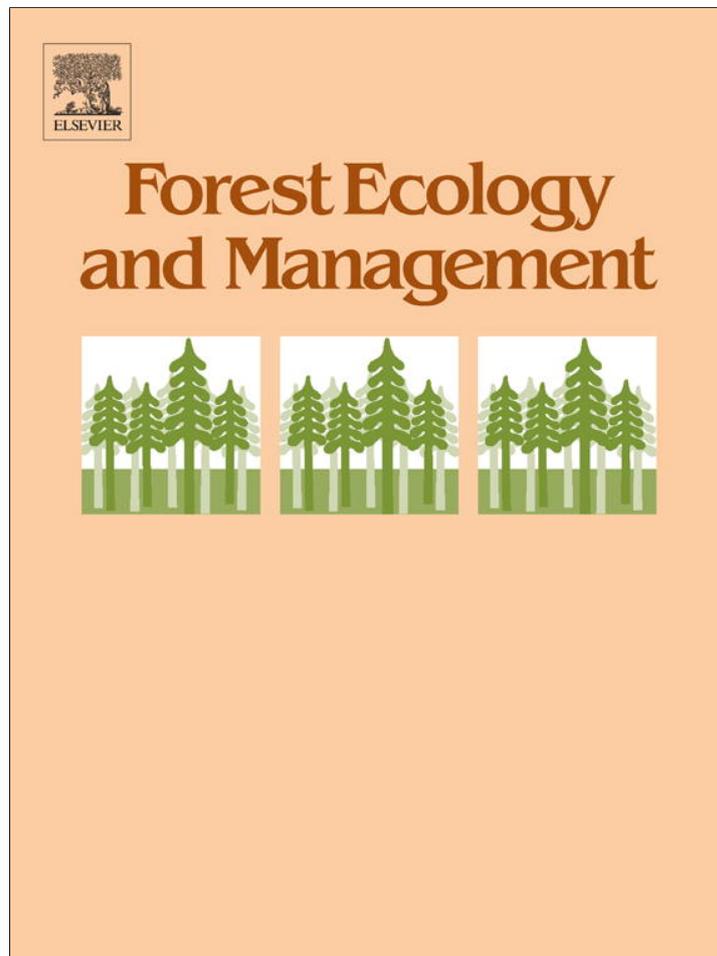


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Modelling seed germination in forest tree species through survival analysis. The *Pinus pinea* L. case study

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ABSTRACT

The direct application of existing models for seed germination may often be inadequate in the context of ecology and forestry germination experiments. This is because basic model assumptions are violated and variables available to forest managers are rarely used. In this paper, we present a method which addresses the aforementioned shortcomings. The approach is illustrated through a case study of *Pinus pinea* L. Our findings will also shed light on the role of germination in the general failure of natural regeneration in managed forests of this species. The presented technique consists of a mixed regression model based on survival analysis. Climate and stand covariates were tested. Data for fitting the model were gathered from a 5-year germination experiment in a mature, managed *P. pinea* stand in the Northern Plateau of Spain in which two different stand densities can be found. The model predictions proved to be unbiased and highly accurate when compared with the training data. Germination in *P. pinea* was controlled through thermal variables at stand level. At microsite level, low densities negatively affected the probability of germination. A time-lag in the response was also detected. Overall, the proposed technique provides a reliable alternative to germination modelling in ecology/forestry studies by using accessible/suitable variables. The *P. pinea* case study highlights the importance of producing unbiased predictions. In this species, the occurrence and timing of germination suggest a very different regeneration strategy from that understood by forest managers until now, which may explain the high failure rate of natural regeneration in managed stands. In addition, these findings provide valuable information for the management of *P. pinea* under climate-change conditions.

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1. Introduction

Natural regeneration is a key process in plant population dynamics consisting of several well-defined stages such as dispersal, predation, germination and establishment (Gomez-Aparicio, 2008). Germination occurrence and germination timing play an essential role in subsequent seedling emergence (Baskin and Baskin, 2001) and therefore, in the overall success of natural regeneration. Hence, the development of tools to predict germination events will lead to an improvement in our understanding of regeneration dynamics, which is a major issue in sensitive ecosystems affected by global change or where natural disturbances have been substituted by management (Dullinger et al., 2004; Castro et al., 2005; Brudvig and Asbjornsen, 2009).

Germination modelling has been developed extensively within an agronomic framework, mainly through the hydrothermal model

(Gummerson, 1986; Bradford, 1990), which is a nonlinear regression approach based on the cumulative effect of water potential and temperature on the cumulative germination rate. However, these methods have been reported to be limited when the true germination time is unknown (Onofri et al., 2010). This is the case in experiments that are revisited at specific intervals (Rees and Long, 1993) or where seeds do not germinate before the end of the experiment (Scott and Jones, 1990). Therefore, rather than the exact moment of germination, the observation data situates the time of germination either within a given interval (interval censoring) or at an undetermined moment after the last measurement (right censoring). Not considering the data as interval censored (e.g. hydrothermal model) leads to misspecifications of the likelihood function, which results in biased parameter estimates (Onofri et al., 2010).

Another constraint of the hydrothermal model in studies concerned with predicting germination under natural conditions is the type of covariate used. In practice, water potential is difficult to obtain, hence it is not feasible to make predictions based on this data type at stand, forest or regional scale. However, there are a number of other variables closely associated with water availability

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which are more readily available and which can be used instead. Soil water content, in particular, can be expressed as a function of time since the last significant rainfall (Roman et al., 2000), depending on the water-holding capacity of the soil. The relative humidity of the air influences water loss through evapotranspiration (Müller, 2000). Additionally, when attempting to identify the thermal requirements for germination under natural conditions, frost occurrence should be taken into account together with temperature. Although low temperatures can induce changes in a hypothetical dormancy state (Baskin and Baskin, 2001), little is known about the effect of frosts on the instantaneous capacity to germinate. In addition, stand structure and density can modify the general climatic conditions at microsite scale (Keyes et al., 2009) as well as limit the possible influence of light as a germination enhancing factor (Seiwa et al., 2009). However, stand-related variables are commonly overlooked in existing germination models.

Germination modelling based on survival time analyses was suggested as an option to cope with censoring in the 1980s (Scott et al., 1984; Pyke and Thompson, 1986). The first applications arose about 10 years afterwards. Rees and Long (1993) developed a likelihood function for interval censoring in order to analyse cohorts of emerged plants at year level. Right censoring was, however, the main concern where data were collected at short enough intervals that made interval censoring negligible (Fox et al., 1995; Evans et al., 1996). Although there are some other subsequent examples of this approach for germination, new improvements have only recently been developed, for instance, to include corrections for seeds that acquire dormancy (Onofri et al., 2011). However, all these models relied on the assumption that time to germination follows a specific distribution, which leads to a time-dependent underlying process for germination (also referred as to “baseline”). Additionally, as these experiments were not designed within a forestry framework, the correspondent models usually neglect to test stand and/or other easily measurable variables, just as in the case of the hydrothermal approach.

Two main families of regression models which include survival time analysis have been commonly used: Accelerated Failure Time (AFT) models and proportional hazard (PH) models (Lawless, 2003). These two types differ in the way that the covariates enter the model. Whereas in AFT models the covariates modify the survival response by altering the time scale, PH models include a fixed survival response (or “baseline”) as a function of time, which is affected by a function of covariates. In the present study, a PH model is developed in order to achieve a clearer understanding and more precise prediction of seed germination in forest tree species under natural conditions.

In order to illustrate (a) the way in which this methodology can be used avoiding strong assumptions on the distribution of time to event and (b) to test the applicability of more accessible variables than those normally utilised, we present a case study of *Pinus pinea* L. in the Northern Plateau of Spain. *P. pinea* is an essential species in Mediterranean ecosystems, often occupying sites where few tree species are able to thrive due to the typical Mediterranean weather conditions, continental winters and sandy soils. In addition, the species provides important economic benefits to local populations from its timber and edible seed production. There are over 50,000 ha of managed *P. pinea* forest on the Northern Plateau. Natural regeneration in these forests is rarely successful using the existing regeneration methods and this situation may worsen under future climate scenarios in Spain according to predictions (De Castro et al., 2005). Although several studies have been conducted to detect possible bottlenecks in the different stages of natural regeneration in *P. pinea* (cone production (Calama et al., 2008b, 2011); seed dispersal (Manso et al., 2012b); or seed predation (Manso et al., 2012a), little attention has been paid to

the importance of germination in the widespread failure of natural regeneration in this species.

The main aims of this study were (1) to test the performance of a proportional hazards model applied to seed germination when no assumptions are made on the distribution of the time to event and when non-standard variables are included; and (2) to quantify the influence of climatic and stand variables on germination in *P. pinea* in the Northern Plateau of Spain. Our key hypothesis is that germination is a climate-mediated process that can be modified by stand conditions.

2. Materials and methods

2.1. Study area

The study area is located in the *Corbejón y Quemados* state forest (41°28'N, 4°43'W) at an altitude of 700 m above sea level. The terrain is flat with sandy soils, typical of the Northern Plateau. The climate is continental-Mediterranean with average monthly temperatures ranging from 4.0 °C in January to 21.7 °C in July. The mean annual precipitation is 435 mm, with a period of summer drought. The site index is 15–16 m at 100 years, typical class II quality (Calama et al., 2003).

The experiment was conducted in a 120-year old even-aged *P. pinea* stand. In 2002–2003, regeneration fellings commenced. In order to reproduce the typical conditions found in mature managed *P. pinea* forests when the regeneration period has started, two different regeneration treatments were applied (the seed tree method (ST) and the shelterwood method (SW)), the fellings in each case resulting in different final stand densities (Table 1).

2.2. Experimental design

The germination trial began in 2006. The experimental design consisted of two 60 m × 80 m (0.48 ha) sample plots that were set up within each regeneration treatment. A 7.5-m buffer area was included around each plot, increasing the overall plot surface to 0.7 ha. All trees within the plots and buffer area were mapped and diameter at breast height (1.3 m; “dbh”) was measured.

In July 2006, 18 seed points were established in each plot. These seed points were distributed so as to include a wide range of light conditions. To minimize rodent predation, the seed points were protected with a 40 cm × 40 cm × 15 cm frame of hard wire mesh (span 0.7 cm × 0.7 cm), which was staked to the ground. The top of the frame could be opened to allow seed manipulation.

On 1 August each year between 2006 and 2010, 50 seeds were placed in each cage. This date was chosen since maximum dispersal rates usually occur at this time. Seeds were obtained from selected stands in the proximity of the study area and tested using the ISTA protocol (viability over 95%). No stratification treatment was applied. The seed points were revisited on a regular basis (average period 18 ± 1 days) either until the next sowing period or until all the seeds had germinated. The number of germinated seeds was recorded at each visit and these were then removed from the cage. Seeds were considered to have germinated when the radicle length exceeded 0.2 cm beyond the nutshell.

In addition to germination measurements, daily climatic variables, such as the mean, maximum and minimum temperatures, mean relative humidity (ratio of the partial pressure of water vapour in the air to the saturated vapour pressure of water), daily precipitation, days with precipitation and days with frost were gathered from a nearby meteorological station (41°17'34"N, 4°40'58"W; data available in www.inforiego.org). To estimate light availability at each seed point, Global Site Factor (GSF) was

Table 1

Summary of stand densities. ST – seed tree method; SW – shelterwood method. Nb/f – Density prior to fellings. After fellings: N – remaining density; BA – basal area; Dg – quadratic mean diameter; H – average height; FCC – forest canopy cover.

Plot	Treatment	Nb/f (ha ⁻¹)	N (ha ⁻¹)	BA (m ² /ha ⁻¹)	Dg (cm)	H (m)	FCC (%)
1	ST	156	46	6.99	44.1	12.6	14
2	SW	192	73	10.82	43.4	14.1	31

calculated from hemispheric photographs taken at each cage. *GSF* is the proportion of global solar radiation (direct plus diffuse) under a plant canopy relative to that in the open. The resulting index was corrected for the angle of incidence relative to a flat intercepting surface. The water-holding capacity of the surface layer of the soil was obtained for each point through texture analysis of two soil samples taken near to each seed point, following the method by Gandullo (1985). Each sample comprised the top 20 cm of the soil profile (soil cylinder of 1.57 dm³).

Finally, in order to take into account the possible influence of trees on microclimate conditions at ground level, we calculated the influence potential (*IPOT*) of all trees located within a 15 m radius of each seed point. None of the seed points were located at distance below 15 m from the outside margin of the buffered area. *IPOT* is a competition index based on the concept of ecological field theory (Wu et al., 1985) and empirically modified by Kuuluvainen and Pukkala (1989) and Barbeito et al. (2011):

$$IPOT_j = 1 - GPOT_j$$

where $GPOT_j = \prod_{h=1}^{n_j} (1 - I_{hj})$ and $I_{hj} = I_{hj}(0) \times \exp(-b \cdot d_{hj})$.

I_{hj} is the potential influence of tree h at seed point j , d_{hj} is the distance from tree h to seed point j , $I_{hj}(0)$ is $dbh_{hj}/(\max dbh_h)$ at each plot), dbh_{hj} is the diameter at breast height of tree h in the influence area of seed point j , b is a parameter, and n_j is the number of trees in the influence area of seed point j . A value of 0.25 was heuristically assigned for b . *IPOT* ranges from 0 (no competition) to 1 (maximum competition).

The range of all variables, along with observed seasonal cumulative germination rates are shown in Table 2.

2.3. Survival analysis

Germination modelling was approached through survival analysis. To illustrate the basis of this technique, let T be defined as a random variable that corresponds to the time until germination

for a particular seed. The cumulative density probability of T at time t is actually the probability that germination occurred at or before time t , i.e. $F(t) = P(T \leq t)$. The survival function $S(t)$ is the probability that a particular seed remains latent after time t , i.e. $S(t) = 1 - F(t)$. The hazard function $h(t)$ represents the instantaneous germination rate, or hazard for a given seed at time t , providing it is still latent at time t . This hazard function is related to the survival function as follows

$$S(t) = \exp\left(-\int_0^t h(x)dx\right) \quad (1)$$

The survival function (Eq. (1)) implies that the probability of a seed remaining latent at time t is a decreasing function of the sum of all hazards prior to time t .

2.4. Hazard function definition

The hazard function includes time-varying and time-independent explanatory variables as well as a number of unknown parameters. Covariates tested in this study were of three types: a) thermal variables (days elapsed from the last frost event (*fr*) and, alternately, daily mean, maximum and minimum temperature (*temp*) in °C); b) water availability-related variables (water-holding capacity (*WHC*), daily relative air humidity (*rh*), daily precipitation (*dp*) and days elapsed from the last precipitation event (*prec*)); and c) stand-related variables: Global Site Factor (*GSF*) and *IPOT*.

For the sake of clarity, let i, j, k, l and m be the indices of the plot, the seed point, the year, the seed and the day, respectively. In order to take into account the time-varying covariates, the hazard function can be expressed on a daily basis, i.e.

$$S_{ijkl}(t) = \exp\left(-\sum_{m=1}^t h(\mathbf{x}_{ijklm}, \boldsymbol{\beta})\right) \quad (2)$$

where t is the number of days since seed l at seed point j in plot i was sown on August 1st of year k ; \mathbf{x}_{ijklm} is a vector of explanatory

Table 2

Summary of variables of interest and observed values for seasonal cumulative germination for the 5 years of study. *WHC*-water holding capacity; *GSF*-Global Site Factor; *IPOT*-overstory influence potential.

	Mean	Max	Min	Stand. error
<i>Spatial covariates</i>				
<i>WHC</i> (mm/cm depth of soil)	104.2267	141.7141	84.0367	2.2125
<i>GSF</i>	0.6654	0.8629	0.4163	0.0169
<i>IPOT</i>	0.4031	0.7174	0.1492	0.0272
<i>Time-varying covariates</i>				
Mean temperature (°C)	11.43	28.03	-5.19	0.15
Maximum temperature (°C)	18.36	38.5	-1.71	0.19
Minimum temperature (°C)	4.41	19.59	-12.02	0.13
Relative humidity (%)	67.14	100	17.83	0.37
Freezing days year ⁻¹	90.4	114	74	8.18
Rainfall days year ⁻¹	128.6	161	90	12.20
<i>Cumulative germination (%)</i>				
		Fall	Winter	Spring
2006–2007		89.74	90.92	-
2007–2008		9.63	22.81	43.35
2008–2009		4.31	7.04	8.3
2009–2010		55.04	63.82	79.61
2010–2011		60.94	65.12	71.13

variables, some of which are measured daily; and β is a vector of unknown but estimable parameters.

From a biological perspective, we can infer that the response of the germination to various explanatory variables is not linear, but rather, nonlinear with some optimal values. Consequently, it can be assumed that the hazard is the product of nonlinear functions of the explanatory variables such that

$$h(\mathbf{x}_{ijklm}, \beta) = \beta_0 \cdot f_1(IPOT_{ij}, \beta_1) \cdot f_2(GSF_{ij}, \beta_2) \cdot f_3(WHC_{ij}, \beta_3) \cdot f_4(rh_{km}, \beta_4) \cdot f_5(dp_{km}, \beta_5) \cdot f_6(prec_{km}, \beta_6) \cdot f_7(fr_{km}, \beta_7) \cdot f_8(temp_{km}, \beta_8) + f_9(h(\mathbf{x}_{ijkl, m-1}, \beta_9)) \quad (3)$$

where $\beta = (\beta_0^T, \beta_1^T, \beta_2^T, \beta_3^T, \beta_4^T, \beta_5^T, \beta_6^T, \beta_7^T, \beta_8^T, \beta_9^T)^T$.

The functions f_1 – f_8 define the effect of each explanatory variable on the daily probability of germination. The independent multiplicative parameter β_0 is a sort of scaling factor that can be understood as a generic model baseline. The function $f_9(h(\mathbf{x}_{ijkl, m-1}, \beta_9)) = \beta_9 h(\mathbf{x}_{ijkl, m-1})$ acts like a lag in the response. In other words, the current daily probability of germination is expected to depend also on the conditions of previous days. In statistical terminology, this lag function is similar to an autoregressive correlation structure (Pinheiro and Bates, 2000, Ch. 5.3).

We defined the functions f_1 – f_8 so that they range from 0 (maximum hazard reduction) to 1 (minimum hazard reduction). Negative values would be inconsistent as they would lead to a survival function $S_{ijk}(t)$ that could exceed 1. In such a context, the product of the functions f_1 – f_8 seemed to be a desirable feature because we expected the effect of the explanatory variables to be multiplicative. Also, from a biological standpoint, this form made more sense as unfavourable conditions from one explanatory variable are likely to reduce the hazard to zero for a particular day if we put aside the lag effect. Therefore, our hazard function can be seen as a product of ecological factors that can reduce the maximum daily germination hazard represented by β_0 . The challenge remained to find a functional form that made sense for each function.

Provided that the potential lowest value for $IPOT_{ij}$, GSF_{ij} , WHC_{ij} , rh_{km} and dp_{km} is zero and that it can be assumed that their influence on germination increases with the value of the explanatory variable, the corresponding functions can be parameterized as

$$f_1(IPOT_{ij}, \beta_1) = 1 - \exp(\beta_1 IPOT_{ij})$$

$$f_2(GSF_{ij}, \beta_2) = 1 - \exp(\beta_2 GSF_{ij})$$

$$f_3(WHC_{ij}, \beta_3) = 1 - \exp(\beta_3 WHC_{ij})$$

$$f_4(rh_{km}, \beta_4) = 1 - \exp(\beta_4 rh_{km})$$

$$f_5(dp_{km}, \beta_5) = 1 - \exp(\beta_5 dp_{km})$$

where the parameters can only adopt negative values.

In the case of rainfall and frost events, we assumed that these have long lasting effects on hazard. We hypothesised that germination hazard was intensified when a precipitation event takes place, the effect decreasing with the number of days since precipitation occurrence. As $prec_{km}$ equals zero on the day of precipitation occurrence, the corresponding function can be defined as $f_6(prec_{km}, \beta_6) = \exp(\beta_6 prec_{km})$, with $\beta_6 < 0$. Inversely, freezing was thought to reduce hazard as a function of the number of days elapsed since the last frost, with maximum reduction taking place on days when frost occurred ($fr_{km} = 0$). Hence it can be parameterized as $f_7(fr_{km}, \beta_7) = 1 - \exp(\beta_7 fr_{km})$, where $\beta_7 < 0$.

Finally, the response to the temperature effect was defined as a non-negative function with a maximum in accordance with existing knowledge on seed ecology (Bewley and Black, 1994). Daily mean, maximum and minimum temperatures were tested under different parameterizations based on those summarized by

Kamkar et al. (2012) plus an additional logistic option. We also considered a strictly non-skewed function through a scaled Normal probability density function that ranged from 0 to 1 (Table 3).

2.5. Likelihood function formulation

The data presented in this study are actually interval censored seed lifetimes since observations were carried out periodically. As a consequence, the exact moment of germination is unknown. For each seed l in year k at seed point j in plot i , we only know that the germination time T_{ijkl} took place within an interval of time where the upper bound is the observation confirming seed germination and the lower bound is the previous observation when the seed had not yet germinated. In other words, we know that $t_{lower,ijkl} < T_{ijkl} < t_{upper,ijkl}$ where $t_{lower,ijkl}$ and $t_{upper,ijkl}$ represent the two limits of the time interval. If the seed fails to germinate during year k , then $t_{upper,ijkl} = \infty$ and T_{ijkl} is said to be right-censored.

Therefore, the marginal likelihood function for this experiment is

$$\ell = \prod_i \prod_j \prod_k \prod_l [S_{ijkl}(t_{upper,ijkl}) - S_{ijkl}(t_{lower,ijkl})]^{q_{ijkl}} \cdot [S_{ijkl}(t_{lower,ijkl})]^{1-q_{ijkl}} \quad (4)$$

where $S_{ijkl}(t)$ is the survival function that sums the daily hazards $h(\mathbf{x}_{ijklm}, \beta)$ (Eq. (3)) in the terms shown in Eq. (2) and q_{ijkl} is a dummy variable that equals 1 when the seed has germinated during a known interval $[t_{upper,ijkl} - t_{lower,ijkl}]$ and zero if it is right-censored in relation to this interval.

2.6. Model fitting and evaluation

In addition to the explanatory variables, we also tried to account for the data structure. In fact, the sampling scheme is likely to result in clustered data and the correlation between observations that belong to the same plot i , seed point j , and/or year k of study might be non-null. In order to prevent this violation of the independence assumption, a vector b of random effects u_i , v_j and w_k for the mentioned levels of variability was included through the expansion of parameter β_0 , the generic baseline. Random effects are assumed to be normally distributed so that $b \sim N(0, D)$, where D is the random effects variance–covariance matrix. Although random effects are commonly included as a multiplicative parameter and they are assumed to follow a distribution adopting non-negative values only (i.e. Gamma; (Lawless, 2003; p. 497)) computing limitations to accommodate our customized hazard function with correction for data correlation forced us to use the present formulation. Also severe problems of convergence arise when different levels of variability are simultaneously considered, especially if it implies crossed effects. Therefore, random effects were tested separately and the resulting models were evaluated in terms of Akaike's Information Criteria (AIC).

In order to illustrate the effect of each explanatory variable and optimal conditions for germination, the marginal hazard function was plotted against the covariate of interest whereas the rest was set to its theoretical maximum. Where possible, a comparison with average climatic conditions in the area was performed. $IPOT$ influence on germination needs to be interpreted in terms of density. Therefore, $IPOT$ was simulated in the central 70 m × 70 m subplot of a 100 m × 100 m plot at a scale of 1 m² for different stocking densities, considering regular spacing and identical diameters for all trees. The $IPOT$ effect, where significant, was then computed based on the resulting model parameterization for each simulated plot.

The model accuracy was evaluated using graphical methods on the training data. Conditional predictions of cumulative germination proportion ($F(t) = 1 - S(t)$) were averaged for each

Table 3

Tested temperature functions. The Segmented and Beta functions comprise the following parameters defining the appropriate thermal interval for germination: μ_o (optimum temperature), μ_b (base temperature) and μ_{max} (ceiling temperature). The logistic function includes intercept (η_{1-2}) and slope (λ_{1-2}) from where μ_o can be deduced. Parameters in the scaled Normal function are μ_o and σ , the latter accounting for the amplitude of the thermal interval. $temp_{km}$ stands for temperature at plot k in day m .

Function	Equation
Segmented	$\begin{cases} f_8(temp_{km}) = \frac{temp_{km} - \mu_b}{\mu_o - \mu_b} & \text{if } \mu_b < temp_{km} < \mu_o \\ f_8(temp_{km}) = 1 - \frac{temp_{km} - \mu_o}{\mu_{max} - \mu_o} & \text{if } \mu_o \leq temp_{km} < \mu_{max} \\ f_8(temp_{km}) = 0 & \text{if } temp_{km} \leq \mu_b \text{ or } temp_{km} \geq \mu_{max} \end{cases}$
Beta	$f_8(temp_{km}) = \left(\frac{temp_{km} - \mu_b}{\mu_o - \mu_b} \times \frac{\mu_{max} - temp_{km}}{\mu_{max} - \mu_o} \right)^{((\mu_m - \mu_o)/(\mu_o - \mu_b))^{\alpha}}$
Logistic	$\begin{cases} \text{logit}(f_8(temp_{km})) = \eta_1 + \lambda_1 temp_{km} & \text{if } temp_{km} \leq \mu_o \\ \text{logit}(f_8(temp_{km})) = \eta_2 + \lambda_2 temp_{km} & \text{if } temp_{km} > \mu_o \end{cases}$ <p>where $\mu_o = (\eta_1 - \eta_2)/(\lambda_2 - \lambda_1)$</p>
Scaled Normal	$f_8(temp_{km}) = \exp\left(-\frac{(temp_{km} - \mu_o)^2}{2\sigma^2}\right)$

interval across all seed points and compared with the corresponding observed values for those intervals. This approach provides evidence of the existence of bias (systematic error in the prediction of the mean) and shows in a simple manner the degree of agreement between model and data (overall agreement between the predicted and observed pattern of germination). Additionally, bias was quantified through the mean error (E) of $F(t)$ for all n individual observations:

$$E = \frac{\sum_{ijkl} (F(t)_{ijkl} - \hat{F}(t)_{ijkl})}{n}$$

All calculations were carried out using PROC NL MIXED in SAS 9.2.

3. Results

3.1. Hazard function

Optimization of the likelihood function shown in Eq. (4) was not straightforwardly achieved under the proposed parameterization. In the selection process we found most of the combinations of parameters to result in severe problems of convergence. This is particularly true in all cases where dp_{km} and/or rh_{km} were included in the model. When the model was fitted without the aforementioned covariates, convergence was achieved either if GSF_{ij} and WHC_{ij} or, alternately, $prep_{km}$ were excluded from model formulation. When fitting the model considering the first possibility, $prep_{km}$ was the only non-significant covariate (p -value 0.2327). In regard to the second possibility, both GSF_{ij} and WHC_{ij} were also found to be non-significant (p -values 0.9315 and 0.9877, respectively) and consequently the model was alternately fitted with each of them at a time. In this process, WHC_{ij} was found to be non-significant, whereas convergence was not attained when the model included GSF_{ij} . As it can be seen, both possibilities led to the same set of parameters when non-significant covariates are dropped of the model. Therefore, germination hazard was finally defined by the overstory competition ($IPOT_{ij}$), number of days elapsed from last frost event (fr_{km}) and daily temperature ($temp_{km}$), all of them significant. These variables modulated the generic base germination through interaction with the baseline (parameter β_0 ; p -value < 0.0106). The autoregressive term was also found to be significant. Out of all the mentioned options for the temperature effect (Table 3), only the scaled Normal approach proved adequate, as the rest implied poorly-resolved estimates with large values in the gradient matrix. Maximum daily temperature produced the best fits and was therefore used in preference to mean and minimum temperature. Selected fixed effects were similar irrespective of the variance structure tested. As regards the random

effects, year level of variability performed better (AIC 24821) than seed point (AIC 24861) or plot (AIC 25218). The AIC value for the marginal model was 25242, which indicates a better performance when random effects are included. Table 4 presents the parameter estimates of the preferred model.

In order to assure we did not have problems of collinearity among the spatial covariates that could yield an incorrect variable selection, the final model was additionally fitted excluding $IPOT_{ij}$ and alternately testing GSF_{ij} and WHC_{ij} . As a result, convergence problems identically arose when GSF_{ij} was considered in absence of WHC_{ij} , whereas the latter was non-significant (p -value 0.9820).

3.2. Optimum conditions for germination

The parameterized hazard function produces a hyperplane, depending on climatic and environmental variables, from which a set of the most favourable conditions can be deduced. Optimum daily maximum temperature was found to be 14.17 °C, the value of parameter μ_o , and the symmetric thermal interval ranges from 12 to 16 °C approximately (Fig. 1a). The hazard-reducing effect of frost occurrence only became negligible after 300 days from the frost event, whereas germination risk is set to zero on days when freezing occurs (Fig. 1b). As regards $IPOT$, only very low values for this variable notably reduced germination risk (when $IPOT$ equals 0.3, hazard reduction with respect to the maximum is only 10.5%). Fig. 2 shows that densities over 25 stems ha^{-1} resulted in a reduction in germination of up to 20% in simulated plots, which implies that only where the overstory is very sparse is there any notable conditioning of germination. Theoretically, germination hazard will be null when overstory competition completely disappears (Fig. 1c). Finally, the autoregressive term showed a notable influence, for example, considerably mitigating the effect of frost (Fig. 1d). In addition, the autoregressive factor causes the hazard

Table 4

Summary of parameter estimates for the selected model. β_0 is the generic baseline; β_1 and β_7 are parameters related to $IPOT$ and frost occurrence, respectively; μ_o stands for optimum daily maximum temperature whereas σ^2 represents the variance of the (scaled) normally distributed germination response to maximum temperature; β_9 is the autoregressive parameter; and σ_{wk}^2 is the variance linked to year random effect.

Parameter	Estimate	Stand. error	df	t Value	p-Value
β_0	0.0250	0.0057	4	4.53	0.0106
β_1	-8.4053	0.7211	4	-11.66	0.0003
β_7	-0.0127	0.0016	4	-7.94	0.0014
μ_o	14.1700	0.0537	4	263.76	<0.0001
σ^2	0.7486	0.0244	4	30.73	<0.0001
β_9	0.9603	0.0023	4	421.8	<0.0001
σ_{wk}^2	0.0001	-	-	-	-

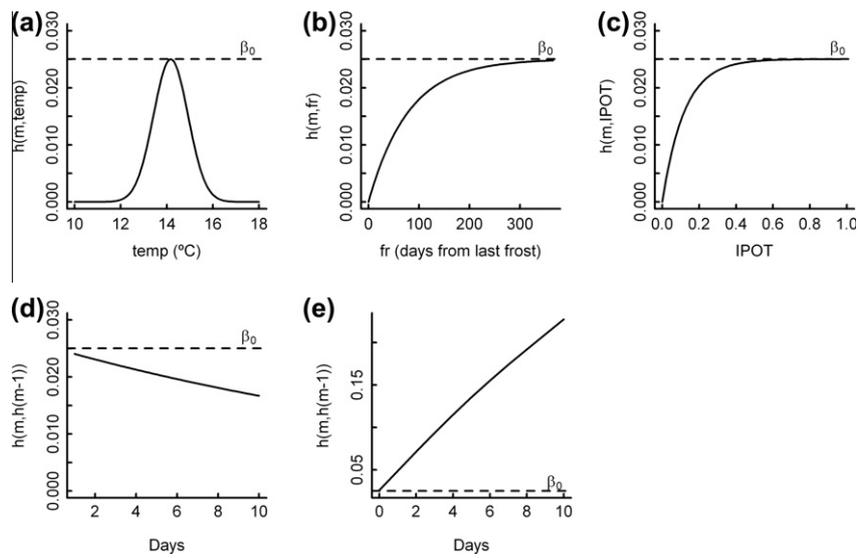


Fig. 1. Marginal effects on hazard of the different covariates included in the model (solid line). (a–c) Daily maximum temperature (*temp*), frost occurrence (*fr*), *IPOT*. The rest of fixed effects were set to 1 and autoregressive term not consider. (d–e) Autoregressive parameter over time (maximum initial hazard) during 10 consecutive freezing days and during 10 consecutive days of optimum temperature. Horizontal dashed line is the theoretical maximum hazard β_0 (autoregressive term excluded).

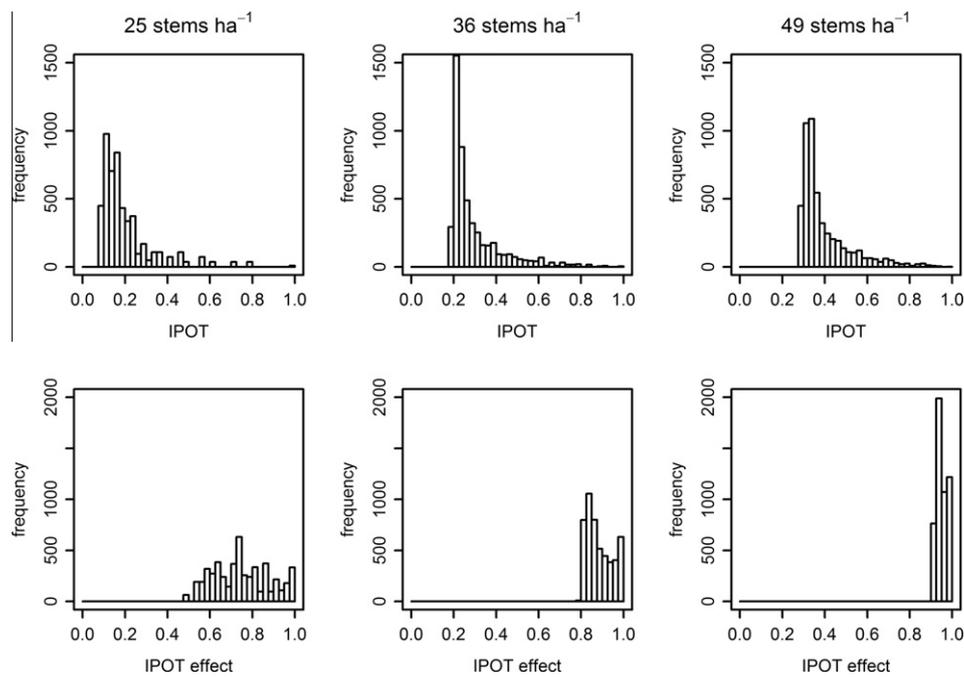


Fig. 2. Histograms of *IPOT* values and corresponding reducing effects on hazard for three different densities. Evaluation was carried out in a simulated 70 m × 70 m plot at a 1 m² scale.

value to increase beyond its theoretical maximum when suitable conditions for germination occur (Fig. 1e).

When considering historic climatic records, Fig. 3 shows how temperatures between June and September over the last 10 years were higher in general than those suitable for germination. In winter, the effect of frost cancels out the potential hazard increase, even if adequate thermal conditions are possible (December and January). Given that frost presents a highly durable effect, spring germination is unlikely in the area and restricted to years with unusually warm winters, such as that of 2007–2008. It can be deduced, therefore, that the optimum germination period in the Northern Plateau are the months of October and November, when

there is a greater probability of favourable conditions and the possible influence of frost is less likely.

3.3. Model evaluation

Predictions of the proportion of germinated seeds over time closely mimic the observed patterns in the two sample plots for all the years of the study (Fig. 4). The model proved to be highly accurate for all years, although displaying a slightly weaker performance in 2009–2010. Furthermore, no bias pattern was apparent, which is confirmed by the low absolute value of the mean error E (–0.0166).

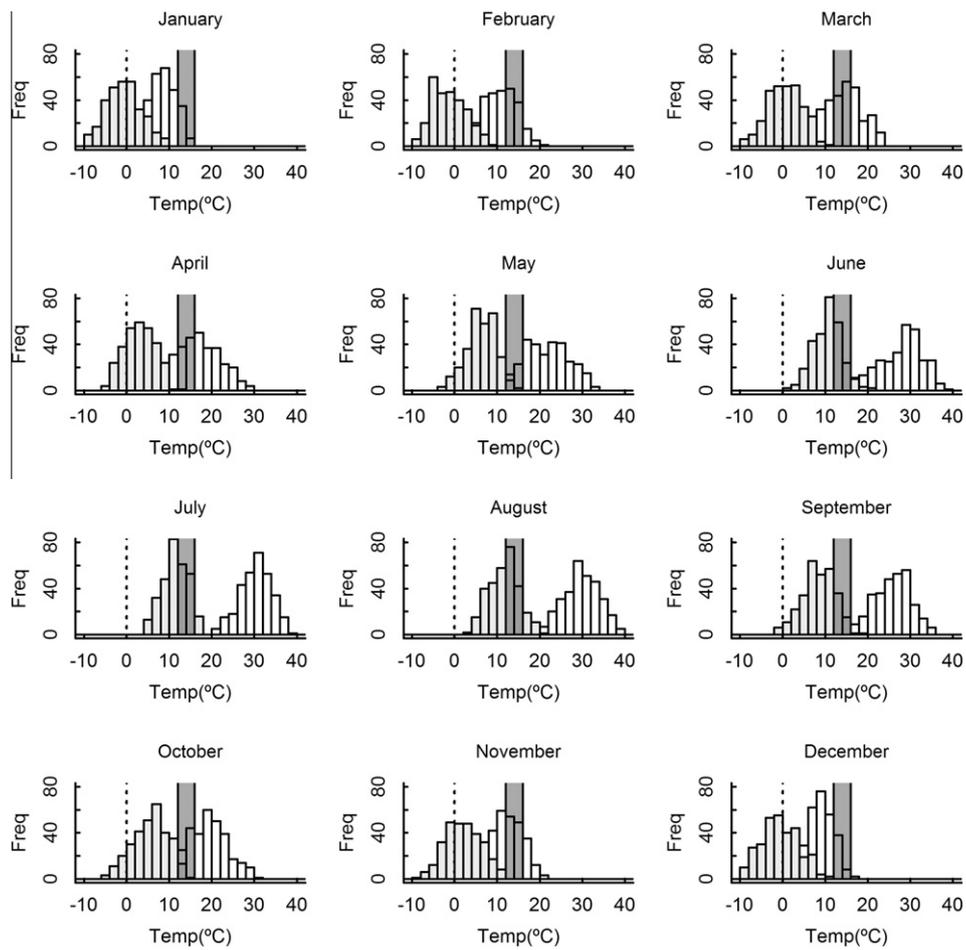


Fig. 3. Histograms of maximum (white) and minimum (light grey) daily temperatures. Dark grey area represents theoretical suitable thermal interval for germination. Vertical dashed line highlights freezing value (0 °C).

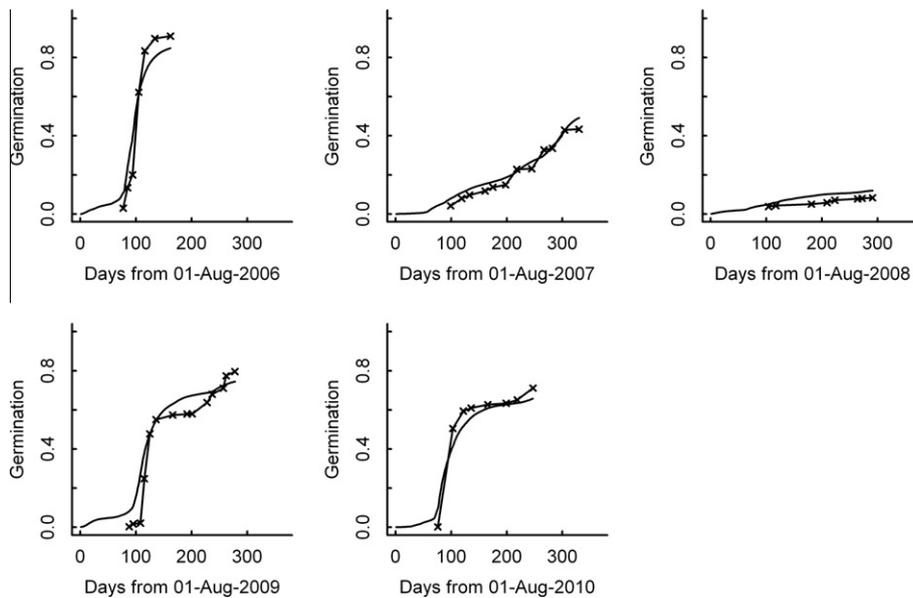


Fig. 4. Predicted (solid line) and observed (crossed-solid line) cumulative proportion of germinated seeds. The abscissa is a 1 year temporal axis starting at the seed deployment date.

4. Discussion

4.1. Modelling approach

Although survival analysis techniques have been scarcely used in the context of seed germination compared to other methodologies, the recent comparative studies carried out by Onofri et al. (2010, 2011) demonstrate the superiority of those models developed through survival analysis. The present paper confirms the potential of survival analysis applied to germination when censored observations are present. We also demonstrate that conventional formulations can be successfully adapted to avoid strong assumptions on the baseline of the process and to include non-standard explanatory variables. Furthermore, the proposed model extends the existing methods to fulfil further model assumptions, such as independence of observations through random effects. Although the use of mixed effect modelling is unavoidable when the independence assumption is violated, it has scarcely been employed in the context of survival analysis (but see Rose et al., 2006; Fortin et al., 2008). In our case, we allowed our generic baseline to vary randomly among years, thus absorbing unnoticed aggregate climatic influences on germination.

A common procedure in survival analysis is to assume a prior behaviour of the process in question (baseline). This prior behaviour can be modified as a function of several covariates. For instance, Onofri et al. (2010) used an AFT model to predict seed germination based on a logistic baseline using water potential as an explanatory variable. However, under non-controlled field conditions, there is no evidence of an existing independent baseline for germination. In our model, we defined the germination hazard as a function of ecological factors with no prior assumptions as regards a baseline for seed germination, with the exception of a technically required scaling parameter.

In the present study we also show that germination can be accurately explained by climatic and stand covariates, at least in this particular case study. The proposed model gained applicability through the set of selected variables in two ways: firstly, they are readily available (as long as daily records are kept) and, secondly, they take into account the effects of forest structure and management on microclimatic conditions (stand variables). The inclusion of time-dependent variables allows predictions to be generated with varying field conditions. Although this paper focuses on the case of *P. pinea*, these possibilities can be explored for most tree species. As a shortcoming, it should be noted that the use of *IPOT* as a stand variable could produce non-comparable results if applied to stands with very different structures.

In contrast to the majority of germination studies, the covariates relating to water availability proved non-significant or convergence was not achieved when included. Concerning water-holding capacity, the notable homogeneity of the variable throughout the two sampled plots could be behind this lack of significance. In regard to precipitation, the low values for water-holding capacity would imply high drainage coefficients (Müller, 2000) and, consequently, little impact of the covariate on water potential reduction. With respect to relative air humidity, it is known to be highly collinear to temperature, which in turn already determines water loss through evapotranspiration (Müller, 2000). Given the high ecological homogeneity of this region, it is presumable that these results would have not notably differed should we have had a replicated design throughout the Northern Plateau. Nevertheless, it is important to note that the non-significance of our water ability-related variables does not necessarily mean that water potential is irrelevant: it only implies that these covariates, under these conditions, could not capture water potential variability

and that the importance of water potential in this context is reduced in contrast to the influence of thermal variables.

Dormancy is a major concern in seed ecology research (Chambers et al., 1999; Baskin and Baskin, 2001). In the case study presented here it was not possible to test dormancy processes, since the seed lots were exposed to natural conditions for a maximum of 1 year. Non-germinated *P. pinea* seeds are assumed to perish in the course of a year due to the high rodent predation rates occurring in the area (Manso et al., 2012a). Under these conditions, distinction between non-germinated seeds and true dormant seeds (if any) is not feasible. However, the proposed methodology allows us to take into account the abovementioned phenomenon in dormancy related experiments. The variables currently included in the model are common for interval and right censored observations, meaning that non-germinated seeds result from an absence of suitable enhancing factors. In order to take into account potentially dormant seeds, the non-germinated fraction should be partitioned into those seeds that simply never abandoned latency and those that acquired dormancy. The latter can be related to a set of covariates z thought to induce the process through the function $\pi(z)$, representing the probability of not germinating due to dormancy. Therefore, the likelihood function in Eq. (4) can be extended as

$$\ell = \prod_i \prod_j \prod_k \prod_l [(1 - \pi_{ijkl}(z)) \cdot (S_{ijkl}(t_{upper,ijkl}) - S_{ijkl}(t_{lower,ijkl}))]^{u_{ijkl}} \times [\pi_{ijkl}(z) + (1 - \pi_{ijkl}(z)) \cdot S_{ijkl}(t_{lower,ijkl})]^{1-u_{ijkl}}$$

similar to those used in zero-inflated model fits (Affleck, 2006; Fortin and DeBlois, 2007; Calama et al., 2011), in this case, a zero-inflated binomial distribution (Tyre et al., 2003). A comparable approach accounting only for the dormant fraction as right censored observations can be found in Onofri et al. (2011), as an extension of an AFT model.

4.2. *Pinus pinea* case study

The proposed model succeeded in determining the importance of germination in *P. pinea* natural regeneration in our sample plots, providing some prior outcomes about regeneration dynamics in the species. The results of this study seem to confirm our key hypothesis of climatic control for seed germination and the importance of forest structure in the process.

The extrapolation of our findings to *P. pinea* stands in the Northern Plateau as a whole must be made prudently, as we did not have a randomized design over the region. However, the high homogeneity of conditions throughout the area leads us to suggest that the role of germination in the *P. pinea* regeneration strategy could be described as follows: *P. pinea* seed dispersal takes place from June to early November (Manso et al., 2012b), whereas maximum rates of seed predation occur in winter months (Manso et al., 2012a) due to higher rodent activity in Mediterranean areas outside the dry period (Díaz et al., 2010). These circumstances lead to a seed-limiting scenario in spring, coinciding with the scarce likelihood of germination predicted for these months. In addition, Calama et al. (2012) found that seedlings established in the fall are more likely to survive the following summer drought period than those produced by spring germinated seeds. Therefore, rapid germination in fall, regardless of site characteristics, could be a key factor for natural regeneration in *P. pinea*, creating a perfectly synchronized post-fructification strategy.

However, this 'window' of optimal conditions in fall is not guaranteed every year. Therefore, such a strategy only makes sense if there is a relatively long period for regeneration and a significant seed source density, which permits both dispersal and germination.

Consequently, a light-independent germination pattern would be expected. Our findings suggest such a pattern (non-significant GSF), at least in the Northern Plateau. Accordingly, Seiwa et al. (2009) stated that large-seeded, non-pioneer species show little sensitivity to environmental signals which enhance germination potential. Non-detection of light as a gap signal along with germination reduction at very low densities leads to a seedling shadow which potentially mimics the seed shadow (Calama et al., 2012). This fact would confirm the medium shade-tolerant behaviour of *P. pinea* reported by Awada et al. (2003) and therefore, the tendency to naturally produce uneven-aged stands (Calama et al., 2008a). In turn, this stand structure ensures long periods for seedling emergence in the absence of large gaps.

In contrast with the strategy described above, the currently-applied regeneration methods in the study area assume a strong shade-intolerant behaviour in stone pine (Montero et al., 2008). For this kind of species, intensive fellings are prescribed to achieve natural regeneration (Smith et al., 1996). However, such practices rapidly lead to seed limitation in *P. pinea* forests (Manso et al., 2012b). If efficient germination has not occurred prior to reducing tree density, natural regeneration will obviously be affected. Additionally, the presence of large gaps in the forest canopy is not favourable to germination.

The future climatic scenario in Spain is characterized by increasing mean and maximum temperatures, particularly during the warm period (De Castro et al., 2005). Minimum temperatures in winter, however, are expected to be more stable. According to these predictions, suitable conditions for germination in terms of daily temperature are likely to shift towards the colder months, although the continued occurrence of frosts during this period will reduce the likelihood of successful germination.

Under the present climatic conditions and management methods employed in *P. pinea* stands in the Northern Plateau, germination can constitute a bottleneck for the natural regeneration of the species. From an ecological perspective, the intensity of regeneration treatments should be reduced and also delayed until a few years after the occurrence of favourable germination/recruitment events in accordance with the light requirements of the species and subsequent natural forest dynamics. In a scenario of global climate change, predictions for future climatic conditions in the Mediterranean basin underline the necessity to consider the recommendations outlined in this paper.

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