies with single (Schupp 1990; De Steven 1991, Herrera et al. 1994; Eriksson and Fröborg 1996; Rey et el. 2004) or multiple species (Jurado and Westoby 1992; Houle 1994; Cornelissen et al. 1996; Saverimuttu and Westoby 1996) have explored these responses at a local scale. They reported differential recruitment patterns in distinct environments (e.g. habitats and microhabitats). However, the exploration of variation in recruitment at different spatial scales remains infrequent, though it would be expected that the major determinants (biotic or abiotic) of seed and seedling ecology changed across spatial scales (Kollmann 2000; García et al. 2005; Gómez-Aparicio et al. 2005a, b).

Mediterranean environments are highly variable both locally (within habitats) and regionally (latitudinal and altitudinal variation), representing particularly adequate systems to explore the consequences of such variation on recruitment patterns (Gómez-Aparicio et al. 2005a, b). A growing body of knowledge on spatio-temporal dynamics in plant recruitment in Mediterranean ecosystems has accumulated in the last two decades (Herrera et al. 1994; Traveset et al. 2003; Gómez 2004; Rey et al. 2004; García et al. 2005; Gómez-Aparicio et al. 2005a). This knowledge has been centered on woody plants, whereas it remains rather scarce for herbs (but see Picó and Riba 2002). Water stress during the summer drought is commonly the major seedling mortality factor in Mediterranean woody plants and it brings about that the transition from seedlings to juveniles becomes the bottleneck in the recruitment dynamics and demography of many species (Herrera et al. 1994; Rey and Alcántara 2000; Traveset et al. 2003). In Mediterranean habitats, the canopy of established vegetation generates environmental heterogeneity, alleviates physical stress, and facilitates seedling survival (Gómez-Aparicio et al. 2004, 2005c). As herbs differ from woody plants in many relevant ecological aspects and in their life cycles, we should expect some particularities in their response to environmental variation and, in turn, in their recruitment dynamics. However, spatio-temporal variations of mortality and seedling recruitment in Mediterranean mountain herbs remains nowadays almost unexplored. For example, it has not been evaluated if the facilitative effect of shrub canopy on seedling establishment applies also to herbs.

This study is aimed at exploring spatial variations in the initial stages (seedling emergence and survival) of the recruitment of a perennial herb (*Helleborus foetidus* L.) in Mediterranean mountain habitats. It has been recently shown for this species that emergence and survival affect recruitment differently at high and low elevations because of altitudinal changes in the weather-related major stressing factor (Ramírez et al. 2006). Now we extend our exploration of the heterogeneity of recruitment to the fine (between 1 m^2 plots), small (between microhabitats within a locality) and large (between localities within a region) scales. Specifically, we address the following questions: (1) Do shrub and tree cover induce small-scale (between microhabitats within a locality) variation in seedling emergence, survival and recruitment in this perennial herb? (2) Is such variation regionally consistent? (3) Does the relative influence of different mortality factors resemble the pattern

found in Mediterranean woody plants? (4) Is the major source of variation in recruitment consistent across spatial scales? We conducted our study of seedling emergence, mortality and recruitment in three distant localities (each in a different mountain system) from south-eastern Iberian Peninsula. Conclusions on this study will add to the growing body of knowledge on plant recruitment patterns in Iberian Mediterranean habitats (Herrera et al. 1994; Rey and Alcántara 2000; Gómez-Aparicio et al. 2005a, and references therein).

Study system and methods

Helleborus foetidus L. (Ranunculaceae), hellebore hereafter, is a myrmecochorous, rhizomatous perennial herb widely distributed in Western Europe and also present in north-western Africa (Werner and Ebel 1994). In the Iberian Peninsula it occurs from 800 m a.s.l. to 2000 m a.s.l., in forest edges and clearings, and open shrublands. Adults reproduce sexually after several seasons of vegetative growth, and many of them die after a season of intense flowering. Flowers have 1–5 carpels (most commonly 2–3), each of which develops 10–15 elaiosome-bearing seeds (see details in Herrera et al. (2001, 2002)). It releases its diaspores during June-early July when many potential ant dispersers are attracted (Garrido et al. 2002). Although rodents consume hellebore seeds, quick ant removal of released seeds generally prevents extensive seed losses (Fedriani et al. 2004). Although the species may propagate asexually by adding new vegetative ramets, its spatial spread and patch colonization are exclusively based on seed dispersal (Rey et al. 2006) and its demography is mainly determined by seedling recruitment (Ramírez and Rey, unpublished matrix projection models). The toxic leaves of adult and juveniles are not consumed by cattle or wild ungulates.

The study was carried out between November 1998 and April 2001 in three localities, each located in a different mountain system (Sierra de Mágina, Sierra de Cazorla and Sierra de Segura). The locality from Sierra de Cazorla (1300 m a.s.l., 37°56′ N-2°52′ W, Cazorla, hereafter) is a *Pinus*

nigra forest with maximum temperature (T_{max}) of 37.1°C, minimum temperature (T_{min}) of –5.7°C, average temperature (T_{ave}) of 11.8°C, and average precipitation (P_{ave}) of 1111 mm (Climatic data from Junta de Andalucía 1996). The locality in Sierra de Mágina (1650 m a.s.l., 37°44′ N-3°28′ W, Mágina, hereafter) is a Mediterranean open shrubland with T_{max} of 37.8°C, T_{min} of 0.0°C, T_{ave} of 15.5°C, and P_{ave} of 695 mm. Finally, the locality at Sierra de Segura (1550 m a.s.l., 38°16′ N-2°32′ W, Segura, hereafter) is a Mediterranean open shrubland with T_{max} of 37°C, T_{min} of –8.4°C, T_{ave} of 11.6°C, P_{ave} of 800 mm. The maximum and minimum distances between localities were 102 km (Mágina-Segura) and 43 km (Cazorla-Segura), respectively. These three localities were chosen as representative of the type of habitat most often occupied by the species (Garrido et al. 2002, for further vegetation details) rather than as replicates; the maximum distance between localities was large enough to allow for exploration of regional variation while still keeping under Mediterranean mountain conditions.

In each location, 100 1 × 1 m permanent sampling plots were distributed along 10 parallel transects. Plots were 2 m from each other and the distance between transects varied from 10 m to 12 m. Fruit production per plant over 1999 and 2000 and the number of reproductive adults were counted at each plot. Plots were monitored at monthly intervals and all emerged seedlings were individually marked. This allowed to account for emergence and survival patterns, and to determine mortality causes. We followed two yearly cohorts of seedlings in each locality, one emerging in 1999 and the other in 2000.

Depending on the presence and nature of plant cover, each plot was assigned to one of three microhabitat types: "covered", microsites with a cover of *Quercus* and/or *Pinus* species (evergreen trees or shrubs offering a permanent cover); "partially covered", microsites with a cover of *Crataegus, Rosa* and *Berberis* species, deciduous spiny shrubs providing cover only part of the year; and "uncovered", microsites in open places without woody cover. The percentage of samples in each microhabitat (covered, partially covered, and uncovered) and locality was, respectively,

58.5, 0, and 41.5, at Cazorla, 12.6, 21.7, and 65.7, at Mágina, and 10.2, 30.0, and 59.8, at Segura.

Seedlings found dead were assigned to one of the following mortality causes: *herbivory*, epicotyl consumed by herbivores, including cotyledons, leaves and buds, or when in previous inspections seedling had lost more than 50% of leaf area; *physical damage*, snapped seedlings due to animal trampling, falling branches and stones, buried, etc; *water stress* (desiccation), dried seedlings presenting no other obvious anomalies on previous checks; *frost*, necrosed seedlings after snow or frosts; and *other causes*: seedling not falling in any of the previously defined categories.

To measure environmental factors, each sampling plot was characterized by herb cover (by visually estimating the percentage of plot surface covered by herbs), litter depth (estimated as the mean, for three points in a plot, of the depth of the litter layer; measured by driving a metric stack into the ground), temperature of the air adjacent to soil surface in the emergence period (measured at less than 1 cm from the soil surface with an air thermocouple thermometer), and PAR [photosynthetically active radiation; using a Quantum Sensor LI-190SA connected to a Datalogger LI-1000 (LI-COR, Nebraska, USA)]. PAR and air temperature measurements were obtained in April 2000 during the seedling emergence period. Three measurements were obtained from each sampling plot during a day (at dawn, midday, and dusk), always with completely clear sky. PAR and air temperature values used in the analyses were the average of three measurements at each plot. This information could not be obtained from Mágina due to persistent unfavourable weather; thus, we obtained information of irradiance at this locality through hemispheric photography taken in April at 10 sites under shrub cover and 10 in open patches. To record summer temperatures by microhabitat, we placed in Mágina and Cazorla, one Omega RH-temp OM-44 data logger in covered and uncovered microsites. Data loggers were set (half meter above ground) to take hourly data from June to August. We averaged data on maximum and mean daily temperature over the summer for each microhabitat.

Data analyses

Most analyses were conducted by fitting generalized linear models (GLIM) to the data with the GENMOD Procedure of SAS (SAS Institute 1999). In each case, we chose the error distribution and the link function according to the nature of the response variable: Poisson error and log link function for seedling counts (seedling emergence and seedling recruitment); binomial error and logit link function for proportions or binary response variables (seedling survival); and normal error and log link function for fruit crop and metric environmental variables. In all these analyses, the spatial variation (Microhabitat and Locality effects) was analysed considering both factors as fixed effects. After a significant interaction between factors was detected, decomposition of the interaction was conducted with planned post-hoc comparisons (option DIFF of the GENMOD), which tested differences between levels of a factor for a given level of the other factor. We conducted separate analyses for each cohort (1999 and 2000) in all cases.

The influence, at the fine-scale of 1 m^2 plots, of different environmental factors (herb cover, litter depth, PAR and air temperature) on emergence, survival and recruitment, was analyzed through Poisson regressions (separately for each cohort and locality). Only plots with hellebore adult plants (i.e. reproductive individuals) were considered in these analyses because we previously confirmed a strong positive effect of presence of an adult hellebore on seedling emergence in a plot (see Results). These analyses were made considering linear relationships between dependent and environmental variables, after checking that there was no evidence of nonlinear relationships. Unless otherwise stated, means are shown along 95% confidence limits.

Results

Regional and microhabitat-induced variation in environment

Air temperature at the time of emergence (April) differed among localities ($F_{1,189} = 75.8$, $P < 0.001$;

Segura = $19.6 \pm 0.3°C$; Cazorla = $17.7 \pm 0.2°C$), but not among microhabitats ($F_{1,189} = 0.28$, $P = 0.60$), this being regionally consistent (nonsignificant locality × microhabitat interaction: $F_{1,189} = 2.0$, $P = 0.16$). In contrast, summer temperature was much higher in the uncovered microhabitat than under shrub cover. Thus, in Mágina, summer mean daily temperature was 22.8 and 19.0°C (uncovered-covered difference = 3.8°), while summer maximum daily temperature was 43.4 and 34.0°C (uncovered-covered difference = 9.4°). In Cazorla, summer mean daily temperature was 21.4 and 19.4 (uncovered-covered difference = 2.0°) while summer maximum daily temperature was 35.0 and 27.3°C (uncovered–covered difference = 7.7°). These data also suggest higher maximum summer temperatures at the open shrubland of Mágina than in the forest of Cazorla (note that a single data logger for each microhabitat and locality precluded statistical analysis for summer temperature).

PAR differed between Segura and Cazorla ($F_{1,189} = 36.5$, $P < 0.001$): it was higher in Segura (397.2 ± 64.3 μmol/m^{2}s) than in Cazorla (165.7 ± 39.6), as expected from the fact that the first is an open shrubland and the second a forest. PAR also varied between microhabitats ($F_{1,189} = 12.9$, $P < 0.001$; uncovered = 350 ± 39.1 μmol/m^{2}s; covered = 212.5 ± 64.7); such variation was regionally consistent (locality × microhabitat interaction not significant; $F_{1,189} = 1.25$, $P = 0.26$). Although not directly comparable with PAR data from Cazorla and Segura, estimates from Mágina (through hemispheric photography) were similar to Segura's and higher than in Cazorla (Mágina is an open shrubland) and also differed between microhabitats ($F_{1,18} = 85.3$, $P < 0.001$; uncovered = 290.6 ± 21.2 μmol/m^{2}s; covered = 158.4 ± 21.3). The higher irradiance characterizing uncovered microsites therefore occurred consistently across localities. Notice that, in order to make Par data directly comparable between localities, and due to the lack of partially covered microhabitat in Cazorla, only data of covered and uncovered microhabitats were considered.

Litter depth differed marginally among localities ($F_{2,334} = 2.9$, $P = 0.06$): it was greater in Cazorla (0.71 ± 0.27 cm) and Segura (0.80 ± 0.23 cm) than in Mágina (0.43 ± 0.24). Litter depth differed among microhabitats ($F_{2,334} = 11.0$, $P < 0.001$), and such differences were consistent across localities (nonsignificant locality × microhabitat interaction; $F_{3,331} = 2.04$, $P = 0.11$). More litter accumulated under shrub cover (1.10 ± 0.28 cm) than in uncovered (0.30 ± 0.27 cm) and partially covered (0.55 ± 0.32) sites (post hocs, $P < 0.05$).

Microhabitats differed in herb cover, though not in a regionally consistent way (significant microhabitat × locality interaction: $F_{1,3} = 3.52$, $P = 0.012$). In Mágina and Segura uncovered microsites had higher herb cover than shrub covered ones (post hocs, $P < 0.05$), whereas in Cazorla there were no differences (post hoc, $P > 0.05$). On the other hand, the only microhabitat differing in herb cover among localities was the uncovered, with lower values in Cazorla (22.9 ± 8.4 %) than in Segura (40.9 ± 6.7%) and Mágina (66.5 ± 6.7%) (post hocs, $P < 0.05$).

Fruit production and seedling emergence

Fruit production per plant varied among localities (Table 1a). No effect of microhabitat on production was detected and this was regionally consistent (interaction locality × microhabitat not significant). The number of fruits per plant was similar in Mágina and Segura (post hoc, $P = 0.12$); however, plants in Cazorla produced substantially fewer fruits than those at the other localities (post hocs, $P > 0.05$ in both cases) (fruits per plant: Mágina = 135.3 ± 45.1; Segura = 141.6 ± 42.3; Cazorla 27.0 ± 66.8). However, the density of plants producing fruits was higher in Mágina ($0.22/m^2$) than in either Segura ($0.17/m^2$) or Cazorla ($0.10/m^2$); consequently, fruit production per plot was higher in Mágina than in Segura and here higher than in Cazorla.

Seedlings emerged between January and May, with a peak from late February to March. Temporal pattern of emergence was similar among localities for the 1999 cohort (Kendall's concordance coefficient, $W = 0.29$, $P = 0.04$, $n = 11$), but not for the 2000 cohort ($W = 0.22$, $P < 0.26$, $n = 11$). Within localities, emergence did not exhibit the same temporal pattern between cohorts

Table 1 Summary of results of GLIM testing differences between localities (Cazorla, Mágina, and Segura) and microhabitats (covered, uncovered, and partially covered) in fruit production (a) and seedling emergence (b) of hellebore seedlings for the two cohorts studied (1999 and 2000). χ^2 refers to Wald's Chi-square. *$P < 0.05$, **$P < 0.001$, ***$P < 0.0001$, ns = $P > 0.05$

| Factors | (a) Fruit production | | | (b) Emergence | | | | |
| | 2-years accumulated | | | 1999 | | | 2000 | |
	df	χ^2	P	df	χ^2	P	χ^2	P
Locality	2	20.8	***	2	15.09	***	12.82	*
Microhabitat	2	0.43	ns	2	6.46	ns	3.04	ns
Loc * micro	2	0.12	ns	3	2.99	ns	3.67	ns

(Cazorla: $W = 0.04$, $P < 0.56$; Mágina: $W = 0.25$, $P < 0.17$; Segura: $W = 0.42$, $P < 0.08$; $n = 6$ for all the cases). Differences between years in the temporal course of emergence were due to the earlier emergence in the second year.

Seedling emergence density varied regionally (Table 1b). This effect was consistent for the two cohorts, with emergence being higher in Mágina than in either Cazorla or Segura in both years (Fig. 1). Microhabitat type did not affect emergence in 2000, and its effect was only marginal in 1999 ($P < 0.1$). Furthermore, the weak influence of microhabitat was congruent across localities

during both years (not significant interaction effect, Table 1b).

The presence of adult hellebore plants in a plot was a major determinant of seedling emergence at all localities (Table 2, Fig. 1), although the magnitude of the effect varied regionally. In Segura the difference between plots with and without adult plants was significant but smaller than in the other localities, and this pattern remained constant between years. Emergence beyond the neighbourhood of adult hellebore plants was generally negligible. Furthermore, fruit production at the fine-scale of 1 m² plots was

Fig. 1 Upper panels: seedling emergence density in the three localities. Lower panels: seedling emergence probability in plots with and without hellebore adult plants. In all cases, model-predicted means and 95% confidence limits are represented
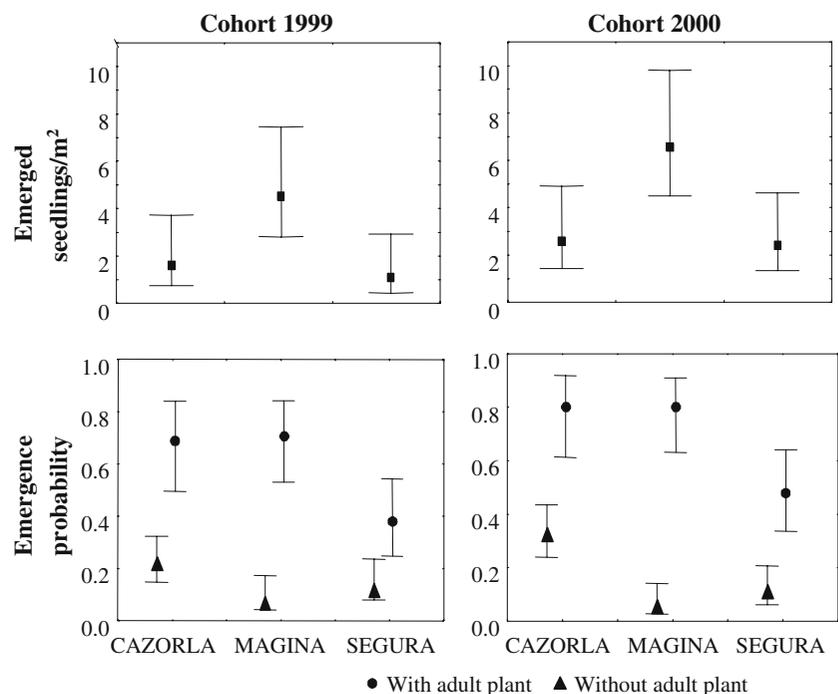
Table 2 Results of generalized linear model testing for the effect of the presence of adult hellebore plants on the probability of emergence of seedlings in individual plots

Factors	1999			2000		
	df	Wald χ^2	P	df	Wald χ^2	P
Locality	2	7.34	*	2	15.25	***
Hellebore presence	1	56.69	***	1	72.00	***
Locality * hell. presence	2	6.87	*	2	7.92	*

Separate analyses were conducted for the two cohorts. *$P < 0.05$, ***$P < 0.0001$

directly correlated at each locality with seedling emergence ($r = 0.37$, $P = 0.04$ in Mágina; $r = 0.41$, $P = 0.01$ en Segura; $r = 0.51$, $P = 0.004$ in Cazorla; only considering plots with reproductive adults).

Survival

Overall, survival probability was low ranging between 0.06 (obtained at the uncovered microhabitat in Cazorla 2000 and in Mágina during both cohorts, and also at the partially covered microhabitat in Segura 2000) and 0.33 (Segura covered in 2000). Variation in survival over small (among microhabitats) and large (among localities) scales depended on each other (i.e., significant interaction of locality × microhabitat on survival for both cohorts; Table 3a). Decomposition of such interaction showed that, at the small scale, survival was influenced by microhabitat differently at each locality. While in Cazorla shrub cover did not increase survival (post hocs, $P > 0.1$), in Mágina it did (post hocs, $P < 0.05$ in 1999, $P < 0.1$ in 2000; both for covered–uncovered and partially covered–uncovered comparisons).

In Segura survival at the uncovered and covered microhabitats did not differ (post hoc, $P > 0.1$), but it was significantly lower at partially covered than at uncovered microhabitats (post hoc $P < 0.05$). At the large scale, variation on survival among localities depended on microhabitat. Localities varied at uncovered microhabitat (seedlings at uncovered in Segura had higher survival than in Mágina during both years and for both cohorts: post hoc, $P < 0.01$ in 1999 and 2000; survival at uncovered in Segura was also higher than in Cazorla in 2000: post hoc, $P < 0.05$) while survival did not differ between covered or partially covered microhabitats ($P > 0.1$, in each case).

The relative importance of different mortality causes differed between cohorts ($\chi^2 = 124.00$, $P < 0.0001$) and localities ($\chi^2 = 60.32$, $P < 0.0001$). However, desiccation prevailed overwhelmingly over all other causes in all regions and years, always accounting for ca. 80% of the total mortality (Fig. 2).

Survival did not depend on the emergence date (Table 4), with the only exception of Mágina in 2000, where survival declined from early-emerged

Table 3 Summary of results of GLIM testing differences between localities (Cazorla, Mágina, and Segura) and microhabitats (covered, uncovered, and partially covered) in survival (a), and recruitment (b) of hellebore seedlings for the two cohorts studied (1999 and 2000)

Factors	(a) Survival					(b) Recruitment						
	1999			2000		1999 (1s yr.)			1999 (2nd yr.)		2000 (1st yr.)	
	df	χ^2	P	χ^2	P	df	χ^2	P	χ^2	P	χ^2	P
Locality	2	1.66	ns	2.89	ns	2	255.83	***	138.04	***	4.15	ns
Microhabitat	2	1.25	ns	2.65	ns	2	285.85	***	188.45	***	2.88	ns
Loc * micro	3	7.89	*	8.33	*	2	460.53	***	282.76	***	6.15	ns

Recruitment results for the 1999 cohort are also dissected into first and second year from emergence. χ^2 refers to Wald's Chi-square. *$P < 0.05$, **$P < 0.001$, ***$P < 0.0001$, ns = $P > 0.05$

Fig. 2 Relative importance of different seedling mortality causes. Data are presented by cohort and locality
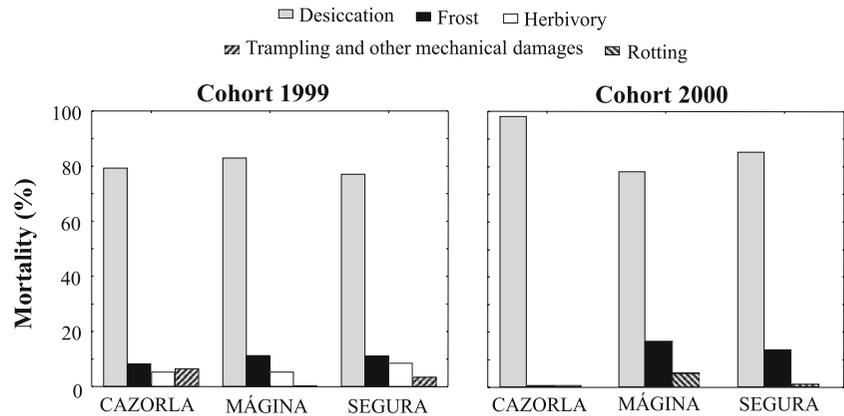

Table 4 GLIM testing the effects of emergence date on survival (treated as a simple binary response variable: seedling surviving or not by the end of summer)

	1999			2000		
	df	Wald χ^2	P	df	Wald χ^2	P
Cazorla	2	4.37	ns	2	5.33	ns
Mágina	2	1.52	ns	1	2550.3	***
Segura	2	0.04	ns	2	1.69	ns

Three phonological categories were considered: early emergence (January and February), middle emergence (March and April), and late emergence (May). Separate analyses were conducted for each cohort. Signs legend: ***$P < 0.0001$, ns = $P > 0.05$

seedlings (survival probability of 0.61) through intermediate (0.33) to late-emerged seedlings (0.05).

Recruitment

Analysis of recruitment, as assessed by the number of seedlings per m^2 after one or two year of emergence, is shown in Table 3b. During the 2-year monitoring of the 1999 cohort, we found a significant microhabitat × region interaction. Decomposition of such interaction (Fig. 3) shows that, at the small-scale of microhabitat, recruitment was strongly dependent on locality: while it was consistently low in uncovered microhabitats, it was regionally variable in the covered microhabitat (due to the failure of recruitment at this microhabitat in Segura). On the other side, the variation at the regional scale was also dependent on the small-scale of microhabitat: whereas in Mágina and Cazorla it was higher in covered or partially covered than

in uncovered, in Segura recruitment was nearly absent in both covered and uncovered sites, being only apparent in partially covered (see Fig. 3). In contrast to the results for 1999, the recruitment of the 2000 cohort did not differ among localities or microhabitats.

Except for a negative influence of herb cover on emergence and survival in Mágina's 2000 cohort (df = 1, $\chi^2 = 7.82$, $P = 0.005$; df = 1, $\chi^2 = 7.64$, $P = 0.006$, respectively), no significant relationship was found at the fine-scale of 1 m^2 plots between environmental variables (herb cover, litter depth, air temperature at emergence and PAR) and recruitment (or its emergence and survival components). Instead, recruitment at fine-scale was positively correlated with seedling emergence in all localities. Such relationship occurred for two cohorts in Mágina and Segura (1999 cohort: $r = 0.65$, $r = 0.83$; 2000 cohort: $r = 0.70$, $r = 0.66$, respectively, $P < 0.001$ for all the cases), while in Cazorla only occurred for the 1999 cohort ($r = 0.47$, $P < 0.01$).
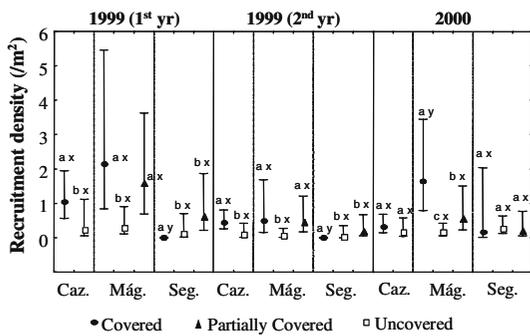
Fig. 3 Seedling recruitment in different localities and microhabitats. Data are the model adjusted means with their 95% confidence intervals for both cohorts. The first letter on each symbol identifies comparisons among microhabitats within a region; the second letter identifies comparisons among regions for the same microhabitat

Discussion

Recruitment is a multiphase sequential process (Garrido et al. 2005). Factors like seed arrival, seed predation, and environmental conditions for germination and seedling emergence and establishment act together to shape the spatial pattern of variation in recruitment (Jordano and Herrera 1995; Garrido et al. 2005; García et al. 2005). This study has shown that recruitment of *Helleborus foetidus* is spatially variable both across and within localities. We will discuss here the extent to which the mentioned factors were responsible for the variation found at different spatial scales and the extent to which recruitment patterns of this perennial herb resemble the common findings for woody plants in the Mediterranean region.

The pervasive influence of seed input in hellebore recruitment

A first putative source of spatial variation in recruitment is seed input and/or arrival. Some results from this and other studies on hellebore prove the importance of seed input on recruitment patterns at different scales. Thus, here we have shown that, at the fine-scale (1 m² plots), recruitment is correlated with seedling emergence density in most localities and years, and seedling emergence density on 1 m² plots depends in turn on *in situ* seed input (as estimated from fruit production). Furthermore, we have shown large

differences in emergence in micro-sites with and without a neighbour reproductive adult (i.e., the seed source; Fig. 1), a pattern that is coherent with the results of seed addition experiments (Rey et al. 2006). Thus hellebore recruitment is seed limited through both fecundity and dispersal limitation (most seed dispersal events take place at very short distances), which ultimately set limits to patch colonization. The pattern of seed arrival differs from that of woody Mediterranean plants where nucleation, both under conspecifics and nonconspecifics, frequently occurs (especially in bird-dispersed plants). However, they have in common that seed arrival shapes the emergence pattern because the relationship between seed arrival and seedling emergence is not disrupted by microhabitat-specific differences in seed germination and/or predation (Herrera et al. 1994; García-Fayos and Verdú 1998; Rey and Alcántara 2000; Verdú and García-Fayos 2002; Traveset et al. 2003; Gómez-Aparicio et al. 2005a). Expanding the scale to the regional level, we also found that the locality with higher seed production (i.e. Mágina) reached higher levels of seedling emergence and recruitment.

Small-scale (within locality) variation in recruitment

Tree and shrub cover has been repeatedly shown to facilitate recruitment in the Mediterranean area through a facilitative effect on germination, emergence and/or survival (Rey and Alcántara 2000; Gómez-Aparicio et al. 2005a). That facilitative effect of plant cover contributes to generate small-scale environmental heterogeneity and spatial variability in microsites adequate for recruitment (Rey et al. 2004; Gómez-Aparicio et al. 2005a, c). This has been shown mainly for the establishment of woody plants, although facilitative effects of shrubs on herbs have been also found in semiarid habitats (Pugnaire et al. 1996; Moro et al. 1997). Here, we show that this small-scale spatial variation in suitability for establishment also applies to perennial herbs, with higher recruitment being associated in most localities with shrub cover (Fig. 3). Microhabitat differences in recruitment might arise from differences in escape of dispersed seeds to predation

(an issue not explored here); however, seed addition and predator exclusion experiments have shown no significant effects of predation on hellebore recruitment (Rey et al. 2006). Since emergence varied little between microhabitats, and this fact was regionally and temporally consistent, we cannot confirm either a facilitative effect of shrub cover for seedling emergence. Experimental sowings of hellebore seeds further supports this contention, showing that germination and emergence are not favoured under shrub cover (Ramírez et al. 2006; Rey et al. 2006). In fact, the pattern of emergence was mainly linked to the presence of adult individuals which was independent of cover type.

The facilitative effect of shrub cover was more apparent on seedling survival. Overall, seedling survival was very low and it was probably the demographic bottleneck in the recruitment of this herb (see also, Garrido et al. 2005; Manzaneda et al. 2005; Rey et al. 2006; Ramírez et al., 2006), as it is usually for woody Mediterranean plants (Herrera et al. 1994; Escudero et al. 1999; Rey and Alcántara 2000; Traveset et al. 2003; Gómez-Aparicio et al., 2005a). The prevailing mortality cause in all regions and microhabitats was desiccation, seedling survival increasing with shrub cover, although this was not always true; while survival was extremely low in uncovered microsites in Mágina and Cazorla (< 0.1), in Segura was high both in uncovered and under shrubs (>0.2). It is generally accepted that water stress is the main seedling mortality agent in the south of the Iberian Peninsula and, more generally, in Mediterranean environments, although this has been confirmed mainly for woody plants (Herrera et al. 1994; García-Fayos and Verdú 1998; Escudero et al. 1999; Rey and Alcántara 2000; see Chaves et al. 2002, for herbs). Mediterranean environments typically experience a severe summer drought, with high temperature and irradiance and absence of rainfall, which results into water stress and photo-inhibition that are usually critical for seedlings. However, the harsh summer conditions are less severe under shrubs because their canopy lessens the intense irradiance and the water stress (Rey et al. 2004; Gómez-Aparicio et al. 2004, 2005c). Though scarcely studied, evidence exists supporting that shrub shadow also favour water economy of herbs during Mediterranean summer drought (Joffre and Rambal 1993; Pugnaire et al. 1996; Gavilán et al. 2002; López-Pintor et al. 2003). In our study, air temperature in summer and PAR (indirect estimators of air and soil relative humidity and irradiance, respectively) were lower under shrubs where examined, and it was probably responsible of the associated higher seedling survival. Herb cover, which was frequently higher at uncovered than under shrubs, might also contribute to make uncovered sites more stressful, through adding a competition effect (De Steven 1991). Other environmental variables like litter depth, which has been shown to negatively influence seedling germination, emergence and survival (Facelli and Pickett 1991; Eriksson 1995, for herbs), also varied between microhabitats, but it did not seem to influence microhabitat differences on survival or recruitment. Contrary to expectations (e.g., Eriksson 1995), survival and recruitment were higher under shrub cover where litter was deeper.

This study has thus confirmed for a perennial herb the trend commonly found in the Mediterranean region for woody species, namely higher mortality at exposed uncovered microsites and increased survival by facilitation under shrubs through a reduction of water and irradiance stress. However, it is worth noting that the advantage for recruitment under shrub cover occurred only in one of the two cohorts considered. The 2-year period of our study is short to conclude about temporal variation in facilitative effects, but other studies in south Iberia have already shown that marked between-year variation in rainfall provokes inter-annual changes in the appearance of such facilitative effects (Gómez-Aparicio et al. 2004, 2005a).

Regional variation in recruitment

Regional variation in recruitment was strongly dependent on differences in microhabitat (i.e., on the smallest spatial scale). Thus, no regional variation was found in uncovered microsites, where recruitment was always nearly zero because of extensive mortality by desiccation. Unfavourable conditions for hellebore recruitment in uncovered sites seem to be the rule in Mediterranean

mountains (see also, Rey et al. 2006; Ramírez et al. 2006), as it is true also for woody species (Gómez-Aparicio et al. 2005a). However, there was regional variation in the covered and partially covered microsites in favour of Mágina. Such regional variation in recruitment seems mainly due to variation in seed input and emergence (since total mortality did not differ between regions in these microhabitats), which were independent of the microhabitat. Seed input was highest in Mágina, and such difference in seed input translated into regional differences in seedling emergence density, explaining why recruitment in covered and partially covered microsites was higher in Mágina than in other localities.

Variation among sites in environmental variables could also contribute to the variation at the regional scale on emergence, mortality and recruitment. For example, litter depth was lower in Mágina (where emergence and recruitment were higher) than in the other two localities. Air temperature in summer and irradiance (as estimated by PAR) were, however, higher in Mágina than in Cazorla, because the greater openness of the habitat in this locality. This might explain locality differences on seedling survival and mortality by desiccation at uncovered, which were particularly high at Mágina. Finally, increased herb cover at the regional level does not seem related to increased negative effect on recruitment through competition with herbs (De Steven 1991), since Mágina had both the highest herb cover and density of emergence and recruitment.

In conclusion, this study shows both small-scale and regional variation in recruitment, with the major source of variation changing across scales. Seed input seems to be important in determining differences in recruitment at the large, regional, scale but also in shaping variation at the 1 m^2 fine-scale of this study. On the other hand, variation between microhabitats within a locality seems mainly related to differences in seedling survival through facilitative effects (alleviation of water and irradiance stresses) of shrub cover, although such effects did not appear in all localities or years. In a certain sense, therefore, each microhabitat behaved as a locally specific environment to recruitment.

References

Augspurger CK, Kelly CK (1984) Pathogen mortality of tropical tree seedlings: experimental studies of the effects for dispersal distance, seedling density, and light conditions. Oecologia 61:211–217

Chaves MM, Pereira JS, Maroco J, Rodrigues ML, Ricardo CPP, Osorio ML, Carvalho I, Faria T, Pinheiro C (2002) How plants cope with water stress in the field: photosynthesis and growth. Ann Bot 89:907–916

Cornelissen JHC, Castro-Diez P, Hunt R (1996) Seedling growth, allocation and leaf attributes in a wide range of woody plant species and types. J Ecol 84:755–765

De Steven D (1991) Experiments on mechanisms of tree establishment in old-field succession: seedling emergence. Ecology 72:1066–1075

Escudero A, Somolinos RC, Olano JM, Rubio A (1999) Factors controlling the establishment of *Helianthemum squamatum*, an edemic gypsophile of semi-arid Spain. J Ecol 87:290–302

Eriksson O (1995) Seedling recruitment in deciduous forest herbs: the effects of litter, soil chemistry and seed bank. Flora 190:65–70

Eriksson O, Fröborg H (1996) "Windows of opportunity" for recruitment in long-lived clonal plants: experimental studies of seedling establishment in *Vaccinium* shrubs. Can J Bot 74:1369–1374

Facelli JM, Pickett STA (1991) Plant litter and effects on plant community structure. Bot Rev 57:1–32

Fedriani JM, Rey PJ, Garrido JL, Guitián J, Herrera CM, Medrano M, Sánchez-Lafuente AM, Cerdá X (2004) Geographical variation in the potential of mice to constrain an ant-seed dispersal mutualism. Oikos 105:181–191

Fenner M (1987) Seedlings. New Phytol 106(suppl):35–47

García D, Obeso JR, Martínez I (2005) Spatial concordance between seed rain and seedling establishment in bird-dispersed trees: does scale-matter? J Ecol 93:693–704

García-Fayos P, Verdú M (1998) Soil seed bank, factors controlling germination and establishment of a Mediterranean shrub: *Pistacia lentiscus* L. Acta Oecologica-Int J Ecol 19:357–366

Garrido JL, Rey PJ, Cerdá X, Herrera CM (2002) Geographical variation in diaspore traits of an ant-dispersed plant (*Helleborus foetidus*): are ant community composition and diaspore traits correlated? J Ecol 90:446–455

Garrido JL, Rey PJ, Herrera CM (2005) Pre-and post-germination determinants of spatial variation in recruitment in the perennial herb *Helleborus foetidus* L. (Ranunculaceae). J Ecol 93:60–66

Gavilán RG, Sánchez-Mata D, Escudero A, Rubio A (2002) Spatial structure and interspecific interactions in Mediterranean high mountain vegetation (Sistema Central, Spain). Israel J Plant Sci 50:217–228

Gómez JM (2004) Importance of microhabitat and acorn burial on *Quercus ilex* early recruitment: non-additive effects on multiple demographic processes. Plant Ecol 172:287–297

Gómez-Aparicio L, Zamora R, Gómez JM, Hódar J A, Castro J, Baraza E (2004) Applying plant facilitation to forest restoration: a meta-analysis of the use of shrubs as nurse plants. Ecol Appl 14:1128–1138

Gómez-Aparicio L, Gómez JM, Zamora R (2005a) Microhabitats shift rank in suitability for seedling establishment depending on habitat type and climate. J Ecol 93:1194–1202

Gómez-Aparicio L, Valladares F, Zamora R, Quero JL (2005b) Response of tree seedlings to the abiotic heterogeneity generated by nurse shrubs: an experimental approach at different scales. Ecography 28:757–768

Gómez-Aparicio L, Gómez JM, Zamora R, Boettinger JL (2005c) Canopy vs. soil effects of shrubs facilitating tree seedlings in Mediterranean montane ecosystems. J Veget Sci 16:191–198

Harper JL (1977) The population biology of plants. Academic Press, London

Herrera CM (2002) Topsoil properties and seedling recruitment: stage-dependence and spatial decoupling of influential parameters. Oikos 97:260–270

Herrera CM, Cerdá X, García MB, Guitián J, Medrano M, Rey PJ, Sánchez-Lafuente AM (2002) Floral integration, phenotypic covariance structure and pollinator variation in bumblebee-pollinated *Helleborus foetidus*. J Evol Biol 15:108–121

Herrera CM, Jordano P, López-Soria L, Amat JA (1994) Recruitment of a mast-fruiting, bird-dispersed tree: bridging frugivore activity and seedling establishment. Ecol Monogr 64:315–344

Herrera CM, Sánchez-Lafuente AM, Medrano M, Guitián J, Cerdá X, Rey PJ (2001) Geographical variation in autonomous self-pollination levels unrelated to pollinator service in *Helleborus foetidus* (Ranunculaceae). Am J Bot 88:1025–1032

Houle G (1994) Spatiotemporal patterns in the components of regeneration of four sympatric tree species: *Acer rubrum*, *A. saccharum*, *Betula alleghaniensis* and *Fagus grandifolia*. Aust J Ecol 82:39–53

Howe HF (1990) Survival and growth of juvenile *Virola surinamensis* in Panama: effects of herbivory and canopy closure. J Trop Ecol 6:259–280

Ida H, Nakagoshi N (1996) Gnawing damage by rodents to the seedlings of *Fagus crenata* and *Quercus mongolica* var. *grosseserrata* in a temperate Sasa grassland-deciduous forest series in southwestern Japan. Ecol Res 11:97–103

Joffre R, Rambal S (1993) How tree cover influences the water balance of Mediterranean rangelands. Ecology 74:407–416

Jordano P, Herrera CM (1995) Shuffling the offspring: uncoupling and spatial discordance of multiple stages in vertebrate seed dispersal. Écoscience 2:230–237

Junta de Andalucía (1996). SinambA Difusión: La Información Ambiental de Andalucía. Consejería de Medio Ambiente, Junta de Andalucía, Sevilla

Jurado E, Westoby M (1992) Seedling growth in relation to seedling size among species of arid Australia. J Ecol 80:407–416

Kitajima K, Fenner M (2000) Ecology of seedling regeneration. In: Fenner M (ed) Seeds. Cabi publishing, Wallingford, UK, pp 331–359

Kollmann J (2000) Dispersal of fleshy-fruited species. A matter of spatial scales? Perspect Plant Ecol Evol System 3:29–51

López-Pintor A, Espigares T, Benayas JMR (2003) Spatial segregation of plant species caused by *Retama sphaerocarpa* influence in a Mediterranean pasture: a perspective from the soil seed bank. Plant Ecol 167:107–116

Manzaneda AJ, Sperens U, García MB (2005) Effects of microsite disturbances and herbivory on seedling performance in the perennial herb *Helleborus foetidus* (Ranunculaceae). Plant Ecol 179:73–82

Moro MJ, Pugnaire FI, Haase P, Puigdefábregas J (1997) Effect of canopy of *Retama sphaerocarpa* on its understorey in a semiarid environment. Funct Ecol 11:425–431

Picó FX, Riba M (2002) Regional-scale demography of *Ramonda myconi*: remnant population dynamics in a preglacial relict species. Plant Ecol 161:1–13

Pugnaire FI, Haase P, Puigdefábregas J (1996) Facilitation between higher plant species in a semiarid environment. Ecology 77:1420–1426

Ramírez JM, Rey PJ, Alcántara JM, Sánchez-Lafuente A (2006) Altitude and woody cover control recruitment of *Helleborus foetidus* in a Mediterranean mountain area. Ecography (in press)

Rey PJ, Alcántara JM (2000) Recruitment dynamics of a fleshy-fruited plant (*Olea europaea*): connecting patterns of seed dispersal to seedling establishment. J Ecol 88:622–633

Rey PJ, Alcántara JM, Valera F, Sánchez-Lafuente, AM, Garrido JL, Ramírez JM, Manzaneda A (2004) Seedling establishment in *Olea europaea*: seed size and microhabitat affect growth and survival. Écoscience 11:310–320

Rey PJ, Ramírez JM, Sánchez-Lafuente AM, Alcántara JM (2006). Seed-vs. microsite-limited recruitment in a myrmecochorous herb. Plant Ecol (in press)

Russo SE, Augspurger CK (2004) Aggregated seed dispersal by spider monkeys limits recruitment to clumped patterns in *Virola calophylla*. Ecol Lett 7:1058–1067

SAS Institute (1999) SAS/STAT Software. SAS Institute, Cary, NC

Saverimuttu T, Westoby M (1996) Seed longevity under deep shade in relation to seed size. J Ecol 84:661–689

Schupp EW (1990) Annual variation in seedfall, post-dispersal predation, and recruitment in a neotropical tree. Ecology 71:504–515

Shibata M, Nakashizuka T (1995) Seed and seedling demography of four co-occurring *Carpinus* species in a temperate deciduous forest. Ecology 76:1099–1108

Traveset A, Gulias J, Riera N, Mus M (2003) Transition probabilities from pollination to establishment in a rare dioecious shrub species (*Rhamnus ludovici-salvatoris*) in two habitats. J Ecol 91:427–437

Verdú M, Garcia-Fayos P (2002) Reproductive ecology of *Pistacia lentiscus* L. (Anacardiaceae): an evolutionary anachronism in the Mediterranean shrubland. Rev Chil Hist Nat 75:57–65

Walters MB, Reich PB (1996) Are shade tolerance, survival, and growth linked? Low light and nitrogen effects on hardwood seedlings. Ecology 77:841–853

Werner K, Ebel F (1994) Life-history of the Genus *Helleborus* L (Ranunculaceae). Flora 189:97–130