

## Article

# Does the Age of *Pinus sylvestris* Mother Trees Influence Reproductive Capacity and Offspring Seedling Survival?

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**Abstract:** We assess how the age of *Pinus sylvestris* mother trees influences seed size, seed viability, germination capacity and later offspring seedling survival under greenhouse conditions. Thirty trees ranging from 30 to 219 years old were selected in the north facing slopes in the Sierra de Guadarrama, where we could find the oldest *Pinus sylvestris* trees in central Spain. Forty cones per tree were harvested to study cone and seed characteristics (size and weight), seed viability and germination capacity related to the mother tree age. In addition, 25 germinated seeds per tree were grown in a greenhouse to assess offspring seedling survival during a death trial, where watering was stopped. Significant differences between trees in cone and seed morphological traits were observed. The age of the mother tree had a significant effect on cone size, seed size, and seed weight, but there was no effect on seed germination capacity and seed viability. Seedling survival was mainly affected by the decrease in water availability. However, a significant effect of the tree age was found once soil moisture had reached 0%. Our results show the ability of overmature *Pinus sylvestris* trees to maintain a relatively high reproductive capacity that assures its persistence.

**Keywords:** old-growth forests; seed size; seed viability; germination capacity; death trial; chlorophyll fluorescence; Scots pine



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## 1. Introduction

The large history of management and use of forests has shaped its landscape and structure over time [1]. The consequence of such prolonged management is the difficulty of finding old-growth forests but also even knowing their level of maturity [2]. In addition to this, we must consider the longevity of some tree species, such as *Pinus*, whose lifespans are over 4000 years (e.g., *Pinus longaeva*), which makes it difficult to elucidate whether senescence has been reached [3].

We must not forget that the last stages of senescence are also part of forest dynamics. Forest dynamics analyzes the changes over time in the structure, composition, functioning and biodiversity of a stand. It includes a cycle with four differentiated stages, which are “Stand Initiation”, “Stem Exclusion”, “Understorey Reinitiation” and “Old Growth” [4]. Research on forest management usually focuses on the two intermediate stages of forest dynamics (i.e., “Stem Exclusion” and “Understorey Reinitiation”). However, under the new paradigms of multifunctionality and close-to-nature management, and considering global change scenarios, it is interesting to study old-growth forests, as well as the decay and mortality-related processes that can condition their functioning and reproductive capacity. In this sense, National Parks are privileged scenarios to study these processes. After the declaration of a National Park, all intensive forest exploitation ceases; thus, forests evolve close to their natural dynamics [5].

The role of old-growth forests in forest dynamics is a well-known process in some aspects. For example, old-growth forests are characterized by harboring high biodiversity [6], providing multiple ecosystem services [7], while increasing stand resilience after extreme climate events. In addition, old-growth forests store carbon in the long term [8]. Although traditionally it has been thought that carbon storage is interrupted as trees get older, different studies have demonstrated that old-growth forests continue storing carbon for centuries and in great amounts. In fact, in temperate and boreal forests in the Northern Hemisphere, old-growth forests provide as much as 10% of the ecosystem's global net productivity [9]. However, there are other old-growth forest aspects that are not so clear, for example, their ability to maintain their reproductive capacity and to produce abundant and viable seeds once they reach senescence [10].

Natural regeneration is a main phase of forest dynamics and the only way to guarantee forest persistence in the long term [11]. The successful regeneration of a forest conforms a temporal succession of phases that should be effectively realized, including: seed production, seed dispersal, seed predation, germination, emergence, seedling survival and seedling initial growth [12]. Regeneration could be seen as a multistage process based in this approach, with underlying consecutive subprocesses that can be identified as successive survival thresholds for potential seedlings [13,14]. Thus, seed availability and viability are key traits necessary for assuring the persistence of old-growth forests. In this sense, there is evidence that, when referring to seed production, tree senescence does not decrease fecundity [15,16]. However, there is not a systematic study on the effect of senescence on progeny viability. For instance, there could be an age-related mother tree effect that reduces some critical stages in the regeneration process, such as seed germination and first-year seedling viability. In this way, senescence will be related to effective reproduction, which is determinant in the biological efficacy of plants and, thus, in the future forest structure and diversity [5]. Results from the few studies available for *Pinus* species about the relationship of seed viability and germination capacity with mother tree age are unclear, being strongly dependent on the species and the senescence stage. For example, no significant mother tree age-related differences in seed weight or seed germinability were found for *Pinus logaeva* [17]. Meanwhile, Tíscar-Oliver [18] found that the oldest *Pinus nigra* trees showed a higher number of empty seeds, with lighter seeds that showed lower germination vigor. Nonetheless, old trees were able to maintain a high germination rate. Moreover, also for *Pinus nigra*, Alejano et al. [10] found significant inter-individual variability in different cone- and seed-related traits, but no mother tree age effect was identified.

Seedling performance once seeds have germinated is also an interesting trait of study. In fact, seed mass, emergence rate and seedling growth rate have been suggested to play important roles in *Pinus sylvestris* early fitness in dry Mediterranean regions [19]. The expected negative influence of climate change on Sub-Mediterranean mountain *Pinus sylvestris* forests [20] makes it necessary to investigate the effect of drought on *Pinus sylvestris* seedling survival and how this effect can be mediated by the senescence stage of trees. Although *Pinus sylvestris* is considered a xerophytic species, the limited plasticity of its xylem and leaf properties may be behind its high vulnerability to the decline in water availability [21].

This study is aimed to assess how the mother tree age in *Pinus sylvestris* influences seed and cone biometric attributes, seed viability and germination capacity, as well as seedling performance (in terms of physiology and survival) in a death trial under nursery conditions. For this purpose, we focused on *Pinus sylvestris* old-growth forests in Sierra de Guadarrama National Park (Central Mountain Range of Spain). Specifically, we hypothesized that: (1) overmature trees maintain their germination capacity and seed viability with age; (2) the inter-individual variability for the studied traits can be greater than the effect of the mother tree age; (3) seed size plays a main role in the reproductive success, thus conditioning the results; (4) seedling performance in terms of survival will be more affected by the water availability level than by the mother tree age.

## 2. Materials and Methods

The study area is located on the north facing slopes of Valsain Forest (40°49' N, 4° 1' W), which is home to the oldest *Pinus sylvestris* in the Sierra de Guadarrama (Central Mountain Range of Spain), at a mean elevation of 1835 m. The climate is Sub-Mediterranean, the mean annual temperature is 8.5 °C, the mean monthly temperatures range between 1.2 °C (January) and 18.3 °C (July) and the average annual precipitation is 1275 mm. The major soil types are moderately deep dystric cambisols and ferric luvisols that have developed over acidic bedrock. The prevailing soil texture type is sandy loam. Ninety percent of the forested area is occupied by pure, even-aged *Pinus sylvestris* forests, with some scattered alpine shrubs in the upper parts.

A sample of 30 trees was chosen over an area of 10 ha, representing all age classes between 30 and 219 years old (See Table 1 for the description of the tree's dasometric characteristics.). Individual tree age was determined following standard dendrochronological procedures [22].

**Table 1.** Dasometric Characteristics of the Selected Trees.

Tree No.	Tree Age (yrs)	Diameter (cm)	Height (m)	Tree No.	Tree Age (yrs)	Diameter (cm)	Height (m)
801	154	62.0	12.9	817	204	82.0	14.2
802	48	34.1	12.1	818	108	37.0	13.1
803	137	29.0	14.4	819	205	62.0	15.5
804	50	29.2	13.7	820	89	37.0	12.1
805	181	64.5	11.1	821	199	76.2	15
806	57	29.2	13.7	822	117	40.0	104
807	215	68.0	14.7	823	204	76.0	14.3
808	46	40.1	14.3	824	185	38.5	13.6
809	216	64.3	14.6	825	194	72.5	13.8
810	30	23.7	15	826	71	31.0	13.7
811	186	58.6	15.1	827	219	57	10
812	62	13.1	7.9	828	203	25	10.4
813	180	66.0	13.8	829	195	56.5	15.6
814	64	28.2	12.5	830	112	29.5	16.1
816	62	35.6	11.3	832	121	41.5	13.7

In December 2019, when cones were mature, but before the start of seed dispersal, an average of 40 cones were collected from each selected tree. Cones were cold stored (3 °C) for a week and then placed in trays at room temperature. The number of cones per tree, together with individual cone fresh weight (in g), cone diameter (in cm) and cone length (in cm), were measured. Cone volume (in cm<sup>3</sup>) was estimated from cone diameter and length, assimilating the volume to an ellipsoid. After cone morphological characterization, cones were oven-dried at 30 °C for 40 h to accelerate their opening. Once cones had opened, the total seed mass per cone (in g) was recorded. Additionally, seed mass was weighed in five random samples per cone, containing 100 seeds in each sample. Each of the 100 seeds in the five samples was individually weighed (in mg), and length (in mm) and diameter (in mm) were measured. The rest of the seeds were stored in sealed glass containers under cold conditions (3 °C) until their use in the experiments described below.

One hundred seeds per tree were randomly selected to be used in the germination tests done at the University of Huelva. First, seeds were disinfected by immersion in a 30 mL L<sup>-1</sup> solution of H<sub>2</sub>O<sub>2</sub> for 10 min. Then, seeds were mixed with wetted perlite and sprayed with a fungicide (a solution of Daconil (Clortalonil) 50%, 1 g L<sup>-1</sup> and Captan 50%, 1.5 g L<sup>-1</sup>). The mixture was placed inside plastic bags under cold conditions (3 °C) and kept in the dark. After one month, 100 seeds per tree were distributed in four 8-cm-diameter Petri dishes (25 seeds per dish). Petri dishes had five sheets of absorbent paper at the bottom that had been previously wetted with distilled water and sprayed with fungicide. Petri dishes were kept at room temperature (18 to 25 °C) with a 14 h light/10h dark photoperiod. Germination

was recorded twice a week for 4 weeks. Different parameters were assessed [23] (FAO 1985): (1) germination energy (GE, which is the percentage of seeds germinated in the first week), (2) germination percentage at the end of the test (GP) and (3) germination values (GVCZ and GVDP) according to Czabator [24] and Djavanshir and Pourbeik [25]. Additionally, an extra sample of 20 seeds per tree was used to perform a tetrazolium test to analyze seed viability [23]. Seeds were soaked in distilled water for 24 h at room temperature and then cut in half with a scalpel. Each half was immersed in a tetrazolium solution (5 g L<sup>-1</sup> of 2,3,5 triphenyl-2H-tetrazoliumchloride) and kept in the dark for 80 min at 20 °C. Viable seeds (VS, i.e., seeds whose embryo and nutritive tissue stained red) from each tree were counted, and the percentage of viable seeds (%VS) was calculated.

In September 2020, an additional sample of 25 seeds per tree was pre-germinated inside Petri dishes in the plant laboratory at INIA, using the same protocol as described above. Ten days after seeding, germinated seeds were transplanted to FP-300 containers filled with a mixture of peat moss and vermiculite (80:20, *v:v*), adding 32 g NUTRICOTE fertilizer per container. The 25 pre-germinated seeds from each tree were distributed in five blocks of five seeds each. Seedlings were grown in the greenhouse and were irrigated to field capacity three days per week. Once the first juvenile needles appeared (37 to 40 days after transplanting), seedlings were left to dry until death. Seedling survival and volumetric water content of the substrate (measured using a portable time-domain reflectometer (TDR) equipped with two 16-cm rod probes (TDR100, Spectrum Technologies, Inc., Aurora, IL 60504, USA) were monitored once per week during 160 days. Volumetric water content at the beginning of the drying process was 15%.

To study the influence of the mother tree age on morphological traits of cones (weight, length, diameter and volume) and seeds (weight, length and diameter), germination capacity and viability (variables GE, GP, GVCZ, GVDP and VS%), we first checked the significance of the between-tree variability by building up a random effect model considering the tree as a random effect. The equation for the model was:

$$y_{ij} = t_j + e_{ij}$$

where  $y_{ij}$  referred to the assessed variable from tree  $j$ ,  $t_j$  is the tree random effect with  $t_j \sim N(0, \sigma^2_t)$  and  $e_{ij}$  is the residual error with  $e_{ij} \sim N(0, \sigma^2_e)$ .

First, we predicted for each measured trait the covariance parameter at the tree level and checked the covariance parameter equal-to-zero hypothesis with a  $\chi^2$  test. We then calculated the percentage of variance explained by the tree effect. In a second step, for those traits with a significant tree effect, we introduced the age of the mother tree as a covariate in a linear and quadratic form. Then, we assessed the significance of the coefficients ( $p < 0.1$ ) with an F test. We introduced the quadratic term to check for non-linear relationships of the mother tree age with the studied traits (morphological traits of cones and seeds, germination capacity and seed viability). The equation for the final model was:

$$y_{ij} = \beta_0 + \beta_1 \text{ age} + \beta_2 \text{ age}^2 + t_j + e_{ij}$$

where  $\beta_0$ ,  $\beta_1$  and  $\beta_2$  are unknown but estimable parameters and  $t_j$  and  $e_{ij}$  are as previously defined.

The influence of the mother tree age on seedling survival during the death trial was assessed considering a survival rate for a given tree  $j$  at a given date  $t$  ( $S_{jt}$ ) as the response variable, following a binomial distribution. As a first step, we considered an independent analysis for each date, using logistic regression with tree age (in a linear and quadratic form) included as explanatory covariates. As a second step, we carried out a repeated measures analysis by means of a generalised linear mixed model, where date was considered a random effect. The final expression of the generalised linear mixed model, assuming a logit link function, was:

$$S_{jt} = \frac{\exp(\theta_{jt})}{1 + \exp(\theta_{jt})}$$

where  $\theta_{it} = \beta_0 + \beta_1 \text{age} + \beta_2 \text{age}^2 + D_t$ ,  $D_t$  is the measurement date random effect with  $D_t \sim N(0, \sigma^2 D)$

For those traits where a significant effect of age was detected, the fitted model was plotted against the observed data in order to describe the pattern of the relationship.

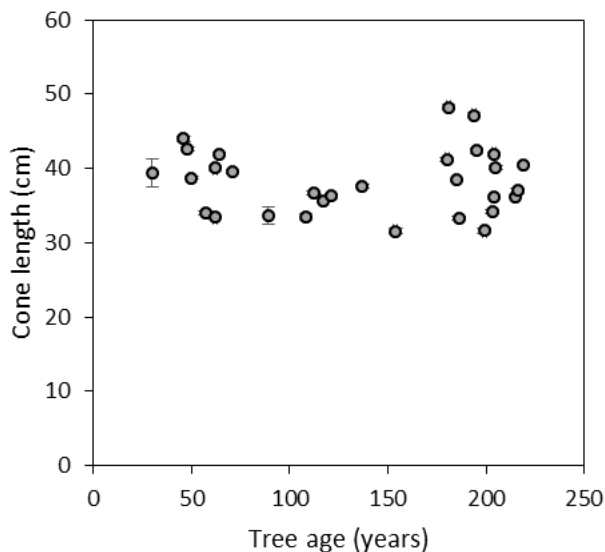
### 3. Results

#### 3.1. Morphological Traits of Cones and Seeds

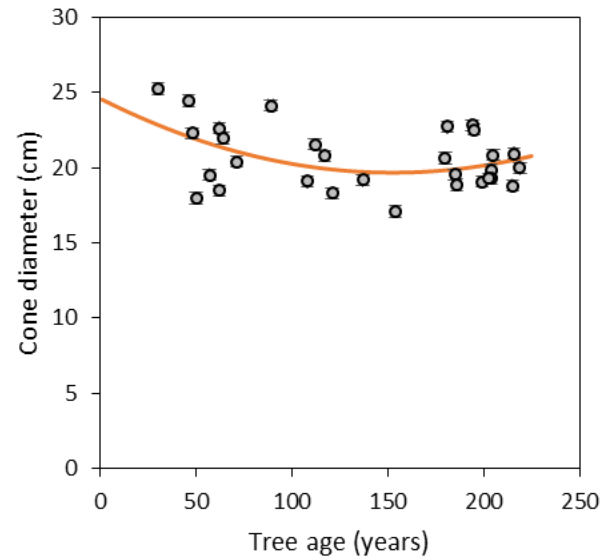
Results for the morphological traits of cones showed significant differences between trees ( $p = 0.0001$ , Table 2). The tree effect accounted for 39 to 47% of the total variability. These four cone traits were highly correlated ( $0.51 < r < 0.93$ ,  $p < 0.0001$ ). Mean values for the studied traits were (SD, range): length = 38.2 cm (4.3, 31.6 to 48.3), diameter = 20.6 cm (2.04, 17.1 to 25.2), weight = 5.8 g (1.29, 3.8 to 9.0) and volume = 71.2 cc (20.0, 39.1 to 113.0). The age of the mother tree had a significant effect on the diameter, weight and volume of cones (Table 2, Figure 1). For these traits, we observed a significant decrease with age, up to a tree age over 150–175 years, when a slight increase in the values was detected.

**Table 2.** Between-Tree Variability and Effect of Tree Age on Morphological Parameters of Cones. When the effect of age is significant ( $p < 0.1$ ), the value is shown in bold.

Variables	Covariance Parameter Estimates			Variance Explained by Tree Effect (%)	$p$ ( $\sigma^2_t = 0$ )	Tree Age Effect	
	Tree ( $\sigma^2_t$ )	Residual ( $\sigma^2_e$ )	Total			Age ( $p > F$ )	Age <sup>2</sup> ( $p > F$ )
Length	18.02	20.14	38.15	47	0.0001	0.16	0.15
Diameter	3.96	6.28	10.24	39	0.0001	<b>0.07</b>	0.19
Weight	1.61	2.01	3.62	44	0.0001	<b>0.07</b>	<b>0.09</b>
Volume	385.71	498.71	884.42	44	0.0001	<b>0.08</b>	0.12

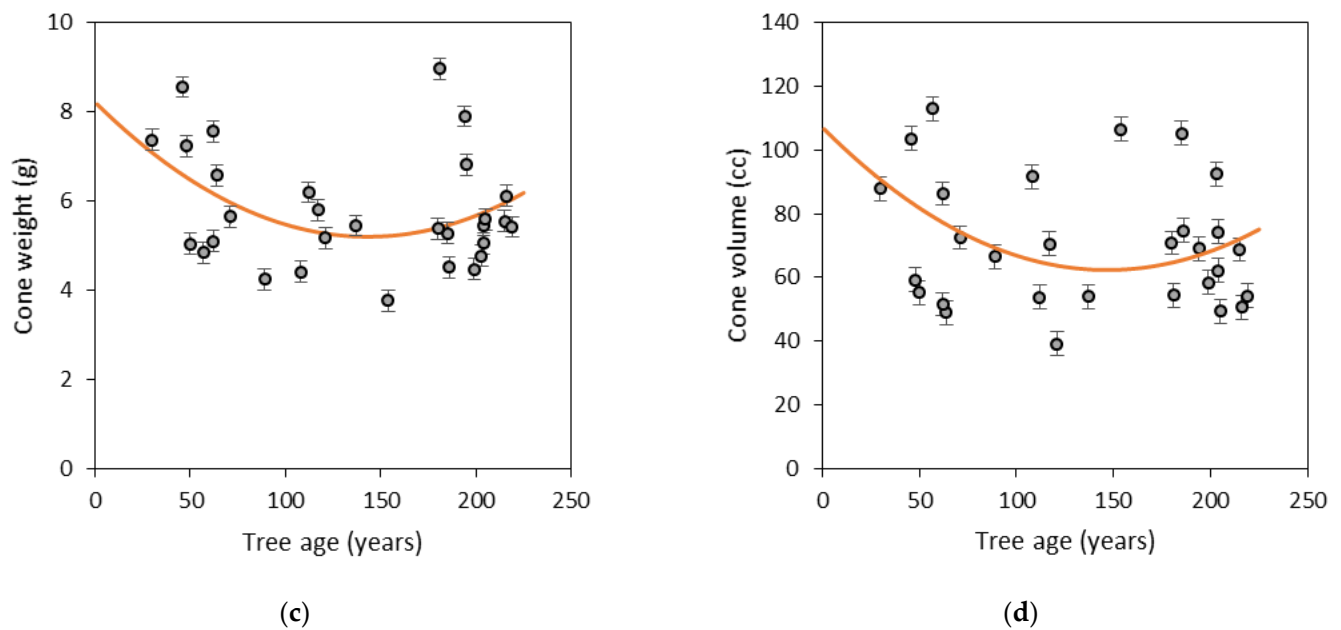


(a)



(b)

**Figure 1.** Cont.



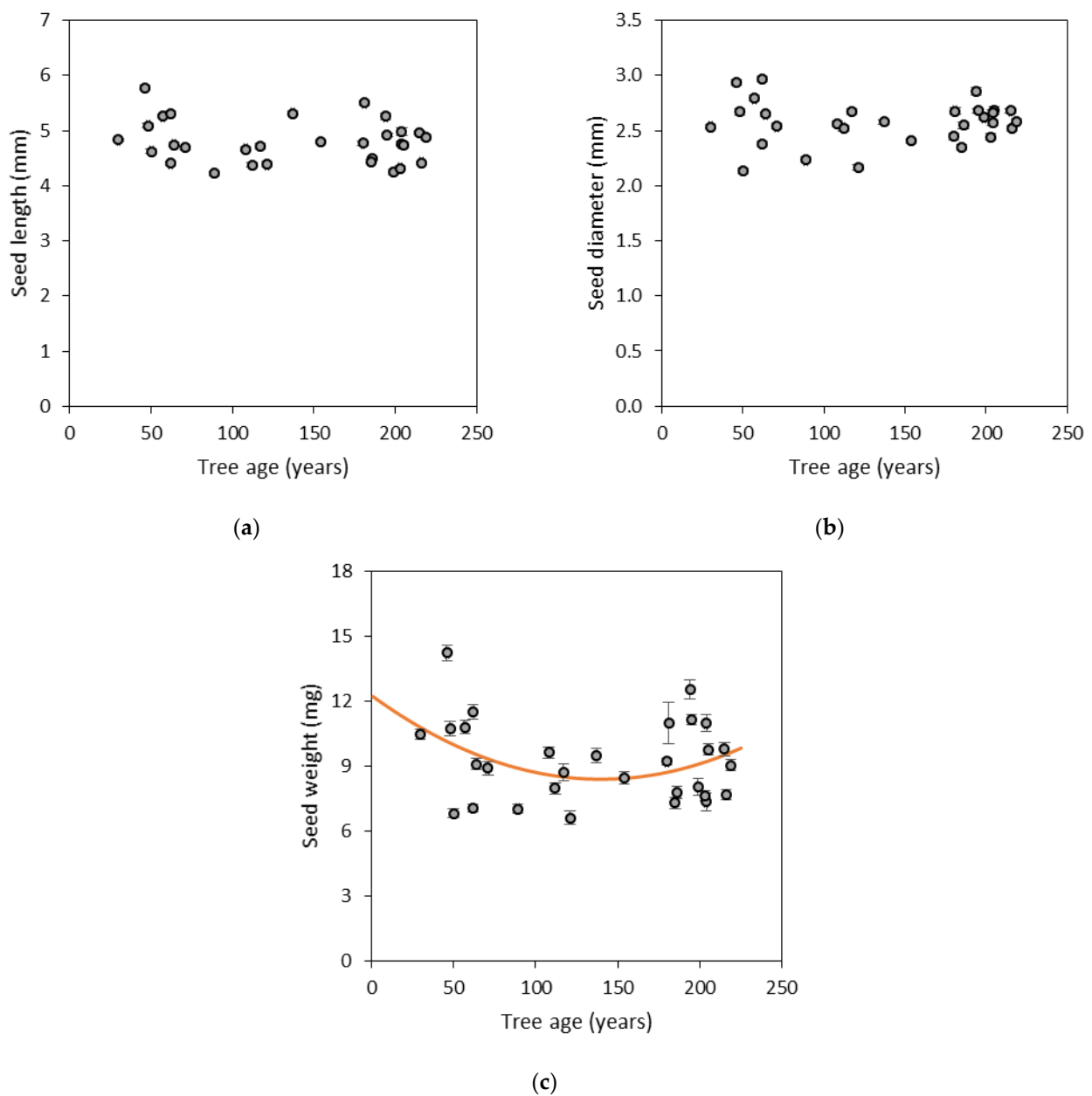
**Figure 1.** Mean values ( $\pm$ SE) (points) and fitted line (red line) for the morphological parameters of cones along the range of tree age analyzed: (a) length (in cm); (b) diameter (in cm); (c) weight (in g) and (d) volume (in cc). Fitted line is only shown for those traits showing a significant relationship with age.

Results for the morphological traits of seeds showed significant differences between trees ( $p = 0.0001$ , Table 3). The tree effect accounted for 22 to 45% of the total variability. These three seed traits were correlated ( $0.38 < r < 0.47$ ,  $p < 0.0001$ ). In addition, we recorded a negative correlation of seed weight ( $r = -0.07$ ,  $p = 0.0003$ ) and length ( $r = -0.12$ ,  $p < 0.0001$ ) with the age of the mother tree. Mean values for the studied traits were (SD, range): length = 4.8 mm (0.4, 4.2 to 5.8), diameter = 2.6 mm (0.2, 2.1 to 3.0) and weight = 9.2 mg (1.8, 6.6 to 14.2). The age of the mother tree had a significant effect on seed weight (Table 3, Figure 2). As in the previous case, for this trait, we also observed a significant decrease with age, up to a tree age over 150–175 years, when a slight increase in the values was detected.

**Table 3.** Between-Tree Variability and Effect of Tree Age on Morphological Parameters of Seeds. When the effect of age is significant ( $p < 0.1$ ), the value is shown in bold.

Variables	Covariance Parameter Estimates			Variance Explained by Tree Effect (%)	$p$ ( $\sigma^2_t = 0$ )	Tree Age Effect	
	Tree ( $\sigma^2_t$ )	Residual ( $\sigma^2_e$ )	Total			Age ( $p > F$ )	Age <sup>2</sup> ( $p > F$ )
Length	0.15	0.18	0.33	45	<0.0001	0.24	0.29
Diameter	0.04	0.07	0.11	36	<0.0001	0.15	0.15
Weight	3.29	11.67	14.95	22	0.0001	<b>0.09</b>	0.12





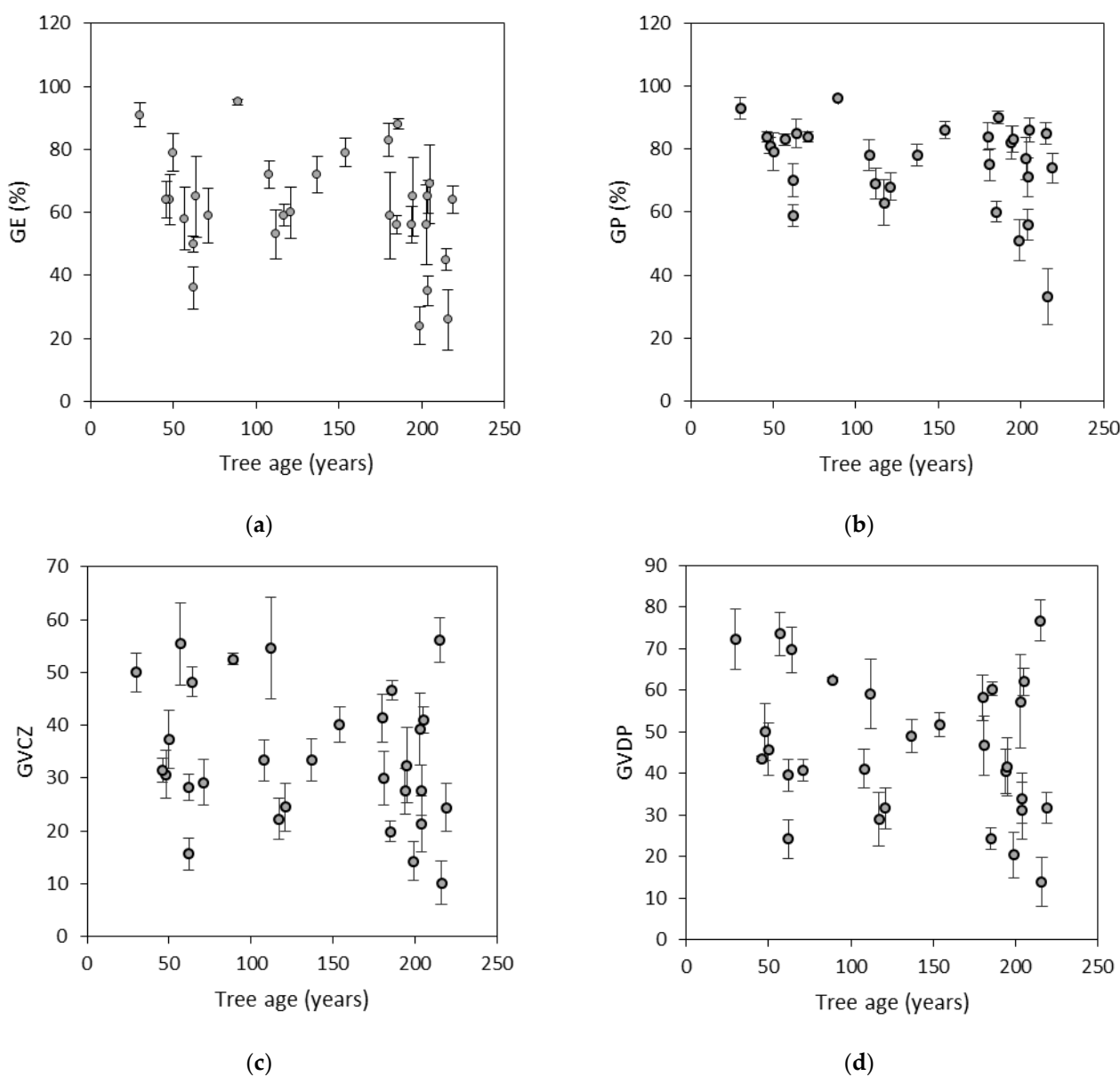
**Figure 2.** Mean values ( $\pm$ SE) (points) and fitted line (red line) for the morphological parameters of seeds along the range of tree age analyzed: (a) length (in mm); (b) diameter (in mm) and (c) weight (in mg). Fitted line is only shown for those traits showing a significant relationship with age.

### 3.2. Germination Capacity and Seed Viability

In the case of seed germination-related parameters (GE, GP, GVCZ and GVDP) significant differences were detected between trees ( $p < 0.001$ , Table 4), the tree effect accounting for 51 to 66% of the total variability. However, the age of the mother tree was not significant (Table 4, Figure 3). These four parameters were highly correlated ( $0.51 < r < 0.96$ ,  $p < 0.002$ ,  $n = 30$ ), the correlation between GVCZ and GVDP being especially prominent ( $r = 0.96$ ,  $p < 0.001$ ,  $n = 30$ ). We observed the following mean values (SD, range): GE = 61.6% (17.3, 24 to 95); GP = 75.4% (13.6, 33 to 96); GVCZ = 34.0 (12.7, 10 to 56) and GVDP = 46.0 (16.7, 14 to 77). Additionally, the percentage of viable seed (VS) ranged from 60 to 100%.

**Table 4.** Between-Tree Variability and Effect of Tree Age on Germination Parameters and Seed Viability. GE: Germination Energy. GP: Germination Percentage. GVCZ: Germination Values according to Czabator. GVDP: Germination Value according to Djavanshir and Pourbeik. VS%: Percentage of Viable Seed.

Variables	Covariance Parameter Estimates			Variance Explained by Tree Effect (%)	$p$ ( $\sigma^2_t = 0$ )	Tree Age Effect	
	Tree ( $\sigma^2_t$ )	Residual ( $\sigma^2_e$ )	Total			Age ( $p > F$ )	Age <sup>2</sup> ( $p > F$ )
GE	234.3	225.5	459.8	51	<0.0001	0.45	0.31
GP	157.9	85.8	242.7	65	<0.0001	0.86	0.92
GVCZ	134.1	85.7	219.8	61	<0.0001	0.94	0.89
GVDP	237.5	122.2	359.7	66	<0.0001	0.49	0.63
VS		0.0698				0.74	0.72

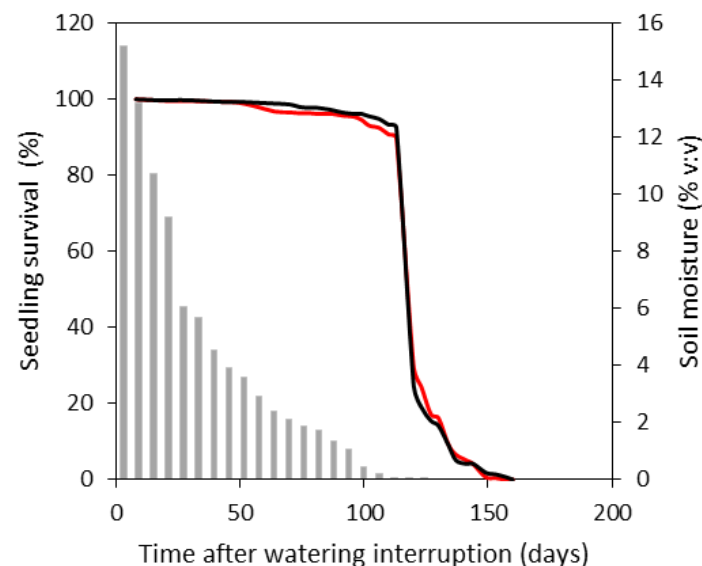


**Figure 3.** Mean values ( $\pm$ SE) (points) for the germination parameters of seeds along the range of tree age analyzed: (a) GE; (b) GP; (c) GVCZ and (d) GVDP. No fitted line is only shown as traits do not show a significant relationship with age.



### 3.3. Seedling Survival in the Death Trial

Results from the repeated measurements analysis of variance showed a significant effect of drought on survival over time ( $p < 0.0001$ ). Irrespective of the age of the mother tree, seedlings showed a survival rate of over 90% during 113 days after watering was interrupted, even if, by then, soil moisture had already reached 0% (Figure 4). From this day, survival drastically decreased to 27.1% in a week, although some seedlings remained still alive for another 47 days until the end of the experiment (day 160).

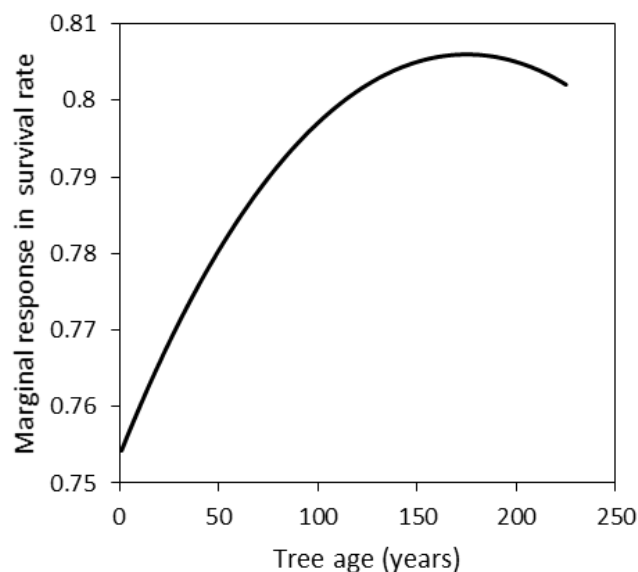


**Figure 4.** Survival (lines) and soil moisture (bars) in the death trial for seedlings from young (red line) and overmature (black line) mother trees. For the sake of clarity, trees are divided into two groups: young (<150 years old) and overmature (>150 years old).

When analyzing each measuring date separately, significant differences ( $p < 0.10$ ) in seedling survival related to the age of the mother tree were recorded in two periods: (i) from day 74 to day 110, when soil moisture ranged from 2.9 to 0.02%, and survival rate was maintained over 90% and (ii) from day 120 to day 144, when soil moisture had already reached 0%, and survival rate decreased from 27.1 to 4.1%. In the first period, higher survival was related to increasing tree age, while in the second period, higher survival was observed for lower tree age. In addition, the generalised linear mixed model revealed a global significant effect of the age of the mother tree on the rate of seedling survival, indicating that the rate of survival increases with mother tree age, up to an age range of 150–200 years old, decreasing from this age (Table 5, Figure 5).

**Table 5.** Parameter Estimates and Level of Significance of the Generalized Linear Mixed Model for the Survival Rate in the Death Trial.

Fixed Effect	Parameter Estimate	Standard Error	<i>t</i> Value	<i>P</i> >   <i>t</i>
Intercept	1.1180	0.7036	1.59	0.1219
Age	0.003500	0.001546	2.26	0.0238
Age <sup>2</sup>	−0.00001	$5.924 \times 10^{-6}$	−1.92	0.0557
Random effects	Covariance estimate	Standard error	<i>Z</i>	<i>P</i> > <i>Z</i>
Date	15.9918	4.1338	3.87	<0.0001



**Figure 5.** Marginal response for the survival rate along the range of tree age analyzed in the death trial.

#### 4. Discussion

Mean values for the morphological parameters of cones and seeds, as well as for seed germination-related parameters for the sampled *Pinus sylvestris* trees from the corresponding age range, were within the average range described for the species in the study area [12,26]. The between-tree differences in cone (between 20 and 60 cones per tree) and seed production (from 162 seeds in a 216-year-old tree to 1871 seeds in a 180-year-old tree) can be expected to be mainly related to the masting habit of the species, irrespective of tree age. For instance, *Pinus sylvestris* produces about two abundant seed yields every seven years [27] that are greatly mediated by the spring precipitation of the two years previous to cone maturation [28]. Additionally, the fact that both the lowest and highest seed production were obtained in overmature trees (216 and 180 years old, respectively) supports the idea that, when referring to seed production, tree senescence does not decrease fecundity with aging [15,16].

The significant differences between trees in the morphological parameters of cones and seeds bring out the high between-tree variability, which can be greatly influenced by species genetics, physiology or the environmental conditions during the collection year, even by cone location in the tree crown, rather than by the age of the mother tree [29–31]. In fact, in our study, the between-tree variability accounts for more than 50% of the total variability. Intraspecific variation in seed mass is common in conifers, whose seed mass could vary 3–5 fold among and within trees [32]. Nevertheless, we found a significant negative effect of the age of the mother tree on different morphological parameters of cones (diameter, weight and volume) and seeds (weight). These negative correlations highlighted lower values for these morphological parameters in trees reaching senescence. The observed nonlinear relationship indicates that this trend tends to be reverted in the oldest trees (>200 years old). Similarly, Kaliniewicz et al. [33] also observed a drop in the weight of *Pinus sylvestris* seeds at increasing ages up to 180 years old. Although, in our study, differences were small in absolute terms, they were significant, indicating that the sampled trees of advanced ages had passed beyond the stage of maturity and entered into the senescence stage [34].

In general, seed germination is determined by both environmental conditions and seed viability [35]. In our study, germination-related parameters and seed viability also showed a high between-tree variability that could point out a mother tree effect. However, these parameters were not related to the mother tree age; thus, there was not likely a decline in seed germination or seed viability in the oldest trees studied, as has been recorded in other species [10,36]. In addition, although seed weight decreased with tree age, no differential

germination rates were observed related to tree age; thus, the hypothesis that postulated that larger seeds generally germinate more rapidly was not checked [37].

Seedling survival in the death trial was mainly affected by the decrease in water availability rather than by the age of the mother tree. Once soil moisture reached 0%, seedlings still maintained survival rates of over 90% for two weeks. After this period, survival drastically decreased to ca. 27% in all seedlings, irrespective of the age of the mother tree. Nevertheless, we found an effect of the age of the mother tree on the survival rate, although the marginal effect of tree age (once the date as a random effect had been discounted) was small, accounting for differences in seedling survival rates of 4.8% (Figure 5). Interestingly, once soil moisture had reached 0%, we observed during 3.5 weeks higher survival rates in seedlings developed from younger trees (from day 120 to day 144). The greater seed weight found in younger trees (Figure 2c) could partially explain such differences in survival rates since larger seed size can confer seedlings with an initial advantage in terms of survival and growth [38,39]. Seed mass strongly influences seedling establishment, with heavier seeds usually exhibiting a higher capacity to survive under hazardous environments due to larger embryo and energy reserves that are efficiently used [32]. In fact, seed mass is a relevant life history trait, affecting crucial processes of species recruitment, including drought [40]. Particularly, seed size effects are expected to be especially relevant in highly competitive environments [41]. The positive association between seed mass and survival under dry conditions has been explained by the fact that larger seeds have more reserves to produce seedlings with larger growths and/or deeper roots (e.g., [42,43]). Thus, seedlings developing from larger seeds containing more nutrients and carbon-based reserves could show an improved ability to uptake the scarce resources and support respiration longer under carbon starvation [44]. For instance, Ramírez-Valiente and Robledo-Arnuncio [45] found a positive association between seed mass and seedling survival rate under dry conditions in *Pinus sylvestris*, which was explained by a lower needle-to-root ratio in seedlings from heavier seeds. However, differences in seedling survival related to the mother tree age were no longer significant when drought was prolonged beyond 144 days. At this point, seedling survival solely depended on the ability of belowground organs to absorb resources available in the soil [33]. Generally, *Pinus sylvestris* seedlings can maintain stable shoot water content under different water conditions. However, the water content collapses under severe drought stress, and seedlings die [46]. During severe drought stress, several physiological adaptation mechanisms allow *Pinus sylvestris* seedlings to obtain the water from the medium and maintain growth processes, though they are obviously affected by water stress [47]. Therefore, until seedlings collapsed, the functioning of PSII remained stable, thus indicating an undisturbed function of PSII even under drought stress. Accordingly, one of the main mechanisms used by *Pinus sylvestris* seedlings to adjust its hydraulic system to climatic conditions has been suggested to be directly linked to stomatal control instead of to the sensitivity of leaf physiology to drought [21].

## 5. Conclusions

For the studied *Pinus sylvestris* trees, ranging between 30 and 219 years old, the age of the mother tree had significantly influenced different cone (diameter, weight and volume) and seed (weight) morphological attributes, but not germination capacity or seed viability. Although the significant effect of mother tree age on the morphological traits of cones and seeds is small in absolute terms, it evidences that the sampled trees of advanced ages had passed beyond the stage of maturity and entered into the senescence stage. In any case, the production of good quality viable seeds, seed germination ability and the production of potentially vigorous seedlings were not affected by the age of the mother tree. Thus, the persistence of the studied overmature stands is guaranteed. It remains to study the proportion of empty seeds that seems to increase with aging. Additionally, in the death trial, seedlings from younger trees showed higher survival rates once soil moisture had reached 0%. Similar to the morphological traits of cones and seeds, the marginal effect of tree age was small. Seedling response, in terms of survival, to the suppression of water

supply revealed the ability of *Pinus sylvestris* seedlings to maintain the functioning of PSII undisturbed even under severe drought stress until the moment when seedlings collapsed and died.

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