Does the recruitment pattern of Spanish black pine (*Pinus nigra* Arn ssp. *salzmannii*) change the regeneration niche over the early life cycle of individuals?

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A B S T R A C T

The abundance and distribution of Mediterranean tree species are determined mainly during early regeneration stages, when seedlings and saplings are most vulnerable to their immediate environment. Initial recruitment of Spanish black pine (*Pinus nigra* Arn ssp. *salzmannii*) was studied under field conditions in a central population (1200 m a.s.l.) and at the upper elevation limits (1500 m a.s.l.) in the Cuenca Mountains (central-eastern Spain). The goal was to assess the effects of (1) overstory density (low density: $15–20$ m^2 ha^{-1}, medium density: $25–30$ m^2 ha^{-1} and high density: $35–40$ m^2 ha^{-1}), (2) soil preparation (scalping and control), and (3) sowing date (S1: February; S2: March; S3: April; S4: May) on seedling survival and initial seedling development (height, basal diameter and crown volume). Results from this study showed that initial recruitment of Spanish black pine depends on the habitat altitude, on the soil preparation and varies with the density of the stand. Site preparation showed a significant negative influence on initial seedling growth, with greater seedling height and basal diameter in the absence of soil preparation. Sowing date had no significant effect on seedling survival or initial seedling growth. The results from this study are discussed within the framework of ontogenetic niche shifts for plant-regeneration dynamics and in the context of the development of management guidelines to improve the natural regeneration of black pine.

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1. Introduction

European black pine (*Pinus nigra* Arn.) forests are included in Annex I of (Habitats) Directive 92/43/EEC, as the priority habitat type "(Sub-) Mediterranean pine forests with endemic black pines". Playing a key protective role against erosion, these forests serve other purposes such as recreation, landscaping, and timber production (Lucas-Borja, 2008). The main threats against black pine forests include insect defoliation, overgrazing, fires, genetic pollution, and unsustainable harvesting (Zaghi, 2008). The Mediterranean-type ecosystems are predicted to be among the most vulnerable to climate change, which will intensify the already limiting conditions for natural regeneration (Christensen et al., 2007; Gómez-Aparicio, 2008). Spanish black pine (*P. nigra* Arn ssp. *salzmannii*) forests are among the most important forests predicted to be seriously affected by climate change within the Central Iberian Peninsula, with a severely declining distribution range (López-Serrano et al., 2009). This imposes a new set of conditions which need to be addressed under an adaptive forest-management perspective, due to climate instability (IPCC, 2007).

Knowing the initial recruitment characteristics of the species provides crucial information to prescribe the best guidelines for enhancing natural regeneration success (and reduce risks). Regeneration can be enhanced by silvicultural treatments, applying appropriate means to ensure optimum seedling density while promoting favourable soil conditions. However, the complexities involved in the interaction between seedling establishment and site factors, can slow down the regeneration process and make it more unpredictable (Zamora et al., 2001). At early stages of plant survival, seedlings are controlled primarily by abiotic factors such as light, soil moisture, and soil temperature (Silvertown and Lovett-Doust, 1993; Castro et al., 1999). Some biotic factors may intervene at this stage as well, such as overstory density, seed predation, and/or seedling and sapling herbivore (Lucas-Borja, 2008; Lucas-Borja et al., 2011).

The stages of seed germination, seedling survival, and early seedling growth, have been recognized as the most limiting stages to natural regeneration and therefore the determinants of forest structure (Dalling et al., 2002). The recent recognition of ontogenetic niche shifts in plant-regeneration dynamics may provide helpful insights into changes in recruitment patterns. For example, factors positively

affecting germination, such as soil preparation or specific ranges of stand density (Del Cerro et al., 2009; González-Alday et al., 2009; Lucas-Borja et al., 2011), may negatively affect seedling survival or initial seedling growth. Schupp (1995) stated that, following seed dispersal, environmental variation imposes further habitat choice on the plant population since available patches vary in different biotic (i.e. abundance of seed predators, pathogens, competitors) and abiotic factors (i.e. light, water or nutrient availability). This potential niche shifting increases the importance of studying the several regeneration stages individually, thus separating (at least) the seed-germination stage from the seedling-survival stage in order to avoid misleading predictions (Schupp, 1995; Quero et al., 2008).

In recent years, several studies have been conducted within Mediterranean ecosystems related to the natural regeneration mechanisms of its main species, including Pinus sylvestris (Gonzalez-Martinez and Bravo, 2001), P. nigra (Del Cerro et al., 2006, 2009; Tiscar Oliver, 2007; Fyllas et al., 2008; Lucas-Borja et al., 2011), Pinus halepensis (López-Serrano et al., 2005), and Pinus pinaster (Ruano et al., 2009). Most experimental studies that include seedling recruitment have focused on only one stage, except for studies such as Herrera et al. (1994), Jordano and Herrera (1995), Clark et al. (1998), and Uriarte et al. (2005). From recent studies, Tíscar and Linares (2011) affirm that natural regeneration and establishment of P. nigra can be facilitated by the canopies of trees and shrubs. Various other authors have taken the most common approach and studied P. nigra natural regeneration as a single process, from the seed-rain stage to the sapling stage (Serrada et al., 1994; Tiscar Oliver, 2007; Del Cerro et al., 2006, 2009). Little attention has been given thus far to the niche-shifting approach, which has great potential to explore more deeply the conflicts occurring during the early life stages of P. nigra.

In this context, we hypothesise that: (i) Spanish black pine seedling survival and initial seedling growth is favoured by greater overstory density; (ii) site preparation enhances the initial seedling recruitment; (iii) sowing date affects seedling survival; and (iv) changing environmental conditions at the Spanish black pine distribution margin could affect the initial recruitment. To test these hypotheses and due to the masting habit of Spanish black pine, a sowing experiment was designed in order to compile a sound database for the research findings. Seeds collected from each experimental site were used in the sowing experiment. Our specific objectives were to quantify the influences of (1) soil preparation, (2) stand density and (3) sowing date on seedling survival and initial seedling growth over time under field conditions. Studying the early recruitment of plant species growing in marginal areas may provide insights into the distribution patterns that could result from climate change (IPCC, 2007). The comparison among contrasting geographical sites is expected to contribute to a fuller understanding not only of the impact that environmental conditions exert on the natural regeneration stages but also of Spanish black pine regeneration responses to different silvicultural management approaches.

2. Materials and methods

2.1. Field site

The study area is located in the Cuenca mountain range (central-eastern Spain). Spanish black pine, naturally distributed in this area between 1000 and 1500 m a.s.l., dominates the forest stand composition Del Cerro et al., 2009. A dense Spanish black pine forest was selected at 1200 m a.s.l. (Palancares site, 40°01′50″N; 1°59′10″W) to represent the core of the distribution range for P. nigra in the study area. A second experimental site was selected at 1490 m a.s.l. (Majadas site, 40° 14′ 30″ N; 1° 58′ 10″ W) to represent the upper elevation limits of the distribution range for black pine. Here, Scots pine (P. sylvestris) sometimes dominates the forest stand composition,

displacing Spanish black pine to isolated or relict populations that are further fragmented into smaller stands. The two experimental sites lie about 50 km apart. Mean annual temperature, mean highest temperatures of the hottest month, and mean lowest temperatures of the coldest month were, respectively, 9.6 °C, 28.3 °C, and −4.5 °C in Majadas, and 11.9 °C, 30.5 °C, and −0.5 at Palancares. The mean three-month drought-period temperature (June, July and August) is 17.7 °C and 15.7 °C for Palancares and Majadas, respectively. Total annual rainfall is 1137 mm (139 mm during summer) in Majadas, and 595 mm (99 mm during summer) for Palancares Instituto Nacional de Meteorología (2001). Soils can be classified as Typical Xerorthent in Majadas, and as Lithic Haploxeroll in Palancares, according to the Soil Survey Staff (1999). Herbaceous vegetation was composed mainly of Eryngium campestre L., Thymus bracteatus L. and Geranium selvaticum L. in Majadas and by E. campestre L., G. selvaticum L., Centaurea paniculata L., and Plantago media L. in Palancares.

2.2. Experimental design

A factorial design was carried out with four factors: experimental site (two levels), stand-density (three levels), soil preparation (two levels) and sowing date (four levels). At the beginning of 2006, nine representative forest stands of about 0.5 ha were selected at each experimental site, three representing low density: 15–20 m^2 ha^{-1} (LD), three representing medium density: 25–30 m^2 ha^{-1} (MD), and three representing high density: 35–40 m^2 ha^{-1} (HD). A permanent plot of 7 × 10 m was set up in each forest stand (Fig. 1). Plots were fenced to prevent any animals from grazing on seedlings and saplings. Inside of each plot, six 3 × 3 m subplots were divided in order to have three replicates for the scalping treatment (organic matter removed using a hoe from 1 to 2 cm of mineral soil) and for control (soil
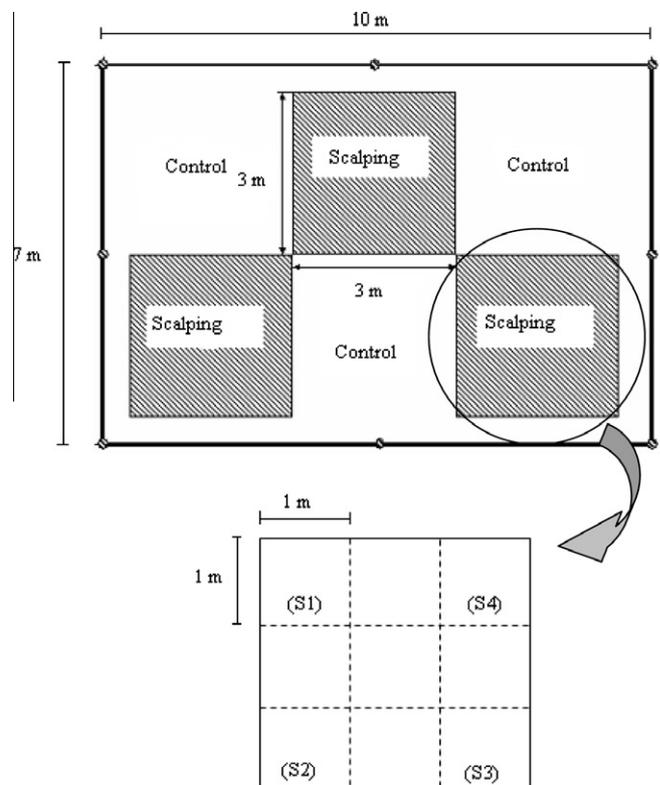
Fig. 1. Experimental design used to quantify the influence of soil preparation, stand density, and sowing date on seedling survival and initial seedling growth under field conditions. Soil treatments were: scalping and control. Sowing dates were: (S1) 11 February; (S2) 15 March; (S3) 12 April; and (S4) 16 May.

without treatment) (Fig. 1). During the natural seed-dispersion period of Spanish black pine in the Cuenca Mountains Del Cerro et al., 2009, four sowing dates were established in 1×1 m sub-subplots within each subplot (3×3 m), where previous soil treatment was assigned (Fig. 1). The 1×1 m sub-subplots were randomly assigned at the vertices of the 3×3 m subplot. The sowing dates were (S1) February 11, 2006; (S2) March 15, 2006; (S3) April 12, 2006; and (S4) May 16, 2006. Sowing dates were replicated three times per soil treatment. Thus, a factorial design was considered with experimental site, soil preparation, a stand density and sowing date as factors. The survey date was considered a split-plot. The seeds used were collected at each experimental site before each sowing date using 30 rectangular seed traps (40 cm long $\times$ 50 cm wide $\times$ 15 cm deep). Seeds naturally dispersed were also considered in order to avoid overestimation or underestimation. Before each sowing date, we calculated the average number of seeds per m^2 naturally dispersed using the seed traps established in each experimental forest and basal-area interval. Then, we completed the number of seeds used at each sub-subplot until reaching the maximum number of seeds naturally dispersed at each sub-subplot for the two experimental sites. The final number of sowed seeds was 107 seeds (c. 2 g of seeds per m^2) at the end of 2006.

2.3. Seedling survival and initial seedling growth measurements

Seedling survival was quantified by counting and labelling all live seedlings in each plot within each subplot (sub-subplot) on different dates: 30-October (2006); 12-October (2007); 12-October (2008) and 3-December (2009). At the end of the experiment (December, 2009), the biometric variables total height (length of the main stem), stem diameter at the base of each seedling (basal diameter), crown length and two perpendicular measures of crown width at the crown base of all surviving seedlings were measured with a calliper (accuracy 0.01 mm). Crown volume was determined using the values of crown length and two perpendicular measures of crown width at the crown base. Climate parameters were recorded daily (at 30-min intervals) in each experimental forest, including air temperature, precipitation, and relative air humidity (from 2005 to 2009) using meteorological stations (model METEODATA 1256C). Climatic variables recorded during the study period are summarized in Table S1 and Figs. S1 and S2 (in the supplementary material).

2.4. Data analyses

Hypothesised differences in seedling survival among experimental sites, stand-density intervals, soil treatments, sowing dates and survey date were tested using a repeated measurement analysis. For the analyses of initial seedling growth, we used multifactor analysis of variance (ANOVA) since seedling measurements were made on one occasion, at the end of the experiment (December 2009). Each representative forest stand was considered spatially independent. A normal probability plot was used to evaluate whether data was normally distributed. The post hoc test applied was the Fisher's Least-Significant Difference (LSD) method. A significance level of $\alpha = 0.05$ was adopted throughout, unless otherwise stated. Statistical analyses used Statgraphics $5.0^®$ and JMP $7.0^®$ software.

3. Results

3.1. Seedling survival and initial seedling growth

Experimental site, soil treatment, stand-density interval, and survey date were statistically significant ($P < 0.05$) with respect to the total values of seedling survival whereas sowing date had no significant effect ($P > 0.05$) (Table 1). Factors tested showed significant interactions affecting seedling-survival percentages. Experimental site $\times$ stand-density interval, and soil preparation $\times$ stand-density interval, were the only significant ($P < 0.05$) second-order interactions with respect to the total values of seedling survival (Table 1). The same factors and second order interactions had a significant ($P < 0.05$) effect in the first growing year (2006) (Table 1). Contrary to this fact, experimental site and the second order interaction soil preparation $\times$ stand-density interval were the only statistically significant ($P < 0.05$) effects with respect to seedling survival in the second (2007) and third (2008) growing years (Table 1). In 2009 (forth growing year), the soil preparation x stand-density interval interaction was the only statistically significant effect. Results showed that seedling-survival percentages were always higher in Palancares (mean ± standard error; $11.1 ± 1.2\%$, $2.4 ± 0.9\%$, $2.0 ± 0.7\%$ and $1.1 ± 0.7\%$) than in Majadas (mean ± standard error; $4.8 ± 0.9\%$, $1.4 ± 0.8\%$, $0.6 ± 0.7\%$ and $0.5 ± 0.3\%$) for the period of study (2006, 2007, 2008 and 2009, respectively) (Fig. 2). With respect to the soil preparation factor, higher seedling-survival percentages were obtained for scalped subplots in 2006 (mean ± standard error; $11.4 ± 1.01\%$, $4.8 ± 0.9\%$, for scalped and control plots respectively) (Fig. 3). Differences between soil preparation were weaker and nonsignificant during the following years (mean ± standard error; $2.2 ± 1.0\%$, $1.6 ± 0.8\%$ for scalped and control subplots, respectively in 2007 and $1.3 ± 1.0\%$, $1.2 ± 0.5\%$ for scalped

Table 1
Results of the repeated measurement analysis for the effects of experimental site (Palancares and Majadas), soil preparation (scalping and control), stand density (LD: 15–20 m^2 ha^{-1}, MD: 25–30 m^2 ha^{-1}, and HD: 35–40 m^2 ha^{-1}), sowing date (S1: 11 February 11; S2: March 15; S3: April 12, and S4: May 16, in 2006) and survey date (October 30, 2006, October 12, 2007, October 12, 2008, and December 3, 2009) on seedling survival. In the table, degrees of freedom (DF), F-ratio (F) and level of significance for the principal effects, second order interactions, effects within each year and second order interactions are represented. Third-order interactions were always not significant.

Effects	DF	Total values	Years			
		F	2006 (F)	2007 (F)	2008 (F)	2009 (F)
Experimental site (E)	1	23.24***	12.98***	3.94*	10.81**	3.04 ns
Soil preparation (Sp)	1	15.91***	15.74***	0.74 ns	0.10 ns	0.88 ns
Stand density (Sd)	2	17.48***	19.07***	0.46 ns	0.40 ns	0.44 ns
Sowing date (Sw)	3	2.23 ns	2.15 ns	0.24 ns	0.30 ns	0.23 ns
Survey date /Sw/Sd/Sp/E	3	3.41***	–	–	–	–
E × Sp	1	1.35 ns	3.18 ns	1.11 ns	0.32 ns	0.10 ns
E × Sd	2	4.17*	6.86**	1.35 ns	0.45 ns	1.78 ns
E × Sw	3	0.54 ns	0.46 ns	0.24 ns	0.37 ns	0.27 ns
Sp × Sd	2	21.31***	14.82***	5.03**	5.70**	3.25*
Sp × Sw	3	1.17 ns	0.42 ns	1.44 ns	0.66 ns	1.00 ns
Sd × Sw	6	1.18 ns	0.57 ns	0.39 ns	0.69 ns	1.45 ns

Significance: ns, not significant.
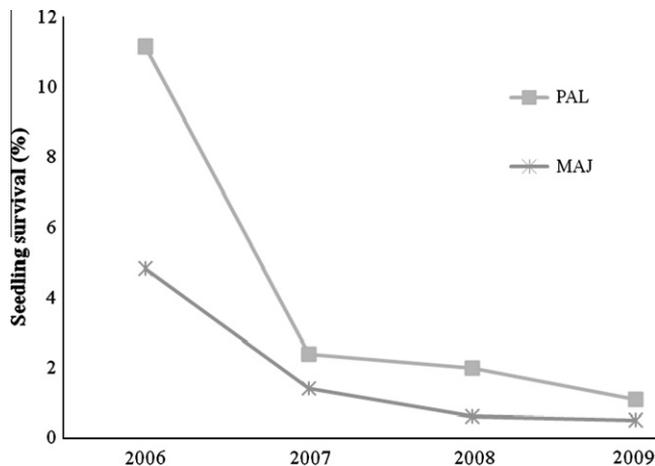* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Fig. 2. Seedling-survival percentage calculated for both experimental sites (MAJ: Majadas and PAL: Palancares) during the period of study (2006–2009).
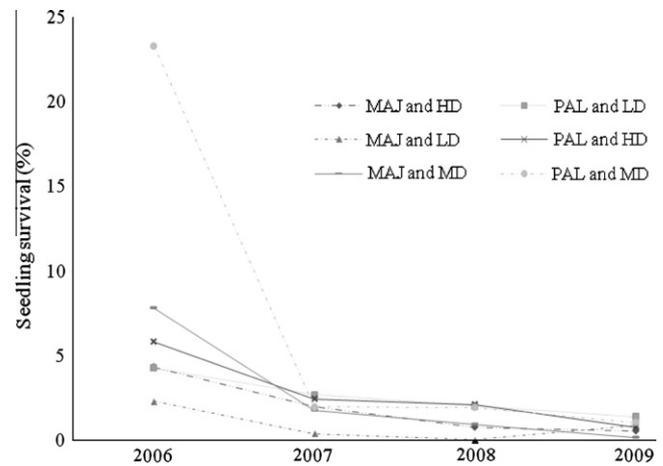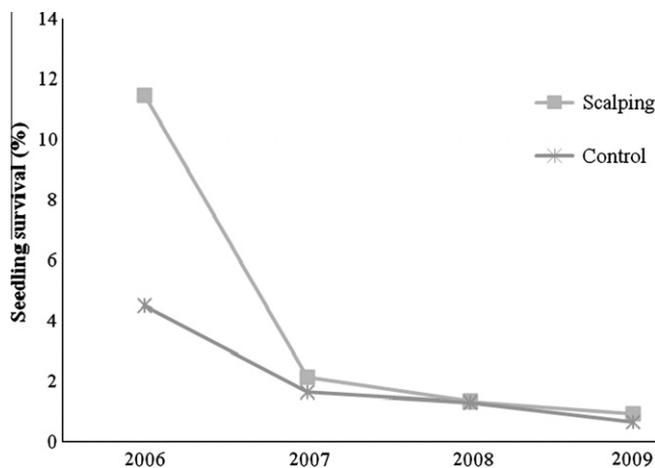

Fig. 3. Seedling-survival percentage calculated for each soil preparation (scalping and control) during the period of study (2006–2009).
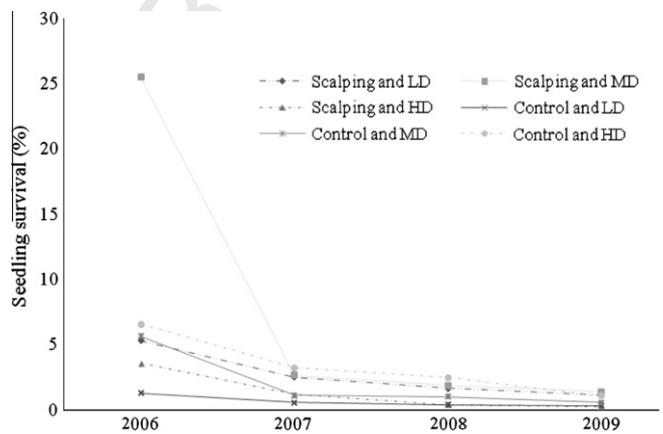

Fig. 4. Seedling-survival percentage calculated for each basal-area interval (LD: 15–20 m^2 ha^{-1}, MD: 25–30 m^2 ha^{-1} and HD: 35–40 m^2 ha^{-1}) and experimental site (MAJ: Majadas and PAL: Palancares) during the period of study (2006–2009).

Fig. 5. Seedling-survival percentage calculated for each basal area interval (LD: 15–20 m^2 ha^{-1}, MD: 25–30 m^2 ha^{-1} and HD: 35–40 m^2 ha^{-1}) and soil preparation (scalping and control) during the period of study (2006–2009).

and control subplots, respectively in 2008), presenting the lowest percentage at the end of 2009 (0.9 ± 0.9%, 0.7 ± 0.4% for scalped and control subplots, respectively) (Fig. 3).

Seedling-survival percentages found at Palancares in 2006 were (mean ± standard error) 4.2 ± 0.9%, 23.3 ± 6.9% and 5.8 ± 0.8% at the lower, medium and higher basal-area intervals, respectively. The same trend but with lower seedling-survival percentages were found in Majadas (mean ± standard error) 2.3 ± 0.5%, 7.8 ± 1.0% and 4.3 ± 0.6% at the lower, medium and higher basal-area intervals, respectively (Fig. 4). As is shown in Fig. 4, differences between basal area intervals and both experimental sites were weaker in 2007 and 2008 (i.e. second and third summer periods, respectively). At the end of the experiment (December 2009), seedling-survival percentages found in Palancares were (mean ± standard error) 1.5 ± 0.4%, 1.1 ± 0.3% and 0.8 ± 0.4% at the lower, medium and higher basal-area intervals, respectively. In Majadas, seedling-survival percentages were (mean ± standard error) 0.9 ± 0.1%, 0.2 ± 0.3% and 0.6 ± 0.3% at the lower, medium and higher basal-area intervals, respectively (Fig. 4).

For the first growing season (2006), soil-preparation subplots located at medium basal-area intervals presented higher seedling-survival percentages (mean ± standard error: 25.5 ± 7.8%) than scalped subplots at low (mean ± standard error: 5.3 ± 0.9%) or higher (mean ± standard error: 3.5 ± 0.8%) basal-area intervals and control plots at low (mean ± standard error: 1.2 ± 0.7%), medium (mean ± standard error: 5.6 ± 0.9%) and high (mean ± standard error: 6.8 ± 0.8%) basal-area intervals (Fig. 5). Seedling survival percentages dramatically decreased from 2007 to 2009 (i.e. for the second, third and forth summer periods), showing soil-preparation subplots located at medium basal-area intervals the statistically significant (P < 0.05) highest values (mean ± standard error: 1.6 ± 0.5%) at the end of the experiment (2009). For this date, seedling-survival percentages were (mean ± standard error) 0.8 ± 0.5% for scalped subplots at low basal-area intervals; 0.2 ± 0.1% for scalped subplots at higher basal-area intervals, 0.3 ± 0.2% for control subplots at low basal-area intervals, 0.5 ± 0.4% for control subplots at medium basal-area intervals and 0.8 ± 0.5% for control subplots at higher basal-area interval.

With regard to initial seedling growth, seedling height and basal diameter were significantly influenced by experimental site, soil treatment and stand-density interval (P < 0.05). Crown volume was affected only by basal-area interval (P < 0.05). Sowing date did not influence any factor, from the variables herein considered for initial seedling growth (Table 2). Spanish black pine seedling height and basal diameter tended to be greater in their common habitat (Los Palancares and Agregados) and on the undisturbed forest floor (Fig. 6). Significant first-order interaction between the

Table 2

Results of the four-factor ANOVA (F-values) for the effects of experimental site (Palancares and Majadas), soil preparation (scalping and control), stand density (LD: 15–20 m^2 ha^{-1}, MD: 25–30 m^2 ha^{-1}, and HD: 35–40 m^2 ha^{-1}), sowing date (S1: 11 February, S2: 15 March, S3: 12 April, and S4: 16 May in 2006) on seedling height, basal diameter and crown volume at the end of the experiment. In the table, degrees of freedom (DF), F-ratio (F) and level of significance for the four principal effects and second order interactions are represented. Third-order interactions were always not significant.

Effects	DF	Height (F)	Basal diameter (F)	Crown volume (F)
Experimental site (E)	1	109.01[***]	83.88[***]	1.92 ns
Soil preparation (Sp)	1	8.01[**]	8.25[***]	1.53 ns
Stand density (Sd)	2	185.83[***]	286.48[***]	112.93[***]
Sowing date (Sw)	3	2.60 ns	2.37 ns	1.22 ns
E × Sp	1	16.03[***]	0.24 ns	1.27 ns
E × Sd	2	21.67[***]	72.23[***]	30.33[***]
E × Sw	3	1.24 ns	0.78 ns	1.47 ns
Sp × Sd	2	19.99[***]	11.94[***]	11.94[***]
Sp × Sw	3	0.73 ns	2.05 ns	1.10 ns
Sd × Sw	6	1.84 ns	0.92 ns	0.50 ns

Significance: ns, not significant; $^*P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.
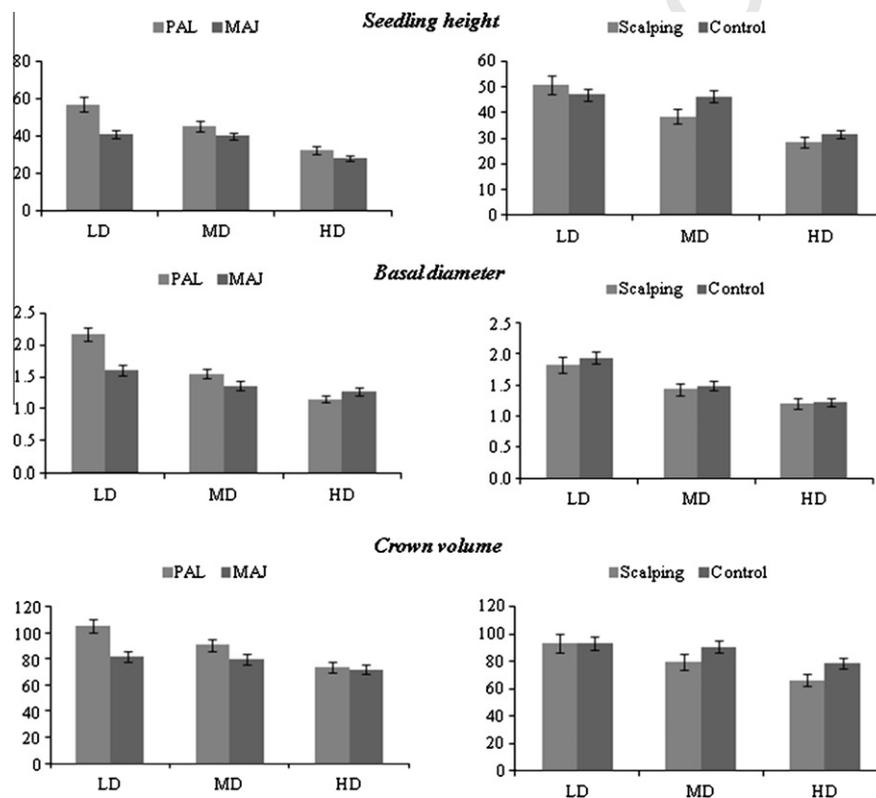
Fig. 6. Spanish black pine seedling dimensions (seedling height, basal diameter and crown volume) calculated for each basal area interval (LD: 15–20 m^2 ha^{-1}, MD: 25–30 m^2 ha^{-1} and HD: 35–40 m^2 ha^{-1}) and soil preparation (scalping and control) at both experimental sites (MAJ: Majadas and PAL: Palancares) at the end of the experiment (December 2009). Error bars indicate the standard error.

experimental site or soil preparation and stand density occurred for the 3 biometric variables analyzed (Table 2). These interactions agree with the general trends shown in Fig. 6, i.e. of undisturbed forest floor and lower basal-area interval or lower basal-area interval in the common species habitat for Spanish black pine (Palancares), resulting in greater seedling height. With respect to seedling height, experimental site x soil preparation were statistically significant ($P < 0.05$) (Table 2).

In relation to the climatic variables (Supporting Information, Table S1 and Figs. S1 and S2), daily mean air temperature (AT), daily mean maximum air temperature (AM), and daily mean precipitation (P) differed for each experimental site ($P < 0.05$) during the study period (from 2005 to 2009). The rest of the climate variables showed no statistically significant differences. Moreover, no differences were found among the years from 2005 to 2009. First-order interactions were not significant for the climatic variables tested.

4. Discussion

The micro-site suitability for a seed emergence may depend on patch conditions when the seed arrives (i.e. seed predation or soil preparation), while suitability for a seedling growth can be related to other different conditions, e.g. light on the forest floor or canopy cover (Schupp, 1995). Consequently, it is possible to find a shifting

niche, where the most suitable micro-sites for seed rain or emergence are not the most suitable for seedling growth, while non-optimal micro-sites for seeds are progressively more suitable for seedlings. Thus, a shifting niche implies conflicts involving the most suitable micro-site conditions for seed emergence and survival, and the most suitable conditions and factors for seedling growth (Schupp, 1995). Previous research by the same authors participating in the current analyses, and using the same experimental design (Palancares and Majadas), showed that soil preparation and high basal-area intervals result in higher seed-germination percentages for the central Spanish black pine population (Lucas-Borja et al., 2011). Environmental conditions at the core of the elevation range appear to be the most suitable for Spanish black pine forests, when compared to the upper altitudinal limits of distribution. Scalping promotes additional suitable conditions by allowing better water conductivity when compared to the organic matter found in control plots (Béland et al., 1999). Moreover, light on the forest floor is a direct consequence of canopy structure and stand density (Catovsky and Bazzaz, 2000), and may adversely affect soil temperature and moisture, which in turn are likely to be the two main factors determining and controlling germination under field conditions (Brown and Neustein, 1972; Lee et al., 2004; Castro et al., 2005). Field experiments conducted on the forest floor under pine stands have indicated that germination depends on the soil–water content (Lee et al., 2004). Overall, however, the timing and rate of germination of other Spanish pines appear to be determined largely by the interaction of soil temperature and soil moisture (Castro et al., 2005).

Seedling survival is one of the most critical stages in a plant's life history, and is often reduced by drought and high temperatures in the Mediterranean basin (Madrigal et al., 2004; Padilla and Pugnaire, 2007), where water deficit in the summer appears to be the dominant cause of seedling mortality (Valladares, 2004). According to our findings, Spanish black pine seedling survival was very low at the end of the experiment (from Figs. 2–5), this representing a problem for sustainable forest management due to the lack of natural regeneration. Contrary to the seed germination results found by the authors for the studied forests using the same statistical design, seedling survival in the present study was favoured by lower basal-area interval, with no difference found between medium and higher basal-area intervals for December, 2009. Simard et al. (1998) stated that increased light favours a longer tap root and longer shoots in seedlings, enabling them to penetrate the soil and survive better. When the roots of Spanish black pine seedlings extend deeper into the soil, they take up sufficient water to increase the chances of seedling survival when the first growing season is compared to others (Lee et al., 2004). At the same time, scalping initially boosted survival percentages, but this trend did not continue later, as no difference was found at the end of the experiment between scalping and control subplots. A probable explanation is that scalped ground may be covered by new vegetation and needles that may compete for soil resources within 2–3 years, in agreement with the findings of Béland et al. (1999).

Spanish black pine seedlings presented a clear trend showing the lowest growth at the marginal distribution of their population (Majadas) and the highest growth in its most common and favourable habitat (Palancares). According to the seedling-survival trends and contrary to patterns observed for the seed germination process, lower basal-area densities promoted higher seedling height and basal diameter; thereby supporting several studies suggesting that stronger light exerts a positive effect on seedling growth (e.g. Madsen and Larsen, 1997; Collet et al., 2001; Lee et al., 2004). In the Spanish black pine seedlings, initial growth allocation to their root system and deeper penetration may generate more vigorous initial seedling growth under canopy openness (Simard et al., 1998; Del Cerro et al., 2006). This result also agrees with other studies and reflects the patterns of various other species growing under similarly harsh conditions (Fisher and Binkley, 1999). Fyllas et al. (2008) reported canopy openness and drought pressure to be the most important factors controlling regeneration density of *P. nigra* ssp. *pallasiana*.

Higher seedling height and basal diameter were found in control plots. Thus, while mineral–soil exposure enhances seed germination and seedling survival, it also seems to reduce overall seedling growth when compared to the undisturbed forest floor. This may be explained by the fact that Spanish black pine seedlings may need to allocate more resources to root expansion in order to avoid competition from ground vegetation and attain sufficient water and resources (Del Cerro et al., 2006). This may explain the positive influence of smaller basal-area intervals, promoting greater seedling height and basal diameter; and thus helping to overcome the negative effects of water deficit and drought.

Ontogenetic niche shifts constitute an ecological concept that can be applied in order to optimise management and restoration programmes involving woody species such as *P. nigra*. Effective restoration activities may broaden the regeneration niche through assisted establishment, since it is strongly needed to ensure viable populations of *P. nigra* in areas with reduced regeneration. This can be achieved by sowing seeds in microhabitats with higher basal-area intervals and a disturbed deep litter layer, promoting both bare ground and dense tree cover in order to increase seed germination. However, once seeds have germinated, tree density needs to be reduced without damaging living seedlings in order to favour seedling survival and early seedling growth. The spatial pattern of *P. nigra* establishment shifts ontogenetically with the stand basal area as the main factor influencing this change.

5. Conclusions

The factors studied (stand density, soil preparation, and experimental site) significantly influenced the recruitment of Spanish black pine. This process appears to be less consistent at the ecological limits for the species, where climatic conditions may not result in the proper conditions for promoting natural regeneration. Data from this study show that Spanish black pine in central-eastern Spain faces conflicting situations among suitable conditions for seed germination and suitable conditions for seedlings survival or for early seedling growth. The effect of the overstory was summarized as being positive for seed germination (as described by Lucas-Borja et al., 2011) but negative for seedling survival and initial seedling growth. Site preparation significantly influenced seed germination, which was encouraged by scalping. At the end of the experiment, seedling survival was no longer significantly affected by soil preparation.

Knowledge of the processes and factors influencing natural regeneration is essential for its success. In this case, optimal forest management should be a compromise between a higher basal-area interval at the beginning of the process and scalping to promote the number of germinated seeds and a lower basal-area interval and undisturbed forest floor in order to maximize early seedling growth. This is not easy to implement in practice, indicating the need of specific silvicultural procedures such as stratifying the forest areas for soil preparation in patches to promote the best seed-germination conditions, and later using selective thinning for density control (basal area) at the earlier stages of the seedlings growth.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foreco.2012.07.042.

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