Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Disponible on line en www.inia.es/srf

Investigación Agraria: Sistemas y Recursos Forestales 2009 18(2), 167-180
ISSN: 1131-7965

Influence of stand density and soil treatment on the Spanish Black Pine (*Pinus nigra* Arn. ssp. *Salzmannii*) regeneration in Spain

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Abstract

Satisfactory results relating to the natural regeneration of the Spanish black pine (*Pinus nigra* Arn ssp. s*alzmannii*) is generally difficult to achieve. The natural regeneration of this pine was studied comparing two types of soil treatment and various overstory densities in six experimental forests. These studies were conducted from 1999 to 2002 and seed rain and germination, as well as seedling survival were observed in a number of specific plots: Brushing, scalping and control plots. In addition various overstory densities were used (measured as base area (m^2/ha). Soil and air temperature together with soil moisture were continuously recorded throughout this summer period.

The results showed that seed germination was higher in plots using the scalping technique, as opposed to the brushed or controlled plots. The best seedling survival percentage was found in scalped plots together with a larger basal area. It was also found that seedling survival was lower during the first year than during the second one. The results have practical implications for management of Spanish black pine forests as well as valuable information which could improve the conditions for regeneration.

Key words: brushing, scalping, basal area, natural regeneration, *Pinus nigra* Arn.

Resumen

Influencia de la densidad de la masa adulta y el tratamiento del suelo sobre la regeneración del pino laricio (*Pinus nigra* Arn. ssp. *Salzmannii*) en España

Generalmente es difícil conseguir una regeneración natural satisfactoria del pino laricio (*Pinus nigra* Arn ssp. *salzmannii*). Esta ha sido estudiada en dos tipos de tratamiento del suelo y bajo diferentes rangos de densidad de masa en seis montes de la Provincia de Cuenca. Entre 1999 y 2000 se ha seguido la lluvia de semillas, germinación y supervivencia de plántulas en las parcelas en las que se realizaron los tratamientos de suelo y bajo diferentes rangos de densidad de masa adulta, medida como área basimétrica (m^2/ha). La temperatura del suelo y aire y la humedad del suelo han sido registradas durante el verano y para las dos primeras épocas de crecimiento de las plántulas. En las zonas decapadas la germinación fue superior a la encontrada en las zonas desbrozadas o testigo. El mejor porcentaje de supervivencia se calculó para las zonas decapadas en combinación con el mayor nivel de área basimétrica medido. La mortalidad de plántulas fue superior durante el primer periodo de crecimiento. Los resultados tienen implicaciones prácticas especialmente en lo relativo a una gestión forestal que mejore las condiciones para la regeneración natural de los bosques de pino laricio.

Palabras clave: desbroce, decapado, área basimétrica, regeneración natural, pino laricio.

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Received: 24-09-08. Accepted: 03-06-09.

Introduction

The European Black Pine (*Pinus nigra* Arn) is a tertiary relictual species belonging to the family of Mediterranean pines (Vidakovic, 1991). These species are an important pine species in forest management of Mediterranean areas (Bogunic, et al., 2007) and their forests are included in the EU endangered habitats listing of natural habitats requiring specific conservation measures (Resolution 4/1996 by the Convention on the Conservation of European Wildlife and Natural Habitats). European black pine shows high morphological, physiological and ecological variability that has led some authors to consider this pine as an aggregate of microgeographical species (Villar, 1947; Svoboda, 1953; Regato y Escudero, 1989; Richardson, 1998; Blanco, 1998). There is still no general consensus on taxonomy of European black pine (Bogunic et al., 2007), but it can be assumed that the Spanish black pine (*Pinus nigra* Arn. ssp *salzmannii*) is mainly distributed and established in Spain. This subspecie has been of interest for foresters and researchers from the late 19th century (Tiscar, 2004) and an enormous amount of information and articles related to the natural regeneration of Spanish black pine have been compiled. Most of this information, however, is based on opinions and observations derived from the authors' own experience throughout their professional lives. They are not backed up by studies which could quantify this topic (Serrada et al., 1994).

In an early diagnosis of the causes responsible for regeneration failure of Spanish black pine, some authors cited the following as the main problems: the masting condition of Spanish black pine (Mackay, 1917; Garcia, 1957; Rubio Jimeno, 1963 and Guardia 1988), dry summers over a period of at least three years (Mackay, 1917), excessive grazing and uncontrolled ploughing activities (Bartola and Esteve, 1957). Other authors placed great emphasis on soil humus as an effective defence against summer drought, thus correcting the possible soil effects which have a negative effect negatively upon the process of natural regeneration (Louro, 1982; Preto, 1983 and Trabaud, and Campant, 1991). Recently, the most important problems for the regeneration of Spanish black pines have been identified as such:-soil compactation, mastings, grazing and attacks by European pine shoot moths on seedlings (Serrada, 2002). Moreover, during many seasons, the establishment and development of initial regeneration depends on microclimatic conditions, particularly the temperature at both the height of seedlings and the soil surface. Soil moisture is also a key factor, as this affects growth directly and indirectly affects biological and chemical processes (Del Cerro et al., 2006). The large amount of information available on this topic as well as its numerous sources could make it difficult to assess the importance of the various factors under similar conditions. Previous studies have been inconclusive and silvicultural models must be improved and more knowledge is needed about seed fall and masting condition or how shelterwood density combined with soil preparation affected seedling emergence and survival of Spanish black pine (Del Cerro et al., 2005).

Seed dispersal links the end of the reproductive cycle of adult plants with the establishment of their offspring, and has profound effects on its vegetation structure (Xiaojun, et al., 2007). High year-to-year variability in seed production, commonly known as masting, also influences the natural regeneration process (Lombardo and McCarthy, 2008) and may infer that selective reproduction using a method of economies of scale could be advantageous.(Kelly, 1994). Masting conditions have been poorly documented for Spanish Black pine in the mountains of Cuenca (Del Cerro et al., 2005).

Soil treatment has proven to give a number of positive effects on the establishment of conifer seedlings, e.g. increased soil temperature (Dobbs and McMinn, 1977; Ritari and Lädhe, 1978; Örlander, 1987) and soil water availability (Bärring, 1965; Pohtila, 1977; Basssman, 1989). In adittion, site preparation is now is becoming of some interest as a means of improving root zone soil water supply (Fleming et al., 1998). This may be accomplished through removal of competing vegetation, increasing soil water storage or encouraging more extensive seedling root development (Flint and Childs, 1987). The effectiveness of site preparation will depend on microclimatic conditions, soil characteristics and the nature of the competing vegetation (Ölander, et al., 1990). Several site preparation methods have been developed but the most commonly used methods in the mountains of Cuenca are scalping and brushing (Del Cerro, 2005). However little is known about which kind of site preparation method is preferable in the natural regeneration of Spanish black pine.

Canopy cover or openness is known to be a critical variable controlling natural regeneration density (i.e. number of seedlings/ha) and growth (Emborg, 1998; Page et al., 2001). By changing the shelterwood density the light climate is greatly influenced, which may affect the performance of the seedlings and the outcome of the regeneration (Agestam, et al., 2003). The relationship between stand density and natural regeneration appears to be site specific (Page et al., 2001) and further research work is needed for Spanish black pine in the mountains of Cuenca.

At that point, we reached the hypothesis that during the early stages of the regeneration process of Spanish black pine in the mountains of Cuenca, the number of seedlings obtained could be improved by using an appropriate combination of soil preparation and overstory density in a year when good seeds were produced. Given the apparent importance of soil surface conditions and of light intensity, the main goal of the study was to determine the effect of different soil treatments and stand density on the germination and survival seedlings of Spanish black pine in the mountains of Cuenca. The understanding of these effects together with any possible physiological interactions plays a vital role in management of natural stands (Van der Meer et al., 1999). Knowledge of process and factors influencing nature regeneration is essential for its successful application (Frey et al., 2007; Pardos et al., 2007). The results have to find practical implications for silviculture, especially concerning choice of regeneration strategy and management of stand structures in order to improve the conditions for natural regeneration of Spanish black pine (Rodriguez Garcia et al., 2007).

Material and Methods

Description of Study Areas

The Spanish black pine has its central distribution core in the dolomite-limestone mountain ranges of the eastern section of Spain, the main forest region of Mediterranean

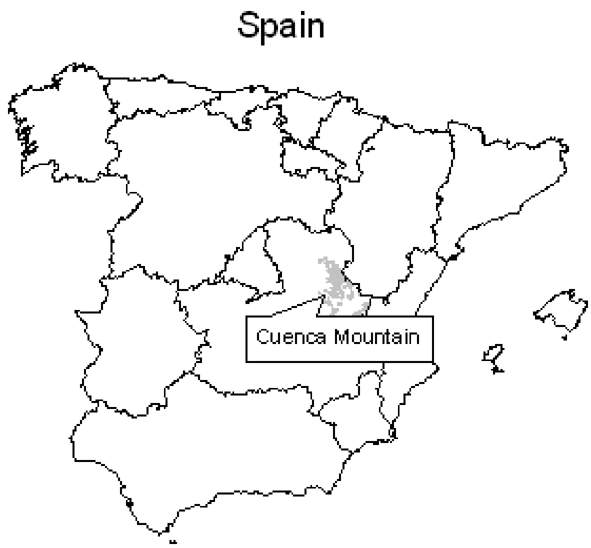

Figure 1. Map of the Study Area.

Spain (Regato and Elena Rosselló, 1995). Within this area lies Cuenca, which is the province with the greatest surface area of Spanish black pine trees.

From 1999 to 2002 six experimental sites within the mountain range of Cuenca (Spain) were set-up. This area is dominated by Spanish black pine, for climatic gradient of this area see (Table 1).

The selected sites were Public Utility Forest nº 131 "Cerro Candalar" (site nº 1), Forest of Public Utility Forest nº 133 "Ensanche de las Majadas" (sites 2 and 3), where forests of Spanish black pine are mixed with Scots pine (*Pinus sylvestris* L.), Public Utility Forest nº 109 "Ensanche de Buenache" (site 4), where Spanish black pine is mixed with sabina (*Juniperus thurifera* L.) and Public Utility Forest nº 106 "Los Palancares y Agregados" (sites 5 and 6), close to pine stands where Spanish black pine is mixed with pino rodeno (*Pinus*

Table 1. Geographical Location and Physiografical and Climatic Characteristics of the Experimental Site (Meteorological station of Enviroment Ministery, Cuenca 1950-2007)

Site	Geographic coordinates	*A*	*p*	*E*	*P*	P_s	S	t_{mm}	t_m	T_{mm}
1	40º15'40" N, 1º56'40" W	1380	2.0	-	1137	139	3	-4.5	9.6	28.3
2	40º14'30" N, 1º58'10" W	1440	19.5	North	1137	139	3	-4.5	9.6	28.3
3	40º16'10" N, 1º58'40" W	1420	10.0	North	1137	139	3	-4.5	9.6	28.3
4	40º06'08" N, 1º55'10" W	1335	3.8	-	1031	111	3	-1.7	9.8	27.4
5	40º01'50" N, 1º59'10" W	1230	4.0	-	600	100	3	-0.5	11.9	30.5
6	40º01'20" N, 1º58'40" W	1200	4.0	-	600	100	3	-0.5	11.9	30.5

A: altitude above sea level (m). *p*: slope (%). *E*: exposure. *P*: mean annual precipitation (mm). P_s: mean summer precipitation (mm). *S*: drought period (months). t_{mm}: mean lowest temperatures of the coldest month (ºC). t_m: mean annual temperature (ºC). T_{mm}: mean highest temperatures of the hottest month (ºC).

pinaster Ait.). According to the soil classification included in Soil Survey Staff (1999), *Calcium haploxerept* and *Lithic haploxeroll* is present in Public Utility Forest nº 106, Los Palancares y Agregados (sites 5 and 6 respectively) whereas *Typical xerorthent* can be found in the others experimental sites. Others forest stand characteristics and more details of each experimental sites are shown in Table 2.

Experimental Design and Treatments

Seed dispersal

Spanish black pine seed fall was estimated using 30 rectangular seed traps, each of them 40 cm in length x 50 width cm x 15 depth cm) regularly distributed in each of six experimental sites from early 1999 to the end of 2001. The top of the traps were protected with a wire netting in order to avoid seed predation. Dispersed seeds were removed monthly from each seed trap using tweezers.

Soil Treatment and Overstorey Density

With respect to soil treatments, three types of seedbeds were once made in early January 2000. Before initiating the dissemination process each plot was randomly assigned a different soil treatment: brushing (removal of ground vegetation), scalping (organic matter removed from up to 1–2 cm of mineral soil) and control (without treatment). Brushing and scalping were made with the straight blade of a Caterpillar D7. Removed vegetation and ground vegetation of control plot was characterized by *Eryngium campestre L., Thymus bracteatus L.* and *Geranium selvaticum L.,* for experimental sites number 1, 2, 3 and 4 and by *Eryngium campestre L., Geranium selvaticum L., Centaurea paniculata* L. *and Achillea odorata* L. for experimental sites number 5 and 6.

Arranged together in two 150 m parallel strips (north-south orientation) 30 rectangular plots were established (10 m length x 3 m width) in early February 2000. Each plot was influenced by a Spanish black pine canopy cover, ranging from plots without any mature tree to plots with 21 trees. Basal area is a convenient indicator of stand density (i.e. number of trees/ha) as it is easy to measure and is used widely by foresters (Philip, 1994). Therefore, in order to estimate the overstory density, we used a prims relascope to calculate the basal area on each rectangular plot established (see Philip, 1984). The diameter at breast height (dbh) was measured for all trees within a circle of 5, 10, 15, 20 and 25 m centred in that plot. The distance from central plot to each tree were determined using a Criterion Laser.

Air Temperature and Soil Moisture

In order to control the soil and air temperature evolution during the vegetative period of Spanish black pine, twelve temperature sensors (Tinytag Ultra; model TGU-0020) were installed. Three temperature sensors were ramdonly placed in each experimental site at a height of 2 m above the surface and covered with aluminium foil to ensure that it was always in the shade (aerial). Nine were placed 2 cm deep (underground) under the different preparation sites (3 sensors by soil treatment randomly chosen in each experimental site). Temperture sensors were capable of measuring temperatures ranging from –40 ºC to +120ºC with a margin of error of ±0.2ºC. A 30-minute data collection interval was established

Table 2. General Characteristics of the Forest stand at each Experimental Site

Site	Experimental site	*n/ha*	*B.A.*	*Dm*	*Hm*	*Ho*	Principal Composition	Stand
1	"Cerro Candalar"	399	12.59	25.54	12.41	13.56	100% Pn	
2	"Ensanche de las Majadas"	265	11.79	24.19	13.12	14.89	75% Pn; 25% Ps	
3	"Ensanche de las Majadas"	387	13.52	26.44	12.72	13.21	70% Pn; 30% Ps	
4	"Ensanche de Buenache"	453	14.56	29.71	15.85	16.45	70% Pn; 30% Jt	
5	"Los Palancares y Agregados"	555	14.02	27.94	13.76	15.35	100% Pn	
6	"Los Palancares y Agregados"	307	11.87	23.16	11.33	13.01	100% Pn	

n/ha: Number of trees per ha; *B.A*: Basal area (m^2/ha); *Dm*: Average stand diametrer (cm); *Hm*: Average stand height (m); *Ho*: Dominant stand height (m).
Pn: *Pinus nigra* Arn. ssp *salzmannii*; Jt: *Juniperus thurifera* L.; Pp: *Pinus pinaster* Ait.; Ps: *Pinus sylvestris* L.

during the period from 26 May 2000 to 23 September 2000 and from 26 May 2001 to 23 September 2001. This way, they were able to control soil and air temperatures for drought periods at all experimental sites.

Soil moisture evolution during vegetative period was also periodically estimated. Five soil pits of 50x50 cm^2 were dug in each experimental plot by mechanical means on a day that had no interference with any rain event during the first year of data collection. The samples were taken rapidly to the laboratory in a cool box for analysis of the soil moisture. It was estimated gravimetrically (Lull and Kenneth, 1955) with a chemical balance (0.01 mg precision) in five samples per experimental plot periodically collected between 9 May 2000 and 26 September 2000. The samples were dried in a 103° C to 110° C oven for about 1 hour and allowed to cool to room temperature in a desiccator. They were then weighed, and heated again for about 30 minutes. The samples were cooled and weighed a second time. The procedure was repeated until successive weighings agree to within 0.3 mg.

Measurements of the Seed fall, Seed germination and Seedling Survival

Dispersed seed were collected twice a month from 1999 to 2001 beginning in early January and continuing until the final collection in late May. Spanish black pine seed fall was observed during this period in the mountains of Cuenca (Orozco, 1998; *personal observation*). Germination was represented by the first visual appearance of the radicle from the outermost structure enveloping the embryo. To estimate seed germination all seedlings were surveyed after seedling emergence in June 2000 on each experimental condition. The survival of seedlings was measured by tallying the number of live seedlings on different dates: 9 and 24 June, 9 and 24 July, 8 and 23 August, 7 and 22 September, 7 and 22 October, 6 November 2000 (first growing season) and 11 November 2001 (second growing season).

Statistical Analysis

The variables seed germination and seedling survival were transformed, $y = (x + 0.5)^{0.5}$ before analysing data statistically to counteract the violations of the normality and constant variation assumptions. This transformation is normally used with data that include many zero values (Greig-Smith, 1983). A normal probability plot, standardised skewness and kurtosis, and the Kolmogorov-Smirnov test were used to determine whether the sample came from a normal distribution. After use of Cochran´s and Bartlett´s test (Neter et al. 1996) to examine the homogeneity of variances between groups, a set of ANOVAs and Least significant distances method (LSD) were carried out to check whether there were significant differences among groups in terms of temperature, soil moisture, seed fall, seed germination and seedling survival. A significant level of $P<0.05$ was adopted throughout, unless otherwise stated.

Multiple regression analyses using indicator or dummy variables were used to detect whether there were significant differences between the intercepts and slopes of the model defined for each experimental site, stand density and soil treatment related to seedling survival. The general linear test statistic (F-test, Neter et al., 1996) was used to test some hypotheses about regression coefficients. See Model 1.

$$Ss_i = b_0 + b_1 E_i + \sum_{i=1}^{2} b_{2i} T_i + \sum_{j=1}^{5} b_{3j} Z_j + \sum_{i=1}^{2} b_{4i} E T_i + \sum_{j=1}^{5} b_{5j} E Z_j + \sum_{i=1}^{2} \sum_{j=1}^{5} b_{6ij} Z_j T_i \quad \text{[Mod. 1]}$$

Where Ss_i is the response variable (seedling survival of each plot; %), E_i is the mature density of each plot (measured as basal area; m^2/ha), T is a dummy variable for soil treatment (three leves, two dummy variables), Z is a dummy variable for each experimental site (six sites, five dummy variables), and b_0 to b_6 are regression coefficients. Collinearity and residuals patterns were assesed both graphically and through the Durbin Watson statistic. A 5% significance level is used throughout, unless stated otherwise. Analyses were performed with Statgraphics 5.0.

Results

Seed Fall

For the tested period, Spanish black pine seed fall has showed uneven patterns from year to year but not between experimental sites (Tab. 3). This was expected due to similar stand ages and stand-structures in all the experimental sites. Seed fall started early January and finished in late May 2000. No conclusion can be given from 1999 to 2001 due to the low number of dispersed seeds. Total annual seed production was (mean ± standard error) 7±2 seeds/m², 212±11 seeds/m² and 8±3 seeds/m² in 1999, 2000 and 2001, respectively. In a good year's seed production (2000), seed were recorded for all traps installed (from 7 seed/traps in January to 44 seed/traps in March) and it can be assumed that seed dispersion was distributed uniformly on the forest soils.

Germination of Spanish Black Pine Seedlings

Soil treatments coincided with abundant dissemination, which was probably one of the most important of recent decades, and, therefore to seed fall research works. The year 2000 can be considered as a mast year for Spanish black pine in the mountains of Cuenca. Average (± standard error) germination was estimated over all sites as 50±6 seedlings/m² in control plots, 55±6 in the brushing plots and 59±3 in the scalping plots.

In relation to experimental sites, the higher germination rate was found in "Los Palancares y Agregados" (36±2 and 32±3 seedlings/m² in site 5 and 6 respectively), followed by Ensanche de Buenache (site 4: 21±6 seedlings/m²), Ensanche de las Majadas (20±7 and 16±22 seedlings/m² in sites 3 and 2 respectively) and Cerro Candalar (site 1: 15±2 seedlings/m²).

With regards to forest stand density, the best area of influence to characterize overstory stand density measured as basal area (m²/ha) in each experimental site was 15 meters around each plot and the range we have been working with was 0,8 to 21,56 m²/ha but not for all experimental forest. In this context, inconclusive relationships were found between basal area and seed germination because not the same basal area intervals were calculated for all experimental sites and because seed predation was not analysed. Nevertheless, in general the seed germination results suggest that the seed germination percentages in each experimental forest can be determined by the overstory density. In this sense a higher number of seedlings were always found with the higher basal area intervals at the end of June in 2000 and for all plots counted.

Table 3. Result of two-factors ANOVA (experimental site and year) for seed fall

DF	Den DF	F-rate	P-value	
Experimental site	6	180	14.56	ns
Year	3	180	76.1	<0.01
Interaction	18	180	9.67	ns

Survival of Spanish Black Pine Seedlings

For the first growing season (from May to October 2000) the number of living Spanish black pine seedlings peaked in July then declined in November (Fig. 2). On the first growing season, Spanish black pine seedling survival from the soil preparation differed in all experimental sites. Mean (±standard error) survival rates of 0,30±0,01%; 2,70±0,07% and 8,82±0,10% were estimated for control, brushing and scalping, respectively in early November 2000 (the differences were significant at level of $P<0.05$). In November 2001, mean survival rate (± standard error) in relation to the initial germination of the year 2000 was 0,20±0,07%, 0,99±0,27% and 6,04±0,34% for control, brushing and scalping, respectively. For the existing seedlings at the start of 2001, mean survival rate was 5.68±1,84%, 27,07±2,67% and 48,51±5,20% for the same soil treatments. If we consider brushing and scalping exclusively, given the low number of control plots in which seedlings remained after the first year, survival was significantly higher in scalping.

Seedling survival also differed significantly ($P<0.05$) among experimental sites for the first and second growing season, which are shown here is ascending order, from the lowest survival to the highest (Mean ± standard error; survival percentage is presented in brackets): site 4, Ensanche de Buenache (3,31±0,09%); site 1, Cerro Candalar (5,35±0,13%); sites 2 and 3, Ensanche de las Majadas (9,31±0,21% and 11,31±0,33%, respectively); and sites 5 and 6, Los Palancares y Agregados (14,01±0,48% and 14,89±0,51%, respectively). In 2000, mean survival rates (± standard error) were 19,24±3,20%, 28,54±5,27%, 32 ,55±4,99%, 34,34±6,11%, 40,26±6,78% and 71,82±9,20% for

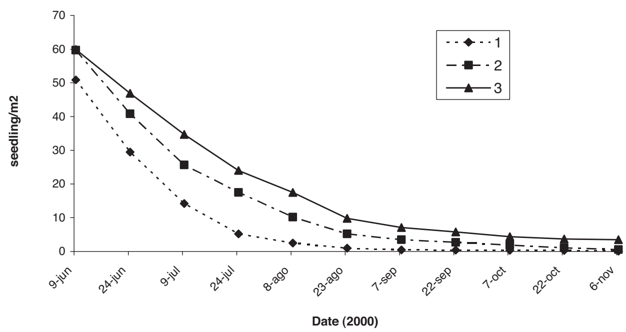

Figure 2. Variation in Spanish black pine seedlings per m² in the vegetative period estimated over all sites and corresponding to the year 2000. The values shown are for the following plots: control (1), brushing (2) and scalping (3).

experimental site 4 (Ensanche de Buenache), site 1 (Cerro Candalar), site 2 (Ensanche de las Majadas), site 3 (Ensanche de las Majadas), site 6 (Los Palancares y Agregados) and site 5 (Los Palancares y Agregados), respectively. The Soil Treatment x Test Site interaction was not significant at level $P<0.05$.

With regards to forest stand density, results showed a positive correlation between this and the basal area within the range we have been working with. An increase of 1 m^2/ha increases survival by 0,29±0,04%. In relation to the experimental sites, the greatest survival rate was seen at site 5, followed by site 6; both sites are in the forest area known as "Los Palancares y Agregados". It should be taken into account, however, that the density range of site 6 is narrower than the rest; a larger density range could alter the model.

The combined effect of soil preparation and basal area for all experimental sites was studied by a multiple regression (model 1) at the end of the second growing season. The definitive model accounted for 57.62% of survival variability. Even though the analysis of variance revealed significant differences among experimental sites 1 (Cerro Candalar), site 2 (Ensanche de las Majadas), site 3 (Ensanche de las Majadas) and site 4 (Ensanche de Buenache), they were all grouped in a single dummy variable since it was considered that the model did not gain in accuracy when these were not grouped. Results from the adjusted model can be observed in Table 4.

The final model was:

$$Ss = -3,93 + 0,29E + 2,40(Brushing) + 8,58(Scalping) + 6,24(Site5) + 4,93(Site6)$$

Due to the climatic conditions of summer 2000 a larger number of seedlings produced from the first year of life of natural regeneration withstood the extreme summer conditions when scalping was combined with a basal area of more than 21 m^2/ha. It should not be discounted, however, that greater survival rates might be obtained with larger densities, at least under these conditions (see Fig. 3).

Air and Soil temperature and Soil moisture

For each soil treatment the mean temperature (±standard error) of the daily means measured at 2 cm depth, during the growth period of 2000 was higher with scalping (21.29±0.31 ºC), somewhat lower with brushing but without significant differences (21.05±0.27 ºC), and significantly lower for the control (20.56± 0.19 ºC). No significant differences between average daily mean temperatures for control and brushing (19.75±0.22 and 19.61±0.17 ºC, respectively) were observed during the growth period of the second year (2001); a higher temperature was seen for scalping (20.46±o.32 ºC). Therefore, the effect that brushing has on the temperature at a depth of 2 cm apparently disappears after the first growth period.

The lowest mean temperature recorded at a height of 2 m above the surface was at "Ensanche de Las Majadas" (site 2) (17.94±1.23 ºC), followed by "Ensanche de Las Majadas" (site 3) (19.52±1.81 ºC), "Cerro Candalar" (site 1) (21.36±1.23 ºC), "Ensanche de Buenache" (site 4) (22.13±1.74 ºC), "Los Palancares y Agregados" (site 5) (22.37±0.83 ºC) and "Los Palancares y Agregados" (site 6) (22.47±1.12 ºC). Soil and air temperature were increasing from June to August and then it went down in September. This circumstance was registered in each experimental forest tested and for all soil treatment (Fig. 4 and 5). Highest soil and air temperatures were recorded in August. No interactions between experimental site and site preparation were found.

Table 4. Parameters estimations of seedlings survival model at the end of the second growing season (October 2001)

Parameter	Estimation	C.I.	*t*	a
Constant	-3.9322	(-5.8725 , -1.9918)	-3.9998	0.0001
E (basal area$_{15}$)	0.2951	(0.1328 , 0.4573)	3.5899	0.0004
Brushing	2.3889	(0.7550 , 4.0228)	2.8857	0.0044
Scalping	8.5758	(6.9416 , 10.2102)	10.3570	0.0000
Site 5	6.2433	(4.3389 , 8.1476)	6.4706	0.0000
Site 6	4.9309	(3.0194 , 6.8427)	5.0914	0.0000

The model was significant ($\alpha < 0.0001$); R^2 = 57.6%, mean value of residues = 0, normal distribution of residues, n=880.
E (Basal area$_{15}$): basal area (m^2/ha) for an area of influence of 15 m around each plot. C.I.: confidence interval for the level of significance of 0.05. t = Student's *t*-test. α= level of significance

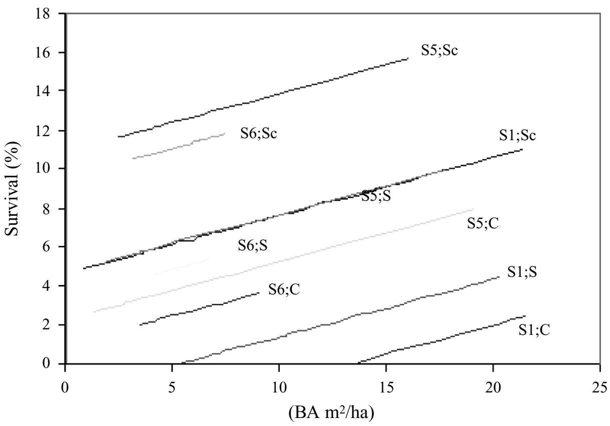

Figure 3. Survival versus basal area for an area of influence (BA) of 15 m around each plot shown by the following indicator variables (S1: sites 1, 2, 3 and 4 grouped. S5: site 5. S6: site 6. C: control. S: Brusing. Sc: scalping).

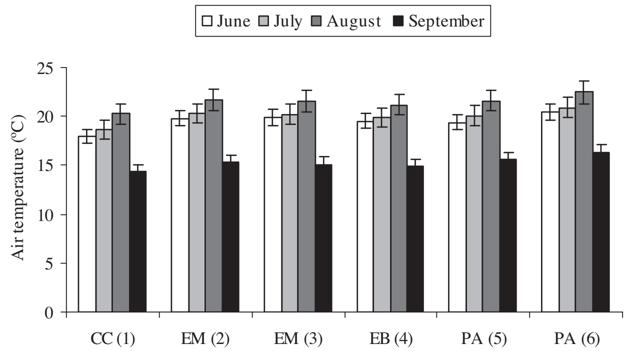

Figure 5. Evolution of the month mean air temperature at 2 metres above ground in each experimental site: 1; "Cerro Candalar", 2 and 3; "Ensanche de las Majadas", 4; "Ensanche de Buenache", 5 and 6; "Palancares y Agregados" during the period from 26 May 2000 to 23 September 2000 and from 26 May 2001 to 23 September 2001. Error bars denote 95% confidence intervals of the mean.

With regard to soil preparation, soil moisture was higher for the control plot (18.25±0.66 %) somewhat lower with brushing but without significant differences (17.41±0.43 %), and significantly lower with scalping (16.15±0.39 %) during the period from 9 May 2000 to 26 September 2000. The lowest soil moisture content was at site 6 (Los Palancares y Agregados; 11.52±1.33 %), and the highest was at site 1 (Cerro Candalar; 23.02±1.76 %). In a four-month- period soil moisture decreased from June to August only to rise in September. This fact was registered for each experimental forest tested and for all soil treatment (Fig. 6 and 7). Lowest soil moisture rates were calculated in August. It is often a drought period and most plants in the Mediterranean basin experience water extress during summer months. No interactions between experimental site and site preparation were found.

Discussion

It is often advocated that ecologically sound silvicultural systems should be based on natural forest process (Attiwill, 1994; Coates and Burton, 1997). In this sense, natural regeneration is a key factor in the forest process, especially, in the case of Spanish black pine, which is very often considered a "delicate species" (Dominguez Lerena, 1994; Tíscar, 2004; Del Cerro et al., 2005; Lucas Borja, 2008). This process does not appear to be as reliable or profuse as for *Pinus sylvestris* L. (Kerr, 2000)

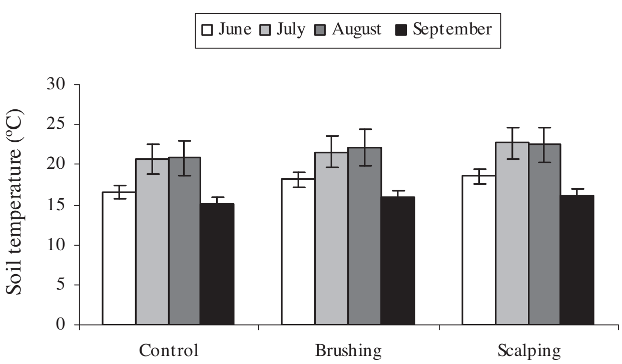

Figure 4. Evolution of the month mean soil temperature at 2 cm depth for each site preparation during the first growing period (From May to October 2000). Error bars denote 95% confidence intervals of the mean.

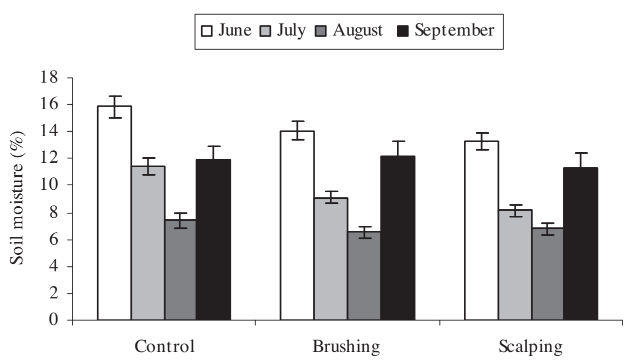

Figure 6. Evolution of the month mean soil moisture for each soil preparation during the period from 9 May 2000 to 26 September 2000. Error bars denote 95% confidence intervals of the mean.

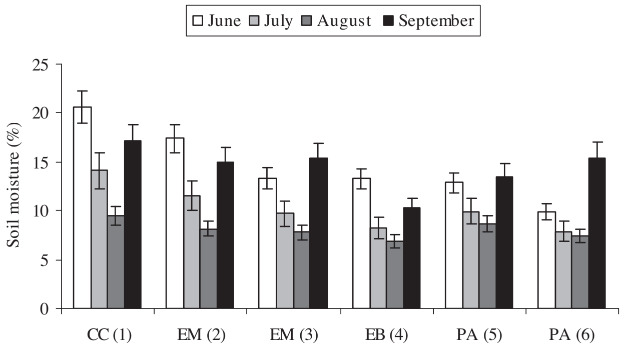

Figure 7. Evolution of the month mean soil moisture for each experimental site: 1; "Cerro Candalar", 2 and 3; "Ensanche de las Majadas", 4; "Ensanche de Buenache", 5 and 6; "Palancares y Agregados" during the period from 9 May 2000 to 26 September 2000. Error bars denote 95% confidence intervals of the mean.

and patience will be often be required and management plans will have to be flexible (Tiscar, 2004 y 2007).

Natural regeneration of forest usually results in high seedling densities at the start of the process (i.e. Madsen and Larsen, 1997; Holgen and Hanell, 2000). This case is not an exception and mean density of regeneration in all experimental sites was higher than the afforestation rates recommended for any of the species (Modrý et al, 2004). However, seedling densities are determined, apart from others factors, by the Spanish black pine masting condition, which has a strong influence on tree recruitment by altering seed predation and seedling densities (Lombardo and McCarthy, 2008). This behaviour has been demonstrated in this research work since seed fall in 2000 was higher than in 1999 or 2001. In earlier works in the mountains of Cuenca, many authors (Orozco, 1998; Del Cerro et al., 2005) found masting condition for Spanish black pine as well, but no mast ranging was described. In accordance with seed fall result of this research work, Tíscar (2007) described a similar mast ranging in other Spanish black pine locations across the Sierra de Cazorla (South of Spain). At least eight hypotheses have been developed to explain mast seedlings (Kelly, 1994), from predator satiation to economies of scale (Norton and Kelly, 1988), but masting needs to be studied and described numerically for Spanish black pine over a long period in the mountains of Cuenca.

Soil temperature and moisture are likely to be the main factors determining and controlling germination under field conditions (Brown and Neustein, 1972; Lee et al., 2004; Castro et al., 2005). Field experiments carried out on the forest floor under pine stand indicate that germination depends on the soil water content (Lee, et al., 2004), but to sum up , the timing and rate of germination of other Spanish pines appear to be largely determined by the interaction of soil temperature and soil moisture (Castro et al., 2005). From the present research work, it can be observed that soil moisture was highly affected by air temperature. Indeed, the experimental sites where the highest temperatures were recorded are the same sites where soil moisture was lowest. At the same time, soil moisture and temperature at 2 cm depth were influenced by soil treatment.

Seed germination differed for each soil treatment throughout the experimental sites. Density of the regeneration increased with the mineral character of the seedbed. These results are in line with other studies of soil preparation effects in natural regeneration. For instance, Kerr (2000) summarized that felling and wood extraction operations, which disturb the surface soil, has beneficial influence on Corsican pine (*Pinus nigra* Arn. ssp. *laricio*) and Castro et al., (2005) found in Southeastern Spain higher seed germination rates in bare soil for Scots pine. Moreover, the seasonal pattern of emergence by conifer seedlings and their preference for mineral soil seedbeds has been noted for some authors as well (Duncan, 1954; Zadasa et al., 1978; Madsen, 1995; Agestam et al., 2003; Del Cerro et al., 2005; Lucas Borja, 2008). Mineral soil is the best seedbed for germination because it allows better water conductivity than organic matter (Riley, 1980; Chrosciewicz, 1987; Béland et al., 2000) and furthermore, the herb layer found in control and brushing plots are a physical barrier preventing fallen seeds from contacting the mineral soil (Gonzalez-Martinez and Bravo, 2001).

With respect to experimental sites, seed germination was higher in Los Palancares y Agregados and Ensanche de Buenache than in the others ones. These findings can be related with the soil temperature, since it is likely a main factor determining germination, as suggested by several research works (Fleming et al., 1998; Castro et al., 2005). The highest monthly mean soil and air temperature registered in the scalping plot and experimental site number 5 and 6 (Los Palancares y Agregados) and 4 (Ensanche de Buenache) were associated with the highest number of seed germination. There are few references which make observations on seedling germination of Spanish black pine and soil or air temperature but some authors (Escudero et al., 1997) argued that the Spanish black pine seed cover only confers a smooth protection to heat shocks or frost, hence high or low temperatures become lethal and seed germination can be prevented. In that respect, soil moisture is also a main factor controlling germination but inside a soil moisture interval, soil temperature seems to be more determining.

Seedling survival is one of the most critical stages in a plant's life history, and is often reduced by drought period and high temperatures in the Mediterranean basin (Madrigal et al., 2004; Padilla and Puignare, 2007) where water deficit in the summer period appears to be the dominant cause of seedling mortality (Valladares, 2004). Our findings show that seedling mortality was particularly severe for the first growing season and summer period (June, July and August) in all experimental sites and soil treatments. During this period, average monthly air and soil temperatures were increasing and soil moisture was decreasing progressively. By contrast, this tend was broken in September 2000 and seedling mortality was lower than the previous three months. Thus, the differences between soil treatments with respect to survival rate were probably an effect of firstly, reduced water availability in the top layer of the upturned mineral soils on top of a humus layer (Agestam et al., 2003) and secondly, the presence of herb layer found in control and brushing plots could have delayed or impeded seedling development (Caccia and Ballaré, 1998). Competition intensity with herbaceous vegetation is likely to increase in environments with low water availability (Davis et al., 1998) and to Fleming et al. (1998), superior seedling survival and growth of *Pinus contorta* Dougl. ex Loud were achieved after three growing season in treatments which removed surface organic layers, change soil porosity or control grass layer. For the second growing season (2001), root seedling could extend into the deeper soil to attain sufficient water (Lee et al., 2004) and seedling survival percentages were higher than in 2000 for all soil treatment and control plots (Caccia and Ballaré, 1998; Gonzalez-Martinez and Bravo, 2001).

Survival rate was significantly higher at site 5 and 6 than at the other sites for the first and second growing season. It is very difficult to answer the question of which factor was responsible for this, since there are a several factors and their interactions could affect seedling survival in each location. However, the trend can be associated with the soil characteristics. Soil type is different according to the soil classification included in Soil Survey Staff (1999) and the differences with regards to seedlings survival rates could be generated by soil type and characteristics. *Calcium haploxerept* and *Lithic haploxeroll* is present in Los Palancares y Agregados (sites 5 and 6 respectively) whereas *Typical xerorthent* can be found in the others experimental sites. Lee et al. (2004) demonstrated that under similar climatic conditions survival can differ from experimental forest to experimental forest based on differences in soil characteristics. Mencuccini et al (1995) argued that edaphic factors appeared to be responsible for the spatial distribution in subalpine Norway spruce (*Picea abies* (L.) Karst.) forest. Furthermore, consistent with results of others studies (i.e. Madsen, 1995; Minota and Pinzauti, 1996; Modrý et al, 2004) soil was found to have a significant effect on seedling survival. This point needs to be clarified and more research work in the topic of soil features and Spanish black pine survival is needed.

Negative effects of a drought period can be corrected by soil preparation and overstorey density in order to obtain higher seedlings survival rates (Feller, 1998; Page et al., 2001; Agestam, 2003; Lucas Borja, 2008). Light intensity has influence in soil temperature and moisture, for that reason, light plays a major role in the regeneration, survival and growth of forest plants (Emborg, 1998; Montgomery and Chazdon, 2002). The effect of light is a direct consecuence of canopy structure and overstorey density, which will determine future recruitment patterns (Catovsky and Bazzaz, 2002). Page et al. (2001) studied the influence of overstorey basal area on density and growth of advance regeneration of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) in variably thinned stands. A significant, positive relationship was found between mean density of the youngest seedlings and basal area. They finally argued that the effect of the overstorey, as characterised by the basal area, can be summarised as being a positive one on its initial density and a negative on the survival and growth of natural regeneration. The present research work, results suggest that high levels of basal area in combination with scalping are positively correlated with seedling survival. This finding demonstrates the preference of Spanish black pine seedlings to moderate light conditions in the mountains of Cuenca, at least during the early stages of growth and mineral soil contact. In this sense, the first overstory removal of shelterwood system should not leave a basal area of less than 20 m^2/ha when a dense blanket of seedlings has become established. Nevertheless, the range studied is too narrow (0.8 to 21.56 m^2/ha) and high basal areas must be studied since lower light levels, mainly under summer drought conditions, can even lead to seedling death because of their susceptibility to solarisation (Feller,

1998) or higher light levels can inhibit seedling germination, survival and growth for all experimental sites (Nyman, 1963; Tillberg, 1992). Thus, in shaded microhabitats, where low light intensity is coupled with lower soil temperature, delayed germination is expected (Castro, et al., 2005).

Conclusions

Data from this study shows that Spanish black pine regeneration can be curbed in different forest stands in the mountains of Cuenca due to the low number of seedling survival. Climatic conditions have a strong influence in the regeneration process but an adequate interval of basal area in combination with soil treatments can improve seedling recruitment in mast years. In this sense, Spanish black pine forest stand treatments need to promote silvicultural flexibility and control during the regeneration process in the mountains of Cuenca. However, this study has to be seen as a preleminary research work. In order to clarify the natural regeneration process of the Spanish black pine more experiments and field work must be carried out. For example, one of important factors that affect regeneration is the availability of seeds. It would be desirable to measure the size and temporal distribution of seed rain for a long time and to fully understand the relationships between variations in annual seed production and weather conditions. Other important aspects to be studied should be what happens with the role of seed predation and mature high densities (higher than 20 m^2/ha). Seed predation has important effects on the availability of seed of pinus species, being a key factor in small size population. Further studies (i.e. observing the effects of daily alternating temperatures, light quality and quantity and cycles of wetting and drying, studing soil properties and seed recruitment interactions) would also be useful in gaining an improved understanding of how these factors and others, interact in conditions associated with natural regeneration.

Acknowledgments

This work was financed by the FEDER funding during 2000-2002 and Castilla La Mancha Regional Government during 2003-2006. Manuel Esteban Lucas Borja was supported by European Forest Institute, Mediterranean Regional Office (EFIMED) during the writing of the manuscript. We are also grateful to two anonymous reviewers for their valuable comments.

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