

Facilitation of tree saplings by nurse plants: Microhabitat amelioration or protection against herbivores?

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Abstract

Question: Positive interactions are predicted to be common in communities developing under high physical stress or high herbivory pressure due to neighbour amelioration of limiting physical and consumer stresses, respectively. However, when both stress sources meet in the same community, the relative importance of the two facilitation mechanisms is poorly understood. We ask: What is the relative importance of abiotic vs. biotic mechanisms of facilitation of tree saplings by shrubs in Mediterranean mountain forests?

Location: Sierra Nevada, SE Spain (1800–1850 m a.s.l.)

Methods: Saplings of four tree taxa (*Acer opalus* ssp. *granatense*, *Quercus ilex*, *Pinus nigra* ssp. *salzmanii* and *P. sylvestris* var. *nevadensis*) were planted following a 2 × 2 factorial design: two levels of herbivory (control and ungulate exclusion) and two microhabitats (under shrubs and in open areas). Sapling survival and growth were monitored for five years.

Results: Shrubs had positive effects on sapling survival both in control and ungulate excluded plots. This effect was species-specific, with shrubs increasing the survival of *Acer opalus* and *Quercus ilex* three and twofold, respectively, but having a minor effect on the *Pinus* species. Herbivory damage was also species-specific, being much higher for *Acer opalus* than for any other species. Shrubs did not protect saplings of any species against ungulates. Thus, all *Acer* saplings (the most damaged species) suffered herbivory outside the enclosures, which largely reduced sapling height.

Conclusions: Protection from abiotic stress (summer drought and winter frost) was much more relevant than protection from biotic stress (herbivory). However, we propose that the final balance between the two mechanisms can be expected to vary strongly between sites, depending on the relative magnitude of the different sources of stress and the intrinsic traits (e.g. palatability) of the species interacting.

Keywords: Abiotic stress; Facilitation mechanism; Herbivory pressure; Mediterranean mountain; Nurse shrub; Sapling survival; Species-specific effect.

Nomenclature: Castroviejo et al. (1986–2001) for tree species, and Molero-Mesa et al. (1992) for shrub species.

Abbreviations: PPFD = Photosynthetic photon flux density; RII = Relative interaction index; VPD = Vapour pressure deficit.

Introduction

The abundance, performance and spatial distribution of plant species are highly linked to the strength and sign of the interactions involving them in their communities (Roughgarden & Diamond 1986; Brown et al. 2001). In 1994, Bertness & Callaway proposed a conceptual model suggesting that strong positive interactions should be particularly common in communities developing under high abiotic stress and in communities with high consumer pressure, whereas competition should be the dominant interaction under mild physical conditions and low consumer pressure. Since then, a large number of empirical studies have supported this hypothesis by reporting that positive interactions are the rule under severe physical conditions (Mulder et al. 2001; Tewksbury & Lloyd 2001; Callaway et al. 2002; Gómez-Aparicio et al. 2004). Consumer driven associational benefits also occur in systems with high consumer pressure (Rousset & Lepart 1999; Meiners & Martinkovic 2002; García & Obeso 2003; Baraza et al. 2006). However, only a few studies have tried to disentangle the relative importance of the two mechanisms of facilitation for the same community (e.g. Callaway 1992).

Mediterranean mountain forests represent an ideal system to simultaneously test the importance of abiotic (microhabitat amelioration) and biotic (herbivory protection) mechanisms of facilitation in plant communities. On the one hand, these systems are characterized by a strong summer drought, which severely limits regeneration of tree and shrub species by causing high seedling and sapling mortality during the early stages of establishment (Rey & Alcántara 2000; García 2001; Traveset et al. 2003; Castro et al. 2004a, 2005). Because of the relevance of drought as a stress factor, the amelioration of extreme summer climatic conditions by shrub canopies has been proposed as a key facilitation mechanism underlying the spatial association between tree propagules and shrubs in many Mediterranean systems (García 2001; Gómez et al. 2001; Castro et al. 2004b; Gómez-Aparicio et al. 2005a). Moreover, the Mediterranean high mountains can

suffer extremely low temperatures in winter, and there is already some evidence suggesting that protection from winter frosts could also represent an important facilitation mechanism in these systems (Castro et al. 2002).

On the other hand, herbivory by ungulates has influenced vegetation patterns throughout the Mediterranean Basin for thousands of years (Callaway & Pugnaire 1999; Zamora et al. 2000). Through a selective consumption of seedlings and saplings, herbivores can alter the course of ecological succession and consequently the species composition of a plant community (Pickett et al. 1987; Huntly 1991; Crawley 1997; Baraza et al. 2006). Thus, the high herbivore pressure characteristic of mediterranean forests has motivated the eradication of deciduous species in favour of the better defended evergreens (Cuartas & García-González 1992; Mesón & Montoya 1993). In this scenario, successful tree establishment could be limited to refuges provided by shrub canopies, especially for palatable species. In fact, some studies have shown shrubs to protect saplings of woody species thanks to deterrents such as toxins or thorns, or simply by hiding them (Brown & Ewel 1987; García & Obeso 2003; Bakker et al. 2004). However, others have shown the opposite pattern (Hjältén & Price 1997; Ibañez & Schupp 2001; Rebollo et al. 2002).

In this work, we experimentally analyse the relative importance of microclimate modification vs. protection against herbivory as mechanisms of facilitation of tree saplings by nurse shrubs. To explore the species specificity of the mechanisms, we chose four of the main tree species inhabiting Mediterranean mountain forests: *Acer opalus* ssp. *granatense*, *Quercus ilex*, *Pinus nigra* ssp. *salzmannii* and *P. sylvestris* var. *nevadensis*. The response of tree saplings to the presence of shrubs, herbivores and their interaction was monitored over five years, which allowed us to examine the variation in time of the relative importance of the two facilitation mechanisms. To our knowledge, no other study is available on a long-term analysis of the relative importance of abiotic vs. biotic facilitation mechanisms on sapling performance of several tree species. The results from this study provide new insights into the mechanisms underlying the spatial patterns of regeneration associated with shrubs as reported for many woody species in stressful environments (Callaway 1992; Rey & Alcántara 2000; García et al. 2000; Gómez et al. 2001).

Material and Methods

Study area

The experiment was conducted between 1997-2001 at the Sierra Nevada National Park (Granada, SE Spain), on the northwestern slope of Loma de los Panaderos (Trevenque area, 37°5' N - 3°28' W; 1800-1850 m a.s.l.). The bedrock is calcareous, with slopes ranging between 5° and 15°. The climate is subhumid mediterranean (871 mm annual mean; 1990-2002). Rainfall is concentrated in spring and autumn, alternating with hot, dry summers (39 mm summer mean, sum of mm in June, July and August; 1990-2002) and cold winters (-0.1°C mean minimum temperature for December-February; 1990-2002). Snow is common during winter, persisting up to two months, and frost occurs from November to April. Weather differed among the study years: 1997 and 1998 had relatively wet summers, with summer rainfall above average for the site (71 mm and 47 mm, respectively), whereas the last three years (1999, 2000 and 2001) had relatively dry summers with rainfall below average (21.8 mm, 2 mm and 10 mm, respectively). In addition, winter was much colder in 1998 than in any other year, with the mean minimum temperature being below zero (-1.7°C in 1998 vs. 0.2-1.5 °C for the other years). The study area was formerly a mixed forest of *P. sylvestris* var. *nevadensis*, *P. nigra* ssp. *salzmannii*, *Q. ilex*, *A. opalus* ssp. *granatense* and *Sorbus aria*. In 1983, a fire in the study area burned ca. 8 ha of the original pine forest. Since then, the area has been recolonized by different successional species of shrubs intermingled with 1 - 3 m diameter interspaces of bare ground. The most abundant shrubs in the study zone are *Salvia lavandulifolia*, an evergreen reaching a maximum height of 50 cm, and several species of deciduous spiny shrubs 1 - 2 m high, mainly *Prunus ramburii*, *Crataegus granatensis* and *Berberis vulgaris* ssp. *australis* (see Castro et al. 2002 for details on habitat structure). The main ungulate in the study zone is the Spanish ibex *Capra pyrenaica*. Saplings used in the experiment were provided by the Consejería de Medio Ambiente (Junta de Andalucía).

Experimental design

The experiment was initiated in March 1997. We selected three blocks (each ca. 0.5 ha) separated by > 200 m in the study site. Two plots (0.25 ha) were marked per block, one being fenced to exclude ungulates from the plants and the other left unfenced as a control. These resulted in three ungulate excluded plots and three control plots. In each of the six plots, we planted two year old saplings of *A. opalus*, *Q. ilex*, *P. nigra* and *P. sylvestris* in two microhabitats: under the canopy of the most

abundant deciduous spiny shrubs (*Prunus ramburii*, *Crataegus granatensis* and *Berberis vulgaris*; hereafter 'shrub microhabitat') and in open interspaces without vegetation (hereafter 'open microhabitat'). We pooled three species of spiny shrubs into the shrub microhabitat due to the fact that none were abundant enough to conduct the experiment. However, the three species have similar effects on soil moisture, nutrient content and understorey light levels (authors' unpubl. data) and therefore the physical environment surrounding the saplings in their understorey can be assumed to be comparable. We planted 15 saplings per tree species per microhabitat per plot ($n = 720$ saplings), with planting points randomly assigned. Planting holes in open interspaces were dug 40 cm deep with a mechanical auger diameter 30 cm. A smaller auger bit (12 cm diameter) was used for planting in the shrub microhabitat to minimize damage to the shrub roots. In June 1997, before the onset of the summer drought, we examined the planted saplings, excluding from the study those that had died (3.5%), presumably due to transplanting shock. As a result, the final number of saplings per treatment combination differed from 15, causing the design to be unbalanced. Sapling height in June 1997 was 7.2 ± 0.5 cm for *A. opalus* ($n = 175$), 8.4 ± 0.6 cm for *Q. ilex* ($n = 173$), 5.0 ± 0.3 cm for *P. nigra* ($n = 175$) and 5.2 ± 0.3 cm for *P. sylvestris* ($n = 172$). Initial height did not differ among microhabitats for any species ($p > 0.05$, one-way ANOVAs).

Abiotic characterization of the microhabitats

Abiotic environmental conditions in each microhabitat were characterized by measuring above and below-ground microclimate and soil water content. Microclimatic conditions were measured with HOBO H8 data loggers (Onset Computer Corporation, Bourne, MA, S US) located at ten sampling stations per microhabitat distributed randomly within the three blocks of the experiment. They recorded air temperature, soil temperature at 5 cm depth and relative humidity every 2 min during three cloudless summer days. Relative humidity and air temperature values were used to calculate the vapour pressure deficit (VPD) as an indicator of the atmospheric evapotranspiration demand. PPFD was recorded with Hall light sensors (EIC SL, Madrid, Spain) cross-calibrated with a LI-190SA quantum sensor (Li-Cor, Lincoln, Nebraska, USA) connected to the data loggers. The same day that HOBO H8 sensors were positioned in the field, volumetric soil water content was recorded using ThetaProbe sensors (Delta-T Devices Ltd., Cambridge, UK). Measurements were made next to the microclimatic sensors at 20 cm depth.

Sapling measurements

Saplings were censused twice a year between June 1997 and October 2001, once after summer (October) and once after winter (April), with the exception of the year 2000 when only the summer census was conducted. For each sapling, we recorded: (1) survival, together with the most likely cause of mortality (distinguishing between summer drought, winter frost and herbivore damage); (2) height, measured from the soil surface to the apical bud and (3) herbivory damage, estimated as the percentage of browsed shoots in relation to the total number of shoots. These data were used to calculate two complementary herbivory indices for each year of the study (Zamora et al. 2001): risk of herbivory (estimated as the percentage of saplings browsed) and damage intensity (estimated only for sapling suffering herbivory as the percentage of browsed shoots in relation to the total number of shoots). In addition, we calculated the frequency of herbivory for the entire study period, estimated as the number of years a sapling was damaged (from 0 to 5), therefore indicating the risk of saplings suffering repeated herbivory.

Data analysis

Abiotic variables were compared between microhabitats using one-way ANOVAs (Proc GLM, Anon. 2002) with microhabitat as a fixed factor. For air and soil temperature and VPD, the mean values per sample point were introduced as dependent variables, in order to avoid pseudoreplication. In the case of photosynthetic photon flux density (PPFD), we considered the mean daily PPFD as a dependent variable.

Survivorship curves were analysed by Cox's Proportional Hazard semiparametric models (Proc PHREG, Anon. 2002), using the maximum partial likelihood as estimation method (Allison 1995). Block, ungulate exclusion, microhabitat, and species were introduced as factors. Cumulative survival at the end of the experiment was analysed using Generalized Logit Models (Proc CATMOD, Anon. 2002). The relationship between the facilitative effect of shrubs and the climatic characteristic of the study year was explored using a relative interaction index (RII, *sensu* Armas et al. 2004). RII was calculated as the difference in survival with and without nurse shrubs relative to the sum of both survival percentages. This index ranges from -1 to 1, with positive values indicating facilitation and negative values competition.

For each species, sapling height was analysed using a multivariate ANOVA (MANOVA, Proc GLM, Anon. 2002). Univariate analyses of repeated measures (rmANOVAs) were not used since models were not comprised of independent orthogonal components (Von Ende 2001). Ungulate exclusion and microhabitat were

introduced as between-group factors, whereas year was introduced as a within-group factor. Block was not considered in the analyses due to the absence of live saplings at the end of the experiment in some combinations of the four factors.

Risk of herbivory was analysed for each year using Generalized Logit Models (Proc CATMOD, Anon. 2002) with block, microhabitat and species as factors. Damage intensity was analysed using three-way ANOVAs (Proc GLM), with microhabitat and species as fixed factors and block as a random factor. Contingency analyses were used to analyse the frequency of herbivory for the entire study period, introducing the number of years with damage (from 0 to 5) as a multinomial dependent variable and microhabitat and species as independent factors. When necessary, data were previously log or arc-transformed. Throughout the paper, we applied the sequential Bonferroni correction to reduce type-I error when multiple comparisons were made (Cabin & Mitchell 2000). Results are expressed as mean \pm 1SE.

Results

Abiotic characterization of the microhabitats

All the microclimatic variables differed significantly among microhabitats (Table 1). Mean daily air and soil temperature were reduced by almost 10 °C under shrubs. VPD was reduced by half, whereas PPFD was decreased by 70%. In contrast, volumetric soil water content did not differ between microhabitats, being in all cases very low (< 4%, Table 1).

Survival

Survival analyses showed significant differences in the temporal sequence of mortality between blocks, microhabitats and species, but not between control and ungulate-excluded plots (Table 2). Similarly, cumulative survival at the end of the study varied between blocks ($L-R \chi^2 = 52.80$, $df = 2$, $p < 0.0001$), microhabitats ($L-R$

Table 2. Results of Cox regression analyses to determine the effects of block, ungulate exclusion, microhabitat, and species on sapling survival. For each significant factor, *ad hoc* comparisons are shown. Levels of all variables were codified using dummy variables.

Source of variation	df	$L-R \chi^2$	p
Block (B)	2	52.05	< 0.0001
Ungulate exclusion (U)	1	0.19	0.664
Microhabitat (M)	1	20.54	< 0.0001
Species (S)	3	67.29	< 0.0001
B \times U	2	6.83	0.033
B \times M	2	3.10	0.212
B \times S	6	19.80	0.003
U \times M	1	0.53	0.467
U \times S	3	0.65	0.885
M \times S	3	13.79	0.003
Model	24	166.07	< 0.0001
<i>Acer opalus</i> vs. <i>Quercus ilex</i>	1	0.001	0.974
<i>Acer opalus</i> vs. <i>Pinus nigra</i>	1	11.18	0.001
<i>Acer opalus</i> vs. <i>Pinus sylvestris</i>	1	27.97	< 0.0001
<i>Quercus ilex</i> vs. <i>Pinus nigra</i>	1	10.84	0.001
<i>Quercus ilex</i> vs. <i>Pinus sylvestris</i>	1	26.66	< 0.0001
<i>Pinus nigra</i> vs. <i>Pinus sylvestris</i>	1	3.04	0.081
Block 1 vs. Block 2	1	12.01	< 0.0001
Block 1 vs. Block 3	1	7.54	0.008
Block 2 vs. Block 3	1	0.36	0.546

$\chi^2 = 26.59$, $df = 1$, $p < 0.0001$) and species ($L-R \chi^2 = 46.25$, $df = 3$, $p < 0.0001$), but not between control and ungulate excluded plots ($L-R \chi^2 = 1.85$, $df = 1$, $p = 0.173$). Cumulative survival in block 1 (49%) was higher than in blocks 2 (23%) and 3 (24%), but in all three blocks survival followed the same among-microhabitat pattern. Saplings of the four species showed higher survival in the shrub than in the open microhabitat (Fig. 1), although differences were significant only for *A. opalus* and *Q. ilex* (significant Microhabitat \times Species interaction term, Table 2). Thus, cumulative survival in the shrub microhabitat was threefold higher than in the open microhabitat for *A. opalus* saplings and almost twofold for *Q. ilex* saplings, both in control and ungulate excluded plots (Fig. 1). The main cause of mortality was summer drought (66.5%), followed by winter frost (32.2%), whereas mortality caused by ungulates was estimated

Table 1. Abiotic conditions measured under nurse shrubs and in open interspaces. Data were recorded every 2 min. for three cloudless summer days. Values are mean \pm SE.

Abiotic variable	Microhabitat		F	p
	Shrub	Open		
Air temperature (°C)	27.5 \pm 0.4	36.9 \pm 0.9	18.08	< 0.0001
Soil temperature (°C)	23.3 \pm 0.3	31.4 \pm 0.6	110.63	< 0.0001
Vapor pressure deficit (KPa)	2.7 \pm 0.7	5.4 \pm 0.2	22.76	< 0.0001
Daily PPFD* (mmoles/m ²)	16.1 \pm 2.7	47.2 \pm 5.2	103.16	< 0.0001
Soil water content (%V)	3.5 \pm 0.2	2.8 \pm 1.2	1.64	0.213

* PPFD = Photosynthetic photon flux density.

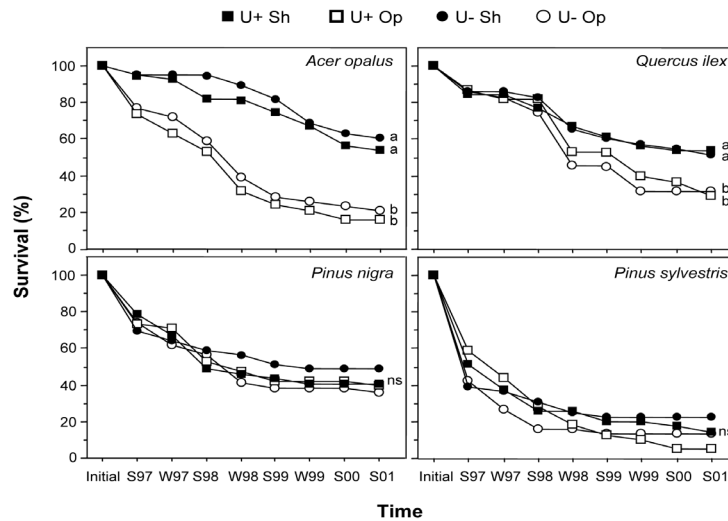


Fig. 1. Survival of experimental saplings throughout 5 years of study in the four experimental treatments. U+ = control plots; U- = ungulate-excluded plots; Sh = shrub microhabitat; Op = open microhabitat; S = summer; W = winter. Different letters indicate significant differences between experimental treatments (after Bonferroni correction) according to pairwise χ^2 tests.

to be only 1.3%. *A. opalus* and *Q. ilex* showed similar survivorship curves, but these differed from those of both *Pinus* species (Table 2). Thus, whereas *P. nigra* and *P. sylvestris* suffered a much higher mortality during the first summer than at any other time during the experiment, *A. opalus* and *Q. ilex* had more sustained mortality, with one peak the first summer and another the second winter (Fig. 1). The beneficial effect of shrubs varied among years and seasons. Despite the greatest overall mortality occurring in summer, the relative advantage of being beneath a shrub was greatest in the winter as shown by higher values of RII (Fig. 2). This was especially true in 1998, an exceptionally cold winter.

Height growth

Herbivory only affected height growth of *A. opalus* saplings (Table 3, Fig. 3). Thus, accumulated height growth after five years was more than twice as high for *A. opalus* saplings inside (18.2 ± 2.6 cm) than outside the exclosures (8.5 ± 1.5 cm) ($F = 7.88$, $df = 1$, $p = 0.015$, one-way ANOVA). The microhabitat did not directly affect height growth of any of the four tree species (Table 3), but it interacted with ungulate exclusion in the case of *A. opalus* (Table 3). Thus, whereas there was no between-microhabitat difference in growth of *A. opalus* saplings in the ungulate-excluded plots, saplings in the control plots grew higher under shrubs than in open interspaces (Fig. 3).

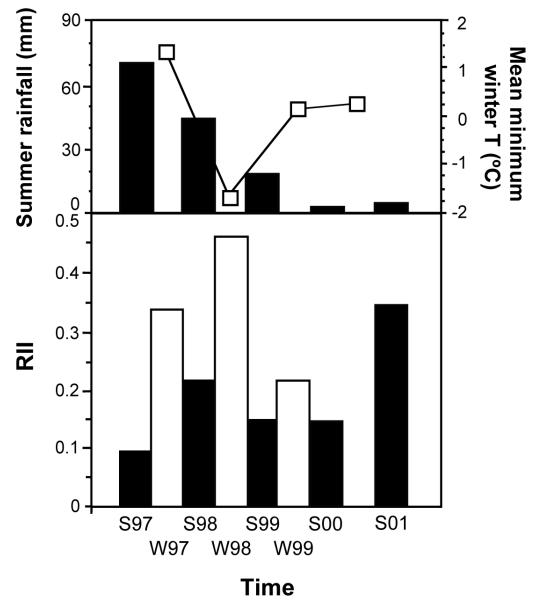


Fig. 2. Temporal variability of the facilitative effect of shrubs on sapling survival, and its relationship with climatic characteristics (climatic data obtained at a meteorological station located in La Cortijuela Botanical Garden, 1 km from the study site). In the upper graph, black bars represent summer rainfall (sum of mm fallen in June, July and August) and open squares represent the mean minimum temperature during winter months (December, January and February). In the lower graph, bars represent the magnitude of facilitation quantified by the RII (Relative Interaction Index). Due to the lack of survival data for winter 2000, RII could not be calculated for that period. Therefore, RII for summer 2001 was calculated with survival data after one entire year.

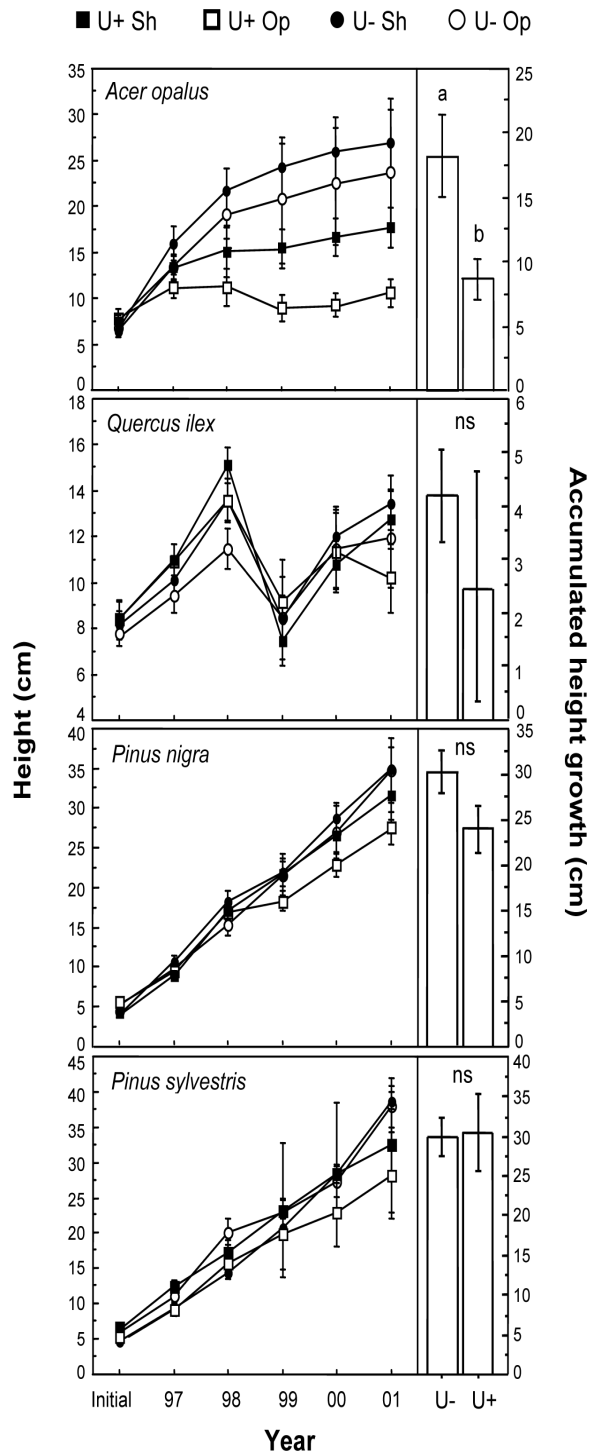


Fig. 3. Change of sapling height over the 5 study years in the four experimental treatments. Bars represent the accumulated growth in height (cm) after 5 years. U+ = control plots; U- = ungulate-excluded plots; Sh = shrub microhabitat; Op = open microhabitat. Values are mean \pm 1 SE

Table 3. MANOVAs analysing the effect of ungulate exclusion and microhabitat on sapling height growth of each of the 4 species throughout the 5 years of the study. Block was not considered in the analysis because not all combinations of the three factors had live saplings at the end of the experiment.

Source of variation	df Num	df Den	λ	F	p
<i>Acer opalus</i>					
Ungulate exclusion (U)	1	43	0.43	5.96	0.014
Microhabitat (M)	1	43	0.99	0.44	0.508
U \times M	1	43	0.49	4.89	0.032
Year (Y)	4	40	0.29	23.69	< 0.0001
Y \times U	4	40	0.87	1.47	0.227
Y \times M	4	40	0.91	1.02	0.407
Y \times U \times M	4	40	0.98	0.24	0.913
<i>Quercus ilex</i>					
U	1	51	0.98	0.58	0.449
M	1	51	0.99	0.61	0.437
U \times M	1	51	0.98	0.77	0.385
Y	4	48	0.12	88.81	< 0.0001
Y \times U	4	48	0.91	1.19	0.326
Y \times M	4	48	0.76	3.73	0.010
Y \times U \times M	4	48	0.83	2.45	0.059
<i>Pinus nigra</i>					
U	1	57	0.98	1.12	0.293
M	1	57	0.98	0.84	0.361
U \times M	1	57	0.99	0.00	0.951
Y	4	54	0.10	114.07	< 0.0001
Y \times U	4	54	0.90	1.46	0.226
Y \times M	4	54	0.88	1.89	0.126
Y \times U \times M	4	54	0.87	1.90	0.124
<i>Pinus sylvestris</i>					
U	1	15	0.98	0.32	0.581
M	1	15	0.95	0.74	0.402
U \times M	1	15	0.98	0.22	0.646
Y	4	12	0.06	49.19	< 0.0001
Y \times U	4	12	0.46	3.47	0.041
Y \times M	4	12	0.88	0.42	0.792
Y \times U \times M	4	12	0.64	1.68	0.217

Herbivory damage

The risk of herbivory differed significantly among blocks in all years, although the pattern changed depending on the year (Table 4). The risk of herbivory varied between microhabitats only in the last three years of study, although significance disappeared after Bonferroni correction (Table 4). As for the block factor, between-microhabitat variation did not present a congruent inter-annual pattern; the risk of herbivory was higher in the open in 1999 and 2000 and higher under shrubs in 2001. The risk of herbivory varied significantly between species throughout the five years of the study (Table 4). *A. opalus* was the most damaged species ($50.3 \pm 8.7\%$ annual mean, Fig. 4), the herbivory risk reaching values of 80% in some years. *Q. ilex* was the least damaged ($6.5 \pm 2.4\%$), whereas *P. nigra* and *P. sylvestris* had intermediate

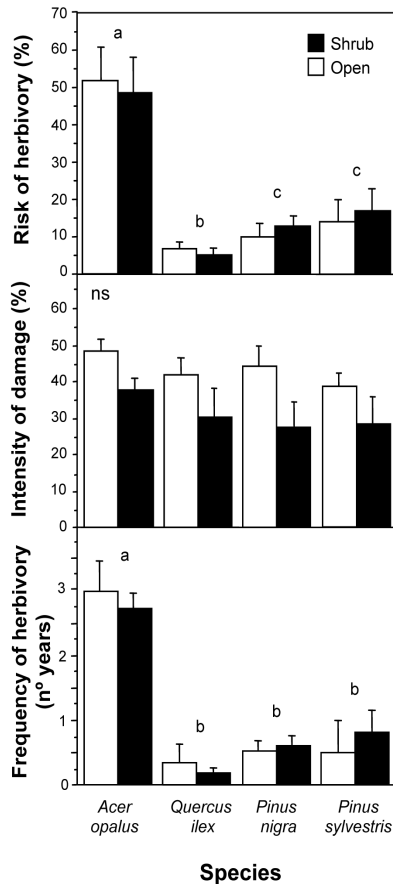


Fig. 4. Risk of herbivory, frequency of herbivory, and damage intensity of saplings alive at the end of the experiment (mean \pm 1 SE for the five years of study). Different letters show significant differences among species after Bonferroni correction. There were no differences among microhabitats in any case.

and similar values ($12.8 \pm 3.4\%$ and $13.4 \pm 5.9\%$, respectively; Fig. 4). The intensity of damage differed between blocks only in the first year (Table 4). It did not differ between microhabitats or among species in any year of study (Table 4), although values were always lower under shrubs than in open interspaces (Fig. 4). The frequency of herbivory did not differ between microhabitats (L-R $\chi^2 = 0.01$, $df = 1$, $p = 0.90$) but it did between species (L-R $\chi^2 = 41.96$, $df = 3$, $p < 0.0001$). Thus, while it was < 1 for *Q. ilex* and both *Pinus* species, it almost reached three for *A. opalus* (Fig. 4). Accordingly, all *A. opalus* saplings alive outside the exclosures at the end of the experiment were damaged in at least one year.

Discussion

Facilitation of sapling survival: The role of microhabitat amelioration

Shrubs had a similar positive effect on sapling survival both inside and outside exclosures. Therefore, neither the sign nor the strength of the shrub-sapling interaction was determined by the presence of ungulates. Instead, microhabitat amelioration was the main facilitation mechanism. Extreme abiotic conditions, primarily summer drought and winter frosts, were the main mortality causes (98.7% of the total mortality). In this scenario, shrub canopies generated a distinctive micro-environment that proved crucial for saplings to survive both critical seasons. Thus, in mid-summer shrubs attenuated radiation by 70%, providing moderate shade able to increase survival and reduce photo-inhibition of

Table 4. Summary of the models that analyse the differences between blocks, microhabitats, and species in the risk of herbivory and intensity of damage of experimental saplings in each of the 5 years of study. Models are Generalized Logit Models for risk of herbivory (L-R χ^2 values) and Generalized Linear Models (F values) for intensity of damage.

Source of variation	df	1997	1998	Year 1999	2000	2001
<i>Risk of herbivory</i>						
Block (B)	4	41.25 ***	11.73 *	22.12 ***	14.51 **	14.28 **
Microhabitat (M)	2	3.27	0.01	9.18 *	8.25 *	7.30 *
Species (S)	6	85.27 ****	98.39 ****	56.52 ****	29.82 ****	40.07 ****
B \times M	8	0.07	1.09	0.98	1.24	1.33
B \times S	12	28.92 **	8.62	10.15	11.53	15.62
M \times S	6	18.25 **	6.95	14.86 *	8.29	10.30
<i>Intensity of damage</i>						
B	2	5.46 **	0.27	0.87	0.12	3.93 *
M	1	1.91	0.22	0.03	0.16	0.67
S	3	1.27	1.59	0.19	0.22	0.14
B \times M	2	0.65	0.45	2.93	0.98	0.78
B \times S	6	9.09	7.89	5.47	9.67	8.80
M \times S	3	0.45	1.28	0.34	1.16	0.21

Note: Asterisks denote the following significant levels: **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Significant values (after Bonferroni correction) are in bold.

woody saplings without significantly limiting carbon gain (Valladares et al. 2005; Gómez-Aparicio et al. 2006). As a result of reduced irradiance, the mean air temperature diminished by almost 10°C, in turn reducing the risk of leaf overheating, an important stress factor in environments where drought limits cooling by transpiration (Larcher 1995). Moreover, VPD under shrubs was about half that in open interspaces. These environmental changes can improve the water status of saplings even in the absence of between-microhabitat differences in soil water content, as happened in this study (Holmgren et al. 1997; Domingo et al. 1999). Although microclimatic differences between microhabitats were not evaluated in winter, shrubs presumably also ameliorate negative temperatures, reducing the risk of frost damage to tissues (Kikvidze & Nakhutsrishvili 1998; Núñez et al. 1999). Moreover, litter accumulation under shrubs might reduce heaving of the soil surface during freeze-thaw cycles, diminishing mortality due to uprooting and/or fracture (Gill & Marks 1991; Gobbi & Schlichter 1998).

Facilitation of sapling survival occurred throughout the five study years, as indicated by the positive values of RII (Fig. 2). This finding suggests that the nurse effect of shrubs, reported mainly for the first year of life of tree seedlings (Maestre et al. 2003; Gómez-Aparicio et al. 2004), is consistent throughout the sapling stage (see also Castro et al. 2004, 2006). At the intra-annual scale, facilitation by shrubs due to microclimatic amelioration in mediterranean environments has been reported mainly during the summer season (Callaway 1992; Rousset & Lepart 1999; Castro et al. 2004a). However, our study shows that, at high altitudes in the Mediterranean mountains, where plants are exposed to a double abiotic stress comprised of drought and frosts (Terradas 2001), protection by shrubs in winter can be even more important than in summer (Fig. 2). Specifically in winter 1998, which was particularly cold, survival under shrubs was four times higher than in open spaces. Moreover, the lowest beneficial effect of shrubs occurred during the first summer, which was unusually wet (see Gómez-Aparicio et al. 2004). In fact, summer 1997 was the second wettest summer of the twentieth-century in the study area (Gómez-Aparicio et al. 2005c). Therefore, our results support the general hypothesis that predicts facilitation to increase with environmental severity (Bertness & Callaway 1994).

Despite sapling survival for the four species being higher under shrubs than in open areas, among-microhabitat differences were significant only for *Q. ilex* and especially for *A. opalus*. In fact, *A. opalus* not only had the greatest increase in survival under shrubs in comparison to bare soil, but this response was highly consistent in space; it was the only species for which

there were no significant qualitative or quantitative differences among experimental blocks. The weak response shown by *P. nigra* to the presence of shrubs agrees with the light-demanding characteristic associated with the genus *Pinus* (Ceballos & Ruíz de la Torre 1971; Keeley & Zedler 1998). Thus, *P. nigra* saplings have been shown to be more tolerant to the strong irradiance characteristic of the open microhabitat than *Q. ilex* and *A. opalus* saplings (Gómez-Aparicio et al. 2006). For these three Mediterranean species, the response to the presence of shrubs ranked inversely to their performance in the sun, which was highest for *P. nigra* (38.1% survival), followed by *Q. ilex* (29.9%) and *A. opalus* (18.1%). In contrast, *P. sylvestris* had both the lowest survival percentage in the sun and the lowest response to shrubs. This finding is probably related to the fact that *P. sylvestris* is a boreal-alpine species that reaches its southernmost distribution limit in Sierra Nevada (Boratynski 1991). Under such extreme abiotic conditions, not even the presence of spiny shrubs effectively altered the micro-environment to increase *P. sylvestris* saplings survival (but see Castro et al. 2004b). In general, our results show that the microclimatic effect of shrubs is more beneficial for stress-intolerant (i.e. *A. opalus*) than for stress-tolerant species (i.e. *P. nigra*) (Gómez-Aparicio et al. 2005b; Rey-Benayas et al. 2005), but that facilitation can wane when environmental conditions are too stressful for a species (i.e. *P. sylvestris*) (Michalet et al. 2006).

Facilitation of sapling growth: the role of herbivore protection

The enclosure of ungulates for five years did not boost sapling survival. However, it significantly affected sapling growth, but only for *A. opalus*. This species-specific effect of the enclosures is connected to the fact that *A. opalus* saplings had more than a threefold higher risk and frequency of herbivory than any other tree species (Fig. 4). This finding agrees with chemical data that show *A. opalus* to be the most palatable species of the four studied here, due to a higher content of foliar nitrogen and a lower content of tannins and phenolic compounds (Baraza 2004). Thus, every *A. opalus* sapling outside the enclosures was damaged at least once during the study period, and saplings accumulated more than twice the height growth inside the enclosures (18.2 ± 2.6 cm) than outside the enclosures (8.5 ± 1.5 cm) (Fig. 3). For small saplings of less palatable species such as *Q. ilex* or *Pinus* spp., even a period of five years of herbivory may be insufficient to appreciate the negative effects of ungulates, which become more relevant when saplings are larger (Zamora et al. 2001; Baraza 2004).

The fact that after five years all *A. opalus* saplings outside the exclosures were damaged, whether in the shrub or open microhabitat, suggests that shrubs did not act efficiently as refuges from ungulates. This result is presumably influenced by the fact that the spiny shrubs used as nurse plants have several characteristics limiting this potential protective role, such as a small to medium size, a growth architecture that leaves open spaces beneath them and an isolated spatial distribution, each individual shrub being surrounded by open space with stunted shrubs. For these reasons, saplings planted beneath shrubs were relatively accessible to ungulates. There is now growing evidence indicating that the protective effect of nurse shrubs is highly dependent on their physical and chemical traits (Bruno et al. 2003; Garcia et al. 2005; Baraza et al. 2006). Therefore, nurse shrubs with different structural characteristics (e.g. shrubs with canopy reaching the soil surface, larger shrubs and/or clumped shrub patches surrounding the saplings, unpalatable shrub species) would presumably have provided a better refuge from ungulates (Gómez et al. 2001).

Although herbivory damage to *A. opalus* did not differ between microhabitats, *A. opalus* saplings outside the exclosures, but not inside, were significantly higher under shrubs than in open interspaces. This result suggests that shrubs had a positive effect on height growth that was not directly mediated by microhabitat amelioration, but rather involved herbivores. Since herbivory damage did not vary between microhabitats, shrubs could have increased the tolerance of *A. opalus* saplings to herbivory by decreasing drought stress, in accordance with the continuum of plant response model (Maschinski & Whitman 1989) that predicts a higher ability of plants to compensate for herbivory in low stress environments (see also Rand 2004). In fact, shrubs are the only microhabitat where *Acer* saplings have a high probability of growing to maturity in the study area (Zamora et al. 2004).

Concluding remarks: Relative importance of microclimate modification vs. protection as a facilitation mechanism

Current theoretical models predict that facilitation increases with environmental stress (Bertness & Callaway 1994), although its importance can decrease at the extreme end of an environmental severity gradient (Michalet et al. 2006). Therefore, with the exception of extremely severe environments, we can expect the importance of the different mechanisms of facilitation to depend on the relative magnitude of the different stresses acting in a system. Furthermore, a proper

evaluation of the final balance among the different mechanisms requires an adequate timing template. In stressful environments such as Mediterranean mountains, tree saplings grow very slowly (Zamora et al. 2001), necessitating long-term studies to fully understand the importance for plant performance of different abiotic and biotic stress sources (e.g. summer drought, winter frosts, herbivory). Our relatively long-term study clearly indicates that, in our study area, abiotic stress was more intense than biotic stress (i.e. herbivory) due to the combination of summer drought and winter frosts. Consequently, microclimate amelioration constituted by far the main benefit for saplings established under shrubs. In summary, the relative importance of the modification of the microclimate vs. protection against herbivory as facilitation mechanisms can be expected to vary strongly among systems, depending on the climatic characteristics of the year, the herbivore pressure and the characteristics of the plant species interacting (see e.g. Turner et al. 1969; Steenberg & Lowe 1969; Valiente-Banuet & Ezcurra 1991; Callaway et al. 1996; Baraza et al. 2006).

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