

Article

# Seed Origin and Protection Are Important Factors Affecting Post-Fire Initial Recruitment in Pine Forest Areas

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**Abstract:** Initial seedling recruitment is one of the most critical stages for plants in the Mediterranean basin. Moreover, wildfires and post-fire environmental conditions might deteriorate regeneration success, which can lead to problems for sustainable forest restoration and forest persistence. On this context, different seed origins and pine species may be better adapted to new environmental conditions remaining after forest fires and seed protection might modulate seedling initial recruitment. This study evaluates the effects of seed origin (*Pinus nigra* Arn. subsp. *salzmannii* Dunal (Franco) from lowland, midland and upland distribution areas), pine species (*Pinus pinaster* Aiton, *Pinus sylvestris* L. and *Pinus nigra* Arn. subsp. *salzmannii* Dunal (Franco)) and seed protection on seed emergence and early seedling survival after forest fires in the Cuenca Mountains. In addition, a greenhouse experiment was set up under controlled conditions to test seedling performance and to compare initial seedling growth of different *P. nigra* seed origins growing in field and greenhouse conditions. Results showed that wetter spring seasons and *P. nigra* seed origins from midland and upland distribution growing in their natural habitat distribution perform better than *P. sylvestris* and *P. pinaster*. Seed protection is an important factor modulating the above-mentioned trend. *P. nigra* seeds growing at the greenhouse experiment showed differences in growth for extreme (upland or lowland) *P. nigra* distribution.

**Keywords:** seed emergence; seedling survival; seedling growth; greenhouse experiment; Mediterranean pine species

## 1. Introduction

Current decreasing precipitation and increasing temperatures, together with the growing likelihood of extreme drought events, may heighten the vulnerability of forest ecosystems in the Mediterranean Basin [1]. Moreover, an increase in the frequency and severity of summer droughts would thus be expected to lead to a large increase in the burned area [1]. Increasing forest fire severity is also expected, affecting forest soils and forest composition, persistence and stability over the Mediterranean region [2]. In this context, knowledge about potential post-fire regeneration of

individual species may be crucial to achieve better post-fire management decisions for forest stand persistence and stability [3]. The regeneration mechanisms allow Mediterranean vegetation to recover naturally after a fire and to persist in these ecosystems [4]. However, recent and predicted fire regimes in the Mediterranean area may restrict the establishment of some species or may result in a change in the species composition [5,6]. These changes have important consequences on plant biodiversity. Plant species poorly adapted to changing conditions may disappear, causing a decline in Mediterranean forest biodiversity.

The initial recruitment of forest tree species is an important subject in both conservation biology and management. The seed and seedling are the most important stages in natural regeneration, since seed dispersal, predation, emergence and survival are the major forces governing regeneration, structure and succession [7]. The regeneration process can be constrained by abiotic factors (e.g., physiographic and climatic variables) as well as by biotic factors [8]. In addition, forest fires resulting in higher heat shock and ash quantity deposition on soil, have direct and dramatic effects on soil properties and organic matter destruction, seed and plant mortality, runoff and water quality, soil loss and erodibility [9]. For species that lack adaptations to severe fire, such as *Pinus uncinata* M., *P. nigra* Arn. ssp. *salzmannii* and *P. sylvestris* L., post-fire recruitment is based on sustaining their stand regeneration by surviving trees [10]. In contrast, *P. pinaster* Ait. and *P. halepensis* M. initial recruitment in the postfire environment is more related to the existence of a large canopy seed bank and a certain degree of serotiny [10]. Thus, differences in climatic characteristics, genetic resources, and soil conditions remaining after fires can be important influences on emergence and seedling survival trends [11]. The use of seed provenances or species better adapted to future climate conditions could help to improve natural regeneration success and to maintain biodiversity [12].

Plants may exhibit many morphological and physiological adaptations to cope with environmental stress in Mediterranean ecosystems [13]. For example, trees can show a plastic response to changing environmental conditions [14,15]. Different studies have shown apparent contradictions suggesting that some demographic processes may mitigate or compensate the negative effects in plant communities induced by extreme climate and forest fires [16,17]. Nevertheless, locally adapted populations have often the highest fitness when compared with other populations at their growing site [18]. For example, Lucas-Borja et al. [19] showed that a better seed emergence and seedling survival were recorded for combination of local seeds with local soils. Thus, local seed sources are most likely to be best adapted to local conditions. In addition, they are unlikely to be best adapted as conditions change. Pine species regeneration process is affected by a set of numerous variables and their interactions, a situation that results in a high spatial heterogeneity of seedling establishment in the central-eastern mountain forests of Spain [20].

Seed predation can limit recruitment not only by reducing seed availability [21] but also by changing the spatial distribution of seeds in the initial seed rain [21]. Post-dispersal seed predation is known to have severe demographic consequences [22] being in some cases a strong “bottleneck” in the natural regeneration process of many forest species [23]. According to other studies of pine species, masting has a strong influence on seedling recruitment by altering seed predation and seedling densities [24,25]. Moreover, Lucas-Borja et al. [26] pointed out that post-dispersal seed protection was an important factor for seed emergence and early survival success after forest fires. Information on plant seed dispersal, natural loss dynamics of seeds (mainly due to post-dispersal seed predation) and emergence is critical for understanding the natural regeneration mechanism for the species and consequently to best manage for sustainability. Planned restoration guidelines and post-fire restoration measures should be based on natural loss dynamics of seed and seedlings.

This study aims to evaluate the effects of seed origin, pine species and seed protection on seed emergence and early seedling survival after forest fires in the Cuenca Mountains (Spain). Here, the distributions of *P. pinaster*, *P. sylvestris* and *P. nigra*, coincide in the central-eastern mountain ranges of Spain. In addition, a greenhouse experiment was set up under controlled conditions to test seedling performance and to compare initial seedling growth of different *P. nigra* seed origins growing in field

and greenhouse conditions. We hypothesised that *P. nigra* seeds coming from warmer and drier forest locations would be better adapted to post-fire environment and greenhouse conditions. Moreover, *P. pinaster*, which is a fire-adapted species, should present better field initial recruitment and initial seedling growth than *P. nigra* and *P. sylvestris*, although seed predation may modulate these trends.

## 2. Methodology

### 2.1. Study Area

The study area is located in the Cuenca Mountain Range (region of Castilla-La Mancha, central-eastern Spain). *P. nigra* is naturally distributed in this area between 1000 and 1500 m above sea level and dominates the forest stand composition [24]. In the Cuenca Mountains, *P. nigra*, *P. sylvestris* and *P. pinaster* show clear altitudinal and latitudinal distribution patterns. *P. pinaster* is present in the low-altitude and southern-most forests of the mountains, whereas *P. nigra* occupies the central and mid-altitude forest zone, and *P. sylvestris* inhabits the northern and upper altitude areas. Consequently, climatic characteristics and species niches are different among these three pine species. A *P. nigra* forest was selected as an experimental site at 1416 m a.s.l. (“Las Majadas” site, 40°15'58" N; 1°56'08" W) to represent a zone affected by a high-severity wildfire (summer 2009). Forest stands in the Cuenca Mountains have traditionally been managed using the shelterwood method, with a shelter-phase of 20–25 years and a rotation period of 100–125 years [27]. This method involves a uniform opening of the canopy for regeneration without site preparation [28]. In the study forest, the mean total annual rainfall is 1137 mm (139 mm during summer season). The mean annual temperature is 9.6 °C and air temperature typically ranges from −4.5 °C (mean lowest temperature of the coldest month) to 28.3 °C (mean highest temperature of the hottest month). The mean three-month drought-period temperature (June, July and August) is 15.7 °C. Daily air temperature and precipitation were recorded during the entire length of the study at a meteorological station (model METEODATA 1256C) located in the same forest near the plots. Calcareous soils are dominant in the Cuenca Mountains area, and the major soil types found in the experimental site are classified as Typical Xerorthent, according to Soil Survey Staff [29], and as Leptosol, according to the Soil Atlas of Europe [30]. Herbaceous vegetation was composed mainly of *Eryngium campestre* L., *Thymus bracteatus* L., *Geranium selvaticum* L., *Festuca rubra* L. and *Cirsium acaule* L.

### 2.2. Experimental Design

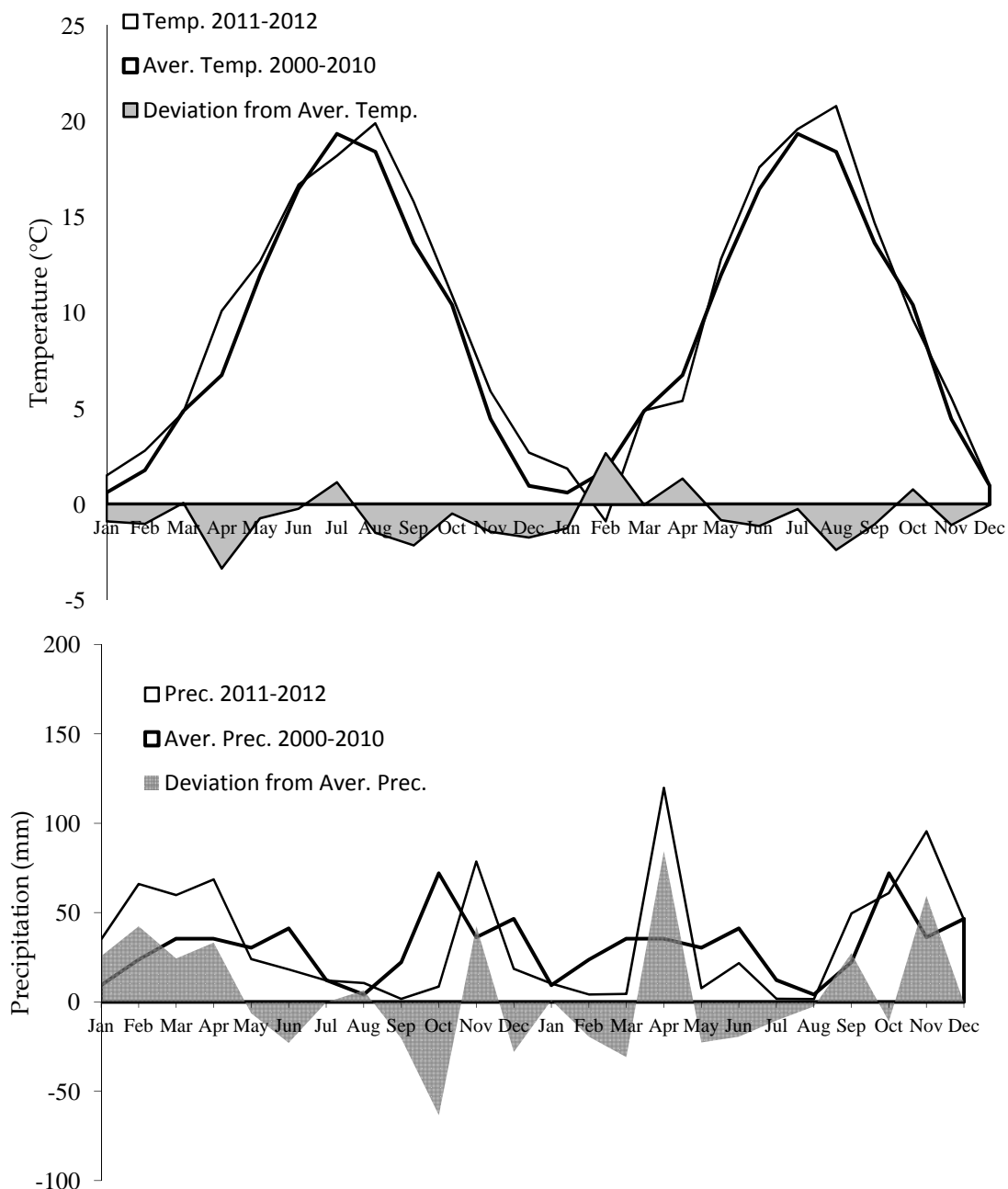
Field experiment: we carried out a sowing experiment from April 2011 to July 2012 at a forest site called Las Majadas (Cuenca Mountains). The experimental area is about 3 ha and was severely burned in 2009. Dead trees killed in the fire were removed before the experiment and the sowing points were randomly established in openings with zero canopy cover. Furthermore, litter was removed before seed sowing. Seeds used in this experiment were manually collected from the Cuenca Mountains. Seed origins were established as follows: (i) *P. nigra* from typical distribution (1200 m a.s.l., MA); (ii) *P. nigra* from upland forests distribution (1500 m a.s.l., HA); (iii) *P. nigra* from lowland forests distribution (950 m a.s.l., LA); (iv) *P. sylvestris* from highlands Cuenca Mountain altitude (1600 m a.s.l., PS) and (v) *P. pinaster* from lowland Cuenca Mountain altitude (900 m a.s.l., PP). An emergence potential test was performed under controlled conditions in the laboratory [28] to check the viability of the collected seeds. Emergence rates exceeded 95% for the five types of seeds. All seeds from each origin (species and provenances) were pooled and kept under refrigeration until the sowing experiment began. Nine blocks of about 10 square meters were randomly distributed on the burned forest area. Site conditions (topography, altitude, soils, etc.) were similar among the selected blocks. Each block was composed of two sowing points (plots) in a grid of 50 × 50 square centimetres, one of which was protected using a wire mesh trap of 1 cm<sup>2</sup> mesh size, to exclude seed predation and seedling herbivory from birds and rodents. The other plot was left unprotected. At each sowing point (plot), 16 seeds from each of the five seed origins were sown 1 cm deep at 3 cm distance between them in March 2011

(5 seed origins  $\times$  9 plots  $\times$  2 seed protection conditions  $\times$  16 seeds). Seed emergence, seedling survival and initial seedling growth were monitored in the field from April 2011 to July 2012. In February 2012, all still alive seedlings were measured (seedling height and seedling diameter). A general view of the totally burned area without canopy cover and a sowing point example can be seen in Figure 1.



**Figure 1.** General view of the totally burned area without canopy cover (A) and a sowing point example in the totally burned area at the beginning of the experiment in April 2011 (B).

Greenhouse experiment: we sowed in commercial soil substrate 24 seeds of each *P. nigra* origin at the greenhouse in April 2011. *P. pinaster* and *P. sylvestris* seeds were not used in the greenhouse experiment. Seed emergence and seedling survival were monitored for the first summer season. In February 2012, seedlings that were still alive were harvested and measured (seedling diameter and seedling height) in order to characterize seedling performance at greenhouse conditions. Precipitation values recorded from 2000 to 2010 (Figure 2) were simulated in the greenhouse experiment, so all seeds were irrigated with the average precipitation amount of the study area.



**Figure 2.** Temperature (Temp.) and precipitation (Prep.) values recorded during 2011 and 2012 in comparison with average (Aver.) precipitation and temperature values of the last 10 years for the study area. Shaded area represents the deviation between average values and 2011–2012 values.

### 2.3. Statistical Analyses

Seedling emergence and seedling survival in the field experiment and greenhouse experiment were modelled using a Weibull survival model. One exception was the analysis of the seed emergence in the field experiment. All seeds emerged in the same time-period, yielding no information on the speed of emergence. Therefore, we analysed this subset used a mixed logistic regression. The covariates reflect the design of the experiments. In the case of the field experiment, this was seed origin, protection and their interaction plus a random effect accounting for the replication of the blocks. In the case of the greenhouse experiment, the only covariate was the seed origin. The Weibull survival model, as fit by an Integrated Nested Laplace Approximation (INLA), has two parameters: the shape and the scale. All observations share the same shape parameter. The scale parameter depends on the covariates. The scale parameter increases as the phenomenon (seed emergence, seedling death, etc.) occurs earlier. Seedling height and seedling diameter were modelled using linear regression using seed origin, experiment (greenhouse and field site) and their interaction as covariates. The variance was allowed to differ among the combinations of seed origin and experiment. All models were fit using INLA, which is a new approach to statistical inference for latent Gaussian Markov Random Field (GMRF) models. The statistical software used was R 3.3.3 [31] and the INLA package version 0.0-1485844051 [32]. Model comparison was based on the Watanabe–Akaike Information Criterion (WAIC) [33]. WAIC decreases with model fit and increases with model complexity. Lower values of WAIC reflect a better model fit balanced with model complexity.

## 3. Results

### 3.1. Climate Records, Seed Emergence and Seedling Survival Rates and Initial Seedling Growth at the Field Experiment

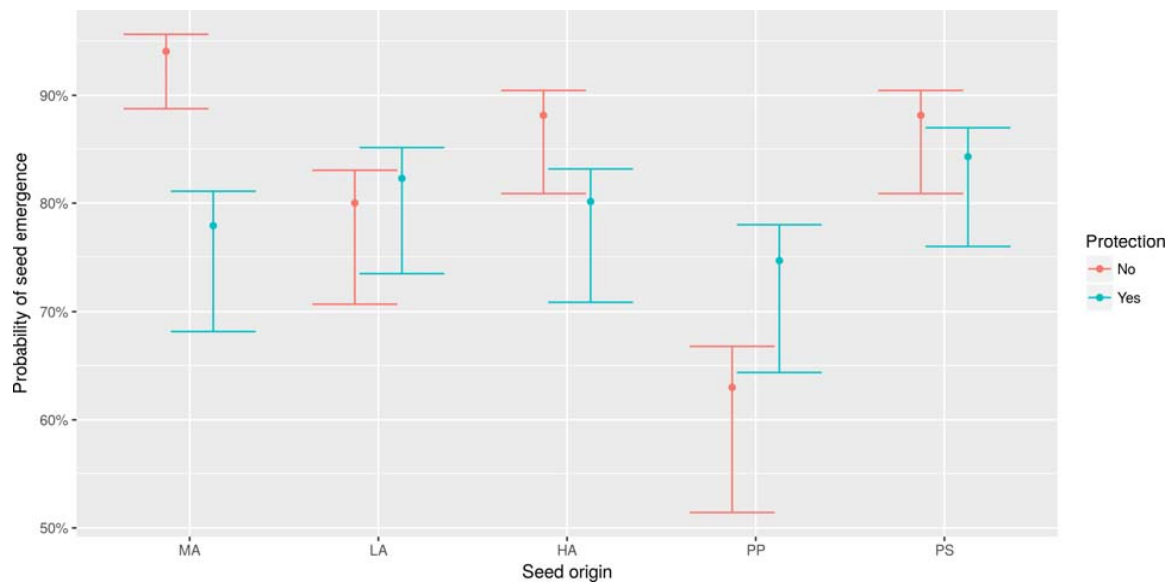
Temperature and rainfall values recorded during 2011 and 2012 varied in comparison with the average precipitation and temperature values of the last 10 years for the study area (Figure 2). Generally, the spring season was wetter and warmer, whereas the autumn was particularly drier during 2011. In addition, the 2011 and 2012 dry seasons were much longer during the experiment (June–October) than in the last 10 years (July–September) (Figure 2).

Seed emergence was higher for *P. nigra* midland (MA), *P. nigra* upland (HA) and *P. sylvestris* (PS), with *P. pinaster* being the least successful species (Table 1). Generally, seed protection did not have a significant effect on seed emergence for all the seed provenances and pine species (Table 1). In relation to seedling survival, *P. nigra* seedlings of midland and upland origins showed the highest seedling survival rate (Table 1). Secondly, in contrast to the seed emergence results, seedling survival was considerably higher with protection (Table 1).

**Table 1.** Number of emerged seeds and survived seedlings for each experimental condition at the field site experiment.  $n = 144$  seeds for each experimental condition.

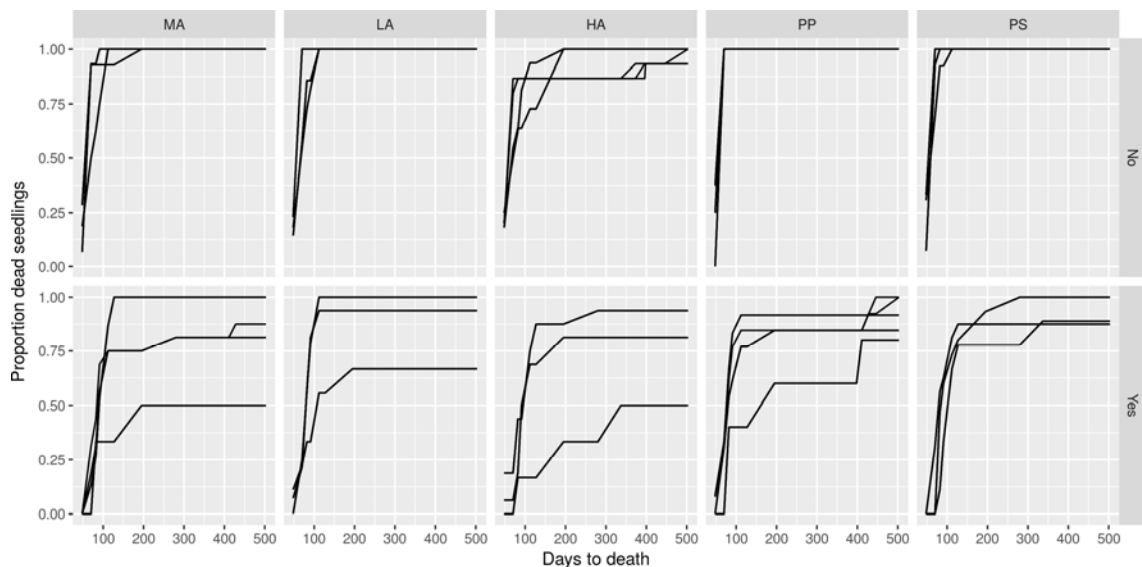
	Protection	<i>P. nigra</i> Lowland (LA)	<i>P. nigra</i> Midland (MA)	<i>P. nigra</i> Upland (HA)	<i>P. pinaster</i> (PP)	<i>P. sylvestris</i> (PS)
<b>Emerged seeds</b>	No	114	135	126	90	126
	Yes	117	111	114	106	120
<b>Seedling survival</b>	No	0	0	2	0	0
	Yes	12	17	21	12	9

We found high variation in emergence among seed origins in the case of no protection, ranging from 63% for *P. pinaster* up to 94% for *P. nigra* midland (Figure 3). The variation among seed origins is much smaller (75–84%). The interaction between seed origin and protection is important because dropping the interaction would increase WAIC from 1331 to 1347. Only the emergence of *P. pinaster*, *P. nigra* lowland and midland benefit from the protection. The emergence for the other origins decreases.



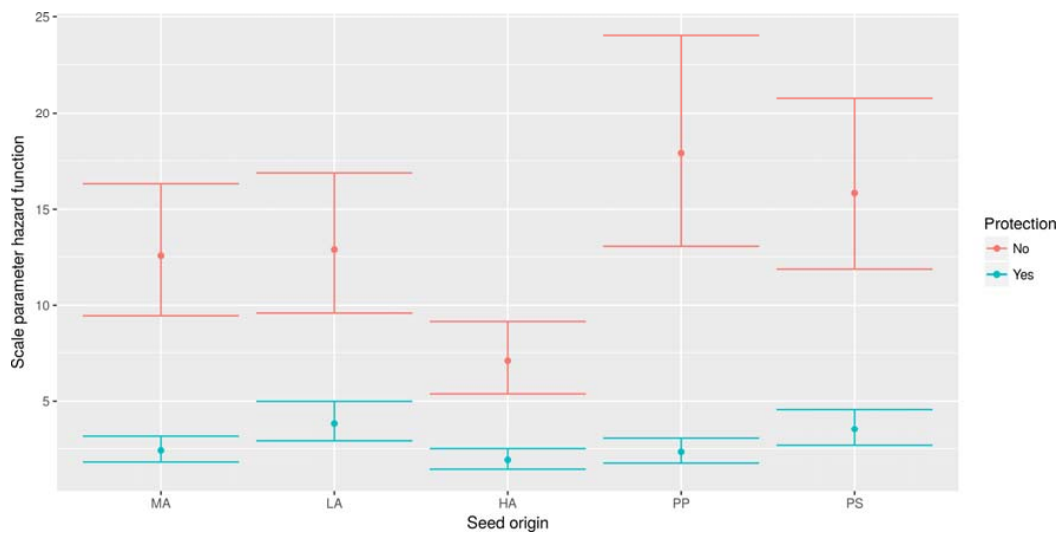
**Figure 3.** Probability of seed emergence for each seed origin (MA: *P. nigra* from typical distribution, HA: *P. nigra* from upland forests distribution, LA: *P. nigra* from lowland forests distribution, PS: *P. sylvestris* and PP: *P. pinaster*) and protection status. The error bars indicate the 95% interval.

Figure 4 illustrates the observed survival pattern of the seedlings. Nearly all unprotected seedlings died within 200 days, except for some *P. nigra* upland seedlings. Although protected seedlings had a higher probability of survival after 500 days, most seedlings died during the first 200 days.



**Figure 4.** Observed survival pattern of the seedlings. Each line represents an experimental block. Subplots on the same row share the protection status. Subplots on the same column share seed origin (MA: *P. nigra* from typical distribution, HA: *P. nigra* from upland forests distribution, LA: *P. nigra* from lowland forests distribution, PS: *P. sylvestris* and PP: *P. pinaster*).

The scale parameter of the hazard function highlights the difference between protected and unprotected seedlings (Figure 5). The model with interaction between seed origin and protection has WAIC = 5514. When we drop the interaction from the model, WAIC increases to 5523, indicating that the interaction is important.



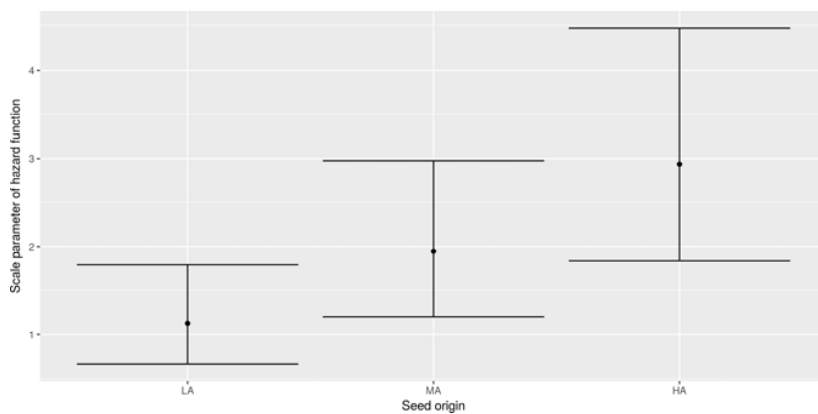
**Figure 5.** Scale parameter of the hazard function for each combination of seed origin (MA: *P. nigra* from typical distribution, HA: *P. nigra* from upland forests distribution, LA: *P. nigra* from lowland forests distribution, PS: *P. sylvestris* and PP: *P. pinaster*) and protection. The error bars indicate the 95% credible interval.

### 3.2. Seed Emergence and Seedling Survival Rates and Initial Seedling Growth at the Greenhouse Experiment

The number of emerged seeds and survived seedlings for each *P. nigra* origin at the greenhouse experiment can be observed in Table 2. The seed origin had an important effect on the emergence (difference in WAIC: 9). Seeds from higher altitude emerged earlier than seeds from lower altitudes (Figure 6). Figure 7 illustrates the observed differences in scale parameter among seed origin. We found no evidence that supports an effect of seed origin on the survival of the seedlings (difference in WAIC =  $-0.7$ ).

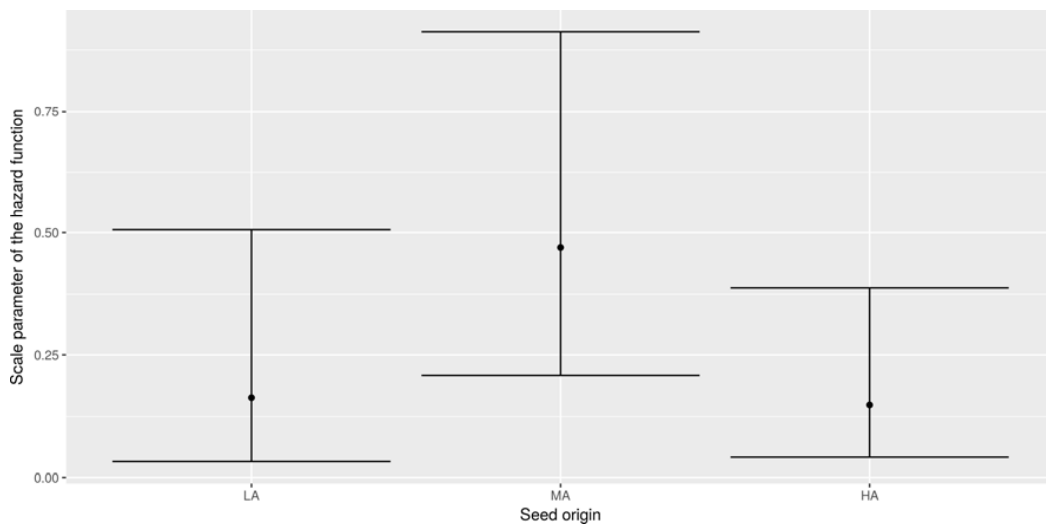
**Table 2.** Number of emerged seeds and survived seedlings for each experimental condition at the greenhouse experiment.  $n = 24$  seeds by *P. nigra* seed origin.

	<i>P. nigra</i> Lowland (LA)	<i>P. nigra</i> Midland (MA)	<i>P. nigra</i> Upland (HA)
<b>Emerged seeds</b>	16	20	24
<b>Seedling survival</b>	14	13	21



**Figure 6.** Scale parameter of the hazard function for emergence at the greenhouse for each seed origin (MA: *P. nigra* from typical distribution, HA: *P. nigra* from upland forests distribution, LA: *P. nigra* from lowland forests distribution). The error bars indicate the 95% credible interval.

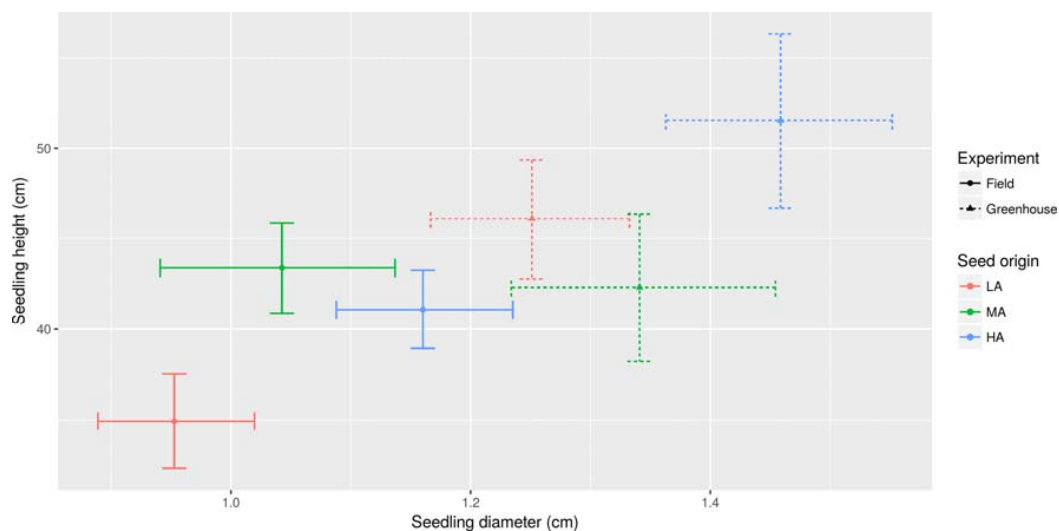




**Figure 7.** Scale parameter of the hazard function for seedling survival at the greenhouse for the different seed origins (MA: *P. nigra* from typical distribution, HA: *P. nigra* from upland forests distribution, LA: *P. nigra* from lowland forests distribution). The errorbars indicate the 95% credible interval.

### 3.3. Comparison of the Greenhouse and Field Experiment in Relation to Seedling Growth

We found an important interaction effect between seed origin and experiment (greenhouse or in the field site) for seedling height (difference in WAIC = 9). This interaction was not important for seedling diameter (difference in WAIC = -0.8). Both the main effects of seed origin (difference in WAIC = 32) and experiment (difference in WAIC = 13) are important for the seedling diameter. Figure 8 displays the modelled seedling height versus the modelled seedling diameter. Seedlings in the greenhouse are taller and thicker than those in the field. The diameter increases with altitude of the seed origin. The midland origin was the tallest in the field but shortest in the greenhouse. Lowland and upland origins display the same pattern in the greenhouse and the field when we take into account that seedling in the greenhouse are globally taller and thicker than in the field.



**Figure 8.** Modeled seedling diameter and seedling height for each seed origin (MA: *P. nigra* from typical distribution, HA: *P. nigra* from upland forests distribution, LA: *P. nigra* from lowland forests distribution), both in the greenhouse and field experiment. The error bars indicate the 95% credible interval.

#### 4. Discussion

The study findings showed that *P. nigra* (all seed provenances) and *P. sylvestris* emergence rates were higher than *P. pinaster* and that seedling survival rates were higher for MA and HA *P. nigra* seed origins for this experiment and for protected seedlings. Nonetheless, seedling survival was very low at the end of the growing season, which represents a problem for sustainable forest management due to the lack of natural regeneration. Seedling protection was an important factor for early recruitment success, as it had a significant influence on survival rates for all seed provenances and pine species in the field [34]. For the greenhouse experiment, seed emergence and survival rates did not differ comparing all *P. nigra* seed origins and initial seedling growth was always higher for seedling growing at the greenhouse except for *P. nigra* MA origin. Thus, initial seedling growth of extreme seed origins (i.e., lowland and upland *P. nigra* distribution) performs differently when growing in field and after fire or greenhouse conditions. These findings indicate the importance of interaction between forest fires, seed origin, pine species and seed predators for understanding initial seedling recruitment in Mediterranean burned areas.

Local climatic data spanning the 20th century show a trend of warming in the Cuenca Mountains, with increased aridity and increasing drought events since the onset of the 1980s [35]. In addition, the natural fire regime observed in high altitudinal areas of the western Mediterranean basin may be changing as a result of changes in climate, plant communities and socioeconomic policies [36]. Under ongoing global change, models fitted to simulate forest occupancy under a climate change scenario project a reduction in the *P. sylvestris* Spanish range and a decline in *P. nigra* forests, whereas *P. pinaster* would maintain or increase the area it occupies [35,37]. Forest fires, which are natural components of Mediterranean forest ecosystems [38] might change pine species distribution through altering post-fire initial recruitment. Fire significantly reduces seedling emergence and survival rates in the short term and seed availability is a crucial variable for post-fire natural regeneration. *P. sylvestris* and *P. nigra* are not serotinous and their post-fire recovery depends mainly on climatic conditions and seed availability from unburned patches for the recolonization of burned areas through seed dispersal [39–41]. In contrast, *P. pinaster* is adapted to regenerate after severe fires. Thus, we conducted a sowing experiment using different *P. nigra* seed provenances and pine species in order to simulate the seeds coming from the surrounding unburned young and mature stands. We assumed that seeds are available after the forest fire, although it could be not possible in many other situations due to masting condition, pine species without serotines cones or unburned patches availability.

In this experiment, upland and midland *P. nigra* seed origins were sown very close to their natural distribution areas. The results show that seedlings coming from upland and midland *P. nigra* seed origins are better adapted to the environmental conditions remaining after fire. *P. sylvestris* seeds were sown closer to their natural distribution area than *P. pinaster*. *P. pinaster* presented the worse emergence and survival rates, indicating that they are not as well-adapted as the other studied types of seeds to the higher and cooler area of study. This is consistent with the findings of Lucas-Borja et al. [19], who showed better results in emergence and survival where the seed and the soil origin coincided in the Cuenca Mountains. As [18] stated, local adaptation occurs since each seed provenance showed a higher performance in its own site. Our results were not surprising since *P. nigra* is considered as a long-lived pine species able to adapt ecologically and physiologically to very different environmental conditions, performing better when the seed and the soil origin coincided Lucas-Borja et al. [19].

As *P. sylvestris* occupies the coolest areas within the study forests, the beneficial effect of the wetter spring 2011 may have been an important factor at the seedling stage for this species. *P. pinaster* is typically classified as a shade-intolerant species and exhibits a high phenotypic plasticity [42]. Several studies have documented that seedlings of this species may require overstorey cover in order to establish successfully [43,44]. In addition, the environmental conditions in which *P. pinaster* normally grows in the Cuenca Mountains range may explain the lower seedling survival rates showed by this species in spite of the post-fire conditions. Tree canopy protection absence, insufficient precipitation, higher evapotranspiration rates because of the higher temperature and light incidence reaching soil

surface after wildfires could imply a strong reduction of survival probabilities for *P. sylvestris* and *P. nigra* seeds and initial recruitment success for *P. pinaster*. In this regard, it is noteworthy that the study occurred under two relatively wetter spring seasons (see precipitation in April 2011 and 2012 from Figure 2). Higher water availability during spring 2011 meant that the seedlings were able to survive without shrub or canopy cover protection as is normally needed in warmer and drier circumstances that characterize the Mediterranean climate [25]. It is possible that with sufficient soil moisture and seed hydration, sunlight favoured a longer taproot and longer shoots in seedlings, enabling them to penetrate the soil and survive better [45]. When the roots of *P. nigra* seedlings and co-existing pine species extend deeper into the soil, they take up sufficient water to increase the chances of seedling survival [46]. In addition, our results might indicate a good adaptation of *P. nigra* seedlings to full light conditions (burned areas) as shown by Gómez-Aparicio et al. [47]. Thus, the micrometeorological and light conditions found in totally burned areas would have generated a better environment for *P. nigra* upland and midland seedling survival in the wetter spring of 2011. Our results also agreed with those of Lucas-Borja et al. [48], who found that survival was higher away from orthogonal shrub projection under favourable climate conditions in 2013, which also suggests a light effect consistent with reports by [49].

At the end of the experiment, only the *P. nigra* HA origin had surviving unprotected seedlings. The wildfire effect was summarized as negative for seed emergence, seedling survival and initial seedling growth in all pine species and seed origins. Thus, post-dispersal seed predation does not predict good expectations for pine species in non-masting years. Contrary to previous studies developed in this area (see [34,38,50]), the high emergence levels of unprotected seeds indicated that seed predation was not significant in our experiment, but post-emergence seedling herbivory was a key factor.

Higher overstorey density can promote taller seedlings and larger basal diameter because negative effects of water deficit and drought period can be ameliorated by canopy shelter. In places where summer drought negatively affects initial seedling recruitment, higher sunlight reaching soil surface can be promoted with lower overstorey densities, and, thus, higher seedling growth can be generated [48]. Data from this study show that *P. pinaster* natural regeneration in midland altitudes from central-eastern Spain is difficult to achieve. In relation to *P. nigra* seed origins, LA showed lower seedling growth, which confirms the idea that lowland seed origin might be worst adapted to burned areas similar to the study site. In relation to greenhouse seedling growth, higher seedlings basal diameter and seedlings height were consistently found for all *P. nigra* seeds origins (only not statistically significant in MA origins). It has been showed that under better conditions found at the greenhouse, seedling roots can extend into the deeper soil to avoid competition from ground vegetation and to attain sufficient water and resources [51]. Results might suggest that biomass production at this age depends mostly on microsite conditions favourable for root development. Moreover, good root development may allow maximum production at reduced fertility levels, and larger root systems may contribute to increased nutrient use efficiency, thus favouring initial seedling recruitment.

## 5. Conclusions

The necessary conditions for a successful seed emergence may depend on specific environmental factors (i.e., seed predation, soil conditions, light on the forest floor or canopy cover), while suitability for seedling survival can be related to different conditions. Moreover, the suitability for each one of these stages implies conflicts involving the most suitable micro-site conditions and factors for seed emergence and survival. Seedling survival is one of the most critical stages for plants in the Mediterranean basin, where water deficit during the summer season appears to be the main cause of seedling mortality. Moreover, post-fire environmental conditions might deteriorate regeneration success, which can be a problem for sustainable forest restoration and forest persistence. Our results suggest that upland and midland *P. nigra* seed origins are best adapted to the environmental conditions in the study area since they showed significantly higher seedling survival rates than seeds from the two

coexisting pine species (*P. sylvestris* and *P. pinaster*) in the Cuenca Mountain Range. Although future climate conditions at the site might be expected to more closely match those of the low-elevation seed source, in the present experiment, the survival of upland and midland *P. nigra* seedlings was the most abundant. *P. sylvestris* and *P. pinaster* survival was null at the end of the experiment. Wetter spring seasons and *P. nigra* seed origins from midland and upland distribution growing in their natural habitat distribution perform better than *P. sylvestris* and *P. pinaster*. Seed protection is an important factor modulating the above-mentioned trend. *P. nigra* seeds growing at the greenhouse experiment showed differences in growth, which might be related to the better soil and water conditions found at the greenhouse.

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