Modelling Spanish black pine postdispersal seed predation in Central-eastern Spain

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Abstract

In this research work, we specifically address post-dispersal Spanish black pine (*Pinus nigra* Arn. ssp *salzmannii*) seed predation at two different locations, one being the most common and representative location for the species and the other located on areas at their ecological limit of distribution in Cuenca Mountain (Spain). A modelling approach was employed to analyze the effect of stand and site characteristics and climate variables on the post-dispersal predation process. Each predator group (ants, rodents and birds) was evaluated by excluding the other two with different experimental devices. Our study demonstrates that the percent of removal was related with the seed rain patterns being lower in the high seed rain year of 2006 (mean $\pm$ se $11.15 \pm 3.7\%$ of removed seeds) than in the low seed rain years of 2005 and 2007 ($81.71 \pm 6.0\%$ and $87.29 \pm 6.6\%$ of removed seeds respectively). In high seed production years, birds were the most important predators and rodents were the less important predator group in both Los Palancares y Agregados and Ensanche de Las Majadas. No conclusion can be obtained in low seed production years due to the high seed removal percentage by all predator groups. Experimental site and overstory density were no significant with respect to seed predation. Modelling approach show that the seed removal percentage is influenced by seed production year (*MastY*) a temporal effect within the year, measured by the day variable (*Day*) and a climatic variable (accumulated averaged maximum air temperatures 20 days before survey date, *AaMAT*). Further developments include the construction of stochastic models to predict the effects of climate-related variables or microhabitat characteristics on seed losses, contributing to reduce uncertainty in post-dispersal Spanish black pine seed predation dynamics and to provide credible tools to decision-makers and forest managers.

Key words: mast year; post-dispersal seed predation; *Pinus nigra*; Mediterranean region.

Resumen

Modelización de predación post-dispersión de semillas de pino laricio en el Centro-este de España

El presente trabajo de investigación se centra en la predación post-dispersión acontecida en dos áreas forestales, una localizada en la zona de distribución característica del pino laricio (*Pinus nigra* Arn ssp *salzmannii)* y otra en su límite ecológico de distribución dentro de la Serranía de Cuenca (España). El efecto que sobre la predación post-dispersión tienen la densidad arbórea, diferentes variables climáticas y varias características de cada masa forestal fue evaluado mediante la creación de diferentes modelos. Cada grupo de predadores (hormigas, ratones y pájaros) fue analizado por exclusión de los otros dos mediante diferentes dispositivos experimentales. Nuestro estudio demuestra que el porcentaje de semilla predada estuvo relacionado con la lluvia de semillas, siendo menor en 2006, año de abundante producción (media $\pm$ estandar error: $11.15 \pm 3.7\%$ de semillas predadas) que en 2005 y 2007, años de escasa producción de semilla ($81.71 \pm 6.0\%$ y $87.29 \pm 6.6\%$ de semillas predadas respectivamente). En años de abundante producción de semilla, los pájaros fueron el grupo de predadores más importantes y los ratones el menos importante en ambos sitios, Los Palancares y Agregados y Ensanche de las Majadas (*P < 0.05*). En años de escasa producción de se-

milla no fue posible extraer conclusiones debido al alto porcentaje de predación que tuvieron los tres grupos de predadores. La zona forestal y la densidad de la masa adulta no generaron un efecto significativo sobre la predación de semillas. Los procesos de modelización demostraron que el porcentaje de semilla predada esta influenciado por la producción de semilla (*MastY*), un efecto temporal dentro de cada año medido por la variable diaria (*Day*) y una variable climática (suma acumulada de las temperaturas máximas en los 20 días previos al conteo de predación, *AaMAT*). En el futuro, se hace necesaria la construcción de modelos estocásticos que predigan el efecto de las variables climáticas o las características de cada microhábitat sobre la predación de semillas, contribuyendo así a reducir el desconocimiento existente en relación a las dinámicas de predación del pino laricio y generando herramientas que ayuden a los gestores de estos montes.

Palabras clave: vecería; predación post-dispersión; *Pinus nigra;* región mediterránea.

Introduction

The Mediterranean area is a well-defined bio-geographical region (Di Castri and Mooney, 1973) where pines are an important, and very often dominant, component of the vegetation generating a multiplicity of products and services for society (Richardson, 1998). *Pinus nigra* Arn is distributed in mountain pines from areas surrounding the Mediterranean (Klaus, 1989) and their populations are divided into several subspecies and numerous varieties. According to different authors (Regato and Escudero *et al.,* 1989; Blanco, 1998; Richardson, 1998) Spanish black pine (*Pinus nigra* Arn. ssp. *salzmannii*) is mainly distributed and established in Spain. Those 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) mainly due to the lack of natural regeneration and the high levels of environmental values.

Forest regeneration is a relatively slow process, with the initial stages of the plant life cycle being critical for natural regeneration dynamics (Harper, 1977). Seed mortality due to predation by animals may affect seedling recruitment, population structure and species composition in forest communities (Schupp, 1990; Ordóñez and Retana, 2004). Moreover, the generalist feeding habits of many post-dispersal seed predators (Hulme, 1998; Fedriani and Manzaneda, 2005) and the limited ability of plants either to compensate for or to respond to post-dispersal seed losses directly suggest that post-dispersal seed predation may have a considerable impact on plant populations (Hulme, 1998).

Post-dispersal seed predation arises once seeds have been released from the parent plant (Fedriani and Manzaneda, 2005). On this moment, ants, birds and rodents have been described as the main post-dispersal seed predators although experimental studies reveal marked differences in both the overall magnitude of post-dispersal seed predation and the relative importance of different guilds of seed predators (Edwards and Crawley, 1999; Hulme, 1998; Hulme and Hunt, 1999; McShea, 2000; Nystrand and Granström, 2000; Alcántara *et al.,* 2000). Ordóñez and Retana (2004) concluded that post-dispersal seed predation by ants, birds and rodents consumed many of the P. *nigra* seeds remaining in different time-since-fire habitats. However, little is known about post-dispersal seed predation in Spanish black pine natural forest ecosystems. Changes in general patterns of post-dispersal seed predation are expected since the predation of these groups of animals may be very variable depending on seed production (Castro *et al.,* 1999; Du *et al.,* 2007), site characteristics (Hulme, 1997; Holt *et al.,* 1995; Ostfeld *et al.,* 1997; Christianini and Galetti, 2007) and forest stand conditions (Hulme, 1998; Bellocq *et al.,* 2005; Christianini and Galetti, 2007; Pérez-Ramos and Marañon, 2008). Seed predation can also depend on masting. The synchronous production of large seed crops, separated by years of greatly reduced seed production is an evolutionary response to seed predation (Kelly, 1994; Li and Zhang, 2007). By storing resource during some years, mast-seeding species can produce massive crops in other years, which swamp local seed eaters with food and allow seed to escape predation successfully establish (Sork, 1993; Li and Zhang, 2007). This hypothesis is one of the best established functional explanations for the mast seeding phenomenon (Kelly, 1994; Kelly and Sork, 2002; Li and Zhang, 2007). On this context, little is known related to post-dispersal seed predation and Spanish black pine masting condition.

Seed removal can also vary spatially because predator and disperser foraging is not random, but usually responds to preferences for certain microhabitats and rejection of others (Pérez-Ramos and Marañon, 2008). Stand density affect microenvironment conditions such as surface soil temperature, soil moisture and atmospheric conditions (Dey and MacDonald, 2001), which

may influence seed predators development and abundance, and thus post-dispersal seed predation (Bellocq *et al.,* 2005). For example, Hulme (1998) found that ants foraged primarily in open microhabitats whereas rodents were least active in open microhabitats. Fedriani and Manzaneda (2005) referred that seeds located in sheltered microsites experienced higher removal by rodents supporting the view that this group respond behaviourally by lessening foraging on microhabitats lacking cover due to higher associated risk of predation. With respect to birds, Ordoñez and Retana (2004) found that bird predation increased with herbaceous cover in burned forest areas. Moreover, seed predation may higher in small, isolated, fragmented or relict forest populations instead of a continuous homogeneous population (Castro *et al.,* 1999; Burgos *et al.,* 2008). Thus, forest stand characteristics and site conditions are expected to affect post-dispersal seed predation although more research work is needed in Spanish black pine natural forest areas. Also, few studies has been developed on the topic of Spanish black pine seed rain and postdispersal seed predation (Ordóñez and Retana, 2004; Tíscar, 2007; Lucas-Borja, 2008).

Seed predation can limit population recruitment not only by a quantitative effect of reducing seed availability but also by a qualitative effect of changing the spatial distribution of seed in the initial seed rain (Castro *et al.,* 1999). Wind or dispersers play an important role by disseminating different numbers of seed in a variety of microhabitats, and thus either influence the spatial distribution of the seedlings or limit recruitment by the deposition of large numbers of seeds in places that are unsuitable for recruitment (Rey and Alcántara, 2000) or where predators can have higher activity and access. With respect to Spanish black pine, seed predation by ants, birds and rodents have rarely been considered in assessing the conservation status of this endangered forest ecosystems although the impact of seed predation has been recognised as an important factor in shaping the structure and composition of plant communities (Brown *et al.,* 1979; Louda, 1982; Heske *et al.,* 1993; Hulme, 1997, 1998).

The current paper focuses on the analysis of different factors that might affect the Spanish black pine post-dispersal seed predation process by the three main predator groups (ants, birds and rodents). These include production year, forest site, stand density and meteorological variables. We hypothesized that the relative proportion of seeds removed on the forest floor by ants, birds and rodents is related to seed production year

(masting or non masting), forest site (A continuous homogeneous Spanish black pine forest and other located at the ecological limit of their distribution in Cuenca Mountain), stand density and climatic conditions of each experimental forest. We used a selective method where each predator group was evaluated by excluding the other two with different experimental devices. A modelling approach was used in order to analyse the effect of environmental conditions and forest stand characteristics on seed removal.

Material and methods

Field site

The study area is located in the region of Castilla-La Mancha (Central-eastern Spain). A continuous homogeneous Spanish black pine experimental forest was selected at the most common and representative location of this specie in Cuenca Mountain (Los Palancares y Agregados, 1,200 m above sea level, 40° 01' 50" N; 1° 59' 10" W). Spanish black pine is naturally distributed on this area between 1,000 and 1,500 m above sea level and dominates the forest stand composition (Del Cerro *et al.,* 2009). A second experimental forest was selected at the ecological limit of their distribution in Cuenca Mountain (Ensanche de las Majadas, 1490 m above sea level, 40° 14' 30" N; 1° 58' 10" W) (Fig. 1) where sometimes Scots pine (*Pinus sylvestris*) dominates the forest stand composition, displacing Spanish black pine to isolated or relict populations that are further fragmented into smaller stands. The experimental sites are separated by 50 km. According to Allué (1990) the climate characteristics of these experimental forests can be classified as Mediterranean humid climate, although in Los Palancares y Agregados present lower annual precipitation and higher temperatures (Table 1). Regarding soil classification, *Typical Xerorthent* is present in Ensanche de las Majadas and *Lithic Haploxeroll* can be found in Los Palancares y Agregados, according to Soil Survey Staff (1999).

Experimental design

Different stand densities, expressed by basal area per hectare, were selected on each experimental site (Los Palancares y Agregados and Ensanche de las Majadas) in order to study the effect of stand density
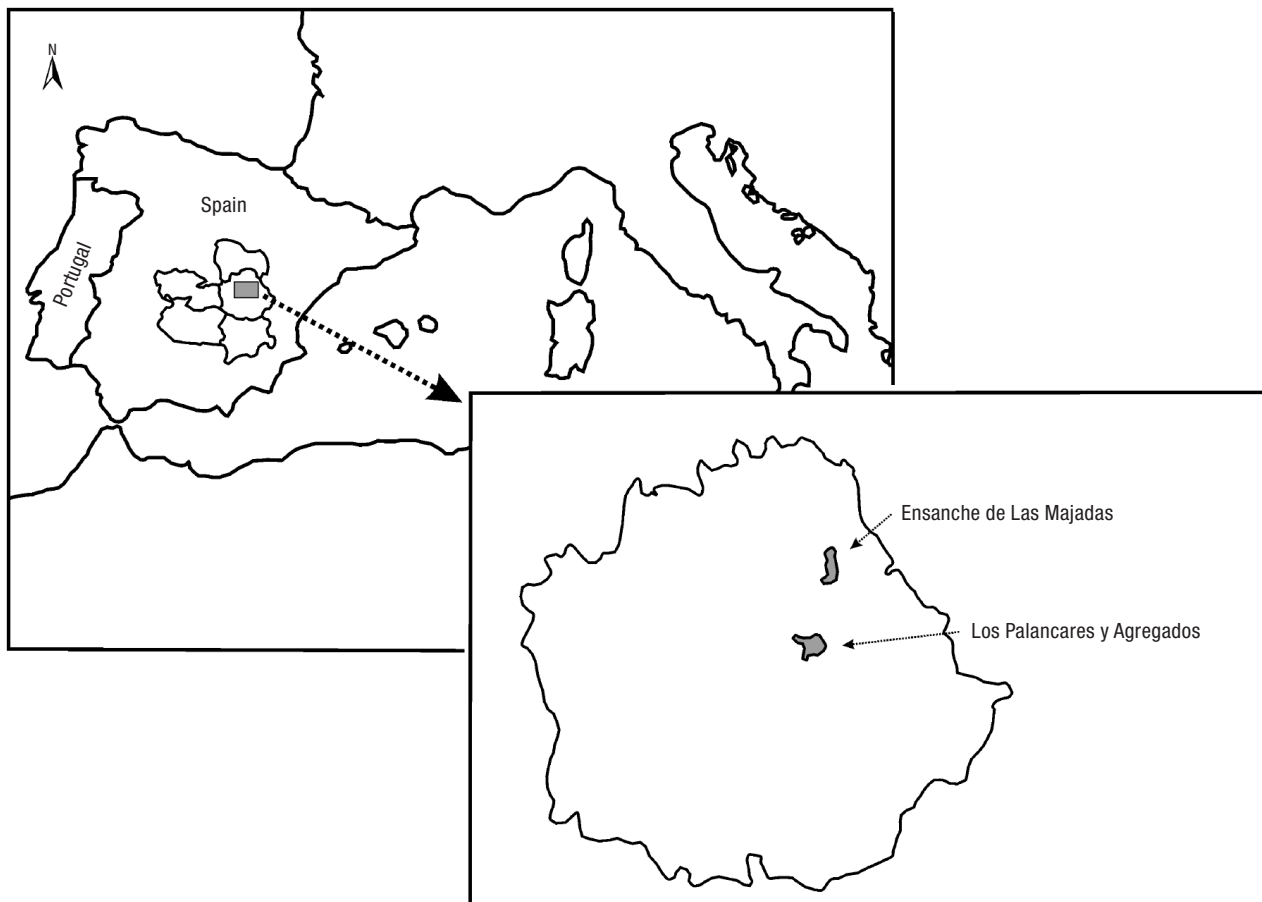
Figure 1. Study area.

on post dispersal seed predation. The basal area intervals of 15-20 m^2 ha^{-1}, 25-30 m^2 ha^{-1} and 35-40 m^2 ha^{-1} were assigned to represent low, medium and high

Table 1. Physiographical and climatic characteristics of the experimental sites

Characteristics	Ensanche de Las Majadas	Los Palancares y Agregados
Altitude above sea level (m)	1,490	1,100
Slope (%)	5.5	4.0
Exposure	North	North
Mean annual precipitation (mm)	1,137	595
Mean summer precipitation (mm)	139	99
Drought period (months)	3	3
Mean lowest temperatures of the coldest month (°C)	–4.5	–0.5
Mean annual temperature (°C)	9.6	11.9
Mean highest temperatures of the hottest month (°C)	28.3	30.5

Note: Climatic variables valus refer to Cuenca Meteorological station records for the period of 1955-2005.

stand densities, respectively, for the studied forests. A meteorological station was used during the experiment on each experimental forest (model METEODATA 1256) to record air temperature, precipitation and relative air humidity (see Lucas-Borja, 2008, for details). Seed rain data used on this paper were obtained during a period of three years (from 2005 to 2007) under different research works developed by Castilla-La Mancha University and Castilla-La Mancha Regional Government (see Lucas-Borja (2008) for details).

In each experimental site and stand densities tested, five 100 m^2 plots were randomly distributed in an area of 1 ha. In each plot, seed removal was analysed depending of the predator group. According to the Cuenca Mountain Forest Service, granivorous species considered as potential predators of Spanish black pine seeds were 1) *Aphaenogaster subterranea*, *Messor capitatus* and *Messor structor* for ants, 2) *Fringilla coelebs*, *Parus caeruleus*, *Parus major*, *Passer domesticus*, *Columba palumbus* and *Serinus serinus* for birds and 3) *Apodemus sylvaticus* and *Mus musculus* for rodents.

Each predator group effect was evaluated by excluding the other two with different experimental devices. One seed was included in each device. Experimental devices used in the seed removal experiment are according to the appropriated methodology and are similar to that proposed by Ordóñez and Retana (2004). The device for ants consisted of transparent plastics tubes (6 cm in length and 0.5 cm in diameter), in which were placed one Spanish black pine seed. In each plot, twenty tubes were fixed to the ground to prevent rodent and bird manipulation. To detect predation by birds, we fenced in one 10 × 10 m subplot inside of each plot with 0.5 cm wire mesh grid diameter. This mesh, of 1 m high and buried 25 cm into the ground, avoided rodent access to seeds. Within each fenced subplot, twenty Spanish black pine seeds were glued to 3 × 3 cm pieces of fine wire mesh to avoid ant removal. To monitor rodent predation, wire cylinders of 0.5 cm grid, 4 cm diameter and 20 cm length open at both ends covered the same 3 × 3 cm pieces of wire with a Spanish black pine seed. Twenty of these structures were placed in each plot and prevented from access to seeds by both ants and birds, but not by rodents. Field observations confirmed that ants found quite easily seeds within these tubes. At the same time, it was confirmed in fields experimental tests that birds (tests were carried out with sparrows, *Passer domesticus*) picked up seeds from these devices without problems caused by the glue and that rodents (the tests were carried out with *Apodemus sylvaticus*, the most common rodent in the areas of study) picked up and eat seeds from these devices in laboratory tests. Seed removal was measured in seven dates in all experimental devices from early January to the end of May in 2005, 2006 and 2007, that is, between seed rain in winter and seed germination in spring, in a total of 7 measurement dates per year. This period corresponds to the period of the year when Spanish black pine seeds might be present in the soil bank. A total of 10 seeds/devices per predator were monitored in each date. For each survey data and once seed was removed by predators, devices were substituted.

Statistical analysis and model structure

The differences in seed removed percentages between experimental forests, basal area intervals, years and predator groups were calculated using ANOVAs. The differences between climatic variables by sites were also calculated using ANOVAs. All plots can be considered as spatially independent. The post-hoc test applied was Fisher's least significant difference (LSD) method. A significance level of $P < 0.05$ was adopted throughout, unless otherwise stated.

Data used in modelling analysis refers to 6,300 observations per each of the three predator groups and experimental forest [three basal area intervals; five plots per experimental forest; twenty devices per predator group; seven survey dates per year; three years of experimental works (from 2005 to 2007)]. Bivariate and multiple regression procedures were used to test for any possible relation between seed removal by groups of predators and stand and site characteristics. The general form of the linear model was:

$$Sp_i = \beta_0 + \beta_1 X_1 + \ldots + \beta_k X_k + \varepsilon$$

where Sp_i is the response variable (percentage of seeds removed by the predator group i (birds, ants and rodents) during the last period of sampling, maintaining always 10 devices available during the 7 measurement dates, X_1 to X_k refers to the independent variables, β_0 to β_k are the regression parameters and ε is the error term. The X's regressors included the meteorological variables (*e.g.* precipitation, air temperature and relative air humidity) evaluated during the sampling period, the stand and the physiographic characteristics (*e.g.* basal area, altitude) and temporal effects (seed production year and the number of days since the beginning of seed rain (*Day,* Julian day). Derived variables as well as interactions between main effects were also considered in a preliminary data analysis. The effect of predation at trap or plot scale considering the possible effect of distance to de closest tree, basal area surrounding the experimental device or similar, is not presented on this work since required data was not collected. For each of the response variables candidate models were developed and residual analysis was conducted to examine the model appropriateness. Automatic procedures (stepwise and «all possible models») were used to help for variable screening. 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 deviation of residuals (s_{yx}). Multicollineary was evaluated through the Variance Inflation Factor (Myers, 1990). A 5% significance level was used throughout, unless stated otherwise. Residual analyses were carried on for detection of problems with the error term. Statistical analyses were carried out with Statgraphics 5.0® and JMP 7.0® software.

Results

According to Lucas-Borja (2008), 2006 was a year of substantial seed rain and coincided with a mast year while 2005 and 2007 had scarce seed rain values. This is in accordance with others studies developed in the same forest area (Del Cerro *et al.,* 2005*,* 2009). Total annual seed rain in Los Palancares y Agregados were (mean ± se) 15 ± 2 seeds m^{-2}, 87 ± 11 seeds m^{-2} and 13 ± 3 seeds m^{-2} in 2005, 2006 and 2007 respectively (Lucas-Borja, 2008). In Ensanche de las Majadas total annual seed rain achieved values of 9 ± 2 seeds m^{-2}, 65 ± 12 seeds m^{-2} and 7 ± 2 seeds m^{-2} respectively in 2005, 2006 and 2007 (Lucas-Borja, 2008). At the present research work, the percent of removal was related with the seed rain patterns being lower in the high seed rain year of 2006 (mean ± se $11.15 \pm 3.7\%$ of removed seeds) than in the low seed rain years of 2005 and 2007 ($81.71 \pm 6.0\%$ and $87.29 \pm 6.6\%$ of removed seeds respectively). The seed removed percentage by each predator group for the period tested (2005 and 2007: lower seed production years; 2006: high seed production year) can be observed in Figure 2 and Figure 3. No significant differences ($P > 0.05$) were found in low seed production year (2005 and 2007) for the three predators group whereas in 2006, birds were the most important predators and rodents were the less important predator group in both Los Palancares y Agregados and Ensanche de Las Majadas ($P < 0.05$) (Fig. 2 and Fig. 3). No differences were found between seed removal percentage for both experimental sites and for each basal area interval tested ($P > 0.05$) although seed predation was higher in Los Palancares y Agregados. With respect to climatic variables, differences by experimental site were found only for accumulated averaged
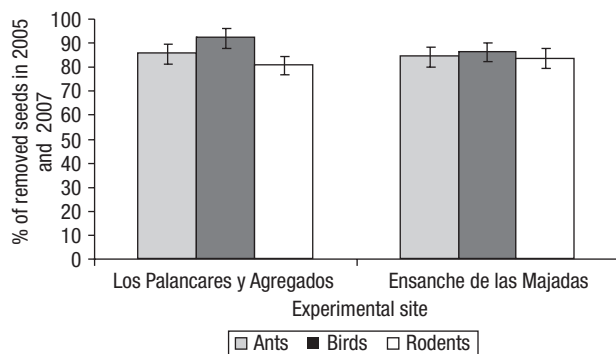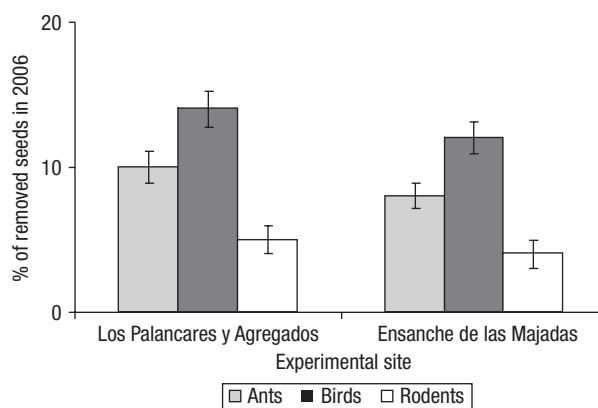
Figure 3. Predated seeds (%) by the three predator groups in the two experimental sites during 2006. Standard errors of the means are indicated by vertical bars.

maximum air temperatures 20 days before survey date (*AaMAT*, °C) (Table 2).

For modelling purposes, three dependent variables referring to the percentage of predated seeds by each predator groups (*Spi*, with *i* representing the group of birds, ants and rodents) were considered independently in the regression analysis. Among the potential explanatory variables tested, the best results were achieved using as independent variables the factors seed production year (*MastY*), a temporal effect within the year, measured by the day variable (*Day*) and a climatic variable (accumulated averaged maximum air temperatures, *AaMAT*). The production year was incorporated as a binary variable (*MastY* variable was coded equal to 1 for «masting year» and zero, otherwise), the *AaMAT* was calculated as the accumulated value of relative air humidity registered for the 20 days period prior to sampling (°C) since it was selected by the statistical procedures used and the day variable corresponds to the elapsed time since January 1st to the date of the evaluation (*Day*, Julian day). The *AaMAT* range values (median ± standard error) were 261.57 ± 6.01 and 236.11 ± 5.37 for Los Palancares y Agregados and Ensanche de las Majadas, respectively. Other variables and respective ranges are expressed in Table 2. The fitted models were parsimonious and did not evidence multicollinearity problems, neither possible omission of important predictor variables. The analysis of residuals was studied informally using Normal Probability Plots. Graphical analyses of the residuals for the new models did not evidence departures from normality. Durbin-Watson test results did not indicate non-independence of the error term. The proposed regression models and goodness-of-fit statistics for

Figure 2. Predated seeds (%) by the three predator groups in the two experimental sites during 2005 and 2007. Standard errors of the means are indicated by vertical bars.

Table 2. Climatic variables calculated (median ± standard error) from the experimental forest selected

	AaMedT (°C)	AaMAT (°C)	AamiT (°C)	P (mm)	H (%)
Palancares y Agregados	112,14 (± 10.25)[a]	261,57 (± 6.01)[a]	−18,97 (± 5.78)[a]	25,14 (± 10.31)[a]	1,475,87 (± 158.77)[a]
Ensanche de las Majadas	98,76 (± 7.05)[a]	236,11 (± 5.37)[b]	−19,63 (± 6.03)[a]	29,94 (± 9.01)[a]	1,458,97 (± 160.29)[a]

AaMedT: accumulated averaged medium air temperatures 20 days before survey date (°C). AaMAT: accumulated averaged maximum air temperatures 20 days before survey date (°C). AamiT: accumulated averaged minimum air temperatures 20 days before survey date (°C). P: total precipitation 20 days before survey date (mm). H: relative air humidity 20 days before survey date (%). For experimental forest, data followed by the same small letter are not significantly different according to the LSD test ($P < 0.05$).

each predator group by experimental site are shown in Table 3.

The main influencing factor on the percentage of seed removal by any of the three groups and on both sites is the occurrence (*MastY* = 1), or absence (*MastY* = 0), of a high seed production year (Table 3). For the two experimental forests and for birds and rodents, comparable goodness-of-fit were obtained with the models that also present as independent variable the accumulated averaged maximum air temperatures 20 days before survey date (*AaMAT*) and the number of days since the beginning of seed rain (*Day*). *AaMAT* representing a negative influence on the percentage of predated seed whereas *Day* showed a contrary trend (Equation 3; Equation 4; Equation 5; Equation 6-Table 3). Thus, the climatic variable and number of days since the beginning of seed rain that consistently,

showed to be significantly related to the quantity of predated seeds but only for birds and rodents in both Ensanche de Las Majadas and Palancares y Agregados.

On the other hand, for pine stands located at favourable areas (Los Palancares y Agregados) and for pine stands located at the altitudinal limit of Spanish black pine distribution in Cuenca Mountains (Ensanche de Las Majadas), the effect of the number of days since the beginning of seed rain (*Day*) is positive whereas the effect of the selected climatic variable (*AaMAT*) is negative for birds and rodents. Thus, seed removal by these two groups increases with the advance of the dissemination period and becomes less intense with the increase of the maximum air temperature. The occurrence of a masting year always showed a negative influence on the percentage of the removed seeds indicating that high seed production years tend to reduce

Table 3. Post-dispersal seed predation models for each predator group by experimental site. The dependent variable is *Spi* (percentage of seeds removed by the predator group i (birds, ants and rodents) during the last period of sampling, maintaining always 10 devices available during the 7 measurement dates). Coefficients (standard errors) and goodness-of-fit statistics ($n = 6,300$ observations for each fitted model)

Equation	Predador group	Local	Intercept	*MastY*	*Day*	*AaMAT*	R^2	s_{yx}
1	Ants	EM	72.857 (3.150)	−68.571 (4.456)			0.855	0.539
2	Ants	PA	53.080 (3.076)	−38.095 (3.202)			0.798	0.649
3	Birds	EM	74.741 (6.413)	−57.522 (4.275)	0.341 (0.084)	−0.129 (0.039)	0.838	0.614
4	Birds	PA	94.610 (6.238)	−77.152 (4.248)	0.382 (0.095)	−0.132 (0.041)	0.902	0.540
5	Rodents	EM	73.753 (5.158)	−67.047 (3.439)	0.352 (0.067)	−0.133 (0.032)	0.879	0.425
6	Rodents	PA	53.522 (5.512)	−42.076 (1.912)	0.421 (0.085)	−0.152 (0.037)	0.884	0.475

MastY: Masting Year (1/0 for presence/absence). Day: Julian day of sampling (from the beginning of the seed rain period). AaMAT: accumulated averaged maximum air temperatures 20 days before survey date (°C). EM refers to site «Ensanche de las Majadas». PA refers to site «Los Palancares y Agregados». All coefficients are significant at P = 0.05.

seed removal by any of the predator groups. The other meteorological and forest stand variables did not showed a significant effect ($P > 0.05$) in the presence of the described main effects.

Discussion

Post-dispersal seed predation is known to have severe demographic consequences (Hulme, 1998) being in some cases a strong «bottleneck» in the natural regeneration process of many forest species (Van der Wall *et al.,* 2005). Taking into account our results and others studies developed in Cuenca Mountains (Del Cerro *et al.,* 2005; Lucas Borja, 2008; Del Cerro *et al.,* 2009), Spanish black pine appears to exhibit a form of masting, in which the populations tends to fruit in alternate years. Moreover, seed deployed in the mast seeding year (2006) showed lower seed removed percentages than seed deployed in the non-masting years (2005 and 2007). This observation conform well to others studies (*e.g.* Theimer, 2001; Jansen *et al.,* 2004; Li and Zhang, 2007). This also supports the prediction of the predator satiation hypothesis: a decrease in removal rates during mast years (Kelly, 1994; Kelly and Sork, 2002).

Abiotic and biotic factors may severely limit chances for regeneration and negative effects may dramatically increase where the species is restricted to marginal populations or at the limit of altitudinal distribution instead of a continuous homogeneous population at the most common and representative location (Castro *et al.,* 1999). Our results shows that seed predation pressure by the three predator groups was higher in the most common location (Los Palancares y Agregados) than at the limit of Spanish black pine altitudinal distribution (Ensanche de Las majadas) for high seed production year (2006) although differences were not significant ($P > 0.05$). Almost all seed can be removed in low seed production years (2005 and 2007) and no conclusions can be obtained with respect to the importance of each predator groups and seed predation rates. Thus, we can assume that experimental forests are not different enough to generate differences with respect to predators' behaviour. In fact, the accumulated averaged maximum air temperatures 20 days before survey date (*AaMAT*) was the only significant climatic variable if both experimental sites were compared (Table 2). Seed removal by the three predator groups was quite constant in 2005 and 2007 for both Ensanche de las Majadas and Los Palancares y Agregados (Fig. 2). With respect to 2006, the three predator groups showed significant differences, presenting birds higher seed removal rates than ants and rodents (Fig. 3).

Predator's density was not evaluated on the present research work and it is clear that those differences among predator's density, food availability and feeding habits may have contributed to changes in the seed removal magnitude (Fedriani and Manzaneda, 2005). However, it is usually assumed that rodents reach their highest abundance in winter (Castro-Díez and Montserrat-Martí, 1998; Ordóñez and Retana, 2004). In spring and when air temperature tends to be higher with respect to winter season, ants increase their abundance, presenting the highest outside activity (Cros *et al.,* 1997; Retana and Cerdá, 2000). In general, birds have larger feeding areas than rodents and ants (Peters, 1983) and can explore larger areas with seed availability. Many bird species have their nesting and breeding periods in spring and summer, when the food requirements of adults increase to supplement the nourishment of the brood (Snow and Perrins, 1997). Thus, seed removed rates showed in 2006 are consistent with the exposed circumstances for each predator group and again, no conclusion can be obtained for low seed production years.

On the other hand, it is known that small mammals and other animals tend to be positively associated with vegetation cover (Hulme, 1993; Fedriani and Manzaneda, 2005), thus fewer seeds are removed from open areas. It is interesting to notice that the opposite appears to be true for ants which forage preferentially in open areas (Hulme, 1998) fact that suggests that the spatial heterogeneity of the vegetation cover will influence the spatial variation in seed removal. However, our results did not showed overstory vegetation density as a significant variable for post-dispersal seed predation models suggesting that for instance the basal area range used have not been wide enough to detect differences on predators' activity or that a different measure of stand density, more related to the cover spacing at local scale, should be used. Moreover, taking into account other studies (*e.g.* Schupp, 1988; Hulme, 1993, 1998; Fedriani and Manzaneda, 2005), the relation of predators is mainly with the shrubland vegetation and not to overstory (trees) vegetation.

According to the models presented for each predator group (Table 3), mast year variable (1/0 for presence/absence) is a main factor in seed predation process. In mast years there are more seed caches and predators are unable to efficiently manage the numerous seeds,

then some seeds have opportunities to establish seedlings (Li and Zhang, 2007). Moreover, seed removal percentages by birds and rodents are positively influenced by the number of days since the beginning of seed rain (*Day*) and negatively influenced by the accumulated averaged maximum air temperatures 20 days before survey date (*AaMAT*) in both experimental forests. It can be explained by the fact that seed removal is increasing when the animals have assimilated the new source of food (Ordóñez and Retana, 2004; Lucas-Borja, 2008). The climatic variable *AaMAT* does not affect seed removal percentage model by ants (Equation 1, Equation 2-Table 3) being an important independent variable for birds and rodents models (Equation 3, Equation 4, Equation 5, Equation 6-Table 3). Rodents reach their highest abundance and activity in winter when air temperatures are lower (Ordóñez and Retana, 2004) and they respond behaviourally by lessening foraging on spring season due to higher associated risk of predation (Fedriani and Manzaneda, 2005). In consequence, lower abundance represents lower seed predator percentages. With respect to birds, *AaMAT* can negatively affect seed removal percentage since higher air temperatures are associated with spring season when the availability of new fruits and food is higher than in summer, autumn or winter and when birds can easily find others sources of food due to their larger feeding areas.

Conclusions

The IUFRO global assessment report on Adaptation of Forests and People to Climate Change indicates natural regeneration as a method of maintaining genetic diversity, and subsequently reducing vulnerability in the face of climate change (GEFP 2009). Additionally, the Forest Expert Panel mention to assist in tree regeneration as an operational-level climate-change adaptation option that may be considered to achieve the management objective of maintaining the productive capacity of forest ecosystems. Although the recognized importance of regeneration processes, information is lacking for a major number of tree species in Mediterranean region. Under a global change scenario interactions of climate-change effects with regeneration dynamics need to be taken into account.

While it is clear that seed predation greatly limits the potential recruitment of many species, an overall conclusion about the impact of seed predation on population dynamics must be made with caution. Our study addressed the local scale process and identified how post-dispersal seed predation occurs. Results demonstrated that Spanish black pine masting condition has a strong influence on seedlings recruitment by altering seed predation and consequently, seedling densities. Results also show that the number of days since the beginning of seed rain (*Day*) and the accumulated averaged maximum air temperatures 20 days before survey date (*AaMAT*) have significant impact of seed removal whereas forest stand location and overstory density do not have a significant effect. Post-dispersal seed predation by ants, birds and rodents is also high, as they consume many of the Spanish black pine seeds remaining in the ground in non-masting years whereas in high seed production years seed predation may not be a problem with respect to initial seedling recruitment. Predator satiation in mast years would result in non interrupted increased seed germination. In low seed production years, this low seed availability is particularly important taking into account that seedling survival is also very low. Thus, to help regeneration at the first stages, foresters should combine natural and artificially seed dispersal in order to provide the minimum levels of seed for the reinitiating stage of *Pinus nigra* stand development in non-masting years.

Finally, analyzing the effect of distance to the closest tree, basal area surrounding the experimental device, or similar could be a good way of improving the present study. Moreover, the use of stochastic dynamic models can also improve our results. By introducing the stochastic variability that characterizes environment variables such as for example air temperature and precipitation it will be possible to simulate the effect of these variables on seed removal. This new approach should include the interaction between simple indicators and habitat resources, with holistic and ecological relevance, and should provide instructive and credible tools to decision-makers and forest managers.

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