Alleviation of summer drought boosts establishment success of *Pinus sylvestris* in a Mediterranean mountain: an experimental approach

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Abstract

We performed an irrigation experiment to study the impact of summer drought on *Pinus sylvestris* establishment at its southernmost distribution limit. Watering was done during the first growing season simulating mesic summer conditions, and we monitored the consequences for survival and growth during the first growing season and the delayed consequences on the second growing season. In addition, we considered the heterogeneity created by the microhabitats where seeds are found after dispersal (bare soil, under shrubs, and under adult pines). Summer drought was the main mortality factor in all the microhabitats. Watering increased emergence and doubled seedling survival compared to non-watered control sites. Differences were even higher when the cumulative effect on emergence and survival was considered, with an overall recruitment of 22.4% in watered plots vs. 7.9% in control. Irrigation increased growth in bare soil and under shrubs, but had scant effect on growth under pines, suggesting that radiation was the limiting factor in this microhabitat. The positive effect of irrigation on growth parameters persisted during the second growing season despite water was not added the second year, showing delayed consequences of drought on seedling performance. Summer drought thus limits *Pinus sylvestris* establishment in these southernmost forests by reducing both recruitment and growth. This might lead to the development of a remnant dynamic in these relict populations under the current regional increase in dryness and rainfall variability associated with global warming.

Introduction

Water is a critical resource limiting distribution, survival and growth of plants (Kozlowski 1968; Grace 1997). Soil moisture exerts a powerful impact on seedling establishment, given that this stage is highly vulnerable to drought stress (Kozlowski 1968). Consequently, water supplementation during the earliest phases of the plant life-cycle increases natural seedling establishment of woody species in a wide range of terrestrial environments, such as coastal sand dunes (De Jong and Klinkhamer 1988; Lichter 2000), semiarid grasslands (Harrington 1991), tropical savannas (Lonsdale and Abrecht 1989; Hoffmann 1996), and temperate forests (Owens et al. 1995; Ibáñez and Schupp 2001).

Mediterranean-type ecosystems are characterised by a sharp seasonality of rainfall and temperature, with hot, dry summers. In addition,

inter-annual variability in precipitation is common, and scattered rainy years are typically followed by dry periods (Rumney 1968; Rodó and Comín 2001). The resulting drought periods bring major stress for plants (Mooney 1983), and limit recruitment by the high seedling mortality ascribed to dryness in these environments (Dunne and Parker 1999; García 2001; Castro et al. 2002a, 2004a). Nevertheless, despite the many reports on the negative effect of summer drought, no experimental studies appear to address the quantitative impact on seedling establishment in Mediterranean environments considering explicitly emergence, survival and growth of seedlings under natural conditions.

Summer drought has prompted the evolution of an array of morphological and physiological traits in the Mediterranean flora that allow plants to cope with shortage and unpredictability of moisture (Turner and Kramer 1980; Margaris 1981). For instance, high root-biomass allocation and the capacity to alter the root:shoot ratio in response to changes in water availability are two features advantageous for survival in drought–prone environments (Hilbert and Canadell 1995; Poorter and Nagel 2000). Nevertheless, the current flora of the Mediterranean basin is also well represented by taxa characteristic of temperate and boreal areas. These species, which have been repeatedly part of the dominant vegetation in the Mediterranean basin during the maximum glacial ages of the Pleistocene (e.g. Bennett 1997), have however lower resistance to summer drought (e.g. Pigott and Pigott 1993; Thomas 2000). Thus, such plants are usually restricted to microclimatic islands, often as relict populations. The *Pinus sylvestris* *Pinus sylvestris* L. is a clear example. Its range has undergone continual latitudinal and altitudinal changes during glacial-interglacial cycles (Willis et al. 1998). The ecological requirements of the species are marked by tolerance to low temperatures and high requirements of soil moisture in relation to pine species characteristic of the Mediterranean region (Ceballos and Ruiz de la Torre 1971; Nikolov and Helmisaari 1992). Consequently, its main distribution area today includes the central and northern parts of the European continent, whereas towards the south, it is restricted to the high mountains of the Mediterranean basin, forming isolated, relict populations (Boratynski 1991).

In this study, we analyse the effect of the alleviation of summer drought on *Pinus sylvestris* seedling establishment in a Mediterranean mountain. An irrigation experiment was performed to simulate conditions of a year with a rainy summer. This is the common situation in the mesic central and northern parts of the continent, where the species has its main distribution area, but is a rare event in the Mediterranean, marginal areas of its range. Owing to the characteristic inter-annual variability in precipitation of the Mediterranean climate, the experiment was conducted by applying the water supplementation only in the first year, and monitoring the delayed consequences for establishment during the second year. In addition, we conducted the experiment considering the spatial heterogeneity created by the most common microhabitats where seedlings may be found in these forests, representing a range of abiotic conditions differing in radiation and soil moisture. Our final aim is to determine the consequences of summer drought for forest regeneration, considering the phases of seedling emergence, survival and initial development. Three questions were put: (1) What is the quantitative impact of summer drought upon seedling emergence, survival and growth in the different microhabitats where seedlings are found? (2) What are the delayed consequences of summer drought for the following years of establishment? (3) What are the overall consequences of summer drought for the regeneration of these *Pinus sylvestris* forests under the strong climatic variability of Mediterranean environments?

Methods

Study site and species

The study was carried out during 1997 and 1998 at the locality of Trevenque (37°05′ N, 3°28′ W; National Park of Sierra Nevada, SE Spain, 1700 m a.s.l.), a mountain area where *Pinus sylvestris* reaches its southernmost distribution range (Boratynski 1991). In these forests, canopy cover is around 30% in a typical stand, whereas the understory consists of several shrub species intermingled with areas of bare soil (see Castro et al. 1999, 2005 for details on habitat structure). The bed rock is calcareous, and the predominant soils are regosols and cambisols (Delgado et al. 1989),
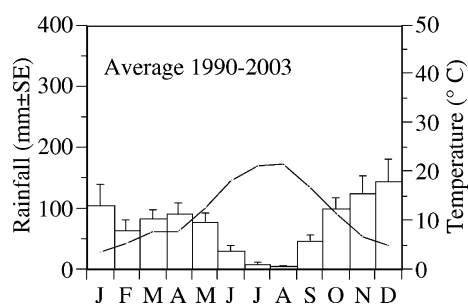
Figure 1. Monthly mean rainfall and temperature in the study area (1990–2000 period). Bars represent rainfall and the line represents the temperature.

with slopes ranging between 10–40°. Seed dispersal occurs between January and March (Castro et al. 1999). Germination spans from April to early May, and emergence from early May to late June, depending on the microhabitat and the year (Castro et al. 2004a). Seedling mortality is concentrated in the first growing season, and the spatial pattern of seedling survival is well established by the second growing season (Castro et al. 2002a, 2004a). The climate is continental Mediterranean, with rainfall (average 830 mm per year; 1990–2003 period) heaviest in autumn and spring, alternating with dry summers (Figure 1). The mean minimum temperature in the coldest month (January) is $-0.9\,°C$, the mean maximum of the hottest month (July) $29.0\,°C$, and the annual average $11.5\,°C$. Year 1997 was rainy (1283 mm) and had a mild summer compared to a normal summer: the mean temperature during July and August 1997 (the driest and hottest months; Figure 1) was $18.6\,°C$ vs. an average of 21.2 for these two months, and the mean maximum temperature during these two months was $24.5\,°C$ vs. an average of 28.5. Precipitation in 1998 was 631 mm.

Experimental design

In an area of ca. 3 ha, we considered the three microhabitats that cover most of the understory in these forests (Castro et al. 1999, 2005): (1) Open, areas of bare ground, separated to the surrounding vegetation a distance large enough to avoid shading. (2) Shrub, under the canopy of deciduous, spiny shrubs (*Berberis hispanica* and *Prunus ramburii*). *B. hispanica* and *P. ramburii* were pooled because they have similar characteristics, being

very abundant deciduous, spiny shrubs of around 1.5 m high and 1 m diameter. Light transmission of these two shrubby species is similar (author's unpublished data). (3) Pine, under the canopy of adult *Pinus sylvestris* trees. For each of the microhabitats, we randomly located 20 sampling stations, in which we established two paired sampling points roughly 75 cm apart (plots, hereafter). At each plot we sowed, on 19 March 1997, 25 seeds in a frame 20×20 cm, the seeds being 4 cm apart and planted 1 cm deep. One of the sown plots of each sampling station was randomly assigned as a control and the other was watered, resulting in a total of 3000 sown seeds (3 microhabitats $\times$ 2 irrigation levels $\times$ 20 sample stations $\times$ 25 seeds). Sown plots were protected with a wire cage (25 cm side, 10 cm height, 1.3 cm mesh) in order to reduce accidental losses as those caused by trampling or hail storms, which are minor causes of mortality in these forests (Castro et al. 2002a, b, 2004a, b).

Plots assigned to irrigation were sprinkler irrigated 12 times at around 10-day intervals during 1997 from the onset of emergence (12 May) to the end of summer drought (15 September, when the first major rainfall was recorded). Water was carefully added to avoid run-off. The surface irrigated (25×25 cm) was greater than the surface of the sown plot in order to prevent border effects. Irrigation consisted of adding 2 l of water at each application time (equivalent to 32 mm at each application). This simulates eventual strong summer storms in the Mediterranean mountain, and fits with the overall summer precipitation in more mesic, northern areas of distribution of the species (Rumney 1968; Catalán 1991; Cañellas et al. 2000). Watered plots of Open microhabitat registered an increase in herb coverage, and were carefully weeded when herbs were still small to levels comparable to the control plots (nearly bare ground).

Seedling monitoring

Seedling emergence (percentage of seedlings emerged from the sown seeds), survival (percentage of seedlings surviving from the emerged seedlings), growth, and cause of mortality were monitored for 2 years. After sowing, plots were visited weekly until first seedlings were detected, and thereafter emergence and survival were

recorded periodically (around 10-day intervals) during the first summer. During the second year, survival was censused at the beginning and at the end of the growing season. At the end of the first growing season (in October 1997), we measured the length of the shoot (± 1 mm) and counted the number of leaves on all the seedlings that survived. Because root collars could not be distinguished, shoot length was considered from cotyledon insertion level to the tip of the shoot. Within 2–3 days, a subsample of 21 randomly selected plots were dug-up and both the root and shoot dry mass of the seedlings in the plot weighed (above-ground mass similarly considered from cotyledon insertion level); we had to sacrifice complete plots because it was not possible to uproot the seedlings without damaging their neighbours in the plot. At the end of the second growing season (October 1998), we harvested all remaining seedlings and measured the dry mass of the shoot; root dry mass was not measured because roots could not be removed intact from the soil.

Dry mass was measured on all occasions after oven drying at 70 °C to constant weight. Causes of mortality were assigned to: (1) 'drought', seedlings turned brown and dried out without any visible damage, mortality occurring during periods of low soil moisture; (2) 'pathogens', damping-off of seedlings, generally with a region of necrosis at root-neck level; (3) 'invertebrate herbivory', seedlings severed by insects; and (4) 'vole tunnels', seedling roots disturbed by voles.

Measurement of abiotic variables

Soil moisture at 1–6 cm depth was measured for 10 randomly selected stations for each microhabitat on 12 June and 5 August 1997, considering only the control level of the irrigation treatment. Moisture content was determined gravimetrically after oven-drying at 110 °C to constant weight. Soil temperature at 3 cm depth was recorded in June for 1 week at 1-h intervals with one or two thermistors per microhabitat. Radiation intensity was measured in the PAR range in June during two clear days at 10-min intervals, using four pyranometers per microhabitat. The magnitude of PAR depression caused by the wire cages was also measured in June by placing, at every microhabitat, two pyranometers under wire cages paired with two located beside the cage, with data being

recorded every 10 min. All loggers and probes were from Onset Computer Corporation (Pocasset, Massachusetts, USA).

Data analysis

The design was a split plot in which microhabitat was considered the *main plot* factor and irrigation the *subplot* factor (Potvin 1993; Von Ende 1993); thus analyses of seedling emergence and survival were performed with univariate repeated-measure ANOVAs in which sampling station was considered the *subject* (whole plot), the microhabitat (with three levels) the *between subject* factor, and the irrigation (with two levels) the *within subject* factor (von Ende 1993). Seedling growth parameters were analysed by two-way ANOVAs considering individual seedlings as replicates; sown plots were not considered as replicates because of the low number of harvested plots, and because of low number of seedlings per plot in some cases. Nevertheless, data of shoot length and leaf number of harvested and non-harvested seedlings were compared, and in no case were there differences (one-way ANOVAs non-significant for every combination of water and microhabitat factors; data not shown), indicating that sacrificed seedlings were representative of the population of seedlings. Causes of mortality were compared with contingency tables. Data were log- or arcsine-transformed to meet assumptions of the analyses. For ANOVAs, we used type III sum of squares, and all factors were considered fixed. The rmA-NOVAs were performed with GLM procedure of SAS 6.12 software (SAS Institute, Cary), and the rest of analysis were performed with JMP 5.0 software (SAS Institute, Cary). Throughout the paper, means are shown ± 1 standard error.

Results

Abiotic variables

Radiation differed among microhabitats, with Open having the highest values and Pine the lowest. Similarly, soil temperature reached the highest and most contrasted values in Open, followed by Shrub and Pine. Soil moisture for control plots also differed among microhabitats, with Open having the

Table 1. Abiotic conditions measured for control plots in the three microhabitats used in the experiment.

Abiotic conditions	Microhabitats			df	*F*	*p*
	Open	Shrub	Pine			
Radiation (μmol m^{-2} s^{-1})	1856 ± 36	336 ± 23	250 ± 18	2, 9	56.98	< 0.0001
Soil moisture (%)						
12/06/1997	11.6 ± 0.3	18.2 ± 0.7	24.1 ± 4.1	2, 27	9.29	0.0009
05/08/1997	1.9 ± 0.1	4.3 ± 0.6	15.8 ± 3.1	2, 27	27.62	< 0.0001
Soil temperature (°C)	20.8 (14.3–29.0)	15.6 (12.7–18.7)	13.0 (11.0–15.1)			

Values are means $\pm$ SE. For solar radiation, the mean values per sampling station (calculations made with records registered during 8 and 16 h, in solar time) were used as a dependent variable. Data of soil temperature could not be analysed due to lack of replication; in brackets, mean minimum and mean maximum values; for microhabitats with two thermistors figures are the mean values.

lowest values and Pine the highest (Table 1). The wire cages caused a 18.2% of reduction of radiation in the Open microhabitat (paired *t*-test, $p < 0.0001$; measurements taken during two consecutive days), but had no effect on the Shrub and Pine microhabitats (paired *t*-test non-significant).

Seedling emergence and survival

A total of 1414 seedlings emerged between the 7th of May and 20th of June 1997 (47.1% of sown seeds). The emergence rate differed among microhabitats (Table 2), being the lowest in Open and the highest in Shrub (Figure 2) and, altogether, was slightly higher in the watered plots than in the control plots (Table 2 and Figure 2).

Irrigation increased seedling survival during the first summer in all the microhabitats, overall surviving 20.3% of non-watered seedlings and 41.9% of watered seedlings (Table 2 and Figure 2). We identified the cause of mortality for 96.4% of dead seedlings. For non-watered seedlings, summer drought was the main mortality factor (72.6%), followed by pathogens (19.4%), vole tunnels (5.2%) and invertebrate herbivory (2.8%), with differences among causes (Goodness of fit test, $\chi^2 = 691.21$, df $= 3$, $p < 0.0001$; all microhabitats pooled). For watered seedlings, summer drought was still the main mortality factor (54.1%), followed by pathogens (38.5%), invertebrate herbivory (5.4%) and vole tunnels (2.0%), with differences among causes ($\chi^2 = 302.57$, df $= 3$, $p < 0.0001$; all microhabitats pooled). Summer drought was the main mortality factor for all the microhabitats and irrigation levels (Figure 3). As a result of the combination of emergence and survival, the recruitment of seedlings by the end of the

Table 2. Summary of the repeated-measurement analysis of variance for seedling emergence, seedling survival and overall recruitment after the first growing season for *Pinus sylvestris* under different microhabitats and irrigation levels.

Source	df	SS	*F*	*p*
Emergence				
Microhabitat	2	4.56	31.29	< 0.0001
Error (between)	56	4.15		
Irrigation	1	0.09	4.22	0.0445
Irrigation × Micro	2	0.06	1.34	0.2693
Error (within)	56	1.24		
Survival				
Microhabitat	2	0.98	3.23	0.0470
Error (between)	56	8.47		
Irrigation	1	2.42	24.59	< 0.0001
Irrigation × Micro	2	0.44	2.24	0.1159
Error (within)	56	5.51		
Recruitment				
Microhabitat	2	0.39	2.99	0.0583
Error (between)	56	3.67		
Irrigation	1	1.45	43.08	< 0.0001
Irrigation × Micro	2	0.38	5.61	0.0060
Error (within)	56	1.88		

Microhabitats are Open, Shrub and Pine. Irrigation levels are control and water supplementation. Recruitment is calculated as the number of surviving seedlings divided by the number of sown seeds (25 per plot).

first growing season (i.e., proportion of live seedlings with respect to the number of seeds planted) differed both among microhabitats and, particularly, between irrigation treatments (Table 2). The highest recruitment occurred for irrigated plots in all the microhabitats, with an overall value of $22.4 \pm 2.4\%$ seedlings vs. $7.9 \pm 1.3\%$ in control plots (the three microhabitats pooled; Figure 2).

Seedling mortality during winter reached 6.8%. During the second summer, mortality rose to 54.1% of available seedlings. Mortality in the second summer did not differ between irrigation
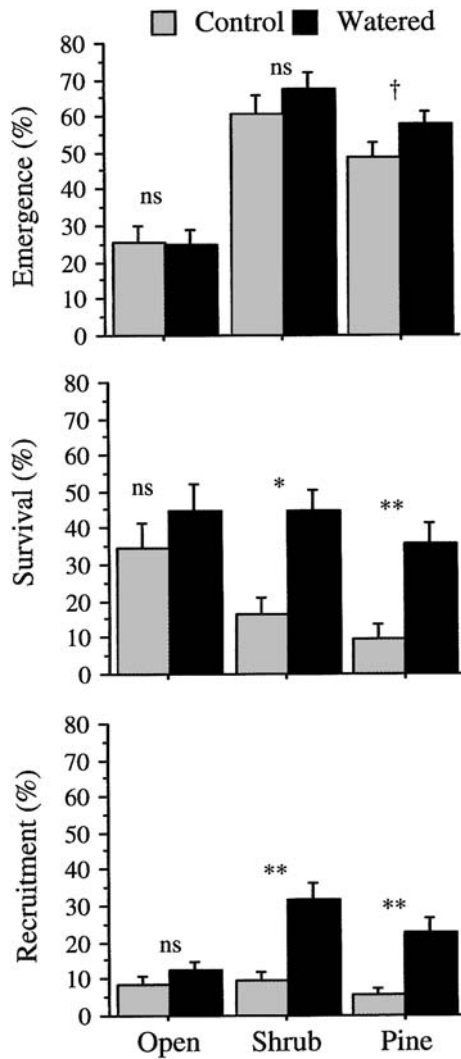
Figure 2. Percentages of seedling emergence, seedling survival, and overall recruitment after the first growing season for *Pinus sylvestris* in different microhabitats and irrigation levels. Microhabitats are Open, Shrub and Pine. Irrigation levels are control and water supplementation. Recruitment is calculated as the number of surviving seedlings divided by the number of sown seeds. Differences between irrigation levels were compared within microhabitat by one-way ANOVAs; df = 1, 36 or 1, 38; *F*-value from 0.03 to 17.26; $^{\dagger}p < 0.1$, $^{**}p < 0.01$, $^{***}p < 0.001$; ns = non-significant).
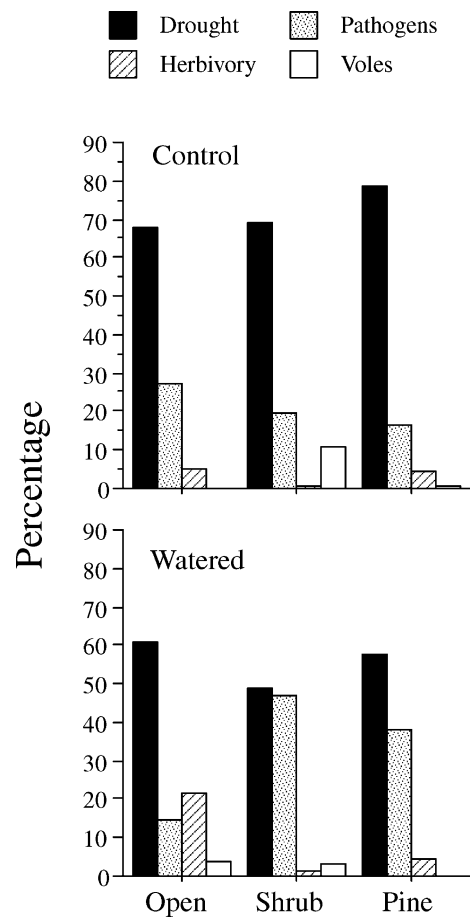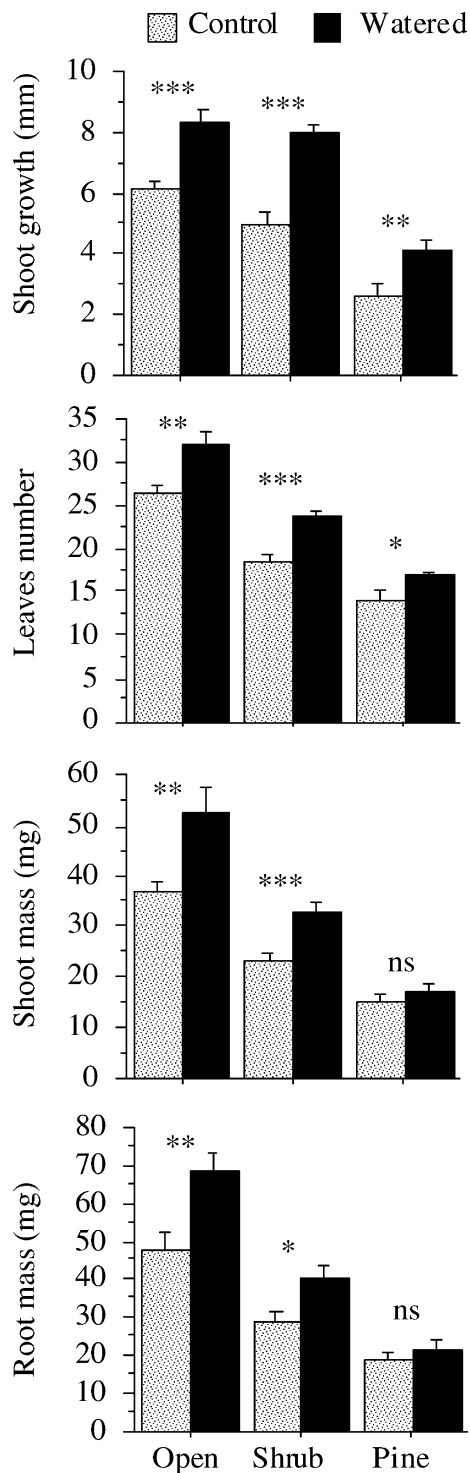

Figure 3. Percentage of causes of mortality registered for *Pinus sylvestris* seedlings during the first growing season in different microhabitats and irrigation levels. Microhabitats are Open, Shrub and Pine. Irrigation levels are control and water supplementation. Herbivory corresponds to invertebrate herbivory (basically insects).

Seedling growth

Irrigation affected all growth parameters measured after the first growing season Table 3), increasing shoot growth, leaf production, shoot dry mass and root dry mass (Figure 4). Microhabitat also affected growth parameters (Table 3), which reached the highest values in Open and the lowest in Pine (Figure 4). The effect exerted by irrigation, however, depended on the microhabitat, rendering the highest increases in Open and Shrub, and the lowest in Pine microhabitat, where irrigation had no effect upon shoot and root mass (Figure 4). Biomass allocation to roots was not affected by water supplementation or by microhabitat (*R/S*

treatments applied during the first growing season (one-way ANOVA, df = 1,60, $F = 0.05$, $p = 0.8185$; all microhabitats pooled). All mortality factors were recognised during the second growing season, summer drought accounting for 99.9% of the cases.

Figure 4. Values for the growth parameters measured after the first growing season for seedlings of *Pinus sylvestris* growing in different microhabitats and irrigation levels. Microhabitats are Open, Shrub and Pine. Irrigation levels are control and water supplementation. Differences between irrigation levels were compared within microhabitat by one-way ANOVAs; df from 1, 22 to 1, 207; *F*-value from 0.07 to 43.30; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; ns = non-significant.

greater for watered seedlings in Open and Shrub microhabitats, but irrigation did not affect the dry mass in microhabitat Pine (Figure 5). Overall, the greatest growth was clearly achieved in microhabitat Open, followed by Shrub, and Pine (Figure 5). Survival and growth did not depend on seedling density per plot in any of the sampling periods or treatments ($p > 0.05$).

Discussion

Seedling emergence and survival

The results show that summer drought was the main mortality factor, being the cause of 73% of seedling deaths in the first growing season for non-watered seedlings, and the cause of almost 100% of deaths during the second growing season (see also Castro et al. 2002a, 2004a for similar results for naturally established seedlings). As a consequence, drought alleviation with water supplementation had a clear-cut effect, increasing emergence and, in particular, boosting survival after the first growing season in all the microhabitats (Figure 2). This effect is further magnified when the cumulative effect of irrigation on emergence and survival is considered, rendering values of seedling recruitment by the end of the first growing season that were 2.9 times higher than for non-watered plots (all microhabitats pooled, Figure 2). The relevance of summer drought is reinforced by the fact that (i) it was the main cause of mortality even in an unusual rainy year with a mild summer (1997), (ii) it also remained the main cause of mortality for irrigated seedlings, and (iii) its detrimental effect was possibly mitigated in open areas given the 18% reduction of radiation caused by the wire cages in this microhabitat. Other factors related to a reduction of summer drought during rainy years, such as a possible increase in herb cover, will not counteract the beneficial effect

value of 1.22 ± 0.03; Table 3). The effect of irrigation persisted during the second growing season, although no water was added in 1998, and showed a similar pattern (Table 3). Thus, shoot mass was

Table 3. Summary of the two-way ANOVAs for growth parameters measured on seedlings of *Pinus sylvestris* after the first and second growing season.

	Source	df	SS	F	p
1st growing season					
Shoot growth ($R^2 = 0.36$)	Microhabitat (*A*)	2	8.79	77.05	< 0.0001
	Irrigation (*B*)	1	2.57	45.01	< 0.0001
	A × *B*	2	0.16	1.38	0.2235
	Error	436	24.88		
Number of leaves ($R^2 = 0.38$)	Microhabitat (*A*)	2	3.39	100.88	< 0.0001
	Irrigation (*B*)	1	0.55	33.07	< 0.0001
	A × *B*	2	0.04	1.31	0.2710
	Error	436	7.34		
Shoot dry mass ($R^2 = 0.57$)	Microhabitat (*A*)	2	3.65	74.40	< 0.0001
	Irrigation (*B*)	1	0.35	14.31	0.0002
	A × *B*	2	0.15	3.08	0.0496
	Error	116	2.86		
Root dry mass ($R^2 = 0.51$)	Microhabitat (*A*)	2	3.53	50.15	< 0.0001
	Irrigation (*B*)	1	0.46	12.96	0.0005
	A × *B*	2	0.07	0.96	0.3871
	Error	100	3.52		
R/S ratio ($R^2 = 0.00$)	Microhabitat (*A*)	2	0.02	1.77	0.1751
	Irrigation (*B*)	1	0.00	0.00	0.9930
	A × *B*	2	0.02	0.13	0.8820
	Error	100		2.67	
2nd growing season					
Shoot mass ($R^2 = 0.53$)	Microhabitat (*A*)	2	5.68	48.12	< 0.0001
	Irrigation (*B*)	1	0.28	4.67	0.0324
	A × *B*	2	0.18	1.54	0.2173
	Error	135	7.97		

Microhabitats are Open, Shrub and Pine. Irrigation levels are control and water supplementation. R^2 is the variance of the response explained by the whole model. Only plants with complete roots were considered for *R/S* ratio analysis.
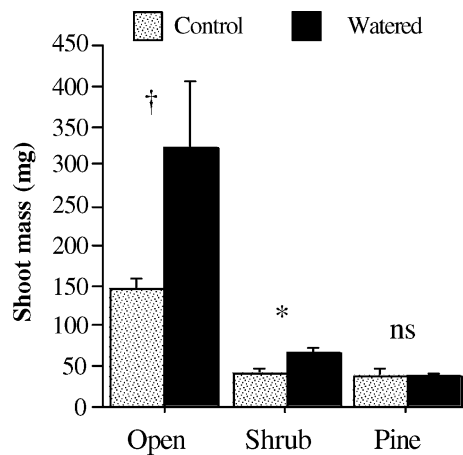

Figure 5. Shoot mass after two growing seasons for seedlings of *Pinus sylvestris* growing in different microhabitats and irrigation levels. Microhabitats are Open, Shrub and Pine. Irrigation levels are control and water supplementation. Differences between irrigation levels were compared within microhabitat by one-way ANOVAs (df from 1, 19 to 1, 66, *F*-value from 0.031 to 3.76; †*p* < 0.1, **p* < 0.05, ns = non-significant).

of higher water availability for seedling establishment, given that (i) herb cover is modest in these ecosystems, and (ii) herb interference in growth of pine seedlings is irrelevant even in grassy meadows (Castro et al. 2002a). On the other hand, seedling recruitment was the highest under the canopy of shrubs, supporting a facilitative effect of shrubs on *Pinus sylvestris* establishment (see also Castro et al. 2002b, 2004a, b).

Seedling growth

The microhabitat had a strong effect on growth. Seedlings had the poorest performance under the pine canopies (the shadiest microhabitat), and the highest under full sun, in accordance with the shade-intolerant nature of the species (Ceballos and Ruiz de la Torre 1971; Nikolov and Helmisaari 1992). In addition, the magnitude of the effect of water addition on seedling growth depended on microhabitat, increasing performance in Open and Shrub but having little effect under pine canopies,

where biomass did not change with irrigation. This suggests that the low amount of radiation blocks the growth under pine trees (e.g., Canham et al. 1996; Lambers et al. 1998; author's unpublished data), and thus seedling establishment is hampered in that microhabitat despite being the site with the highest soil moisture.

Water supplementation also had a strong effect on seedling growth during the first growing season, increasing available photosynthetic tissue (longer shoots and more leaves), shoot mass, and root mass. In addition, growth of watered seedlings was also higher during the second growing season, despite irrigation not being provided in that period. This must be a consequence of the larger shoot size and larger root systems of irrigated plants after the first growing season (Figure 4), allowing seedlings to increase carbon acquisition and to explore a higher volume of soil for water and nutrients in the next growing season (e.g., Canadell and Zedler 1995; Lloret et al. 1999). In any case, summer drought had therefore delayed consequences for recruitment, constraining both current and future seedling performance.

Allocation to roots, however, was affected neither by water supplementation nor by the microhabitat, despite the adaptive advantage of changing biomass allocation in response to drought (Turner and Kramer 1980; Poorter and Nagel 2000). Allocation to roots in *Pinus sylvestris* has already being shown to be scarcely affected by growing conditions, as reported by Schultz and Gatherum (1971) for seedlings growing in contrasting moisture regimes, or Colpaert et al. (1996) for different conditions of nitrogen availability and mycorrhizal colonisation. In addition, root allocation was low (R/S ratio = 1.22; see also Castro (1999) for similar values under nurse conditions), in accordance with previous studies showing even lower values (Schultz and Gatherum 1971; Lippu 1994; Colpaert et al. 1996; Norgren 1996). This contrasts with patterns found for seedlings of Mediterranean woody species of similar age, which (i) usually show sharp changes in R/S ratio (even above two-fold) in response to altered environmental conditions (Gulmon and Chu 1981; Broncano et al. 1998), and (ii) may reach higher R/S ratios, with values of up to 2.5 (Broncano et al. 1998; Lloret et al. 1999). All this suggests that the *Pinus sylvestris* has low plasticity for changing nutrient allocation in response to environmental conditions (see also Karlsson and Nordell 1987; Broadmeadow and Jackson 2000), and that the species is less able to cope with drought than are other Mediterranean species.

In short, the *Pinus sylvestris* seedlings show a combination of traits favourable for survival in northern environments characterised by low moisture stress and high competition for light, such as preferential allocation to shoots and sharp positive response to light (see also de Chantal et al. 2003). These traits, however, are less favourable in a Mediterranean scenario, and seedlings are therefore particularly subjected to the detrimental effect of summer drought.

Consequences for regeneration

The effect of summer drought has severe consequences for regeneration in these Mediterranean *Pinus sylvestris* forests at both spatial and temporal scales. In a spatial context, the combination of summer drought and light intensity creates a gradient from high radiation and low soil moisture in the Open microhabitat to deep shade and higher soil moisture in Pine microhabitat (see Castro et al. 2002b, 2004a; Gómez-Aparicio et al. 2004, for a similar pattern in abiotic conditions) that uncouples the requirements for survival and growth. Recruitment under pine canopies is hampered because of deficient radiation levels despite being the microhabitat with the highest soil moisture (Castro et al. 2004a; this study). Areas of bare soil (Open) are adequate for seedling growth, but needs the mitigation of summer drought to ensure seedling recruitment. However, seedling mortality in open areas during the first growing season is very high, even reaching 100% in years with a typical dry summer (Castro et al. 2004a; see also Castro et al. 2002b, 2004b). The higher seedling establishment in open areas reported in this study compared to a typical year is likely due to the unusual climatic conditions during 1997, with a precipitation above the average and a mild summer that reduced drought stress for seedlings. In fact, soil water content during summer at 15 cm depth during 1997 in the study area was ca. 4 times higher than during typical dryer summers (Gómez-Aparicio et al. 2004). Open areas are therefore suitable for establishment only during occasional rainy years with a mild summer, as was the case in

1997, and even then, irrigation benefited establishment in this microhabitat by increasing seedling growth. Finally, recruitment under shrub canopies is possible both during a typical year (Castro et al. 2002b, 2004a) and during a year with a mild summer (this study), and in any case mitigation of summer drought boosts establishment, as demonstrated by irrigation. The notable effect of irrigation on establishment in this microhabitat as compared to open areas (Figure 2) was presumably due to the effect of other abiotic variables that are ameliorated under the canopy of shrubs but not in open areas, such as a higher air relative humidity or lower soil temperature (Castro et al. 2002b, 2004a; Gómez-Aparicio et al. 2004). This adds to the beneficial effect of shrubs upon recruitment, reinforcing their facilitative effect. In short, during a typical year recruitment will be restricted to areas underneath shrub canopies, whereas in unusual years with a rainy summer recruitment is possible in both, under shrub canopies and in open areas.

In a temporal context, summer drought restricts recruitment to scattered years with an extended period of rainfall at the end of the spring, making *Pinus sylvestris* seedling establishment episodic in the Mediterranean mountain (see also Rojo et al. 1994). In fact, seedling survival is nil in these forests in years with below-average precipitation (Castro 2000). Given the current and future scenario of climatic change, with an increase in both dryness and variability of rainfall regime for the Mediterranean region (IPCC 2001; Rodó and Comín 2001), the already rare event that barely allows recruitment in unusual rainy years may become even more sporadic, a situation which may reduce the ability of these forests to regenerate naturally. These processes operating at the seedling level may be a major factor limiting this species to microclimatic islands in the Mediterranean mountain during the interglacial periods, exacerbated by the present increase of aridity in the Mediterranean basin. This might eventually lead to the development of a remnant dynamic (*sensu* Eriksson 1996) in these relict, southernmost *Pinus sylvestris* populations.

Acknowledgements

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