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Ecology of seed germination of *Pinus sylvestris* L. at its southern, Mediterranean distribution range

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Abstract

Seed germination of *Pinus sylvestris* L. in south eastern Spain was studied under field and growth chamber conditions to assess the effect of the most representative microhabitats of these forests. Under growth chamber conditions, germination was high (almost 95%) in the litter from all microhabitats. Germination under field conditions was highest (up to 95%) in shade-free microhabitats and therefore higher soil temperature (e.g. areas of bare soil and meadows), and lowest (down to 62%) in microhabitats with dense canopy shade and thus lower soil temperature (e.g. under the canopy of pines and under the canopy of juniper). Nevertheless, germination was high also in shaded microhabitats in a year with high rainfall during the germination period, supporting the hypothesis that germination was determined by a combination of appropriate levels of soil temperature, moisture, and light intensity. In contrast, biotic species-specific characteristics of the microhabitat were not relevant. Germination may thus reach high percentages in all the microhabitats of the understory during rainy years that ensure appropriate soil moisture during the germination period. However, during dry years germination will concentrate in sunny microhabitats because they reach higher soil temperature early in the season before soil desiccation. These patterns have implications for forest management and stand regeneration via direct seeding.

Key words: abiotic *versus* biotic factors, Mediterranean mountains, microsite, Scots pine, seed ecology.

Resumen

Ecología de la germinación de la semillas de *Pinus sylvestris* L. en el límite sur de su distribución

Se analiza la germinación de las semillas de pino silvestre en el límite sur de su distribución (Sierra Nevada) mediante experimentos de campo y de laboratorio en una muestra de diez microhábitats que representan la mayoría de los lugares en los que se encuentran las semillas tras la dispersión en estos bosques. En condiciones de laboratorio, las semillas mostraron una alta tasa de germinación (en torno al 95%) en la hojarasca de todos los microhábitats. En el campo las semillas mostraron una tasa de germinación mayor (en torno al 95%) en microhábitats directamente expuestos al sol y por tanto con mayor temperatura (e.g. suelo sin vegetación y prados de herbáceas) y una menor tasa bajo plantas con copa espesa que generan un microhábitat sombreado y con menor temperatura (bajo copa de pinos adultos y bajo copa de enebros). No obstante, durante un año lluvioso la tasa de germinación en microhábitats sombreados se incrementó considerablemente, sugiriendo que la germinación está controlada por una combinación adecuada de radiación, humedad y temperatura. Por el contrario, no se detectó ningún efecto biótico relacionado con el microhábitat, como alelopatía. La germinación de las semillas de pino silvestre en estos bosques puede por tanto alcanzar valores altos en todos los microhábitats durante años con primavera lluviosa que asegure la confluencia de humedad y temperatura apropiada en el suelo. Sin embargo, en años con primaveras más secas la germinación se concentrará en microhábitats con alta radiación, ya que la mayor temperatura del suelo permitirá una rápida germinación antes de que se produzca la desecación del sustrato. Estos patrones deben tenerse en cuenta a la hora de planificar la regeneración del bosque mediante la siembra de semillas.

Palabras clave: ecología de semillas, factores bióticos y abióticos, microhábitat, montaña mediterránea, pino silvestre.

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Introduction

Seed germination represents a risky transition from the stage most tolerant to environmental conditions (i.e., resting seed) to the weakest and most vulnerable stage in plant development, the seedling (Harper, 1977). In addition, germination is an irreversible process (Bewley and Black, 1994), and thus wrong timing or location of germination may cause the death of the individual, impacting population recruitment (Harper, 1977; Silvertown and Charlesworth, 2001). Different environmental factors may determine seed germination, although the essentials are an appropriate combination of temperature, moisture and light (Mayer and Poljakoff-Mayber, 1989; Bewley and Black, 1994; Baskin and Baskin, 1998). In addition, the chemical environment surrounding the seed must be suitable (Karssen and Hilhorst, 1992), and the presence of allelochemicals inhibitors released by the surrounding vegetation may also determine germination success (Rice, 1984; Friedman, 1995).

Seeds are dispersed across a wide variety of microhabitats covering a range of abiotic and biotic conditions affecting germination. Consequently, germination under field conditions can be spatially and temporally variable, as some microhabitats may provide better conditions than others, while different microhabitats may provide appropriate conditions for germination at different times (Bisigato and Bertiller, 1999; Guariguata, 2000; Nilsson *et al.,* 2000; Oleskog and Sahlén, 2000; Isselstein *et al.,* 2002). In addition, the temporal pattern of rainfall also affects the germination pattern. For example, in Mediterranean environments germination is often restricted to short periods in the wetter spring or autumn, but is unlikely to occur during the dry summer (García-Fayos *et al.,* 2000; Quilichini and Debussche, 2000). This may be especially critical for species whose seeds do not undergo dormancy and thus do not form a persistent seed bank. Regeneration in such cases is particularly limited to appropriate timed pulses of moisture to germination, and thus is particularly vulnerable to climatic hazards.

Scots pine (*Pinus sylvestris* L.) is a wind-dispersed species with little to no seed dormancy (Nyman, 1963; Castro, 1999). It is widely distributed in central and northern Eurasia, while the Mediterranean region represents the southernmost limit of its natural distribution (Boratynski, 1991). In southern Spain, in an area well below the end of Euro-Siberian domain, two relictic Scots pine populations grow in the Sierra Nevada and Sierra de Baza mountains (ca 80 km apart), under extreme abiotic conditions for this boreal species (Catalán, 1991). Factors controlling seed germination of Scots pine in field conditions have been well studied in boreal populations. In such sites, microhabitat greatly influences seed germination through biotic interactions such as allelopathy (Hytönen, 1992; Steijlen *et al.,* 1995; Zackrisson *et al.,* 1997; Nilsson *et al.,* 2000), while soil moisture in these wetter regions is not a main factor blocking germination [Zasada *et al.,* 1992; Zackrisson *et al.,* 1998; and see Douglas (1995) for other boreal tree species]. In contrast, factors affecting Scots pine seed germination at its southern limit, under Mediterranean climatic conditions, are largely unknown (Rojo and Montero, 1996), despite the species' significance to the Mediterranean silviculture and despite that germination is a critical stage for forest regeneration (Cañellas *et al.,* 2000). In the present study, the ecology of Scots pine seed germination at its southernmost Mediterranean boundary was investigated, aiming to determine the role of germination in the regeneration ability of these forests. We used a combination of field and growth chamber experiments focusing on the environmental variability provided by the most common microhabitats of the understory. Three questions were posed: 1) What is the magnitude of seed germination of Scots pine in different microhabitats? 2) What are the factors determining germination in the different microhabitats? and 3) What are the consequences for regeneration?

Materials and Methods

Study area

Scots pine forests of Sierra Nevada (37°05'N, 3°28'W) and Sierra de Baza (37°23'N, 2°51'W) mountains form the treeline at an altitude of 1,600-2,200 m a.s.l., and have similar environmental conditions and species composition. The forest has ca 25% tree cover in a typical stand, whereas the understory is rich in several shrub species that are mixed with areas of bare soil and scattered grassy meadows (Table 1: see Castro *et al.,* 2002a for species composition of meadows). A typical forest stand has ≈ 100 reproductive individuals per hectare, predominating trees with 30-60 cm trunk diameter at breast height (see Castro *et al.,* 1999 and Zamora *et al.,* 2001 for details). Seed dispersal spans from January to March (Castro *et al.,* 1999). The climate

Table 1. Habitat structure at the most representative Scots pine populations at its southern European limit, *viz.,* Sierra Nevada mountain (Barranco del Espinar site) and Sierra de Baza mountain (Boleta site) (SE Spain)

		Sierra Nevada		Sierra de Baza	
		Woodland	**Treeline**	**Woodland**	**Treeline**
A	**Canopy cover**				
	Pinus sylvestris	21.3	2.5	16.0	4.0
	Understory cover				
	Juniperus communis	16.9	4.8	13.8	7.9
	Juniperus sabina	11.8	1.0	19.6	28.3
	Genista versicolor	6.4	3.4		
	Salvia lavandulifolia	0.2	9.5		
	Prunus ramburii	3.5	6.3	1.8	4.8
	Berberis hispanica	11.6	9.3	4.8	6.1
	Herbaceous	11.7	5.5	3.6	3.0
	Soil	11.6	22.4	38.2	28.4
B	**Rocks**	2.7	6.5	5.1	17.9
	Other tree species(1)	4.7	0.0	4.9	0.4
	Other shrubs > 1 m(2)	6.6	1.7	0.2	0
	Other shrubs < 1 m(3)	15.8	28.7	9.4	8

«Woodland» represent the interior of the forest, and «Treeline» represent timberline areas above forest stands. A: microhabitats used in the stuy of seed germination. B: remaining microhabitats. Habitat structure was determined by sampling 1,000 points per habitat (woodland and treeline) and population (10 randomly established transects of 100 points). Numbers are percentage of cover. (1) *Pinus nigra, Acer granatense,* and *Taxus baccata.* (2) Mostly *Amelanchier ovalis, Cotoneaster granatense, Crataegus granatensis, Lonicera arborea* and *Rosa* spp. (3) Mostly *Ononis aragonensis, Astragalus granatense, Erinacea anthyllis, Vella spinosa, Hormathophylla spinosa* and *Prunus prostrata.* «Herbaceous» corresponds to scattered herbaceous species plus grassy meadows.

is continental Mediterranean, with hot, dry summers and cold winters. The mean minimum temperature of the coldest month (Januray) is –1.2° C, and the mean maximum of the hottest month (July) is 28.5° C. Annual rainfall, concentrated mainly in autumn and spring, is 860 mm (average 1990-2002; Figure 1. Data for Sierra Nevada population). Field experiments were conducted in the Sierra Nevada National Park (Barranco del Espinar site, ~ 1,700 m a.s.l.) due to logistic support available in that area. The selection of microhabitats, however, was done considering microhabitat availability in both pine forests at Sierra Nevada and Sierra de Baza (Table 1).

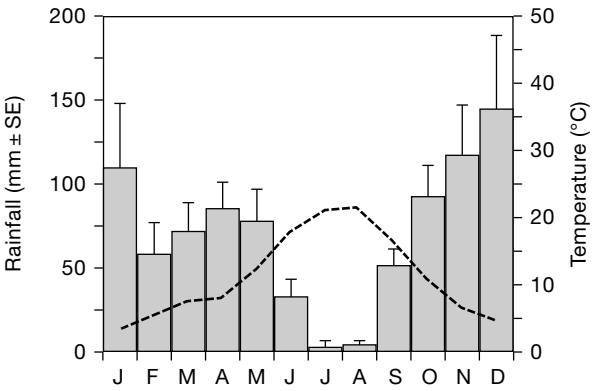

Figure 1. Monthly mean rainfall and temperature in the study area (1990-2000 period; data from a meteorological station placed at 1650 m a.s.l.).

Experimental design

Two field experiments and one growth chamber experiment were performed to study the impact of the microhabitat on seed germination and the possible factors determining patterns of germination. Seed viability was around 95% in all cases according to a prior germination test in a growth chamber (unsounds seeds previously discarded manually). Seeds used in the experiments were from the current year, collected from a large number of trees (> 30) just before cone opening (early January), and were stored under room conditions until the start of the experiments. Seeds were considered germinated when the radicle protruded at least 2 mm. Experiments started at the end of the period of natural seed dispersal.

Field experiments

In 1996, we performed an experiment designed to test the effect of microhabitat on Scots pine seed germination under field conditions. Nine of the most common microhabitats, representing most of the canopy and understory composition in these forests (Table 1), were selected: 1) Open, areas of bare soil; 2) Salvia, under the canopy of *Salvia lavandulifolia*; 3) Sloe, under the canopy of *Prunus ramburii*; 4) Barberry, under the canopy of *Berberis hispanica*; 5) Juniper, under the canopy of *Juniperus communis*; 6) Sabina, under the canopy of *Juniperus sabina*; 7) Genista, under the canopy of *Genista versicolor*; 8) Grass, grassy meadows; and 9) Pine, under the canopy of Scots pine. In addition, we selected a tenth microhabitat, Moss (spots of the moss *Cratoneuron commutatum*), that is restricted to streams and, although not abundant in the study site, it was included in the design as it represents a microhabitat of high moisture at all times. In each microhabitat, we randomly selected 10 sample stations. At each station, on April 4 we buried at 2 cm depth and 25 cm apart two 1.5 mm mesh nylon bags, each bag (10 × 6 cm) containing 35 seeds. Bags rested flat, so that all seeds were in direct contact with the substrate of the microhabitat. Mesh size allowed the radicle to grow through the bag. Germination was monitored *in situ* 45 days after sowing (20 May) by digging up the bags, removing the germinated seeds and immediately burying the bags again with the remaining seeds. The length of the radicle of germinated seeds was measured in the laboratory in 65 seedlings per microhabitat to check whether patterns of germination were coupled with patterns of radicle growth. The experiment ended 70 days after sowing (15 June), when the bags were again exhumed, the germinated seeds counted, and radicle length was measured in 40 seedlings per microhabitat.

In 1997, we repeated the experiment with a subset of the microhabitats used in 1996 representing the range of germination variability found the previous year: 1) Open, areas of bare ground (which reached the highest percentage in 1996); 2) Shrub, under the canopy of barberry or sloe (which reached intermediate values); and 3) Pine, under the canopy of adult Scots pines (which reached low values in 1996). Barberry and sloe were pooled because they have similar characteristics, being deciduous, spiny shrubs of around 1.5 m high very abundant in these forests. At each of the microhabitats, we randomly assigned 20 sampling plots for burial of two seed bags at 2 cm depth, separated by about 75 cm each other. Bags contained 25 seeds each and were sown on 19 March. Bags were exhumed on May 10 and brought to the laboratory to count the number of germinated seeds. Radicle length was also measured in the laboratory in a sub-sample of germinated seeds (n = 50 per microhabitat).

Several abiotic variables potentially influencing seed germination were recorded under field conditions. In the 1996 field experiment, soil moisture at 1-6 cm depth was determined for each microhabitat at the beginning of the experiment (4 April), at the first sampling (20 May) and 15 days after the end of the experiment (29 June), with sampling stations (10 per microhabitat) randomly chosen at each sampling period. Samples were collected with a 4 cm diamater × 5 cm deep augur, and the percentage of moisture was determined after oven drying at 110°C to constant weight. In the 1997 field experiment, soil temperature was recorded at 3 cm depth from 17 April to 4 May 1997 at 1 h intervals in all the microhabitats, using one or two thermistors per microhabitat. In addition, light conditions were estimated 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. All loggers and temperature and radiation probes were from Onset Computer Corporation (Pocasset, Massachusetts, USA).

Growth chamber experiment

In 1996 an experiment was designed to test the effect that litter of different microhabitats exerted upon seed germination. Seeds were placed in 12-cm diameter glass Petri dishes containing litter of the microhabitats in which germination was checked under field conditions, thus: 1) Salvia, litter of *Salvia lavandulifolia*; 2) Sloe, litter of *Prunus ramburii*; 3) Barberry, litter of *Berberis hispanica*; 4) Juniper, litter of *Juniperus communis*; 5) Sabina, litter of *Juniperus sabina*; 6) Genista, litter of *Genista versicolor*; 7) Grass, litter and green leaves collected in grassy meadows; 8) Pine, litter of Scots pine and; 9) Moss, live moss *Cratoneuron commutatum*. As a control (tenth treatment) we used seeds spread over paper-disk filters resting on a single layer of 5 mm glass beads, adding 15 ml of water to the dishes. The microhabitat Open (areas of bare ground) was not included in this experiment as it does not contain litter. Litter, collected from the different microhabitats on April 4 in the upper 4 cm of the litter layer, was spread

on trays in the laboratory, left to dry at room temperature protected from direct solar radiation in order to minimize chemical alteration (Inderjit *et al.,* 1999), and mixed to have an homogeneous sample of the 4 cm depth. The germination experiment started two weeks later, coinciding with the period of germination under field conditions. We used 5 g of dry litter per Petri dish, which filled the dishes. After that, dishes were watered with 20 ml of sterilized, distilled water, and left to full imbibition for 24 h at 20 °C prior to seed addition. We used 7 replicates per treatment containing 25 seeds each, that were immersed in the litter content of the dishes. The photoperiod within the growth chamber was set at 16 h light and 8 h darkness. Water was replenished when needed by adding 5 ml, simultaneously to all the treatments. Germination was recorded at days 6 and 12. Seeds were therefore germinated in contact with litter from the upper 4 cm of soil depth, where most seeds are found after dispersal, according to previous inspection. The temperature of the growth chamber was kept at 20 °C, and light was provided by fluorescent tubes emitting a photon flux density of 135 μmol m^{-2} s^{-1} in the photosynthetically active radiation (PAR) range (measured using a Li-Cor LI-200 sz pyranometer sensor; Li-Cor Inc., Lincoln, Nebraska, USA).

Data analysis

Analyses were performed with one-way ANOVAs, with data being log or, for ratio values, arc-sin transformed (Zar, 1996). For analyses of germination under field conditions, the two seed bags of each sampling station were pooled. Analyses were performed with JMP 5.0 software (SAS Institute). Throughout the paper, values are mean ± 1SE.

Results

Field experiments

Field germination in 1996 differed among microhabitats at 45 days after sowing ($F = 56.61$, df = 9, 89, $P < 0.0001$), with Open, Grass and Moss microhabitats having greater germination than all the other microhabitats (Table 2). After 70 days, the cumulative

Table 2. Seed-germination percentages and radicle length at 45 and 70 days for seeds buried in different microhabitats in 1996, and for seeds buried in 1997 at 50 days after sowing

	Germination (%)		Radicle length (mm)	
Microhabitat	**45 days**	**70 days**	**45 days**	**70 days**
Year 1996				
Open	94.0 ± 0.8^{a}	94.2 ± 0.8^{ag}	29.2 ± 1.2^{a}	—
Salvia	54.4 ± 4.7^{b}	86.2 ± 1.3^{abg}	10.3 ± 0.8^{b}	30.6 ± 2.0^{ab}
Sloe	59.4 ± 5.6^{b}	84.7 ± 3.4^{acg}	12.5 ± 1.2^{b}	32.6 ± 1.7^{ab}
Barberry	42.4 ± 6.7^{bc}	75.7 ± 5.2^{abd}	7.2 ± 0.7^{c}	34.6 ± 2.2^{ab}
Juniper	12.1 ± 2.4^{d}	61.7 ± 6.2^{bdh}	4.2 ± 0.3^{d}	35.3 ± 1.5^{a}
Sabina	27.7 ± 5.5^{cd}	83.9 ± 7.0^{agi}	5.6 ± 0.5^{cd}	32.8 ± 1.6^{ab}
Genista	19.1 ± 3.5^{d}	85.3 ± 2.4^{aegh}	4.3 ± 0.3^{d}	34.3 ± 1.0^{a}
Grass	85.4 ± 1.7^{a}	91.6 ± 0.9^{afg}	16.9 ± 1.1^{e}	—
Pine	23.1 ± 2.8^{cd}	69.6 ± 5.7^{bcefi}	5.6 ± 0.3^{cd}	26.8 ± 1.8^{b}
Moss	84.3 ± 2.0^{a}	94.9 ± 1.0^{g}	21.8 ± 1.4^{e}	—
	50 days		**50 days**	
Year 1997				
Open	94.6 ± 0.7^{a}		39.6 ± 0.8^{a}	
Shrub	94.7 ± 0.9^{a}		35.0 ± 1.1^{a}	
Pine	85.6 ± 1.9^{b}		17.3 ± 1.4^{b}	

For microhabitats Open, Grass and Moss, radicle length in 1996 was not measured after 70 days because most of the seedlings had germinated by day 45. For each year and day of sampling, different letters denote differences among treatments according to Bonferroni-Dunn test at α-level of 0.05 after one-way ANOVA. Germination percentages at 70 days in 1996 are cumulative values.

germination also differed among microhabitats ($F = 5.95$, df = 9, 89, $P < 0.0001$), with Juniper and Pine having the lower germination compared to the other microhabitats. Nevertheless, cumulative germination percentages increased considerably in all microhabitats, reaching values above 80% in most of the cases (Table 2). Radicle length followed a similar trend to that observed for seed germination. There were differences among microhabitats after 45 days ($F = 79.67$, df = 9, 640, $P < 0.0001$), with longest radicles recorded in Open, Grass and Moss microhabitats (Table 2). After 70 days, there were also differences among microhabitats ($F = 2.93$, df = 6, 273, $P = 0.0087$; microhabitats Open, Grass and Moss excluded from the analysis due to lack of ungerminated seeds by that date), although these differences were much smaller, with all radicle lengths ranging from 25 to 35 mm (Table 2). Seed germination in 1997 also differed among microhabitats ($F = 15.17$, df = 2, 56, $P < 0.0001$; one sampling station of microhabitat Pine was lost), with greater germination in Open and Shrub than in Pine (Table 2). Root length also differed among microhabitats ($F = 66.74$, df = 2, 147, $P < 0.0001$), seeds sowed in the Open and Shrub microhabitats having longer roots than in Pine (Table 2).

Soil moisture measured in the 1996 field experiment differed among microhabitats in all three sampling periods (one-way ANOVAs, F from 59.05 to 163.13, df = 9, 89, $P < 0.0001$). The highest moisture was found in Moss and the lowest in the Open microhabitat in all cases (Fig. 2). As expected, soil moisture decreased with time in all the microhabitats except for Moss, where moisture remained constant throughout the experiment (Fig. 2). Soil temperature sampled in 1997 field experiment had the highest and most contrasting values in the Open (mean = 13.4; mean minimum = 7.6; mean maximum = 21.8), followed by Shrub (mean = 10.6; mean minimum = 8.0; mean maximum = 13.8) and Pine (mean = 8.9; mean minimum = 7.5; mean maximum = 10.6; data not analysed due to lack of replicates). Global site factor followed a similar trend, being highest in the Open (0.79 ± 0.08) followed by Shrub (0.58 ± 0.14) and Pine (0.28 ± 0.01; df = 2, 57, $F = 126.34$, $P < 0.0001$).

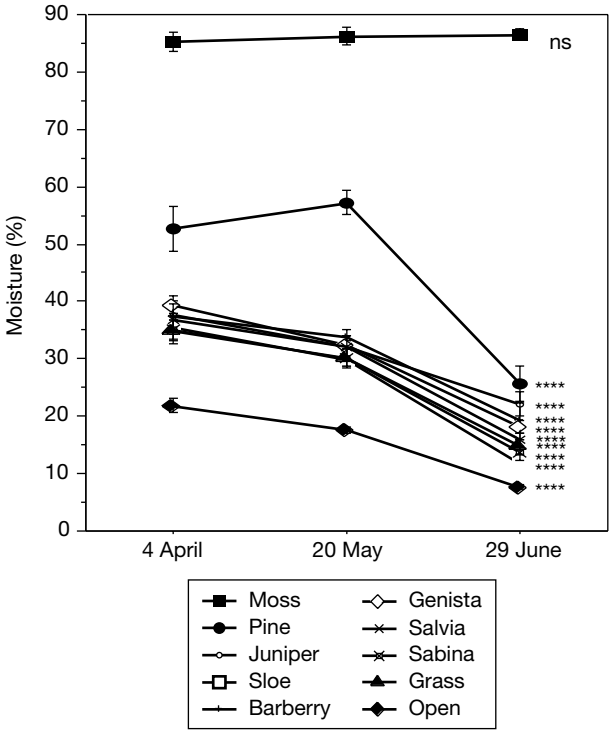

Figure 2. Moisture at different dates (measured at 1-6 cm depth) in the microhabitats where seed bags were buried in 1996. Differences among sampling dates were compared for each microhabitat with one-way ANOVAs. df from 2, 24 to 2, 27. ns: non-significant. **** $P < 0.0001$.

Growth chamber experiment

Seed germination was rapid in all the litter types tested. After 6 days, the germination percent ranged between 66.9% in the Moss treatment and 89.0% in the Control, with significant differences among treatments ($F = 4.24$, df = 9, 60, $P = 0.0003$). Differences among treatments persisted after 12 days ($F = 3.53$, df = 9, 60, $P = 0.0014$), although germination percentage was very high in all litter types (Fig. 3).

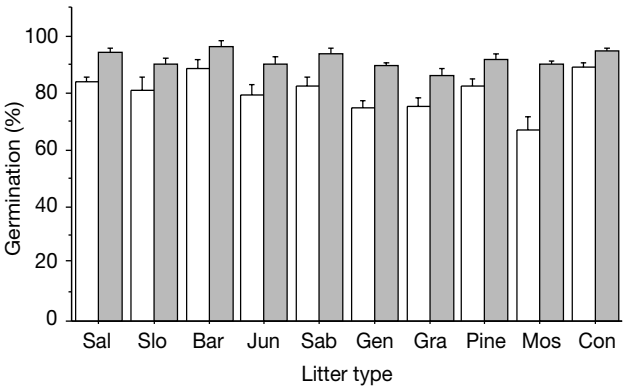

Figure 3. Percentage of germination of Scots pine seeds (in a growth chamber) placed on litter from different microhabitats at 6 (empty bars) and 12 (full bars) days after sowing. X-axis: Sal = salvia, Slo = sloe, Bar = barberry, Jun = juniper, Sab = sabina, Gen = genista, Gra = grass, Pinne = pine, Mos = moss, Con = control.

Discussion

The results show that seed germination of *Pinus sylvestris* in the Sierra Nevada mountain occurred within a short time interval and reached high percentages at all microhabitats, while under growth chamber conditions was similarly high. This indicates lack of seed dormancy (see also Nyman, 1963; Castro, 1999), meaning firstly that, in the absence of inhibitory factors, germination under field conditions will proceed as soon as appropriate environmental conditions are available (Bewley and Black, 1994; Vleeshouwers *et al.,* 1995), and secondly that the species will not form a persistent seed bank (Baker, 1989; Bewley and Black, 1994). Nevertheless, germination differed among microhabitats, being lower under the canopy of adult pines, higher in areas of bare soil, and intermediate under the canopy of shrubby species.

Factors determining among-microhabitat differences in germination

Soil temperature was likely a main factor determining germination, as suggested by several findings. 1) Germination was fastest in full-sun microhabitats (Open, Grass, and Moss, where soil temperature may reach maximum values), intermediate in moderately shaded microhabitats (deciduous spiny shrubs and *Salvia lavandulifolia*, with low leaf density), and slowest in microhabitats with a thick canopy (Juniper, Sabina, Genista and Pine, where temperature reach the lowest values; see also Gómez-Aparico *et al.,* 2004; Castro et al., 2002b, 2004 for a similar gradient of temperature variation among microhabitats). 2) Germination after 70 days in 1996 reached roughly 85% in most microhabitats with respect to lower values at 45 days, indicating that the rise of ground temperatures through time allows germination in the cooler microhabitats. And 3) the rate of root elongation followed the same pattern as germination did, supporting an effect of abiotic conditions such as temperature and not of allelopathy in the microhabitats with lower germination percentages [Rice, 1984; see also Chambers *et al.* (2001) for a correlation between microhabitat temperature and seed germination].

Moisture was also likely a main factor controlling germination under field conditions. The beneficial effect of temperature can be negated by desiccation with heating (Probert, 1992; Bewley and Black, 1994). Thus, higher temperatures found in shaded microhabitats later in spring would not promote germination if the soil has dried at that point. This can explain the higher germination percentage found beneath Shrub and Pine microhabitats in 1997 relative to 1996: rainfall during the period of germination (April) in 1997 was unusually high (130 mm *versus* a mean of 84 mm; see Fig. 1), and in addition was distributed homogeneously through the month. This would result in an overlap of high moisture availability and suitable warm temperatures in 1997 germination period, allowing higher germination percentages in shaded microhabitats. Similarly, where soil moisture was always at saturation, such as in Moss microhabitat in 1996 field experiment, germination was maximal (Table 2). Furthermore, germination percentage in the laboratory with moisture at saturation was similarly high in all litter types tested.

The effect of soil temperature and moisture is in addition affected by light intensity. Germination of Scots pine seeds is delayed under low light intensity (Nyman, 1963; Tillberg, 1992). Thus, in shaded microhabitats, where low light intensity is coupled with lower soil temperature, delayed germination is expected. The effect of light intensity upon germination contributes therefore to differences between shaded, cooler microhabitats and sunny, warmer microhabitats. In summary, the timing and rate of germination of Scots pine under field conditions in these forests appear to be largely determined by the interaction of soil temperature and soil moisture, which in turn are related to the radiation received in each microhabitat.

Lack of biotic effects

Extensive seed germination in the growth chamber as well as under field conditions in all microhabitats suggests that allelopathy was not involved, or had a weak effect, in Scots pine germination at the area of study. This contrasts with results from northern areas, where several plant species, particularly in the Ericaceae, inhibit germination of Scots pine (e.g. Hytönen, 1992; Zackrisson and Nilsson, 1992; Jäderlund *et al.,* 1996; Nilsson *et al.,* 2000; and references therein). One explanation could be a lack of phytotoxicity of plants in our forests, although this seems unlikely for all cases, since some species belong to genera with known allelopathic effects (Rice, 1984). Moreover, in some cases phytotoxicity has been demonstrated (e.g. extracts of fresh Scots pine needles have autotoxic

effects; Hytönen, 1992). An alternative explanation could be the temporal separation between leaf fall in the Mediterranean high mountains (autumn for deciduous species, and mainly late summer and autumn for evergreen species; e.g. Martín *et al.,* 1996; Moro *et al.,* 1996), and the timing of germination for the Scots pine (April). Allelopathic compounds may be released in a period of weeks at the early stages of decomposition, and then be rapidly broken down by microorganisms or leached and lost from the litter layer (Rice, 1984; Jadërlund *et al.,* 1996; Dalton, 1999). Thus, allelopathy would be critical only if germination overlaps with the time of allelochemical compound release (Rice, 1984). In the Mediterranean ecosystems, litter decomposition occurs mainly during the autumn and spring, and in relatively mild periods during winter (Gallardo, 2001; Fioretto *et al.,* 2001; and references therein). Decomposition occurring from leaf fall to the onset of the next year's seed germination on next spring could be enough for the leaching or inactivation of potential allelochemicals in the litter. This situation would contrast to that found in northern areas, where the low winter temperatures limits litter decomposition mostly to the wet and mild summer, coinciding with the period of seed germination.

Consequences for regeneration

The margin for germination in these southernmost populations is extremely restricted to a short period in spring. Germination before spring is blocked by low winter temperatures. Germination in autumn, the next period with appropriate moisture and temperature once finished the summer drought, is also limited given the high rates of post-dispersal seed predation in these forests (Castro *et al.,* 1999; 2002a) coupled with the lack of seed dormancy, which prevents the formation of a transient seed bank (see also Archibold, 1989; Johnson and Fryer, 1996). Germination during spring could potentially reach maximal values in all microhabitats of the understory under field conditions, but only if there is sufficient soil moisture still available when the cooler microhabitats have warmed sufficiently. Thus, if moisture remains high in the cooler, shaded microhabitats, extensive germination will be achieved, but much of it will be late germination. If, however, soil water is rapidly depleted, germination in cooler microhabitats will be limited. Programmes of management and stand regeneration should consider this among-microhabitat variability in germination ability as well as among-year variability in precipitation to ensure natural regeneration success of Mediterranean Scots pine forests. During an unusual year with a wet summer, regeneration via seeding may occur in both open areas and under shrub canopies. In a typical year with a dry summer, regeneration via seeding should be restricted to areas beneath shrub canopies, because although high germination percentages in open areas occur, seedling survival is almost restricted to sites under the canopy of shrubs (Castro *et al.,* 2004). Either in wet or dry years, regeneration via seedings should avoid areas beneath pine cover, as both germination and survival reach low values (Castro *et al.,* 2004; this study).

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