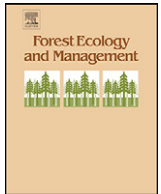




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Modelling Spanish black pine seedling emergence: Establishing management strategies for endangered forest areas

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ABSTRACT

Changes in climate may reduce the success of natural regeneration and hence require adjustments to silvicultural practices. Special attention is required for species such as Spanish black pine (*Pinus nigra* Arn. ssp. *salzmannii*) because of impediments to achieve successful natural regeneration. Spanish black pine seed germination was studied under field conditions at a normal altitude for the species and at the upper altitude limit of its distribution in the Cuenca Mountains (southeastern Spain). The aim was to assess the effect of location, overstorey density, soil treatment, sowing date and climatic characteristics on regeneration success. ANOVA results indicated a significant interaction of location by overstorey density on germination rates. A logistic model containing the temperature variable Tem_{minAc} , the light radiation effect (*Rad* variable) and the interaction term between soil treatment (*Soil Treat*) and radiation (*Soil Treat* × *Rad*) correctly predicted the germination success in 94% of cases. Moreover, two Poisson regression models (one for each experimental site) showed that the number of germinated seeds depends on stand basal area (*G*), soil treatment (*Soil Treat*) and sowing date (*Seed Season*). Conservation management could increase initial seedling recruitment by promoting soil preparation and higher basal area levels. The populations at higher altitudes are particularly endangered due to the unfavourable environmental conditions for the development of this species, which seriously affect seed rain density and germination rates.

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1. Introduction

Pinus nigra Arn. is a southern European pine species with a wide natural range of distribution (Varelides et al., 2001). Differentiation of populations in categories is not fully agreed by the various authors although it can be assumed that Spanish black pine (*Pinus nigra* Arn. ssp. *salzmannii*) is located in Spain (Regato Pajares and Elena-Rosselló, 1995). This species is one of the most important pines in the Mediterranean forest areas because of its utilization for landscaping and soil protection. Moreover, Spanish black pine 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), because of their environmental values and difficulties to achieve successful natural regeneration.

Since the end of the 19th century, Spanish black pine forest stands have been managed under different systems in Spain that have not always promoted forest stand persistence (Serrano, 2002). A shelterwood system with a 100- to 120-year rotation and a 20- to 30-year regeneration period is one of the most prescribed methods in Spanish black pine forest management plans. Initial seedling recruitment has always been difficult to achieve due to factors such as soil compaction, masting condition, inadequate overstorey density, seed predation, grazing and attacks by European pine shoot moths on seedlings (Serrada, 2002; Tíscar Oliver, 2003, 2007; Del Cerro et al., 2005, 2009; Lucas-Borja et al., 2010). In this context, knowing the state and limitations of regeneration in populations of endangered species is necessary for improving future conservation strategies (Kollmann et al., 2008) and further studies would be useful.

Germination, represented as the first visual appearance of the radicle from the outermost structure enveloping the embryo, is an important phenological event that influences the success of the initial seedling recruitment. Soil temperature and soil water content are the main environmental factors affecting seedling emergence

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(Kerr, 2000) and some microhabitats or certain periods of the year may provide better conditions than others with respect to seed germination (Castro et al., 2005; Del Cerro et al., 2005; Lucas-Borja, 2008). Elucidating their relative importance in controlling initial seedling recruitment of endangered species has management value (Kollmann et al., 2008), because it may be possible to manipulate seed availability and microsite characteristics to prevent further decline of Spanish black pine. Also, soil temperature and soil water availability can be modified indirectly through silvicultural operations (Del Cerro et al., 2005, 2009). The amount of light that reaches the soil surface is greatly influenced by shelterwood density, which may affect the performance of the seedlings and the outcome of the regeneration (Agestam et al., 2003). On the other hand, soil treatment has proven to give several positive effects for the establishment of conifer seedlings, e.g. increased soil temperature (Dobbs and McMinn, 1977; Ritari and Ladhe, 1978; Del Cerro et al., 2009) and soil water availability (Pohtila, 1977; Bassman, 1989).

Seed germination is 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). The success of conservation strategies depends on detailed knowledge of the key processes associated with plant regeneration (Kollmann et al., 2008). Modelling seed germination seems to be a promising approach to better understand the initial phases of the seedling recruitment process. Furthermore, its inclusion in the context of ecological models can help to synthesize the pieces of ecological knowledge, emphasizing the need for a holistic view of certain environmental problems (Mitsch and Jørgensen, 1989) such as natural regeneration failure. Constructions of models may be useful in dealing with such complexity of ecological systems and in better understanding the factors that influence germination dynamics of Spanish black pine.

At this point, we hypothesized that (i) Spanish black pine seed germination is favoured by increasing overstory density, (ii) site preparation enhances the amount of seedling emergence, (iii) sowing date affects the number of germinated seeds and (iv) changing environmental conditions at the Spanish black pine distribution margin could affect the germination. The principal aims of this study are to test the proposed hypotheses, to develop a model that predicts the probability of successful seed germination, and to construct an equation that forecasts the number of germinated seeds where successful germination occurs. Simple but effective empirical models that combine soil preparation, sowing data inside of the natural seed rain period, overstory density and climatic variables can provide vital information for Spanish black pine management decisions.

2. Materials and methods

2.1. Field site

The study area is located in the Cuenca mountain range (Region of Castilla-La Mancha, east-central Spain), where two experimental sites were selected. The first site is a continuous homogeneous Spanish black pine forest (the central population), which is called Los Palancares y Agregados (PAL) forest (1200 m above sea level, 40°01'20"N, 1°58'40"W). The second site occurs at the upper altitude limit of the species distribution (the peripheral population), which is called Ensanche de las Majadas (MAJ) forest (1490 m above sea level, 40°14'30"N, 1°58'10"W). The shelterwood uniform system is the most widely used regeneration method in both mixed and pure even-aged Spanish black pine stands in the Cuenca Mountains. This method involves a uniform opening of the canopy for regeneration purposes without soil preparation. The stand is

opened gradually over a period of at least 30 years and thinning is done when the stand has reached the stem exclusion stage, approximately at the age of 40–50 years (Cuenca local forest authority). Some seed trees still remain after the last cutting. The rotation in PAL is 130 years whereas in MAJ it is 120 years.

In Los Palancares y Agregados forest the mean annual rainfall is 600 mm, the mean temperature is 11.9 °C and air temperature typically ranges from –0.5 °C to 30.5 °C. In Ensanche de las Majadas forest the mean annual rainfall is 1137 mm, the mean temperature is 9.6 °C and air temperature typically ranges from –4.5 °C to 28.3 °C. The major soil types are Lithic haploxeroll to Typical xerorthent according to Soil Survey Staff (1999). Slopes at PAL are less than 5°. The dominant understory grasses are *Eryngium campestre* L., *Geranium selvaticum* L., *Centaurea paniculata* L. and *Plantago media* L. Slopes at MAJ are inferior to 25°. Spanish black pine is sometimes mixed with Scots pine (*Pinus sylvestris* L.) and the dominant understory grasses are *Eryngium campestre* L., *Thymus bracteatus* L., *Geranium selvaticum* L., *Festuca rubra* L. and *Cirsium acaule* L.

2.2. Experimental design

In January 2005, nine representative forest stands were selected in each experimental site, three representing low density: 15–20 m² ha⁻¹ (LD), three representing medium density: 25–30 m² ha⁻¹ (MD), and three representing high density: 35–40 m² ha⁻¹ (HD). A 7 m × 10 m permanent plot was set up in each forest stand (Fig. 1). Plots were fenced in order to avoid the impact of grazing on seedlings and saplings. Inside of each plot, six 3 m × 3 m subplots were installed in order to have three replicates each of scalping soil preparation (organic matter removed with the straight blade of a Caterpillar D7 from up to 1–2 cm of mineral soil) and control (without treatment) (Fig. 1). During the natural dispersal period of Spanish black pine seed in the Cuenca Mountains (Del Cerro et al., 2009), four sowing dates were established in 1 m × 1 m sub-subplots of each 3 m × 3 m subplot where previously a soil treatment was assigned (Fig. 1). Sowing dates were: (S1) 11 February 2006; (S2) 15 March 2006; (S3) 12 April 2006; (S4) 16 May 2006. Each seed sowing was composed of 2 g (approximately 107 Spanish black pine seeds). Sowing dates were replicated three times on each soil treatment. The seed used were previously collected before each sowing date using 90 rectangular seed traps (40 cm length × 50 cm width × 15 cm depth) regularly distributed in each experimental forest and basal area interval. Seeds naturally dispersed were also taken into account so as not to overestimate the number of germinated seeds on each experimental condition. Before each sowing date we calculated the average number of seeds per m² naturally dispersed using the seed traps established in each experimental forest and basal area interval. Then, we completed the number of seeds until it reaches the quantity of 107 seeds (approximately 2 g of seeds per m²).

Germination was defined as the first visual appearance of the radicle from the outermost structure enveloping the embryo. To estimate seed germination, each experimental condition was surveyed after seedling emergence on 1 June 2006. All the seedlings were labelled and counted. Dead seedlings observed in the sampled plots were also counted in order to account for early mortality events. One meteorological station was set up on each experimental forest in early January 2005 (model METEODATA 1256C). Air temperature, precipitation and relative air humidity were recorded daily. Sums of the mean, minimum, and maximum daily air temperature and sums of the mean daily precipitation 30 days before and after each sowing date were calculated. Light radiation was also recorded using a light meter (model LI-COR 250).

Collected seeds were tested in a laboratory. A controlled seed germination experiment was done in order to check if seed germi-

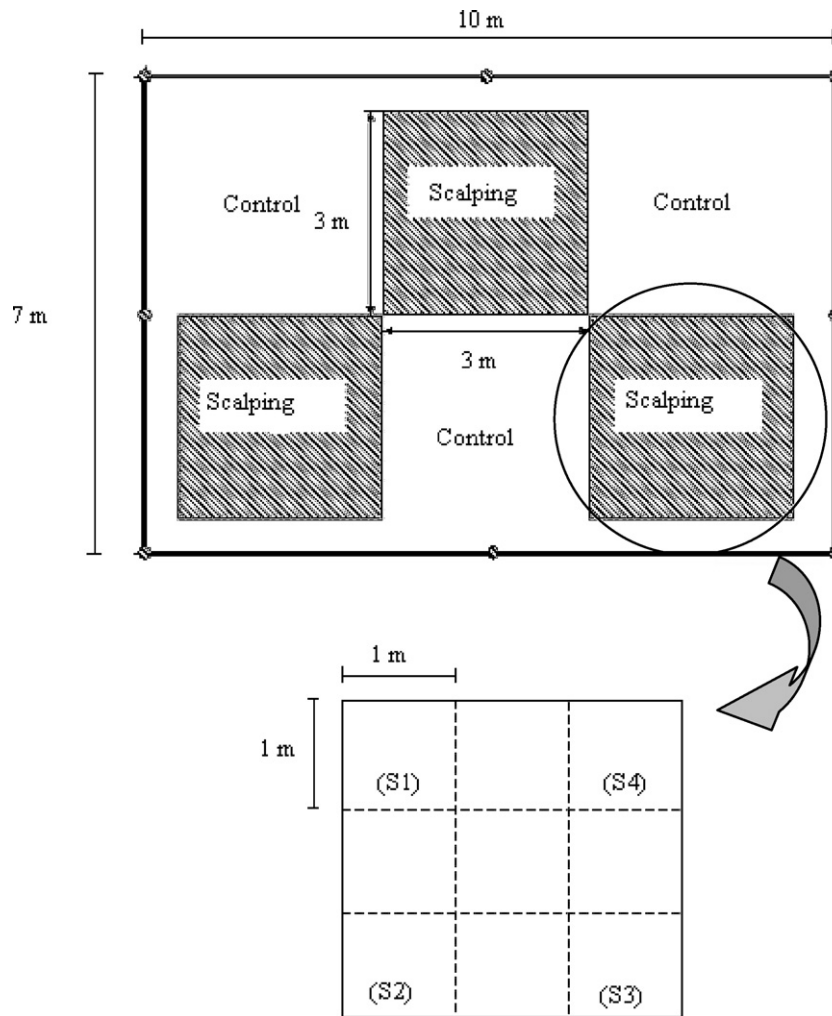


Fig. 1. Main characteristics of the experimental plots used. Soil treatments were: scalping and control. Sowing dates were: (S1) 11 February 2006; (S2) 15 March 2006; (S3) 12 April 2006; (S4) 16 May 2006.

nation differences between experimental forests were due to seed characteristics or to forest and environmental conditions. Three samples of twenty-five seeds, collected before each sowing date, were put in a germination chamber (Model GR-36L) and night/day temperatures were set at 7°/24° interval during four weeks. At the end of this time, the percentage of seed germination was calculated for each experimental forest and each collected date.

2.3. Statistical analysis

Hypothesized differences in seed germination percentage among experimental sites (random effect), stand density intervals (fixed effect), soil treatments (fixed effect), and sowing dates (fixed effect) were tested using analysis of variance (ANOVA). Nested within each experimental site was a split-split-plot design with stand density as the whole plot, soil treatment as subplot, and sowing date as sub-subplot. Each representative forest stand can be considered as spatially independent. The laboratory based seed germination experiment was evaluated using ANOVAs. The post hoc test applied was Fisher’s least significant difference (LSD) method. In order to conform to the assumptions of the parametric tests, percentage data were transformed using the inverse sine transformation, i.e. $X' = \sin^{-1} \sqrt{X}$.

Finally, the data used in modelling germination success come from 144 experimental units measured on PAL and MAJ. We assigned $Y = 1$ if germination was successful (defined as ≥ 10 germinated seeds per m^2), otherwise $Y = 0$.

An appropriate methodology for analyzing binary response data is the logistic model (Neter et al., 1996).

$$\text{logit}(p) = \beta_0 + \beta_1 X_1 + \dots + \beta_k X_k + \varepsilon \tag{1}$$

where $\text{logit}(p)$ corresponds to $\ln[p/(1 - p)]$ and p indicates the probability of the event to occur (that is, $Y = 1$). The X 's are independent variables, the β 's are model parameters (usually estimated by maximum likelihood procedures) and ε is the error term. After being fitted, model (1) transformed into original units becomes:

$$\hat{p} = [1 + \exp(-(\hat{\beta}_0 + \hat{\beta}_1 X_1 + \dots + \hat{\beta}_k X_k))]^{-1} \tag{2}$$

The accuracy of classification by a logistic model is measured by the ability to correctly predict the observed events and the non-events, for a given cut off probability (usually set at $p = 0.5$).

Where successful germination occurs, it is desired to estimate the number of germinated seeds. Poisson regression was used to model number of germinated seeds against the studied factors, stand and site characteristics. Poisson regression differs from linear least squares regression in assuming that at values of the independent variables, the dependent variable is Poisson distributed as opposed to being normally distributed. In addition, the dependent variable is assumed to be a count (discrete variable).

The general form of the Poisson regression model is:

$$\ln G_s = b_0 + b_1 X_1 + \dots + b_k X_k + e \tag{3}$$

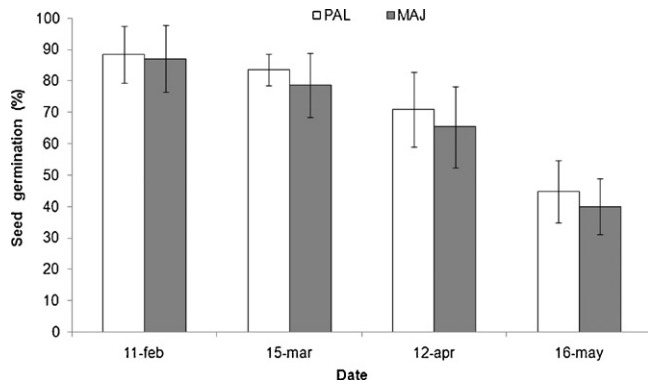


Fig. 2. Seed germination percentage at the end of four weeks for seeds collected before each sowing date during 2006.

where G_s is the response variable (germinated seeds; number per m^2), X_1 to X_k refer to the independent variables, β_0 to β_k are regression parameters and ε is the error term.

The X variables considered for both analyses included meteorological variables (e.g. precipitation, air temperature and humidity values, evaluated at different moments), soil treatment, stand and physiographic characteristics (e.g. basal area, number of trees per hectare, altitude) and time effects (sowing date; seed production year) (Appendix A). Derived variables as well as interactions between main effects were also considered in the data analysis procedures. Candidate models were developed and residual analysis was conducted to examine the model appropriateness. Final model selection among candidate models was based on logical criteria and on the summary statistics of fit criteria, such as the coefficient of determination (R^2) and the standard residual deviation ($S_{y,x}$) for the Poisson regression models and the generalized R^2 statistic (Nagelkerke, 1991) for the logistic model. A 5% significance level was used throughout, unless stated otherwise. Statistical analyses were made with Statgraphics 5.0® and JMP 7.0® software.

3. Results

Results of the laboratory seed germination experiment showed a decreasing pattern of seed germination percentage with time for both experimental sites (Fig. 2). Seed germination percentage was lower for seeds collected before the last sowing date tested (percent of germinated seeds in 16 May were $44 \pm 5\%$ and $40 \pm 4\%$ for PAL and MAJ, respectively) but no existing significant differences were found for the other three sowing dates analyzed (Fig. 2).

In terms of main effects, experimental site, stand density interval, soil treatment and sowing date all significantly affected the seed germination percentage. Experimental site \times stand density was the only significant second-order interaction (Table 1). This

Table 1
Results of the ANOVA test (F -values and P -values) for the effect of experimental site, stand density, soil treatment, and sowing date on seed germination.

Source of variation	DF	F	P
Experimental site (E)	1	23.81	<0.001
Stand density (S)	2	24.75	<0.001
Soil treatment (Y)	1	57.23	<0.001
Sowing date (W)	3	10.84	<0.001
$E \times S$	2	56.38	0.001
$E \times Y$	1	0.26	0.612
$E \times W$	3	2.40	0.072
$S \times Y$	2	2.55	0.083
$S \times W$	6	1.34	0.248
$Y \times W$	3	0.65	0.584

Data were transformed using the inverse sine transformation, i.e. $X' = \sin^{-1} \sqrt{X}$.

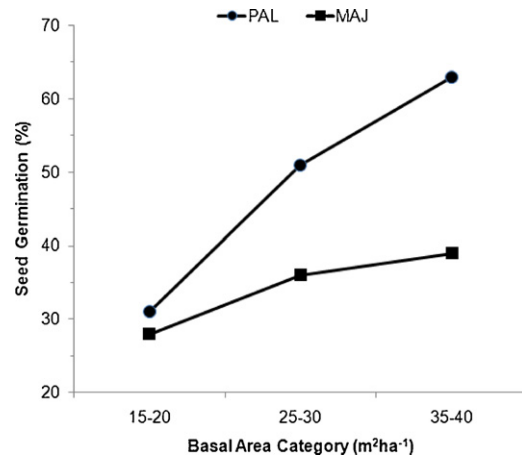


Fig. 3. Experimental site \times stand density interaction effect on the response of Spanish black pine seed germination in PAL and MAJ sites in the Cuenca Mountains, Spain (PAL: Los Palancares y Agregados; MAJ: Ensanche de las Majadas).

interaction reveals some interesting trends and will be examined closely.

Let us look at the results of the main effects. Not surprisingly, seed germination percentage at the end of 2006 was higher in PAL (mean \pm standard error, $40 \pm 2\%$) than in the high elevation site MAJ ($26 \pm 3\%$). For overstorey density, stands with low basal area values ($15\text{--}20 m^2 ha^{-1}$) had significantly lower percentage of germination than stands with medium ($25\text{--}30 m^2 ha^{-1}$) and higher basal area intervals ($35\text{--}40 m^2 ha^{-1}$) (LD: $16 \pm 1\%$; MD: $42 \pm 2\%$ and HD: $43 \pm 2\%$). Soil preparation was also a significant factor, with higher seed germination percentage occurring in scalping subplots ($41 \pm 3\%$) than in subplots without soil treatment ($25 \pm 2\%$). The percentage of germinated seeds was significantly lower for the last sowing date (S4—middle of May) and no differences were found for the other three sowing dates tested (S1: $35 \pm 4\%$; S2: $37 \pm 2\%$; S3: $38 \pm 3\%$ and S4: $22 \pm 2\%$).

The seed germination trend on the basal area categories differed between the two experimental sites resulting in a significant interaction. For Los Palancares y Agregados the values are: LD $31 \pm 1\%$, MD $51 \pm 2\%$, and HD $63 \pm 2\%$. On the high elevation site Ensanche de las Majadas the values are: LD $28 \pm 1\%$, MD $36 \pm 2\%$, and HD $39 \pm 3\%$. We see in Fig. 3a graph of the seed germination trends between the two sites. As is readily seen in the graph the response slope is much steeper on the PAL site versus the MAJ site. At the low density the mean germination values are very similar, only a 3% difference. As we move to the medium density category the mean germination response between the sites is separated by 15%. Finally at the high density the difference is 24%. Most likely environmental and geophysical factors between PAL and MAJ account for the differences.

With respect to the logit model, values (minimum, maximum, mean, standard deviation and number of observations) are presented in Table 2. The probability of success of seed germination was related with light radiation (Rad), which varied from 116.33 to $706.82 \mu mol s^{-1} m^{-2}$ (average $Rad \pm$ standard error; 431.31 ± 28.80 and $425.99 \pm 22.47 \mu mol s^{-1} m^{-2}$ for PAL and MAJ, respectively), soil treatment ($Soil Treat$, coded 0/1 for absence/presence), and the sum of minimum daily air temperatures for the last 30 day period after sowing (Tem_{minAc}), which varied from 23.08 to $132.00^\circ C$ (average $Tem_{minAc} \pm$ standard error; 71.47 ± 4.22 and $83.52 \pm 5.26^\circ C$ for PAL and MAJ, respectively). The logistic function coefficients indicate that the probability of successful germination decreases with greater values of light radiation and with higher sums of minimum daily air temperatures in stands where no soil treatment is the usual practice (Fig. 4). In presence

Table 2

Values (minimum, maximum, mean, standard deviation and number of observations) of the models presented for Ensanche de Las Majadas and Los Palancares y Agregados.

	Minimum	Maximum	Mean	Standard deviation
Ensanche de las Majadas (72 obs.)				
G ($m^2 ha^{-1}$)	20.00	39.00	30.00	7.84
Rad ($\mu mol s^{-1} m^{-2}$)	203.40	666.36	425.99	190.74
Tem_{minAc} ($^{\circ}C$)	23.80	132.00	83.52	44.67
G_s (no seeds)	4.00	93.00	44.36	24.37
Los Palancares y Agregados (72 obs.)				
G ($m^2 ha^{-1}$)	16.95	45.00	34.65	12.72
Rad ($\mu mol s^{-1} m^{-2}$)	116.33	706.82	431.31	244.37
Tem_{minAc} ($^{\circ}C$)	37.50	120.50	71.47	35.85
G_s (no seeds)	2.00	107.00	67.15	47.28

Tem_{minAc} : the sum of minimum daily air temperatures for the last 30 day period after sowing, Rad : light radiation, G : basal area interval, G_s : number of germinated seeds per m^2 .

Table 3

Model parameters and statistics for $\text{logit}(p)$ ($n = 144$).

Variable	Estimate	Std error	Chi square	Prob > Chi square
Intercept	16.094	6.064	7.04	0.0080
Tem_{minAc} ($^{\circ}C$)	-0.021	0.010	4.04	0.0444
Rad	-0.021	0.008	5.99	0.0144
$Soil\ Treat \times Rad$	0.005	0.001	8.79	0.0030

Tem_{minAc} : the sum of minimum daily air temperatures for the last 30 day period after sowing ($^{\circ}C$), Rad : light radiation ($\mu mol s^{-1} m^{-2}$), $Soil\ Treat$: soil treatment coded 0/1 for absence/presence.

of soil treatment, the negative effect of radiation is reduced as evidenced by the positive sign of the interaction term between soil treatment and light radiation ($Soil\ Treat \times Rad$) (Table 3). The logit model correctly predicts the germination success in 94% of the cases, using a cut off probability of 0.5. The generalized R^2 statistic for the logistic model is 0.552.

With regard to the seed germination model, percentages differed between the two experimental sites (Fig. 3). Hence, separate Poisson regression models were fitted, one for each site. In both Los Palancares y Agregados and Ensanche de Las Majadas the number of germinated seeds was positively influenced by the stand basal area (G), soil treatment ($Soil\ Treat$, coded 0/1 for absence/presence) and sowing season ($Seed\ Season$, coded 1 for February, March or April and 0 for May) (Table 4). The positive effect of overstorey density is attenuated in the presence of soil scalping as evidenced by

the negative sign of the interaction term between soil treatment and stand basal area ($Soil\ Treat \times G$) (Table 4). The standard residual deviation ($s_{y,x}$) expressed in the original units is equal to 30.4 plants per m^2 in PAL and 16.2 plants per m^2 in MAJ. The Poisson regression model for PAL explained 61% of the observed variation in number of germinated plants per unit area ($R^2 = 0.608$) whereas the model for MAJ explained 58% of the observed variation ($R^2 = 0.582$).

In Fig. 5 one can see the positive effect of soil scalping for increasing germination as well as increased germination from the early seed season (February–April) and the additive effect of scalping and the February–April seed season (i.e., shift of intercept up the y-axis). Whereas scalping increases the intercept it also decreases the slope. This results in crossover of some of the lines (Fig. 5A and B), that is to say, as basal area increases the soil scalping effect on the (log of) number of germinated plants diminishes. For Ensanche de las Majadas and in stands with low values of basal area, the model estimates a greater number of germinated seeds for scalped soils than in untreated soils in both May and February to April periods (Fig. 5B). Moreover, Ensanche de las Majadas presented higher number of germinated seeds in low values of basal area than Los Palancares y Agregados. However, scalping seems to diminish the effect of stand density for medium and high densities (Fig. 5B).

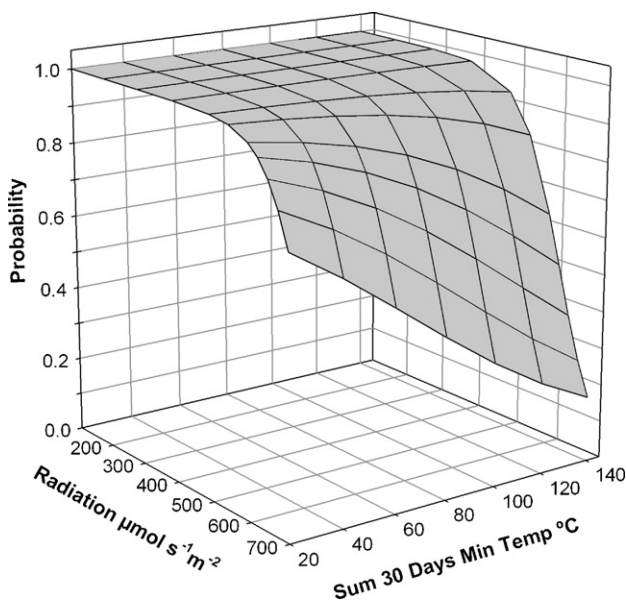


Fig. 4. Logistic regression response surface illustrating probability of successful Spanish black pine seed germination (≥ 10 per m^2) against light radiation and sums of minimum daily air temperatures in the Cuenca Mountains, Spain.

4. Discussion

Seed germination was higher in the laboratory than in the field experiments. This result was expected because field conditions are less favourable than laboratory conditions for seed germination since reasons such as seed predation, herbivore damage, pests and diseases can limit population recruitment (Schupp, 1995; Tiscar Oliver, 2003; Gómez-Aparicio et al., 2005). No differences were found in germination rates from the chamber experiment for seeds collected in Los Palancares y Agregados and Ensanche de las Majadas (see Fig. 2). Thus, differences in seed germination percentage detected in field experiments are necessarily related to environmental and forest conditions. Ensanche de las Majadas is located at the upper altitude limit of the Spanish black pine distribution in the Cuenca Mountains, where environmental conditions are less favourable to initial recruitment (Del Cerro et al., 2009). As Mencuccini et al. (1995) argued, a complex of climatic

Table 4
Models parameters and statistics for the number of germinated seeds using the Poisson regression model at each experimental site.

Local	Variable	Estimate	Std error ^a	Lower CL	Upper CL
Los Palancares y Agregados (72 obs.)	Intercept	1.396	0.125	1.146	1.636
	Soil Treat	1.078	0.146	0.794	1.368
	Seed Season	0.343	0.037	0.271	0.416
	G	0.059	0.003	0.054	0.065
	Soil Treat × G	-0.016	0.004	-0.023	-0.009
Ensanche de las Majadas (72 obs.)	Intercept	1.444	0.142	1.162	1.719
	Soil Treat	1.614	0.163	1.296	1.935
	Seed Season	0.743	0.051	0.644	0.846
	G	0.046	0.004	0.039	0.054
	Soil Treat × G	-0.035	0.005	-0.044	-0.025

G: basal area ($\text{m}^2 \text{ha}^{-1}$), Soil Treat: soil treatment coded 0/1 for absence/presence and Seed Season: sowing season coded 1/0 for February, March, April/May.

^a Approximated values. CL: Asymptotic 95% confidence limits.

and edaphic factors appeared to be responsible for the reduction in initial seedling recruitment along the elevation gradient mainly due to the unfavourable conditions at the upper altitude limit of the species distribution.

Seedling establishment is a critical step for the regeneration of plant populations, both because of the commonly high mortality rates during this stage and because of the potential to alter and fix the spatial and temporal patterns of recruitment (Castro et al., 1999; Greenberg and Parresol, 2002). Many factors are involved in this process although soil temperature and moisture are likely to be the main factors determining and controlling germination under field conditions (Lee et al., 2004; Castro et al., 2005; Del

Cerro et al., 2009). To sum up, the number of germinated seeds of other pine species appears to be largely determined by the interaction of soil temperature and soil moisture (Castro et al., 2005). Indirectly, these factors can be altered through silvicultural operations promoting better conditions to favour initial recruitment (Del Cerro et al., 2005, 2009). Our results indicate that stand density and soil preparation have a positive influence on the number of germinated seeds (Table 4 and Fig. 5). However, the magnitude of the effect depends on conditions at each forest location. Overall, germination was not as profuse at Ensanche de las Majadas (the peripheral population located at the upper altitude limit of the species distribution where rainfall/snowfall is high and the topography is rugged and steep). Scalping allows better water conductivity than organic matter (Riley, 1980; Chrosiewicz, 1987; Béland et al., 1999) and furthermore, the herb layer found in control plots is a physical barrier preventing seeds from contacting the mineral soil (González-Martínez and Bravo, 2001). With respect to stand density, the effect of light, which also affects soil temperature and moisture, is a direct consequence of canopy structure (Catovsky and Bazzaz, 2002; Del Cerro et al., 2009). Lower densities did not show significant differences in germination rate when we compared both forest locations (a nonsignificant 3% difference). However, medium and high basal areas ($25\text{--}40 \text{m}^2 \text{ha}^{-1}$) generate the highest seed germination percentage, obtaining always a higher number of germinated seeds in Los Palancares y Agregados than in Ensanche de las Majadas (Fig. 3).

Factors affecting successful seed germination at a locale (i.e., probability of success) differ from those affecting the number of germinated seeds, although soil treatment is a common variable for both processes. The probability of sufficient seed germination (≥ 10 per m^2) is negatively influenced by solar radiation and by the sum of minimum daily air temperatures for the last 30 day period after sowing. The negative effect of light radiation is reduced by soil scalping. In the Mediterranean basin, soil moisture availability during summer drought is often crucial to the establishment of seeds, and small amounts of water, usually only two to three times the weight of the seed, are necessary to soften seed coats and stimulate metabolic processes (Kozłowski, 2002). Thus, light radiation in combination with soil preparation generates a better response in Spanish black pine seed germination because scalping allows better water conductivity and hydraulic contact between soil particles and seeds than organic matter found in control plots (Riley, 1980; Chrosiewicz, 1987; Béland et al., 1999). Moreover, germination is very responsive to temperature and much higher temperature regimes are required to stimulate the metabolic activity necessary for germination (Kozłowski, 2002). The variable Tem_{minAc} (sum of minimum daily air temperatures) has a negative influence on Spanish black pine seed germination probability indicating that germination under field conditions would take place when the field temperature is higher and enters the thermal range within which germination can occur (Batlla et al., 2003).

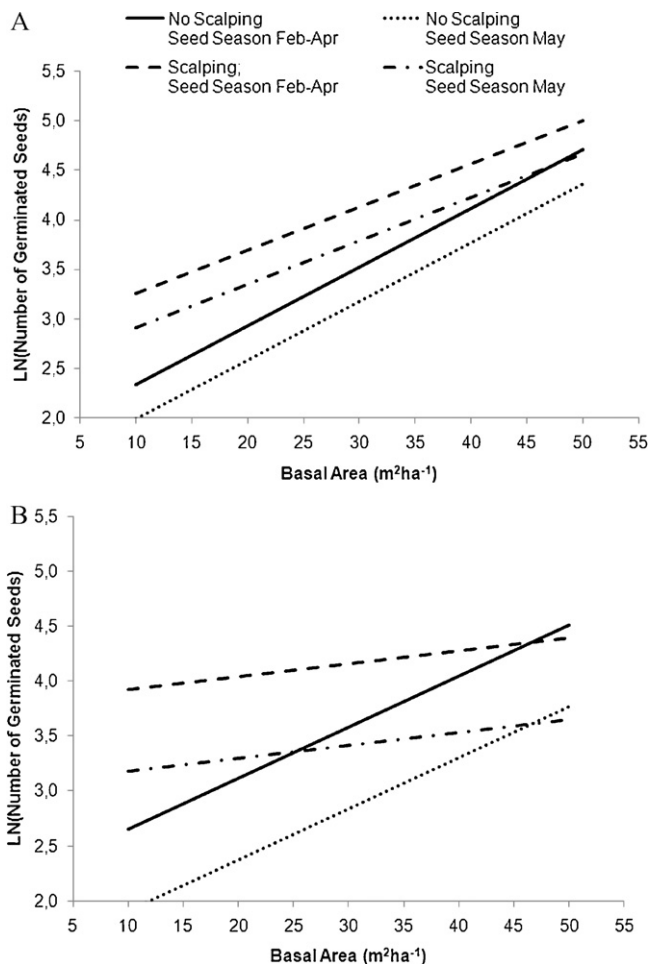


Fig. 5. Spanish black pine seed germination response against basal area in Los Palancares y Agregados forest (A) and in Ensanche de las Majadas forest (B), the Cuenca Mountains, Spain.

With regards to the number of germinated seeds, models presented in Table 4 showed that overstorey density (measured as basal area) and soil treatment are important variables to consider. As can be seen in Fig. 5A and B, soil scalping generally improves germination. However, soil preparation directly affects the basal area influence on seed germination, reducing or even regulating the overall positive density effect. The importance of density is in accordance with other studies carried out in the Cuenca Mountains (Del Cerro et al., 2005, 2009; Lucas-Borja, 2008). Increasing canopy cover reduces radiation and soil temperature, and increases soil moisture and air humidity, which should improve the water status of seed and seedlings (Castro et al., 2005). This fact is especially important in the Mediterranean climate where drought and water availability is one of the most limiting factors for initial seedlings recruitment (Pardos et al., 2005). At the same time, soil preparation can improve water availability as was discussed above. Scalping improves aeration of soil whereas ground litter found in control plots does not warm rapidly and impedes penetration of radicles, thereby preventing contact with mineral soil (Facelli and Pickett, 1991). The presence of an herb layer found in control plots could have delayed or impeded seedling development (Caccia and Ballaré, 1998). Moreover, competition intensity with herbaceous vegetation is likely to increase in environments with low water availability (Davis et al., 1998). However, in higher basal area intervals, where protection from surrounding trees and water availability is higher than in the lower basal area intervals, unaltered soils provide better conditions for seed germination. This agrees with the pattern shown in Fig. 5B. The untreated soils for both sites present lower initial values of number of germinated seeds than the soils with treatment. But after these baseline differences the untreated soils show a steeper increase on the germinated seeds number with increasing values of basal area comparatively to what is observed in the stands with soil scalping.

Sowing date is another important variable to take in account. According to the analysis, seeds sowed in the final sowing period (S4; 16 May 2006) showed a negative influence in relation to the number of germinated seeds whereas no differences were found for the other three sowing dates tested. At the end of the spring season, where temperatures become higher and precipitation is lower, seed germination is more difficult to achieve due to unfavourable conditions (Castro et al., 2004). This pattern was also found in laboratory experiments. The chamber experiments showed that the lowest Spanish black pine seed germination percentage was found for seeds collected at the end of spring season (Fig. 5). Thus disseminated seed at the end of the seed fall period are less likely to germinate than the others disseminated before, from January to the end of April. This fact cannot be also related to the environment and forests conditions since it has been detected in field and laboratory experiments. It has to be related to seed characteristics disseminated at the end of the seed rain period too (e.g. seed resources, immature seeds, empty seeds, etc.). Nevertheless, keeping in mind the results, stands with low density located in marginal locations for the specie might present higher values of germinated seeds later on in the spring season than earlier, depending on the presence/absence of soil treatment. This was shown for Ensanche de Las Majadas (Fig. 5B).

5. Conclusion

This study showed that the amount of current seed germination of Spanish black pine at its upper altitude limit is lower than the densities obtained from the central population. Thus, forest stands of Spanish black pine at the species distribution limit are particularly endangered due to less favourable site conditions. Conservation efforts should focus on these populations and proper

silvicultural operations should be applied. For MAJ, scalping should be recommended especially if stand basal area is low since the effect of soil preparation tend to disappear when stand basal area is higher. In PAL, we recommend scalping combined with medium or higher basal area intervals during the uniform opening of the canopy for regeneration purposes. These areas should be strictly protected to promote germination of this endangered conifer. Moreover, when sowing will be planned in order to obtain higher seedling densities, the last period of the Spanish black pine seed rain season should be generally avoided since seed dissemination at this time showed the lowest seed germination percentages. Results presented in this study are a major step towards the development of Spanish black pine natural regeneration dynamics models that allow simulation and prediction of the ecological consequences of resource levels alterations due to silvicultural practices and environmental changes. The proposed model in Table 3 estimates a reduction of the probability of germination with the increase of minimum daily temperatures, holding daily light radiation and the category of soil treatment variable constant. Studying the regeneration of Spanish black pine provides an understanding of how the ecological conditions affect initial recruitment. Modelling the process may help to suggest strategies for adaptive forest management and forest conservation of endangered habitats.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foreco.2011.03.023.

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