

# Seedling establishment of a boreal tree species (*Pinus sylvestris*) at its southernmost distribution limit: consequences of being in a marginal Mediterranean habitat

JORGE CASTRO, REGINO ZAMORA, JOSÉ A. HÓDAR and  
JOSÉ M. GÓMEZ

*Grupo de Ecología Terrestre. Departamento de Biología Animal y Ecología, Facultad de Ciencias, Universidad de Granada, E-18071 Granada, Spain*

## Summary

**1** We analyse the factors controlling seedling establishment of Scots pine at its southernmost geographical limit (southern Spain), by monitoring emergence, survival and growth for up to 4 years in the microhabitats to which seeds are dispersed. Naturally established seedlings were monitored in two mountain ranges, and experimental sowings were performed both in woodlands and in adjacent successional shrublands into which the forest could expand.

**2** Emergence was high in all microhabitats, although it was highest under the canopy of shrubs. Overall survival was low, with *c.* 90% of seedlings dying in the first growing season (*c.* 98% after several growing seasons). Survival differed among microhabitats, being highest under shrubs and extremely low (or zero) under pines or in bare soil.

**3** Seedling growth was the highest in areas of bare soil, intermediate under shrubs, and very low under pines.

**4** Establishment under pines was prevented by both mortality and poor performance, and good performance cannot counteract high mortality in the open. Shrubs, however, acted as nurse plants, buffering summer drought without reducing radiation to levels critical for growth, and protecting seedlings from ungulate trampling, hail and frost heave.

**5** Patterns of recruitment were similar for woodland stands and successional shrublands. In addition, patterns of survival for naturally established seedlings were similar to those of seedlings originating from experimental sowings.

**6** Juveniles were positively associated with shrubs but negatively with bare soil or areas below pine canopies. The facilitative effect of shrubs on seedling survival therefore changes the spatial pattern of recruitment from that determined by germination.

**7** Overall, processes controlling seedling establishment in these southern Scots pine forests differ sharply from those operating in its main distribution area. The comparison among contrasting geographical ranges may contribute to an understanding of the role of environmental conditions in the balance between competition and facilitation, and assist in forecasting plant regeneration responses to global climate change.

*Key-words:* facilitation, geographical range, microenvironment, spatial heterogeneity, tree recruitment

Journal of Ecology (2004) **92**, 266–277

## Introduction

Seedling establishment is a critical step for the regeneration of plant populations, both because of the commonly high mortality rates during this stage and

because of the potential to alter and fix the spatial and temporal patterns of recruitment (Harper 1977; Silvertown & Lovett-Doust 1993; Clark *et al.* 1999). Seeds are dispersed to a variety of microhabitats that constitute the starting environment for all subsequent recruitment stages (Harper 1977; Schupp 1995). This provides a range of differences in biotic and abiotic conditions affecting seedling emergence, survival or growth, hence

influencing establishment success (Collins 1990; Schupp 1995; Castro *et al.* 2002a).

In plant species with a wide distribution, different populations are usually subjected to different biotic and abiotic conditions, and therefore to environments with contrasting limitations on seedling establishment. This is the case of boreo-alpine species in the northern hemisphere. Their range has undergone continuous latitudinal and altitudinal changes during glacial-interglacial cycles of the Pleistocene. They had a wider distribution more southerly at the peak of the last glacial period than they do in the current interglacial (Peñalba 1994; Bennet 1997; Taberlet *et al.* 1998). Their main area is now in the northern part of the continents, persisting in the south only in microclimatic islands within a region whose climate is generally adverse, often in refuges at high altitude. Given the impact of climate on establishment success (e.g. Milton 1995; Marone *et al.* 2000), factors controlling seedling recruitment of these species may differ sharply in contrasting areas of their geographical range.

In Europe, the Scots pine, *Pinus sylvestris* L., is distributed mainly in central and northern parts, and, in the south, is restricted to the high mountains of the Mediterranean basin (Ceballos & Ruiz de la Torre 1971; Boratynski 1991). Here, it persists as isolated nuclei facing ecological conditions very different from those of the main distribution area and from those considered to be optimum (Ceballos & Ruiz de la Torre 1971; Catalán 1991; Hódar *et al.* 2003). Most information concerning the ecology of Scots pine seedling establishment comes from central and northern Europe, where the main abiotic constraints for establishment are low temperatures (James *et al.* 1994; Ryyppö *et al.* 1998; Domisch *et al.* 2002), and the major biotic factors are invertebrate herbivores (Vaartaja 1950; Hagner & Jonsson 1995; Nystrand & Granström 1997, 2000), pathogens (Burdon *et al.* 1994), or interference with existing vegetation (e.g. Kuuluvainen & Juntunen 1998; Nilsson *et al.* 2000; Valkonen *et al.* 2002). The interactions between these factors exerts a powerful impact on the spatial pattern of recruitment, concentrating regeneration in some microhabitats and preventing seedling establishment in others (Burdon *et al.* 1994; Steijlen *et al.* 1995; Zackrisson *et al.* 1995, 1997). Factors controlling seedling establishment of Scots pine in Mediterranean areas, however, are largely unknown, despite the relevance that this may have for forest regeneration.

Contrary to the situation in central and northern Europe, the main abiotic factor constraining establishment of woody species in the Mediterranean region, as well as in Mediterranean-type ecosystems in general, is usually summer drought (Dunne & Parker 1999; Rey & Alcántara 2000; Castro *et al.* 2002a,b). Similarly, biotic factors influencing seedling establishment in the Mediterranean region may also differ from those of more mesic, northern areas. For instance, in temperate environments, the typically dense vegetation cover often competes with establishing seedlings (e.g. Lorimer *et al.*

1994; Kolb & Robberecht 1996; Buckley *et al.* 1998). However, in drought-stressed Mediterranean ecosystems, the presence of vegetation may protect establishing seedlings against high radiation, high temperatures and losses of soil moisture, thereby increasing survival (Callaway 1995; Castro *et al.* 2002a; Gómez *et al.* 2004). Knowledge of the factors determining seedling establishment of Scots pine in southern areas could thus aid the understanding of the patterns and processes involved in the recruitment of plant populations growing in contrasting ecological habitats.

This study explores the factors controlling establishment of Scots pine in Sierra Nevada and Sierra de Baza (south-east Spain). These two mountain ranges, some 80 km apart, constitute the southernmost geographical limit of Scots pine, and are separated from the nearest population (in central Spain) by *c.* 500 km (Ceballos & Ruiz de la Torre 1971; Boratynski 1991). The relict, isolated populations have also experienced decline in recent centuries due to human activity (Willkomm 1882; Voigt 1889; Blanca *et al.* 1998; Hódar *et al.* 2003), resulting in a mosaic of woodland interspersed with areas of successional shrublands (Catalán 1991; Castro 2000). Our aim is to determine the spatial pattern of seedling recruitment and its consequences for forest regeneration. Our study was performed in woodland stands as well as in adjacent shrublands into which the forest could expand. We monitored seedling survival, growth and cause of mortality in the field, and performed sowing experiments. Given the impact of habitat structure on seedling recruitment, we also considered the performance of seeds and seedlings in the most common microhabitats where seeds are found after dispersal, i.e. areas of bare soil, under the canopy of abundant shrubby species and under the canopy of adult pines. The differences in environmental conditions suggest that factors controlling seedling establishment in southern areas will differ from those in northern and Fennoscandian areas, and that, as a result, the mechanisms underlying the spatial pattern of recruitment will also differ. We predicted that summer drought would be the main abiotic factor constraining seedling survival in the Mediterranean mountain and that existing vegetation would protect seedlings against such stress, resulting in differences in microhabitat suitability for establishment. Juveniles were therefore expected to be associated with microhabitats providing this nurse effect. We asked:

- 1 What is the magnitude of population losses during seedling emergence and establishment in the different microhabitats?
- 2 What are the biotic and abiotic agents that cause seedling mortality of Scots pine at its southern geographical limit?
- 3 What is the effect of the stand type (woodland vs. successional shrublands) and microhabitat type on seedling recruitment and performance?
- 4 Are the patterns of seedling survival and performance coupled?

5 What is the resulting spatial pattern of seedling recruitment and how does this affect forest regeneration under Mediterranean drought-stressed ecosystems compared with more mesic northern and Fennoscandian areas?

6 Does the pattern of seedling establishment translate to the pattern of juvenile distribution?

## Methods

### STUDY SITES

The study was conducted in the Trevenque area (Barranco del Espinar site, 37°05' N, 3°28' W, Sierra Nevada National Park) and in the Sierra de Baza (hereafter Baza, 37°22' N, 2°51' W, Sierra de Baza Natural Park), where Scots pine grows between 1600 and 2200 m a.s.l., forming the treeline. The two localities have similar characteristics, i.e. soil type, climate, altitude and vegetation type. The climate is continental Mediterranean, with hot, dry summers and cold winters. Mean annual rainfall is 830 mm, mean temperature of the coldest month (January) is 3.5 °C, and mean temperature of the hottest month (August) is 21.6 °C (data from 1990 to 2000 for Trevenque). Snow is common during winter, persisting for up to 2 months, and frost occurs from November to March. Rainfall in 1996, 97, 98 and 99 was 1793, 1283, 632 and 755 mm, respectively. The bedrock is calcareous, and the predominant soils are regosols and cambisols. Natural recruitment of seedlings was monitored in both sites, while experimental manipulations were restricted to Trevenque.

Scots pine typically forms a canopy of *c.* 25% cover, with a few, scattered individuals of *Taxus baccata*, *Pinus nigra* ssp. *salzmannii*, and *Acer granatense* (constituting less than 5% of the canopy cover). The understorey is composed of areas of bare soil with intermingled shrubby species, mostly junipers (*Juniperus communis* and *J. sabina*), tall spiny shrubs (basically *Prunus ramburii* and *Berberis hispanica*) and several scrub species (see below). Successional shrublands are covered by scrub intermingled with open areas, *Salvia lavandulifolia* being particularly abundant in these habitats (see Castro *et al.* 2002a). Dispersal spans January to March (Castro *et al.* 1999), and seeds germinate quickly when warmth and moisture are adequate (in late April under field conditions; Castro 1999). Wild ungulates (*Capra pyrenaica*, Spanish ibex), and domestic sheep and goats browse heavily on Scots pine saplings, retarding their growth but rarely causing mortality (Hódar *et al.* 1998; Zamora *et al.* 2001). Sown seeds had a viability of around 95% (checked in a growth chamber before sowing) and were from the current year (collected in January and stored under room conditions until sowing).

### NATURAL RECRUITMENT OF SEEDLINGS

The natural pattern of recruitment was monitored in woodland stands at Trevenque and Baza by marking

seedlings in different microhabitats during the period of emergence. Only seedlings that emerged during the year of sampling were considered, and all therefore had the same age as seedlings from experimental sowings. Microhabitats used were: (i) bare soil (Open); (ii) under the canopy of shrubs (Shrub); and (iii) under the canopy of adult Scots pine (Pine). In 1996, we marked 205 seedlings at Trevenque (75 in Open, 19 in Shrub and 111 in Pine), and their survival and cause of mortality were monitored four times during the first growing season, and thereafter twice per year (at the beginning and at the end of the growing season, except October 1999) until 15 July 2000 (i.e. well into the fifth growing season). In 1997 we marked 417 seedlings at Baza (302 in Open, 20 in Shrub and 95 in Pine), and monitored them at the beginning and end of each growing season until 11 July 1999 (well into the third season). In addition, we sampled the density of seedlings in different microhabitats at the beginning of the summer by counting the number of live seedlings in 2 × 2 m quadrats (50, 60 or 70 per microhabitat, randomly placed) on 12 July 1996 (Trevenque) and 27 July 1997 (Baza).

### EXPERIMENT 1. EFFECT OF THE MICROHABITAT ON SEEDLING ESTABLISHMENT ON WOODLAND STANDS

This experiment was set up at Trevenque in 1996. We haphazardly assigned 60 sampling stations in each of four microhabitats: (i) interspaces of bare soil (Open); (ii) under the canopy of deciduous, tall (*c.* 1.5 m in height) spiny shrubs, either *Berberis hispanica* or *Prunus ramburii* (Spiny); (iii) under the canopy of *Juniperus communis* (Juniper), and (iv) under the canopy of pines (Pine). On 12–13 April we sowed 25 seeds at each point, in a frame of five by five seeds, with a distance of 4 cm between seeds (a total of 6000 sown seeds). Because we were interested in studying performance, sampling stations were protected with a cage made of 1.3-cm wire mesh in order to reduce seedling losses from trampling.

Sampling stations were censused at *c.* 10-day intervals during the first growing season, noting emergence, survival and cause of mortality. After that, censuses were performed at the beginning and at the end of the growing season (May and October, respectively). Seedling growth was estimated *in situ* after three growing seasons (October 1998) as the length of the shoot. In addition, we harvested a subsample of seedlings (cut off at ground level) to estimate dry mass of the shoot after oven-drying at 70 °C to constant weight. Seedlings from Spiny and Juniper microhabitats were randomly taken from among the surviving seedlings. For Pine and Open microhabitats, however, we had to collect all the seedlings in order to have an adequate sample size. Survival of the remaining seedlings (Spiny and Juniper microhabitats) was monitored until July 2000 (well into the fifth growing season). At that time, we harvested a subsample of seedlings to measure the

length and mass of the shoot. Because the root collar was difficult to distinguish, shoot length and mass were measured from the cotyledon insertion, i.e. without hypocotyl. Sampling stations that were damaged by ungulate trampling or wild boar grubbing were eliminated from the experiment from the time of damage.

#### EXPERIMENT 2. EFFECT OF THE MICROHABITAT ON SEEDLING ESTABLISHMENT ON SUCCESSIONAL SHRUBLANDS

This experiment was set up in 1997 at Trevenque to study patterns of recruitment in the areas of potential expansion for the forest. We used a gap of around 8 ha, resulting from a small fire in 1983, now excluded from grazing and having abundant shrub cover composed mainly of spiny shrubs and *Salvia lavandulifolia* (Castro *et al.* 2002a). We tried to simulate the natural distribution of emerged seedlings in this successional habitat by sowing seeds at a high number of sites but at low density. Furthermore, the seedlings were not protected by exclosures, and were allowed to grow without harvest. We established 150 points in each of four microhabitats: (i) open; (ii) spiny shrub south (hereafter Spiny S); (iii) spiny shrub north (hereafter Spiny N); and (4) *Salvia*. *Salvia* sites were located under the canopy of individuals of *Salvia lavandulifolia*, an evergreen reaching around 30 cm high. In this experiment we divided the spiny habitat into northern (less exposed) and southern (more exposed) sides of tall shrubs (*Prunus ramburii* and *Berberis hispanica*) in view of the impact that exposure to solar radiation has on seedling establishment in open, dry habitats (e.g. Fisher & Gardner 1995; Castro *et al.* 2002a). On 14–17 April, we sowed 10 seeds *c.* 8 cm apart in a line (6000 seeds in total). Sampling stations were censused at *c.* 15-day intervals during the first growing season, noting emergence, survival and cause of mortality. Afterwards, censuses were made at the beginning and at the end of the growing season (May and October, respectively) until 10 July 2000 (well into the fourth growing season). At that date, we harvested the seedlings and estimated their dry weight (from cotyledon insertion) after oven-drying at 70 °C to constant weight. Low seedling survival in some microhabitats since the first growing season, however, hampered comparisons of growth parameters.

#### CAUSES OF MORTALITY

Seedling death was assigned to the most evident cause. Seedlings that became brown and dried out during the summer without any visible damage were classed as dying due to summer drought. Seedlings dying shortly after emergence, when the soil was wet enough to rule out desiccation, often with root-collar necrosis, were assigned to pathogens. Seedlings attacked by insects or rodents (voles and woodmice) were assigned to herbivory, which was lower in caged seedlings. Vole tunnels

caused seedling roots to be disturbed. Trampling by ungulates caused death either by direct stepping on seedlings or by burial as a consequence of displacement of soil over steep slopes (absent in caged seedlings). Hailstones caused seedlings breakage (absent in caged seedlings). Finally, frost heave lifted seedlings and loosened them from the soil. Seedlings that could not be observed near the time of death were assigned no cause of mortality and were excluded from the analyses of causes of mortality.

#### ABIOTIC VARIABLES

Soil moisture during the growing season was determined at 1–6 cm depth for experiment 1 and experiment 2 on several dates, and also at 10–15 cm depth for experiment 2 in August 1997. Sample size was 10–12 per microhabitat, with stations randomly selected at each date. Moisture was determined gravimetrically after oven-drying at 110 °C to constant weight. For experiment 2, we measured total radiation and air humidity, both at 10 cm from the soil, using a Li-Cor LI-200 sz pyranometer sensor (for radiation) and a Rotronic YA-100 humidity sensor (Rotronic AG, Zürich, Switzerland) connected to a LI-1000 data logger (Li-Cor Inc., Lincoln, Nebraska, USA). Sampling was performed on 6 August 1997, a clear day with no clouds, at 1-hour intervals from 10.00 to 16.00 solar time in the four microhabitats (six sample stations per microhabitat, seven cycles completed). Light conditions were also estimated for a subset of the microhabitats as the ‘global site factor’ from hemispherical photographs taken at 0.5 m in height with a fish-eye camera lens in 20 stations per microhabitat. Soil temperature at 5 cm depth was recorded at 1-hour intervals by using thermistors (one or two per microhabitat) connected to a Hobo XT temperature logger (Onset Computer Corporation, Pocasset, Massachusetts, USA).

#### SPATIAL PATTERN OF JUVENILE DISTRIBUTION

To assess whether the pattern of seedling establishment determined by microhabitat suitability was maintained in the distribution of juveniles, we monitored the distribution of juvenile pines in relation to microhabitat availability. Between 1996 and 1997 we tagged all juveniles between 10 and 75 cm height in an area of *c.* 2 ha in woodland stands at both Trevenque and Baza (see Zamora *et al.* 2001 for details). This height interval ensures that we monitored juveniles between *c.* 7 and 16 years old (Zamora *et al.* 2001), after which juvenile mortality is almost nil (Zamora *et al.* 2001). We therefore recorded a recruitment pattern that was already set. For each juvenile, we determined the microhabitat in which the sapling was growing according to vegetation cover, considering Open, Shrub and Pine microhabitats (as defined for natural recruitment of seedlings). Microhabitat availability was determined by sampling vegetation

cover using 10 50-m long transects randomly located in the 2 ha area (Bullock 1996). At each meter the identity of any vegetation cover at two points, each 1 m on opposite sides of the transect, was recorded ( $n = 100$  sampling points per transect, 1000 points in total). The proportion of juveniles growing in each microhabitat was compared with microhabitat availability.

**DATA ANALYSIS**

Seedling emergence was analysed with ANOVA. Survival was analysed either with ANOVA (GLM procedure, SAS 6.12) or with a failure-time analysis (LIFEREG procedure, SAS 6.12), depending on the data set. For experiment 1, we used an ANOVA. For experiment 2 and for naturally established seedlings, survival was analysed with a failure-time approach, which measures the time to failure (death) of each individual (Fox 1993; Allison 1995). A Weibull distribution was used, based on previous graphic inspection and because we expected a continuous decrease in failure rates (Pyke & Thompson 1986; Allison 1995). In addition, cumulative survival was analysed using contingency analysis in order to explore the final result without influence of the shape of the survival curve. Seedling growth was analysed with ANOVAs (experiment 1), considering individual seedlings as replicates. Causes of mortality were compared with contingency tables. The pattern of juvenile distribution was analysed with a contingency test. Because the sum of canopy plus understorey cover exceeds 100%, the coverage of each microhabitat as well as the number of juveniles per microhabitat were recalculated on the basis of a theoretical cover of 100% for graphic presentation. Eleven of the juveniles found were located under both shrubs and pine canopy; they were assigned to microhabitat Pine for analysis.

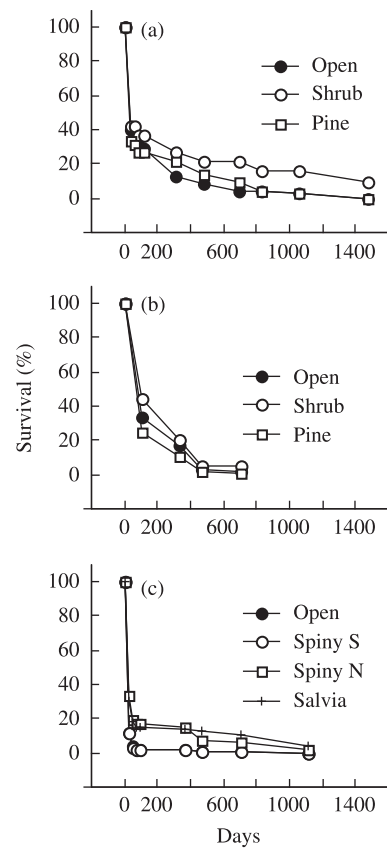
For ANOVAs, data were log- or (for ratio values) arcsine-transformed to meet assumptions of the analysis, and the non-parametric Kruskal–Wallis test was used when required (Zar 1996). We used type III sum of squares, and all factors were considered fixed. Mean values are presented  $\pm$  standard error.

**Results**

**NATURAL RECRUITMENT OF SEEDLINGS**

Seedling density at Trevenque differed among microhabitats ( $H = 19.29$ , d.f. = 2,  $P < 0.0001$ , Kruskal–Wallis test), Open having  $1.2 \pm 0.9$  seedlings per 100 m<sup>2</sup>, Shrub  $1.7 \pm 0.8$ , and Pine  $6.2 \pm 2.4$ . Seedling density also differed among microhabitats at Baza ( $H = 23.09$ , d.f. = 2,  $P < 0.0001$ ), Open having  $31.7 \pm 4.9$  per 100 m<sup>2</sup>, Shrub  $6.2 \pm 1.6$ , and Pine  $21.7 \pm 4.9$ .

Seedling survival at Trevenque after 4 years was 0.9% (all microhabitats pooled). The failure-time analysis did not detect differences among survival curves. Nevertheless, there were differences among microhabitats for cumulative survival, with Shrub having the



**Fig. 1** Percentage of Scots pine seedling survival for (a) naturally emerged seedlings at Trevenque (after 4 years of study), (b) naturally emerged seedlings at Baza (after 2 years of study), and (c) seedlings emerged from sown seeds in successional shrublands at Trevenque (experiment 2, after 3 years of study).

final highest survival (10.5% after 4 years) compared with no survival in Open or Pine (Fig. 1a;  $\chi^2 = 7.89$ , d.f. = 2,  $P = 0.019$  by May 1999, i.e. after 3 years; cumulative survival at the end of the study could not be analysed due to lack of seedlings in Open and Pine microhabitats). Causes of mortality were recognized in 93.6% of cases, with summer drought the most important (69.1%), followed by trampling (28.9%) and herbivory (2.0%). Causes of mortality were affected by microhabitat; thus, of the two most important causes, trampling had a stronger effect on Open (38.0% due to trampling vs. 62.0% to summer drought) and Pine (26.2% due to trampling vs. 73.8% to summer drought) than on Shrub (12.5% due to trampling vs. 87.5% to summer drought;  $\chi^2 = 5.24$ , d.f. = 2,  $P = 0.07$ ).

Seedling survival at Baza after 2 years was 2.0% overall. Differences in survival curves were marginally significant (Table 1a), Shrub having the highest cumulative survival (5.0%), followed by Open (2.0%) and Pine (1.0%) (Fig. 1b; cumulative survival not analysed due to frequencies being too low in some cells). Causes of mortality were recognized in 95.4% of cases and were in the same order as at Trevenque: summer drought (74.1%), trampling (22.3%) and herbivory (1.0%). Causes of mortality were again affected by the

**Table 1** Summary of the results of failure-time analyses for Scots pine seedling survival (LIFEREG procedure) for (a) naturally established seedlings at Baza (from 1997 to 1999), and (b) seedlings emerged in 1997 from sown seeds at Trevenque (experiment 2; 1997–2000)

(a) Natural recruitment					
Variable	d.f.	Estimate	SE	Chi-square	<i>P</i>
Intercept	1	5.56	0.07	5756.66	0.0001
Microhabitat	1	−0.07	0.04	2.93	0.0868
Scale	1	0.71	0.02		
Log likelihood = −448.65					
(b) Experiment 2					
Variable	d.f.	Estimate	SE	Chi-square	<i>P</i>
Intercept	1	3.02	0.09	1496.32	0.0001
Microhabitat	1	0.48	0.03	305.00	0.0001
Scale	1	1.34	0.02		
Log likelihood = −3232.50					

microhabitat. Of the two most important causes, trampling had a higher effect in Open (23.8% due to trampling vs. 76.2% to summer drought) and Pine (25.6% due to trampling vs. 74.4% to summer drought) than in Shrub (0.0% due to trampling vs. 100% to summer drought; data not analysed due to violations of assumptions of contingency analysis).

#### EXPERIMENT 1

Emergence was concentrated from mid-May to late June, with a total of 2685 seedlings. Six more seedlings emerged in late autumn (November 1996), but all of these died within a few days, and were not considered in the analyses. Seedling emergence differed among microhabitats ( $F_{3,236} = 14.22$ ,  $P < 0.0001$ ), with Pine having the lowest value ( $28.1 \pm 2.8\%$ ), whereas the rest of the microhabitats had similar values (Open =  $49.2 \pm 2.8$ ; Spiny =  $51.3 \pm 3.3$ ; Juniper =  $50.8 \pm 3.4$ ).

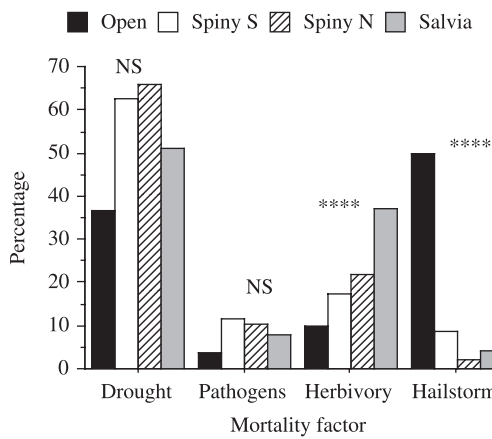
Mortality in the first growing season was 90.1%, rising to 96.2% after three growing seasons with survival differing among microhabitats ( $F_{3,211} = 5.10$ ,  $P = 0.002$ ; 25 sampling stations eliminated from analysis). The highest survival was in Juniper ( $8.2 \pm 2.2$ ), followed by Spiny ( $3.3 \pm 1.4$ ), Open ( $2.4 \pm 1.3$ ) and Pine ( $1.2 \pm 0.6$ ). The cause of mortality was distinguished for 2518 of the seedlings monitored until the third growing season (94.7% of the dead seedlings). There were significant differences in the three main causes ( $\chi^2 = 2020.61$ , d.f. = 2,  $P < 0.0001$ ), summer drought being the most important (75.8%), followed by pathogens (17.7%) and herbivory (4.3%). Vole tunnels (1.3%) and frost heave (0.9%), caused lesser amounts of damage. The latter mostly occurred in Open, where 61.3% of the deaths caused by this mortality factor were observed. Of the seedlings remaining in microhabitats Juniper and Spiny after the 1998 harvest, 69.3% had died by July 2000 (cause of mortality recognized in 96.2% of the cases). Summer drought was again the main source (64% of deaths), and vole tunnels caused the rest of the

deaths. Survival did not depend on seedling density per sampling station in any of the sampling periods or microhabitats (regressions of survival percentage vs. number of seedlings;  $P > 0.05$  in all cases).

Shoot length after three growing seasons differed among microhabitats ( $F_{3,45} = 3.19$ ,  $P = 0.0326$ ). Seedlings from Pine microhabitat were the smallest ( $24.0 \pm 2.9$  mm), whereas the rest of the microhabitats had seedlings of similar size (Open =  $37.4 \pm 7.0$ , Spiny =  $34.4 \pm 2.0$ , Juniper =  $39.8 \pm 2.2$ ). Differences in seedling performance after three growing seasons were particularly high for shoot dry mass, with seedlings from Open ( $328.1 \pm 96.2$  mg) having a mass several-fold greater than seedlings from shrubby microhabitats (Spiny,  $91.5 \pm 12.6$ ; Juniper,  $84.3 \pm 8.6$ ) and markedly greater than seedlings from Pine ( $48.5 \pm 6.9$ ;  $F_{3,45} = 8.92$ ,  $P < 0.0001$ ). The growth of the seedlings remaining after the 1998 harvest did not differ between Juniper and Spiny microhabitats (final dry mass of  $249.2 \pm 0.1$  mg;  $P > 0.05$ ; i.e. still below that of Open seedlings 2 years earlier).

#### EXPERIMENT 2

Emergence spanned mid-May to late June, with a total of 1861 seedlings. Emergence differed among microhabitats ( $F_{3,596} = 18.12$ ,  $P < 0.0001$ ), with Salvia having the highest value ( $41.7 \pm 1.6\%$ ), whereas the rest of the microhabitats had similar values (Open =  $28.7 \pm 1.8$ , Shrub S =  $25.6 \pm 1.6$ , Shrub N =  $28.1 \pm 1.6$ ). Survival curves after 3 years differed among microhabitats (Table 1b), with Salvia showing the highest cumulative survival (4.2%), and Open and Spiny S the lowest (0% survival by the third growing season; see Fig. 1c) (cumulative survival not analysed due to lack of seedlings in Open from the second growing season). The first summer was the season with the highest mortality (90.3% of the seedlings). At the end of the experiment (July 2000), the cause of mortality was recognized for 1397 seedlings (76.4% of the dead seedlings). Summer



**Fig. 2** Causes of mortality in different microhabitats after three growing seasons for Scots pine seedlings emerged from seeds sown in 1997 at Trevenque (experiment 2). Mortality by hailstones occurred on 26 May 1997. Minor causes such as vole tunnels, trampling or frost heave are not considered. Comparisons among microhabitats were performed independently for each of the causes by using a chi-square analysis ( $\chi^2$  from 1.14 to 444.57, d.f. = 3; NS = non-significant; \*\*\*\*  $P < 0.0001$ ). Drought = summer drought.

drought was the most important (49.5%), followed by herbivory (20.6%), hailstones (18.0%) and pathogens (7.4%), with significant differences among these major causes ( $\chi^2 = 569.94$ , d.f. = 3,  $P < 0.0001$ ). Summer drought and pathogens had similar effects on all the microhabitats (Fig. 2). However, mortality by hailstones was particularly high in Open and low in all microhabitats with a cover that could buffer their impact (Fig. 2). Mortality by herbivores also differed among microhabitats, being the highest in Salvia and

the lowest in Open (Fig. 2), although the early death by summer drought and hailstones of most of the seedlings in Open and Shrub S may bias this difference. Shoot dry mass of seedlings located in Salvia by 10 July 2000 (i.e. in the fourth growing season) was  $165.5 \pm 47.5$  mg, thus still lower than seedlings located in Open after three growing seasons (measured in experiment 1).

**ABIOTIC VARIABLES**

Soil moisture differed among microhabitats during the summer in woodland stands, Open having the lowest values and Pine the highest. However, moisture rose in all the microhabitats once the first rains arrived, with differences disappearing by the beginning of autumn (Table 2). In successional shrublands, the four microhabitats also differed markedly in abiotic conditions. Radiation and soil temperature were the highest in Open and Shrub S, whereas air humidity and soil moisture followed the opposite trend, having the highest values in Shrub N and Salvia (Table 2). Global site factor was  $0.79 \pm 0.08$  for Open microhabitat,  $0.58 \pm 0.14$  for Spiny and  $0.28 \pm 0.01$  for Pine, with significant differences among microhabitats ( $F_{2,56} = 126.34$ ,  $P < 0.0001$ ).

**SPATIAL PATTERN OF JUVENILE DISTRIBUTION**

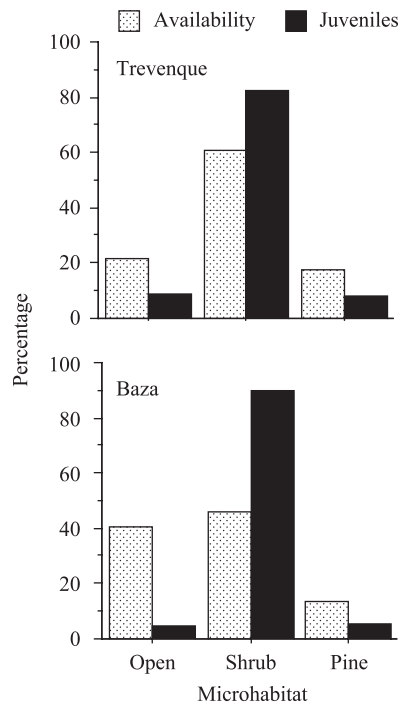
Microhabitat Pine covered 21.3% of the surface at Trevenque. In the understorey, Open covered 26.0%, whereas Shrub covered the remaining 74.0% (the most common species being *Juniperus communis* (16.9%), *J. sabina* (11.8%), and spiny shrubs, mostly *Prunus ram-*

**Table 2** Soil temperature and moisture registered at different microhabitats in woodland stands (experiment 1) and successional shrublands (experiment 2). Different letters indicate between-microhabitat differences at  $\alpha = 0.05$  according to Bonferroni-Dunn test. Soil moisture was measured at 1–5 cm depth (except \*, measured at 10–15 cm depth.) Data of radiation and air humidity are analysed with mean values recorded per sample station throughout the day used as the dependent variable. Data of soil temperature could not be analysed due to lack of replicates; the table shows mean values and, in brackets, mean minimum and mean maximum values; when two thermistors were placed per microhabitat, their data were pooled for calculations. RH = relative humidity; NS = non-significant

Woodland stands	Variable	Date	Microhabitat				d.f.	F	P
			Open	Spiny	Juniper	Tree			
	Soil moisture (%)	02/07/96	4.2 ± 0.4 <sup>a</sup>	6.3 ± 0.8 <sup>a</sup>	7.1 ± 0.7 <sup>ab</sup>	12.1 ± 2.6 <sup>b</sup>	3, 40	7.33	0.0005
	Soil moisture (%)	03/08/96	4.0 ± 0.7 <sup>a</sup>	6.2 ± 0.4 <sup>ab</sup>	7.9 ± 1.3 <sup>b</sup>	9.1 ± 1.3 <sup>b</sup>	3, 36	5.73	0.0026
	Soil moisture (%)	03/09/96	19.0 ± 1.0	23.3 ± 1.0	21.9 ± 1.4	24.6 ± 2.2	3, 37	2.72	0.0582
	Soil moisture (%)	07/10/96	22.3 ± 1.3	26.7 ± 1.5	22.3 ± 1.4	23.8 ± 1.8	3, 43	1.84	NS

Successional shrublands	Variable	Date	Microhabitat				d.f.	F	P
			Open	Spiny S	Spiny N	Salvia			
	Soil moisture (%)	25/06/97	8.3 ± 0.7 <sup>a</sup>	13.0 ± 1.1 <sup>b</sup>	15.2 ± 0.9 <sup>b</sup>	14.2 ± 0.7 <sup>b</sup>	3, 43	13.70	< 0.0001
	Soil moisture (%)	21/08/97	4.1 ± 0.4 <sup>a</sup>	5.6 ± 0.9 <sup>ab</sup>	6.9 ± 0.9 <sup>b</sup>	6.9 ± 0.4 <sup>b</sup>	3, 36	4.34	0.0103
	Soil moisture (%)*	06/08/97	8.6 ± 0.9 <sup>a</sup>	10.6 ± 0.3 <sup>ab</sup>	11.1 ± 0.5 <sup>b</sup>	11.7 ± 0.3 <sup>b</sup>	3, 36	5.42	0.0103
	Radiation (W/m <sup>2</sup> )	06/08/97	916.9 ± 3.2 <sup>a</sup>	895.7 ± 26.2 <sup>a</sup>	340.2 ± 40.4 <sup>b</sup>	473.1 ± 61.1 <sup>b</sup>	3, 20	23.33	0.0035
	Air RH (%)	06/08/97	22.3 ± 0.4 <sup>a</sup>	24.3 ± 0.3 <sup>b</sup>	26.4 ± 0.3 <sup>bc</sup>	26.5 ± 0.8 <sup>c</sup>	3, 20	17.57	< 0.0001
	Soil temperature (°C)	19/08–04/09 1997	18.5 (10.6–32.9)	16.7 (11.0–27.1)	14.7 (10.8–20.0)	15.0 (13.7–16.3)			



**Fig. 3** Comparison between the availability of different microhabitats at the study areas and the proportion of juvenile Scots pine growing in them. There were more juveniles growing under shrubs than expected from microhabitat availability. Percentage cover and juveniles number were re-adjusted to a theoretical cover of 100% (see Methods).

*burii* and *Berberis hispanica* (15.1%). At Baza, Pine covered 16.0% of the surface, and in the understorey Open covered 46.9% and Shrub the remaining 53.1% (*J. communis* = 13.8%, *J. sabina* = 19.6%, and spiny shrubs = 6.2%).

There were 133 juveniles between 10 and 75 cm height at Trevenque (12 in Open, 110 in Shrub and 11 in Pine). At Baza, the 108 juveniles were distributed as 5 in Open, 97 in Shrub and 6 in Pine. Saplings were associated with shrubs more than expected according to shrub cover, and less associated than expected with Open or Pine microhabitats ( $\chi^2 = 26.42$  for Trevenque,  $\chi^2 = 85.07$  for Baza; d.f. = 2,  $P < 0.001$ ; Fig. 3).

## Discussion

### SEEDLING EMERGENCE AND SURVIVAL

Seedling emergence was generally above 20% and rose up to 50%. This suggests that emergence is not a major constraint for regeneration of these Mediterranean Scots pine forests, provided that seeds are available when conditions are suitable for germination. In fact, these values are similar to emergence rates for Scots pine in boreal forests with successful establishment (Ohlson & Zackrisson 1992; Winsa 1995; Karlsson & Örlander 2000). Emergence, however, differed among years, presumably owing to temporal variability in the coincidence of appropriate environmental conditions

such as rainfall and temperature (Ohlson & Zackrisson 1992; Milton 1995; Paynter *et al.* 1998). Nevertheless, despite the overall high values, it is noticeable that seedling emergence was consistently highest under the canopy of shrubby species.

Seedling mortality, however, was very high, being around 90% during the first growing season and reaching up to 100% in some microhabitats after several growing seasons. This, together with the low seed production of these marginal Scots pine forests (Castro *et al.* 1999) and the high rates of seed predation (Castro *et al.* 1999, 2002b), results in extremely low values of seedling density. This contrasts with reports from northern areas, where high seed production (Koski 1991) coupled with survival rates commonly above 50% after several growing seasons (Ohlson & Zackrisson 1992; Mäkitalo 1999; Karlsson & Örlander 2000) ensures that there is an abundant bank of seedlings in the understorey. In our study area, the main mortality agent is the summer drought, which causes between 50 and 75% of deaths, and continues to cause high mortality up to 4 years after emergence (see also Hódar *et al.* 1998; Castro *et al.* 2002a,b, 2004). This situation again contrasts with northern areas, where soil moisture is usually high enough during most of the summer to ensure seedling survival (Ohlson & Zackrisson 1992; Zasada *et al.* 1992; Zackrisson *et al.* 1998). Pathogens or invertebrate herbivores, such as insects and slugs, which are the main mortality factors for Scots pine seedlings in Fennoscandia (Burdon *et al.* 1994; Hagner & Jonsson 1995; Nystrand & Granström 1997, 2000), had little impact here (see also Hódar *et al.* 1998; Castro *et al.* 2002a,b). Thus, different regional climatic conditions may underlie the contrasting patterns of establishment, either directly, via moisture and temperature, or indirectly, e.g. by providing conditions (mostly dry summers in the Mediterranean mountains) that discourage certain mortality agents such as pathogens and invertebrate herbivores.

### NURSE-SHRUB EFFECT

Seedling survival was consistently highest under the canopy of shrubs (5–10% after several growing seasons), compared with low, or zero, values under pine canopies or in areas of bare soil. Shrubs therefore acted as nurse plants during Scots pine recruitment (Callaway 1995), facilitating not only emergence but, particularly, survival. Shrubs reduced radiation and soil temperature, and increased soil moisture and air humidity, which would improve the water status of seedlings (see also Gómez *et al.* 2001a, 2004; Castro *et al.* 2002a). In fact, seedling survival on the southern side of shrubs was similar to that in Open (whose microclimatic conditions were similar), whereas survival was higher in the north side of shrubs, supporting a direct effect of shading (Kitzberger *et al.* 2000; Rousset & Lepart 2000; Castro *et al.* 2002a, 2004). Shrubs also offered protection against ungulate trampling (see also Gómez *et al.*



2001b, 2003), the impact of hailstorms (a common phenomena in the Mediterranean mountains that caused high mortality in Open, see also Castro *et al.* 2002b), and frost heave. The relevance of this facilitation is shown by the fact that very few seedlings survived in Open despite 2 years of sowing (1996 and 1997) coinciding with high rainfall: in dry years no seedlings at all survive in open areas (Castro 2000). Similarly, facilitation by shrubs occurred in both woodland stands and adjacent successional shrublands, indicating that this nurse effect is critical for both within-stand regeneration and colonization of new habitats.

The nurse effect of shrubs, however, had a cost in terms of reduced growth, suggesting that the establishment of Scots pine seedlings in Mediterranean mountains is largely controlled by a gradient of abiotic conditions, determined by radiation and moisture, that uncouples the requirements for survival and growth. Areas below pine canopies provide the highest soil moisture and cooler temperatures that encourage survival, but the low radiation hampers seedling growth. Areas of bare soil, however, offer full radiation, as required for optimum seedling performance, but the low soil moisture and high temperatures cause extremely high mortality. Areas below shrub canopies show intermediate values of radiation and moisture, ensuring higher survival rates and adequate seedling performance (see also Gómez *et al.* 2001a, 2004; Castro *et al.* 2002a, 2004). In short, the recruitment of Scots pine seedlings in Mediterranean drought-stressed ecosystems requires moderate shade despite the shade-intolerant nature of the species (Ceballos & Ruiz de la Torre 1971; Nikolov & Helmisaari 1992), in contrast to northern ecosystems where its regeneration is associated with open gaps receiving full sunlight (Nikolov & Helmisaari 1992; Zasada *et al.* 1992; de Chantal *et al.* 2003).

#### CONSEQUENCES FOR REGENERATION

Spatial heterogeneity may exert a varying influence upon different stages of recruitment, giving rise to conflicts of regeneration that change microhabitat suitability (Harper 1977; Jordano & Herrera 1995; Schupp 1995; García 2001). In our Scots pine forests, phases up to germination will lead to a higher initial proportion of seedlings under the canopy of pines, given that most seeds fall under maternal plants (Castro *et al.* 1999), post-dispersal seed predation is similarly high in all the microhabitats (Castro *et al.* 1999), and germination and emergence, although slightly lower under pine canopies (Castro 2000; this study), is high in all microhabitats. Although initial seedling density under the canopy of shrubs is thus expected to be low, these seedlings have higher chances of survival. In fact, the pattern of juvenile distribution is positively associated with shrubby species (Fig. 3) and, considering that sapling mortality is very low (Hódar *et al.* 1998; Zamora *et al.* 2001), this pattern must be fixed by the spatial distribution created by seedling survival. Seedling survival

must therefore be regarded as the critical phase for Scots pine recruitment in Mediterranean mountains not only because of the high losses of reproductive potential occurring at this stage, but also because of its strong influence on latter phases.

The spatial pattern of seedling establishment was consistent across habitats, with both current woodland stands and successional shrublands having similarly high rates of seedling mortality and higher survival under the canopy of shrubs. This implies, on one hand, that both within-stand regeneration and expansion to adjacent areas is seriously constrained, which is exacerbated by the low seed production and high seed predation in both habitats (Castro *et al.* 1999, 2002b). On the other hand, the facilitative effect of shrubs indicates that they are key microhabitats for reforestation and conservation programmes in these southernmost Scots pine forests. Management strategies concerning either seeding or planting of seedlings should therefore consider shrubby species as regeneration niches to ensure establishment success (see also García *et al.* 2000; Gómez *et al.* 2001a, 2004; Castro *et al.* 2002a, 2004).

#### REGENERATION PATTERNS ACROSS GEOGRAPHICAL RANGES

This study shows that the factors determining establishment success in southern, Mediterranean areas of the Scots pine distribution are remarkably different from those operating in central and northern Europe. These differences are ultimately determined by differences in climatic conditions, which affect seedling establishment either directly (e.g. drought) or indirectly through the modulation of biotic interactions. In this sense, the facilitative effect of shrubs contrasts with their predominantly negative effect in Fennoscandia (e.g. Dolling 1996; Kuuluvainen & Juntunen 1998; Nilsson *et al.* 2000; references therein), an area characterized by oceanic, temperate-type climatic conditions and a dense understorey (Ohlson & Zackrisson 1992; Kuuluvainen & Juntunen 1998). This agrees with predictions that competition for resources will predominate in environments with low environmental stress, whereas facilitative interactions will predominate in harsher environments where association with other species can offset the negative consequences of competition (Callaway 1995; Brooker & Callaghan 1998; Bruno *et al.* 2003). Central and northern areas of the Scots pine distribution range would represent mesic environments prone to antagonist interactions (mostly competition for nutrients and light). On the contrary, the Mediterranean southern boundary is marked by a harsh summer drought and is therefore a stressful environment liable to facilitative interactions (see also Pugnaire *et al.* 1996; Castro *et al.* 2002a, 2004; Gómez *et al.* 2004).

Strong efforts are currently being made to assess the role of facilitation in structuring plant communities (Bruno *et al.* 2003). The outcome of plant–plant interactions is compared under contrasting ecological

settings differing in environmental stress at local scale, such as south- vs. west-facing slopes (Callaway *et al.* 1996), dry vs. mesic adjacent sites (Holmgren *et al.* 2000), high vs. low altitudes (Pugnaire & Luque 2001; Callaway *et al.* 2002), wet vs. dry years (Casper 1996; Tielbörger & Kadmon 2000; Ibáñez & Schupp 2001), or summer vs. winter periods (Hastwell & Facelli 2003). At much larger spatial scales and over integrated temporal scales, different environmental conditions are represented by the less stressful main areas of distribution vs. the more stressful marginal areas. The results reported here show that comparing the regeneration of populations growing under contrasting geographical ranges is a complementary way of exploring the impact of environmental conditions on the balance between competition and facilitation. In addition, studying the regeneration of plant species growing in marginal, southern distribution areas may provide a scenario for the ecological conditions that may develop in northern areas, given the current trends of global warming (IPCC 2001). This may help to assess the impact of climate change on plant communities, and to suggest strategies for conservation and restoration.

### Acknowledgements

We thank the Consejería de Medio Ambiente, Junta de Andalucía, and the Directors of the National Park of Sierra Nevada and Natural Park of Sierra de Baza for permitting our fieldwork. Fernando Valladares made the hemispherical photographs and calculated global site factors. We also thank Daniel García for field assistance and David Nesbitt for linguistic advice. This study was supported by a grant PFPI-MEC to JC and projects AGF98-0984, 1FD97-0743-CO3-02 and REN2001-4552-E to RZ.

### References

- Allison, P.D. (1995) *Survival Analysis Using the SAS® System. A Practical Guide*. SAS Institute, Cary.
- Bennett, K.D. (1997) *Ecology and Evolution: a Pace for Life*. Cambridge University Press, Cambridge.
- Blanca, G., Cueto, M., Martínez-Lirola, M.J. & Molero-Mesa, J. (1998) Threatened vascular flora of Sierra Nevada (Southern Spain). *Biological Conservation*, **85**, 269–285.
- Boratynski, A. (1991) Range of natural distribution. *Genetics of Scots Pine* (eds M. Giertych & C. Mátyás), pp. 19–30. Akadémiai Kiadó, Budapest.
- Brooker, R.W. & Callaghan, T.V. (1998) The balance between positive and negative plant interactions and its relationship to environmental gradients: a model. *Oikos*, **81**, 196–207.
- Bruno, J.F., Stachowicz, J.J. & Bertness, M.D. (2003) Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution*, **18**, 119–125.
- Buckley, D.S., Sharik, T.L. & Isebrands, J.G. (1998) Regeneration of northern Red oak: positive and negative effects of competitor removal. *Ecology*, **79**, 65–78.
- Bullock, J. (1996) Plants. *Ecological Census Techniques: a Handbook* (ed. W.J. Sutherland), pp. 111–138. Cambridge University Press, Cambridge.
- Burdon, J.J., Wennström, A., Müller, W.J. & Ericson, L. (1994) Spatial patterning in young stands of *Pinus sylvestris* in relation to mortality caused by the snow blight pathogen *Phacidium infestans*. *Oikos*, **71**, 130–136.
- Callaway, R.M. (1995) Positive interactions among plants. *Botanical Review*, **61**, 306–349.
- Callaway, R.M., Brooker, R.W., Choler, P., Kikvidze, Z., Lorite, C.J., Michalet, R. *et al.* (2002) Positive interactions among alpine plants increase with stress. *Nature*, **417**, 844–848.
- Callaway, R.M., DeLucia, E.H., Moore, D., Nowak, R. & Schlesinger, W.H. (1996) Competition and facilitation: contrasting effects of *Artemisia tridentata* on desert vs. montane pines. *Ecology*, **77**, 2130–2141.
- Casper, B.B. (1996) Demographic consequences of drought in the herbaceous perennial *Cryptantha flava*: effects of density, associations with shrubs, and plant size. *Oecologia*, **106**, 144–152.
- Castro, J. (1999) Seed mass versus seedling performance in Scots pine: a maternally dependent trait. *New Phytologist*, **144**, 153–161.
- Castro, J. (2000) *Dinámica de la regeneración de los pinares autóctonos de Pino silvestre (Pinus sylvestris L. var. nevadensis. Christ) de Sierra Nevada Y Sierra de Baza*. PhD thesis, University of Granada, Granada.
- Castro, J., Gómez, J.M., García, D., Zamora, R. & Hódar, J.A. (1999) Seed predation and dispersal in relict Scots pine forests in southern Spain. *Plant Ecology*, **145**, 115–123.
- Castro, J., Zamora, R. & Hódar, J.A. (2002b) Mechanisms blocking *Pinus sylvestris* colonization of Mediterranean mountain meadows. *Journal of Vegetation Science*, **13**, 725–731.
- Castro, J., Zamora, R., Hódar, J.A. & Gómez, J.M. (2002a) The use of shrubs as nurse plants: a new technique for reforestation in Mediterranean mountains. *Restoration Ecology*, **10**, 297–305.
- Castro, J., Zamora, R., Hódar, J.A., Gómez, J.M. & Gómez, L. (2004) Benefits of using shrubs as nurse plants for reforestation in Mediterranean mountains: a 4-year study. *Restoration Ecology*, **12**, in press.
- Catalán, G. (1991) *Las Regiones de Procedencia de Pinus sylvestris L. y Pinus nigra Arn. Subsp. Salzmannii (Dunal) Franco en España*. ICONA, Madrid.
- Ceballos, L. & Ruiz de la Torre, J. (1971) *Árboles Y Arbustos de la España Peninsular*. Instituto Forestal de Investigaciones y Experiencias, Madrid.
- de Chantal, M., Leinonen, K., Kuuluvainen, T. & Cescatti, A. (2003) Early response of *Pinus sylvestris* and *Picea abies* seedlings to an experimental canopy gap in a boreal spruce forest. *Forest Ecology and Management*, **176**, 321–336.
- Clark, J.S., Beckage, B., Camill, P., Cleveland, B., HilleRis-Lambers, J., Lichter, J. *et al.* (1999) Interpreting recruitment limitation in forests. *American Journal of Botany*, **86**, 1–16.
- Collins, S.L. (1990) Habitat relationship and survivorship of tree seedlings in hemlock-hardwood forest. *Canadian Journal of Botany*, **68**, 790–797.
- Dolling, A.H.U. (1996) Interference of bracken (*Pteridium aquilinum* L. Kuhn) with Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* L. Karst) seedling establishment. *Forest Ecology and Management*, **88**, 227–235.
- Domisch, T., Finér, L., Lehto, T. & Smolander, A. (2002) Effect of soil temperature on nutrient allocation and mycorrhizas in Scots pine seedlings. *Plant and Soil*, **239**, 173–185.
- Dunne, J.A. & Parker, V.T. (1999) Species-mediated soil moisture availability and patchy establishment of *Pseudotsuga menziesii* in chaparral. *Oecologia*, **119**, 36–45.
- Fisher, M. & Gardner, A.S. (1995) The status and ecology of a *Juniperus excelsa* subsp. *polycarpus* woodland in the northern mountains of Oman. *Vegetatio*, **119**, 33–51.
- Fox, G.A. (1993) Failure-time analysis: emergence, flowering, survivorship, and other waiting times. *Design and Analysis*

- of *Ecological Experiments* (eds S.M. Scheiner & J. Gurevitch), pp. 253–289. Chapman & Hall, New York.
- García, D. (2001) Effects of seed dispersal on *Juniperus communis* recruitment on a Mediterranean mountain. *Journal of Vegetation Science*, **12**, 839–848.
- García, D., Zamora, R., Hódar, J.A., Gómez, J.M. & Castro, J. (2000) Yew (*Taxus baccata* L.) regeneration is facilitated by fleshy-fruited shrubs in Mediterranean environments. *Biological Conservation*, **95**, 31–38.
- Gómez, J.M., García, D. & Zamora, R. (2003) Impact of vertebrate acorn- and seedling-predators on a Mediterranean *Quercus pyrenaica* forest. *Forest Ecology and Management*, **180**, 125–134.
- Gómez, J.M., Hódar, J.A., Zamora, R., Castro, J. & García, D. (2001b) Ungulate damage on Scots pines in Mediterranean environments: effects of association with shrubs. *Canadian Journal of Botany*, **79**, 739–746.
- Gómez, L., Zamora, R., Gómez, J.M., Hódar, J.A., Castro, J. & Baraza, E. (2004) Applying plant facilitation to forest restoration in Mediterranean ecosystems: a meta-analysis of the use of shrubs as nurse plants. *Ecological Applications*, **14**, in press.
- Gómez, L., Zamora, R., Hódar, J.A., Gómez, J.M. & Castro, J. (2001a) Facilitation of tree seedlings by shrubs in Sierra Nevada (SE Spain): disentangling the mechanisms. *Forest Research: a Challenge for an Integrated European Approach*, I (ed. K. Radoglou), pp. 395–400. NAGREF, Forest Research Institute, Thessaloniki.
- Hagner, M. & Jonsson, C. (1995) Survival after planting without soil preparation for pine and spruce seedlings protected from *Hylobius abietis* by physical and chemical shelters. *Scandinavian Journal of Forest Research*, **10**, 225–234.
- Harper, J.L. (1977) *Population Biology of Plants*. Academic Press, New York.
- Hastwell, G.T. & Facelli, J.M. (2003) Differing effects of shade-induced facilitation on growth and survival during the establishment of a chenopod shrub. *Journal of Ecology*, **91**, 941–950.
- Hódar, J.A., Castro, J., Gómez, J.M., García, D. & Zamora, R. (1998) Effects of herbivory on growth and survival of seedlings and saplings of *Pinus sylvestris nevadensis* in SE Spain. *Ecological Basis of Livestock Grazing in Mediterranean Ecosystems* (eds V.P. Papanastasis & D. Peter), pp. 264–267. Office for Official Publications of the European Communities, Luxembourg.
- Hódar, J.A., Castro, J. & Zamora, R. (2003) Pine processionary caterpillar *Thaumetopoea pityocampa* as a new threat for relict Mediterranean Scots pine forests under climatic warming. *Biological Conservation*, **110**, 123–129.
- Holmgren, M., Segura, A.M. & Fuentes, E.R. (2000) Limiting mechanisms in the regeneration of the Chilean matorral. *Plant Ecology*, **147**, 49–57.
- Ibáñez, I. & Schupp, E.W. (2001) Positive and negative interactions between environmental conditions affecting *Cercocarpus ledifolius* seedling survival. *Oecologia*, **129**, 543–550.
- IPCC (2001) *The Intergovernmental Panel on Climate Change (IPCC) Third Assessment Report*. Cambridge University Press, Cambridge.
- James, J.C., Grace, J. & Hoad, S.P. (1994) Growth and photosynthesis of *Pinus sylvestris* at its altitudinal limit in Scotland. *Journal of Ecology*, **82**, 297–306.
- Jordano, P. & Herrera, C.M. (1995) Shuffling the offspring: uncoupling and spatial discordance of multiple stages in vertebrate seed dispersal. *Écoscience*, **2**, 230–237.
- Karlsson, C. & Örlander, G. (2000) Soil scarification shortly before a rich seed fall improves seedling establishment in seed tree stands of *Pinus sylvestris*. *Scandinavian Journal of Forest Research*, **15**, 256–266.
- Kitzberger, T., Steinaker, D.F. & Veblen, T.T. (2000) Effects of climatic variability on facilitation of tree establishment in northern Patagonia. *Ecology*, **81**, 1914–1924.
- Kolb, P.F. & Robberecht, R. (1996) *Pinus ponderosa* seedling establishment and the influence of competition with the bunchgrass *Agropyron spicatum*. *International Journal of Plant Science*, **157**, 509–515.
- Koski, V. (1991) Generative reproduction and genetic processes in nature. *Genetics of Scots Pine* (eds M. Giertych & C. Mátyás), pp. 59–72. Akadémiai Kiadó, Budapest.
- Kuuluvainen, T. & Juntunen, P. (1998) Seedling establishment in relation to microhabitat variation in a windthrow gap in a boreal *Pinus sylvestris* forest. *Journal of Vegetation Science*, **9**, 551–562.
- Lorimer, C.G., Chapman, J.W. & Lambert, W.D. (1994) Tall understorey vegetation as a factor in the poor development of oak seedlings beneath mature stands. *Journal of Ecology*, **82**, 227–237.
- Mäkitalo, K. (1999) Effect of site preparation and reforestation method on survival and height growth of Scots pine. *Scandinavian Journal of Forest Research*, **14**, 512–525.
- Marone, L., Horno, M.E. & González del Solar, R. (2000) Post-dispersal fate of seeds in the Monte desert of Argentina: patterns of germination in successive wet and dry years. *Journal of Ecology*, **88**, 940–949.
- Milton, S.J. (1995) Spatial and temporal patterns in the emergence and survival of seedlings in arid Karoo shrubland. *Journal of Applied Ecology*, **32**, 145–156.
- Nikolov, N. & Helmisaari, H. (1992) Silvics of the circumpolar boreal forest tree species. *A System Analysis of the Global Boreal Forest* (eds H.H. Shugart, R. Leemans & G.B. Bonan), pp. 13–84. Cambridge University Press, Cambridge.
- Nilsson, M.-C., Zackrisson, O., Sterner, O. & Wallstedt, A. (2000) Characterisation of the differential interference effects of two boreal dwarf shrub species. *Oecologia*, **123**, 122–128.
- Nystrand, O. & Granström, A. (1997) Forest floor moisture controls predator activity on juvenile seedlings of *Pinus sylvestris*. *Canadian Journal of Forest Research*, **27**, 1746–1752.
- Nystrand, O. & Granström, A. (2000) Predation of *Pinus sylvestris* seeds and juvenile seedlings in Swedish boreal forest in relation to stand disturbance by logging. *Journal of Applied Ecology*, **37**, 449–463.
- Ohlson, M. & Zackrisson, O. (1992) Tree establishment and microhabitat relationships in north Swedish peatlands. *Canadian Journal of Forest Research*, **22**, 1869–1877.
- Paynter, Q., Fowler, S.V., Memmott, J. & Sheppard, A.W. (1998) Factors affecting the establishment of *Cytisus scoparius* in southern France: implications for managing both native and exotic populations. *Journal of Applied Ecology*, **35**, 582–595.
- Peñalba, M.C. (1994) The history of the Holocene vegetation in northern Spain from pollen analysis. *Journal of Ecology*, **82**, 815–832.
- Pugnaire, F.I., Haase, P., Puigdefábregas, J., Cueto, M., Clark, C. & Incoll, L.D. (1996) Facilitation and succession under the canopy of a leguminous shrub, *Retama sphaerocarpa*, in a semi-arid environment in south-east Spain. *Oikos*, **76**, 455–464.
- Pugnaire, F.I. & Luque, M.T. (2001) Changes in plant interaction along a gradient of environmental stress. *Oikos*, **93**, 42–49.
- Pyke, D.A. & Thompson, J.N. (1986) Statistical analysis of survival and removal rate experiments. *Ecology*, **67**, 240–245.
- Rey, P.J. & Alcántara, J.M. (2000) Recruitment dynamics of a fleshy-fruited plant (*Olea europaea*): connecting patterns of seed dispersal to seedling establishment. *Journal of Ecology*, **88**, 622–633.
- Rousset, O. & Lepart, J. (2000) Positive and negative interactions at different life stages of a colonizing species (*Quercus humilis*). *Journal of Ecology*, **88**, 401–412.
- Ryppö, A., Iivonen, S., Rikala, R., Sutinen, M.-L. & Vapaavuori, E. (1998) Responses of Scots pine seedlings to

- low root zone temperature in spring. *Physiologia Plantarum*, **102**, 503–512.
- Schupp, E.W. (1995) Seed-seedling conflicts, habitat choice, and patterns of plant recruitment. *American Journal of Botany*, **82**, 399–409.
- Silvertown, J.W. & Lovett-Doust, J. (1993) *Introduction to Plant Population Biology*. Blackwell Science, Oxford.
- Steijlen, I., Nilsson, M.-C. & Zackrisson, O. (1995) Seed regeneration of Scots pine forest stands dominated by lichen and feather moss. *Canadian Journal of Forest Research*, **25**, 713–723.
- Taberlet, P., Fumagalli, L., Wust-Saucy, A.G. & Cosson, J.F. (1998) Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, **7**, 453–464.
- Tielbörger, K. & Kadmon, R. (2000) Temporal environmental variation tips the balance between facilitation and interference in desert plants. *Ecology*, **81**, 1544–1553.
- Vaartaja, O. (1950) On factors affecting the initial development of pine. *Oikos*, **2**, 89–108.
- Valkonen, S., Ruuska, J. & Siipilehto, J. (2002) Effect of retained trees on the development of young Scots pine stands in Southern Finland. *Forest Ecology and Management*, **166**, 227–243.
- Voigt, C. (1889) Wanderungen in der S. Nevada. *Ent. Ztg. Stettin*, **50**, 356–412 (Spanish edition by Caja de Granada Foundation, 1995, Granada).
- Willkomm, M. (1882) *Aus den Hochgebirgen von Granada*. (Spanish edition by Caja de Granada Foundation, 1995, Granada).
- Winsa, H. (1995) Influence of rain shelter and site preparation on seedling emergence of *Pinus sylvestris* L. after direct seeding. *Scandinavian Journal of Forest Research*, **10**, 167–175.
- Zackrisson, O., Dahlberg, A., Norberg, G., Nilsson, M.-C. & Jäderlund, A. (1998) Experiments on the effects of water availability and exclusion of fungal hyphae on nutrient uptake and establishment of *Pinus sylvestris* seedlings in carpets of the moss *Pleurozium schreberi*. *Écoscience*, **5**, 77–85.
- Zackrisson, O., Nilsson, M.-C., Dahlberg, A. & Jäderlund, A. (1997) Interference mechanisms in conifer-Ericaceae-feathermoss communities. *Oikos*, **78**, 209–220.
- Zackrisson, O., Nilsson, M.-C., Steijlen, Y. & Hörnberg, G. (1995) Regeneration pulses and climate-vegetation interactions in nonpyrogenic boreal Scots pine stands. *Journal of Ecology*, **83**, 469–483.
- Zamora, R., Gómez, J.M., Hódar, J.A., Castro, J. & García, D. (2001) Effect of browsing by ungulates on sapling growth of Scots pine in a Mediterranean environment: consequences for forest regeneration. *Forest Ecology and Management*, **144**, 33–42.
- Zar, J.H. (1996) *Biostatistical Analysis*, 3rd edn. Prentice Hall, New Jersey.
- Zasada, J.C., Sharik, T.L. & Nygren, M. (1992) The reproductive process in boreal forest trees. *System Analysis of the Global Boreal Forest* (eds H.H. Shugart, R. Leemans & G.B. Bonan), pp. 85–125. Cambridge University Press, Cambridge.

Received 2 May 2003 revision

accepted 5 November 2003

Handling Editor: Chris J. Peterson