

## Article

# Fertilization and Shading Trials to Promote *Pinus nigra* Seedlings' Nursery Growth under the Climate Change Demands

Marianthi Tsakalimi <sup>1,\*</sup> , Panagiota Giannaki <sup>1</sup> , Vladan Ivetić <sup>2</sup> , Nikoleta Kapsali <sup>1</sup>  and Petros Ganatsas <sup>1</sup> 

<sup>1</sup> Laboratory of Silviculture, Department of Forestry and Natural Environment, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece; pgiannaki@for.auth.gr (P.G.); nkapsali@for.auth.gr (N.K.); pgana@for.auth.gr (P.G.)

<sup>2</sup> Faculty of Forestry, University of Belgrade, 11030 Belgrade, Serbia; vladan.ivetic@sfb.bg.ac.rs

\* Correspondence: marian@for.auth.gr

**Abstract:** *Pinus nigra* is one of the most widely used tree species for reforestation within its geographical distribution, as well as being a potential substitute for other tree species in Central Europe under future climate scenarios. *P. nigra* is transplanted into the field as two-year or three-year old seedlings because of its relatively low growth rate in the nursery. This study investigated the effects of fertilization programs and shading on *P. nigra* seedlings, aiming to accelerate early growth, and thus to reduce the nursery rearing time. The experiment (a completely randomized block design) was conducted in an open-air nursery by sowing seeds from Grevena, Northern Greece, in Quick pots filled with peat and perlite in a 2:1 ratio. The seedlings were subjected to two levels of fertilization—5 and 10 g L<sup>−1</sup> NPK (30-10-10)—and two shading levels: 50% and 70%. At the ends of the first and second nursery growing season, we recorded the seedlings' above- and below-ground morphology and biomass data. The results show that the application of all of the treatments produced seedlings which met the targeted quality standards for outplanting. However, the combination of a high fertilization rate and low shading level resulted in seedlings of a higher morphological quality, which is often considered to be an indicator for a successful seedling establishment in the field.

**Keywords:** black pine; reforestation; climate change; nitrogen; shade; seedling morphology



**Citation:** Tsakalimi, M.; Giannaki, P.; Ivetić, V.; Kapsali, N.; Ganatsas, P. Fertilization and Shading Trials to Promote *Pinus nigra* Seedlings' Nursery Growth under the Climate Change Demands. *Sustainability* **2021**, *13*, 3563. <https://doi.org/10.3390/su13063563>

Academic Editors:

Michelle Serapiglia and Imre Holb

Received: 18 January 2021

Accepted: 12 March 2021

Published: 23 March 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Wildfires are a common phenomenon worldwide, and their frequency is expected to increase under the climate change [1,2]. They usually occur in forest ecosystems which are adapted to them, but in recent years, many fires have occurred in global forest ecosystems which have not developed adaptation mechanisms to wildfires, especially to crown fires. Some typical cases are the ecosystems of black pine (*Pinus nigra* J.F. Arnold) and fir forests (Greek fir—*Abies cephalonica* Loud. and King Boris fir—*Abies borisii regis* Mattf). The seeds of both pine and fir species mature during the autumn and disperse in the spring, so in the case of a summer fire there are no mature seeds to ensure regeneration [3]. At the same time, these species cannot regenerate asexually and, as a result, there is the risk of the forest not being able to re-establish itself [4], causing significant consequences such as loss of biodiversity and natural resources (i.e., soil due to erosion) and landscape degradation.

*Pinus nigra* J.F. Arnold is a conifer species with a relatively wide, but fragmented, distribution [5] across Europe and Asia Minor, predominantly in mountain areas. Because of its ecological flexibility and economic importance in southern Europe, it is one of the most widely used tree species for reforestation within its geographical distribution [6–8]. Furthermore, it is considered to be a potential substitute for other indigenous coniferous species in Central Europe under future climate scenarios. After a wildfire in a black pine forest, secondary succession results in the development of a non-forest ecosystem.

Following a fire in a black pine forest in Turkey, secondary succession led to the dominance of the shrub species *Cistus laurifolius* in the burnt pine areas [9]. Retana et al. [10] also reported that there is a decline of black pine presence in burnt areas in Spain due to the lack of regeneration of the species in post-fire conditions, and a great percentage of burnt black pine forests has a significant probability of turning into shrubland. Fyllas et al. [11] reported that, 13 years after the fire (in 2007) in a black pine forest at Olympos Mountain on Lesbos island, the burnt area was dominated by mixed stands of *Pinus brutia* and evergreen broadleaves. Similar observations were made in Greece for many burnt black pine forests [6].

Based on the available data, the recurrence of large wildfires is threatening the conservation of *P. nigra* ecosystems, as very little natural regeneration occurs in these species' forests after wildfires. Because the black pine is a non-serotinous pine, and because it has no adaptive mechanisms to crown fires, the natural regeneration of the species is limited to a short distance from the unburnt stands [3,10,12]. Seed dispersal models have shown that the natural regeneration of the black pine occurs at distances no greater than 100 m, depending on the following factors: the side seeding potential, site characteristics (soil fertility, moisture, slope, etc.), germination success, competing vegetation, survival and growth of the germinants, and anthropogenic interventions (fires, grazing, etc.) [10,13,14].

The ecosystems formed by black pine are a priority habitat type on a European level according to the Habitats Directive (Directive 92/43/EEC). Tackling the problem of the species' conservation is imperative, because a greater number of fires are expected to appear in these ecosystems in the future, due to climate change [15]. This gives rise to an urgent need to prepare and perform effective reforestation projects for the restoration of the burnt black pine forests within its geographical distribution. The preferred option for black pine reforestation is planting [3].

In forestry practice, *P. nigra* seedlings are transplanted into the field as two or three-year old seedlings because of their relatively low growth rate during the first few years [16–19]. Larger planting stock has a higher nutrient and carbohydrate content, which can promote field performance [20]. In the study of Devetaković et al. [21], taller and slender *P. nigra* seedlings survived at a higher rate, because in environmental conditions with a lack of vegetation control, the greater seedling height can be considered as an advantage [22,23].

Fertilization at the nursery phase is of great importance in producing seedlings of high quality with the potential for favorable field performance [23–25], due to good growth, nutrient storage reserves, and resistance to biotic and abiotic stresses [20]. However, unlike the vast literature on the effect of fertilization on pine seedling nursery growth [26–29], the effects on seedling above- and below-ground-morphology of *P. nigra* seedlings are poorly known [29,30].

*P. nigra* is a semi-shade tolerant tree species, and is considered to have similar behavior to that of shade-tolerant species [6,31]. Shading is used in Mediterranean nurseries as a common technique to protect seedlings from leaf damage and water loss due to the excessive radiation during the summer months. During the nursery phase, shading increases the shoot:root ratio, the leaf area, and the shoot height of the seedlings [26,32–34]. Therefore, identifying the tolerance degree of the species to specific shading conditions during the seedlings' production could provide information for both the species' plasticity and the best level of shading to produce vigorous high quality seedlings in a shorter time [35].

The specific main objectives of this study are to evaluate any possible effect of the application of different nitrogen-concentration fertilizers, different levels of shading, and their combined effects at the nursery phase, on the above- and below-ground growth of *P. nigra* seedlings, and accordingly, to identify whether the above treatments effectively accelerate the early seedlings' growth and reduce the time needed for them to gain a plantable size and quality.

## 2. Materials and Methods

The experiment was conducted in an open-air nursery of the Laboratory of Silviculture of the Aristotle University of Thessaloniki, northern Greece (40°53'85618" N, 22°9'9705299" E). Undamaged *P. nigra* seeds originating from the Grevena area, Northern Greece (Figure 1), were sown in March (2017) in plastic trays (Quick pots with 24 cavities each, a cavity volume of 330 cm<sup>3</sup>, and a depth 16 cm). All of the pots were filled with peat and perlite (2:1, v/v).



**Figure 1.** Location of the sampled *Pinus nigra* seeds from the Grevena area in Greece.

The seedlings were subjected to the following treatments: two fertilization regimes with two high doses (5 g and 10 g) [36] of water-soluble fertilizer N:P:K 30:10:10 + micronutrients per liter of substrate (486 mg seedling<sup>−1</sup> and 972 mg seedling<sup>−1</sup> respectively), and two shading regimes: 50% and 70%. Half the amount of fertilizer was incorporated into the substrate prior to sowing, and the other half was applied by watering during the rapid growth period of the seedlings. The shading treatments were applied from the beginning of June to the end of September. The four treatments (applied as a 2 × 2 factorial) were arranged in a completely randomized block design, and there were 10 blocks (i.e., 10 trays) per treatment, and a total of 222 seedlings per treatment. All of the seedlings were irrigated by an overhead irrigation system as needed.

At the end of the first growing season in the nursery (October 2017), the seedlings' above- and below-ground morphology and biomass were measured and recorded [8,37]. The measurements included: the shoot height (SH ± 0.1 cm), root collar diameter (RCD ± 0.1 mm), and percentage of mature needles of all of the seedlings. Furthermore, for a number of the sampled seedlings (seven seedlings per treatment) [26], and after a destructive sampling, the following quality parameters were measured: the central root length (CRL), number of first-order lateral roots longer than 1 cm (FOLR), number of root tips, and above and below-ground biomass. For the destructive sampling, the seedlings were extracted from the pots, and the root system was carefully separated from the substrate, under a gentle water jet, using a sieve to collect any root fragments detached from the system [37]. Then, the shoot and roots were separated (cut) at the root collar. The CRL, FOLR, and number of root tips were manually measured, as accurately as possible. Then, the shoots and the separated roots were dried at 70 °C for 72 h for the dry biomass measurements (accuracy of 0.01 g).

During the second growing season, 14 and 20 months after sowing (May 2018 and December 2018, respectively), the shoot height (SH) and root collar diameter (RCD) were measured on all of the seedlings.

A two-way ANOVA was used to determine the main treatment effects and their interaction. We considered the treatment's effect to be significant when  $p < 0.05$ . The trend of the seedlings' SH and RCD growth over time, during the 20 months after sowing in

the nursery in the two doses of fertilization and two shade regimes, were investigated by regression analysis. Several linear and non-linear models were tested, and we selected the simplest significant ( $p < 0.01$ ) models that best interpreted each relationship. All of the models were evaluated for goodness of fit by the graphical analysis of residuals. The best fitting model was selected with the highest coefficient of determination ( $R^2$ ), and the lower root mean square error in prediction (RMSE) [37]. The statistical analysis was performed using the SPSS program (v. 23, SPSS Inc., Chicago, IL, USA).

### 3. Results and Discussion

The *P. nigra* seedlings at the end of the growth period (seven months after sowing, October 2017), reached a size ranging from 8.5 ( $\pm 0.5$ ) to 10.1 ( $\pm 0.6$ ) cm in height, and from 2.0 ( $\pm 0.1$ ) to 2.4 ( $\pm 0.008$ ) mm in root collar diameter. The produced one-year-old container seedlings subjected to the specific fertilization and shade regimes were found to be shorter but thicker in diameter (Table 1), and with a greater number of FOLR (Table 2) than those of the same age reported by Kolevska et al. [38], whose SH fluctuated between 11.2 and 13.0 cm, RCD was between 1.90 and 1.97 mm, and number of FOLR was between 12.4 and 15.9. Furthermore, Ivetić et al. [18] reported much lower dimensions for the same age of black pine container seedlings (RCD 1.92 mm and SH 7.25 cm) than those of the current study. However, in both studies, the black pine seedlings were raised in smaller containers in volume and depth compared to those used in the present study. Larger containers generally result in better seedling nursery growth and field survival [39–41]. Therefore, the greater diameter and number of FOLR of the current study seedlings could be attributed to the influence either of the N fertilization and shading regimes, or to the greater container capacity.

**Table 1.** Effects of fertilization and shade regimes, and their interaction on *P. nigra* seedlings' shoot morphology and biomass at the end of the first growing season in the nursery, 7 months after sowing (October 2017).

Shade 50%					
Fertilization Level per Lit Substrate	Shoot Height SH, (cm)	Root Collar Diameter, RCD, (mm)	Percentage of Mature Needles (%)	Fresh Weight (g)	Dry Weight (g)
5 g	9.3 $\pm$ 0.47	2.1 $\pm$ 0.08	72.0 $\pm$ 4.6	1.7 $\pm$ 0.10	0.5 $\pm$ 0.03
10 g	8.5 $\pm$ 0.49	2.4 $\pm$ 0.08	87.5 $\pm$ 6.7	1.6 $\pm$ 0.13	0.6 $\pm$ 0.04
<i>p</i> value	0.267	* 0.043	* 0.045	0.708	0.079
Shade 70%					
5 g	10.1 $\pm$ 0.53	1.9 $\pm$ 0.09	57.0 $\pm$ 3.4	1.3 $\pm$ 0.15	0.4 $\pm$ 0.04
10 g	10.1 $\pm$ 0.60	2.0 $\pm$ 0.10	90.9 $\pm$ 8.8	1.6 $\pm$ 0.17	0.4 $\pm$ 0.05
<i>p</i> value	0.985	0.207	* 0.049	0.166	0.369
Shade effect	* 0.024	** 0.001	0.090	0.252	* 0.016
<i>p</i> value					
Fertilization effect	0.458	* 0.047	* 0.032	0.334	0.066
<i>p</i> value					
Fertilization X Shade	0.442	0.294	0.087	0.160	0.625
<i>p</i> value					

The means are followed by the  $\pm$  standard error of the mean, ( $n = 222$  and  $n = 7$  for fresh and dry weight). The symbols \* and \*\* mean significant effect at  $p < 0.05$  and  $0.01$ , respectively.

At this point, all of the studied seedlings cannot be considered plantable [42], according to the Serbian standard SRPS D.Z2.111:1968), as no treatment resulted in the seedling's RCD being higher than the crucial limit of 3 mm (Turkish Standards Institute TS 2265/March 1976 and TS 2265/February 1988, [43]). This is not surprising for black pine seedlings, taking

into consideration the eco-physiological attitudes of the species and the low endogenous growth rate during the first year [43].

**Table 2.** Effects of the fertilization and shade regimes, and their interaction on *P. nigra* seedlings' root morphology and biomass at the end of the first growing season in the nursery, 7 months after sowing (October 2017).

Shade 50%					
Fertilization Level per Lit Substrate	Central Root Length (cm)	FOLR	Number of Root Tips	Fresh Weight (g)	Dry Weight (g)
5 g	17.6 ± 0.68	38.5 ± 2.08	661.4 ± 80.7	0.9 ± 0.10	0.3 ± 0.03
10 g	18.3 ± 0.72	29.6 ± 2.21	723.9 ± 85.7	1.0 ± 0.10	0.4 ± 0.03
<i>p</i> value	0.278	** 0.001	0.627	0.733	** 0.007
Shade 70%					
5 g	18.4 ± 0.77	36.9 ± 2.36	658.1 ± 91.6	0.7 ± 0.10	0.2 ± 0.03
10 g	16.7 ± 0.87	24.4 ± 2.67	323.5 ± 103.3	0.6 ± 0.11	0.2 ± 0.03
<i>p</i> value	0.279	* 0.010	* 0.011	0.499	0.449
Shade effect	0.619	0.150	* 0.030	* 0.010	** 0.000
<i>p</i> value					
Fertilization effect	0.503	** 0.000	0.140	0.820	0.119
<i>p</i> value					
Fertilization X Shade	0.116	0.461	* 0.033	0.483	* 0.013
<i>p</i> value					

The means are followed by the ± standard error of the mean, (n = 7). The symbols \* and \*\* mean significant effect at  $p < 0.05$  and 0.01, respectively.

The seedlings' RCD and mature needles significantly increased under the higher fertilization dose, while the number of FOLR was significantly reduced (Tables 1 and 2). Toca et al. [29,30] reported for black pine seedlings supplied with 150 mg 20N–20P<sub>2</sub>O<sub>5</sub>–20K<sub>2</sub>O fertilizer that they developed larger root systems by maintaining a greater number of growing roots, rather than by increasing the elongation rate of the individual roots. Puértolas et al. [40] found that SDW, RDW, RCD, and SH increased with the fertilizer application rate in *P. halepensis* seedlings. However, we must mention that the effect of the nursery treatments (nutrient supply and growing density) in the root development of *P. nigra* seedlings is difficult to interpret, because they present a high variability of root length and number of root tips [44].

Regardless of the fertilization dose, the shade level affected the majority of the seedlings' attributes. The seedlings raised under 50% shade were significantly shorter, but presented significantly greater RCD, shoot dry mass, root fresh and dry mass (and therefore a greater root:shoot ratio), and number of root tips than those under 70% shading. Santelices et al. [36] also reported that *Nothofagus leonii* seedlings grew more efficiently under 50% shade. Puértolas et al. [34], who studied two Mediterranean species with contrasting shade tolerance, found that the quality of the seedlings of *Quercus ilex* (a shade tolerant species) and *P. halepensis* (shade intolerant) grown under 60% shade was not affected. Meanwhile, a previous study [33] on *Quercus ilex* seedlings concluded that 45% shading during the nursery growth significantly affected only the shoot:root ratio.

The shoot fresh weight and CRL showed no change with the applied treatments. *P. nigra*, as with most pine species, is tap-rooted at a young age, and the development of the central root of the seedlings was limited by the container depth [45], resulting in a similar length for all of the treatments.

Seedling root growth and fibrosity (FOLR, root tips) are valuable indicators of seedlings' quality [39,46]. However, in this study, even though the interpretation of the treatments effect on root branching is complex, it is quite clear that the seedlings raised under 50%



shade presented significantly greater RCD, root biomass, and number of root tips than those under 70% shading.

During the second growth period, 14 months after sowing, the shade regimes significantly affected the seedlings' SH and RCD, while the higher N fertilization dose significantly increased the seedlings' RCD in both shade regimes (Table 3). The seedlings raised under 50% shade were found to be thicker and higher. However, almost all of the seedlings could be considered plantable 14 months after sowing, because they had a diameter of 3.5–4.0 mm—a decisive factor for transplanting success—and a satisfactory height of approximately 14 cm. This may be interesting in some cases, when reforestation concerns areas of high elevation in an oro-Mediterranean climate, where plantings are carried out in spring [47].

**Table 3.** Effects of fertilization and shade regimes, and their interaction on *P. nigra* seedlings' shoot morphology 14 months after sowing (May 2018).

Fertilization Level per Lit Substrate	Shade 50%	
	Shoot Height (cm)	Root Collar Diameter (mm)
5 g	13.8 ± 0.5	3.6 ± 0.10
10 g	13.9 ± 0.6	4.0 ± 0.11
<i>p</i> value	0.932	* 0.033
Fertilization Level per Lit Substrate	Shade 70%	
	Shoot Height (cm)	Root Collar Diameter (mm)
5 g	14.0 ± 0.4	3.4 ± 0.07
10 g	12.9 ± 0.7	3.6 ± 0.16
<i>p</i> value	* 0.022	0.358
Shade effect <i>p</i> value	* 0.041	** 0.003
Fertilization effect <i>p</i> value	* 0.040	* 0.026
Fertilization X Shade <i>p</i> value	0.273	0.315

The means are followed by the ± standard error of the mean (n = 215). The symbols \* and \*\* mean significant effect at  $p < 0.05$  and 0.01, respectively.

At the end of the second growth period (twenty months after sowing) the produced seedlings achieved the dimensions needed for transplanting into the field. Almost all of the applied treatments produced seedlings of a satisfactory size, because their RCD exceeded 5 mm, and their SH exceeded 25 cm (Table 4). These studied container seedlings were found to be thicker and taller, and to have a greater number of FOLR than two-year-old container [8,18,43,48,49] and bareroot or tubed [50] black pine seedlings. It is of great importance that the achieved seedlings' size, in all of the treatments applied in this study, is considered well balanced in terms of diameter and height, and quite high for an effective field transplant [42], as they excel the minimum standard values proposed as suitable for black pine seedlings by the Greek Forest Service, as well as by the Turkish Standards Institute [43] and Serbian Standards [18]. The produced seedlings can not only be considered plantable but they are also morphologically improved Grade 1 seedlings, because almost all of the seedlings presented RCD greater than 5 mm, a value which is considered as fundamental for that characterization [42]. These seedlings usually have a higher root:shoot ratio, and are characterized by more fibrous roots [51]. Jinks and Kerr [49], who studied the field performance of *P. nigra* var. *maritime* seedlings, concluded that the initial seedling size was the most important factor determining the future growth in the field. Similarly, many previous studies have shown that the seedling performance after outplanting in the field was related to the initial seedling size at planting, even in the Mediterranean environments [18,20,37,52,53]. According to Villar-Salvador et al. [23], a seedling's size is an important attribute because it strongly determines the plant's photosynthesis and nutrient storage capacity and, consequently, resource mobilization and growth capacity. The above

responses could be critical in enhancing the ability of seedlings with larger root and shoot systems to grow quickly and occupy site resources during the establishment phase in the field [39,54].

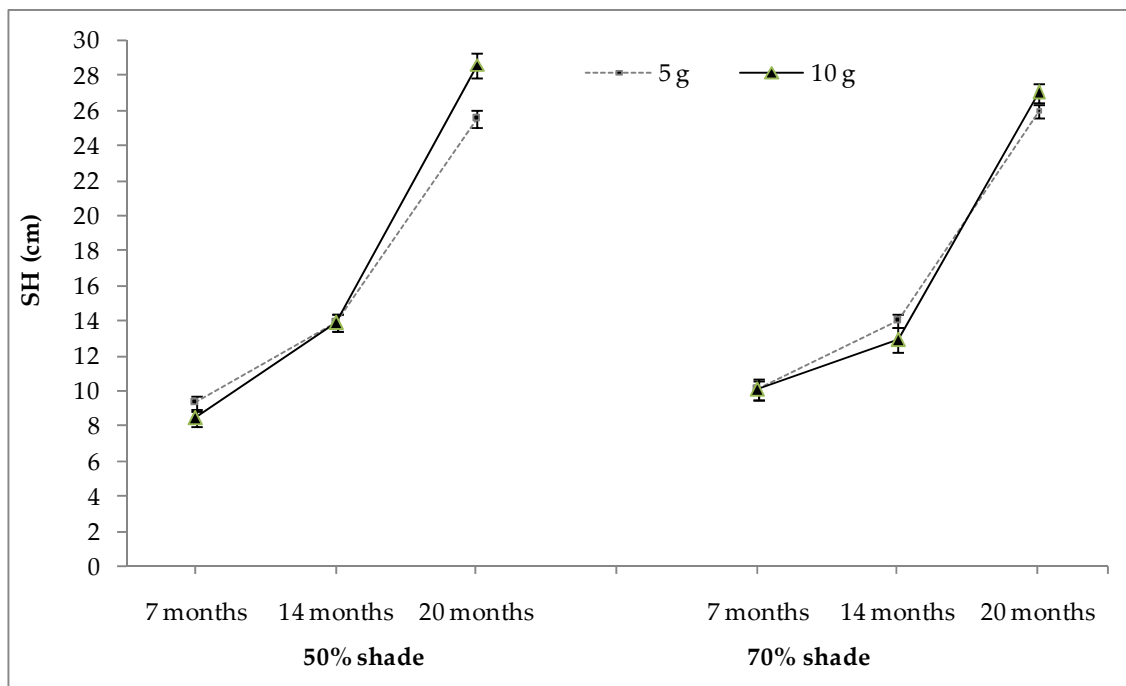
**Table 4.** Effects of the fertilization and shade regimes, and their interaction on *P. nigra* seedlings' shoot morphology 20 months after sowing (December 2018).

Shade 50%		
Fertilization Level per Lit Substrate	Shoot Height (cm)	Root Collar Diameter (mm)
5 g	25.5 ± 0.5	5.9 ± 0.16
10 g	28.6 ± 0.7	6.5 ± 0.18
<i>p</i> value	** 0.001	** 0.003
Shade 70%		
5 g	26.0 ± 0.6	5.2 ± 0.13
10 g	27.0 ± 0.7	5.4 ± 0.15
<i>p</i> value	0.598	0.504
Shade effect <i>p</i> value	* 0.020	** 0.000
Fertilization effect <i>p</i> value	* 0.018	* 0.009
Fertilization X Shade <i>p</i> value	* 0.011	* 0.046

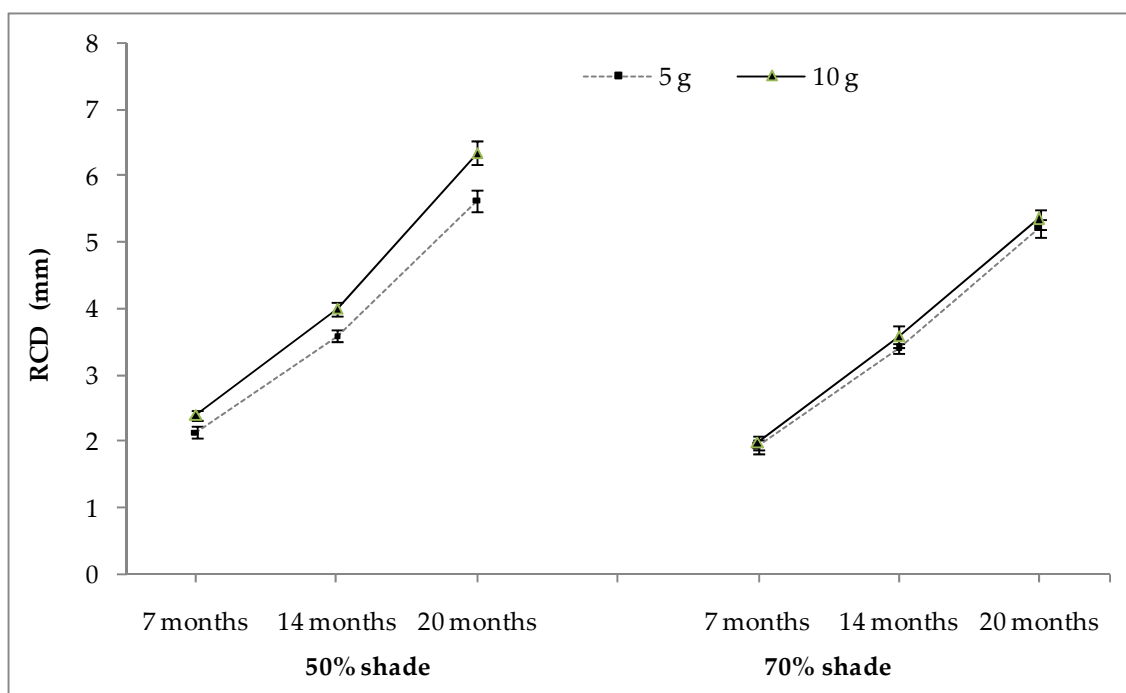
Means are followed by the ± standard error of the mean (n = 215). The symbols \* and \*\* mean significant effect at  $p < 0.05$  and 0.01, respectively.

Regardless of the shading treatment, twenty months after sowing, the higher fertilization dose was favorable for the seedlings' size (Table 4). This is evident from the seedling's growth trend during the whole period in the nursery (Figures 2 and 3): the higher the N fertilization dose, the larger the seedlings. However, the seedlings' SH and RCD were significantly greater only under 50% shade (Table 4). Our observations agree with the previous studies which found that the SH and RCD of pine seedlings increased with increasing fertilization rates applied during the nursery phase [28,55]. Similarly, González Orozco et al. [27] found that a high dose with 8 g L<sup>-1</sup> of Multicote TM (18-6-12) per liter of substrate improved the seedlings' height, diameter, total biomass, and Dickson quality index of *Pinus cooperi* Blanco. Many studies have indicated that an increase in seedling size and tissue nutrient concentration improves plants' survival in Mediterranean forest plantations [20,33]. The seedlings' SH and RCD were also significantly affected by the applied shade regimes (Table 4). During the same nursery period, regardless of the fertilization dose, the shade effect was significant. The 50% shade regime better favored the growth of the seedlings' RCD. Similarly to the first growing season, the combination of a higher fertilization dose (10 g per lit substrate) and 50% shade produced significantly thicker (RCD 6.35 mm) and taller seedlings (SH 28.6 cm).

Concerning the seedlings' growth trend during the 20 months after sowing in the nursery, 50% shade contributed to the exponential growth of the seedlings' SH ( $R^2 = 0.90$ ,  $p < 0.01$ ) and RCD ( $R^2 = 0.97$ ,  $p < 0.01$ ) (Figures 4 and 5). In case of 70% shade, the seedlings' SH also increased exponentially with the time ( $R^2 = 0.85$ ,  $p < 0.01$ ), while their RCD increased linearly ( $R^2 = 0.94$ ,  $p < 0.01$ ). It is worthwhile to point out that the seedlings grew not only during the typical growth period (May to October, for northern temperate regions), but also during the rest of the months of the year. This type of growth was apparent for the seedlings' height and diameter. However, there was more evidence for diameter increment.

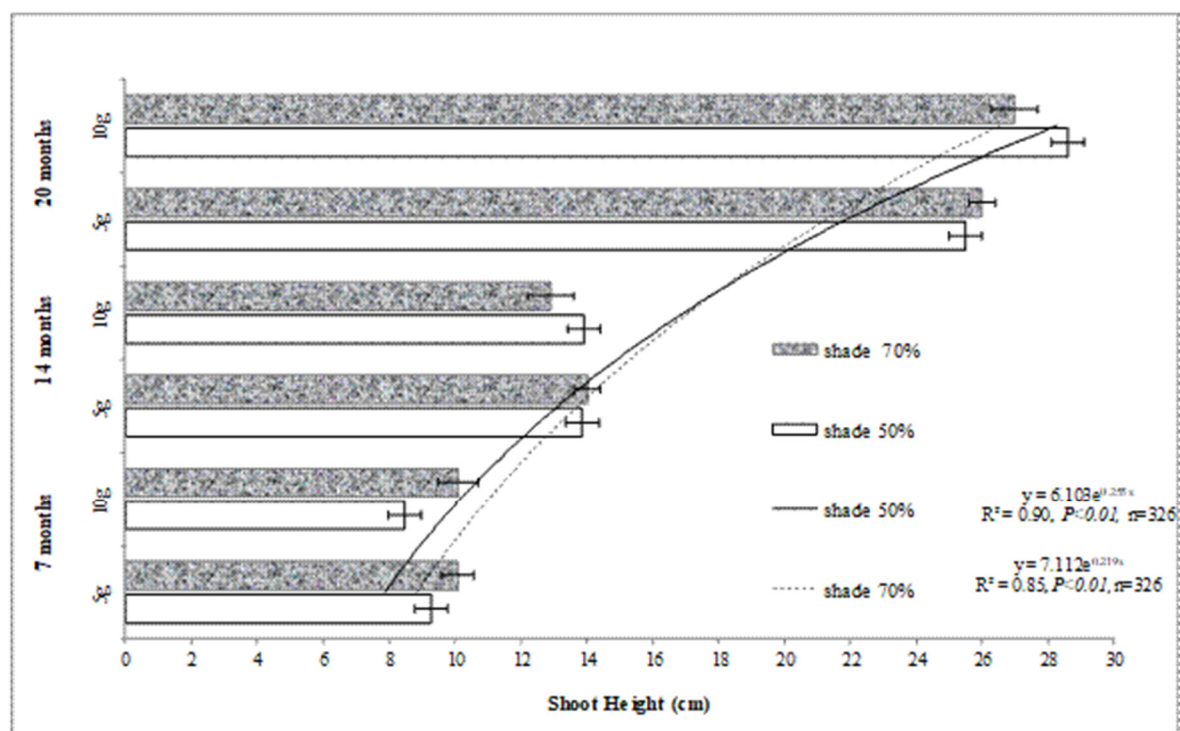


**Figure 2.** Seedlings' SH growth trend (means  $\pm$  standard error) during the 20 months after sowing in the nursery, in the two shade regimes (50% and 70%). Continuous line: 10 g fertilization dose; dashed line: 5 g fertilization dose.

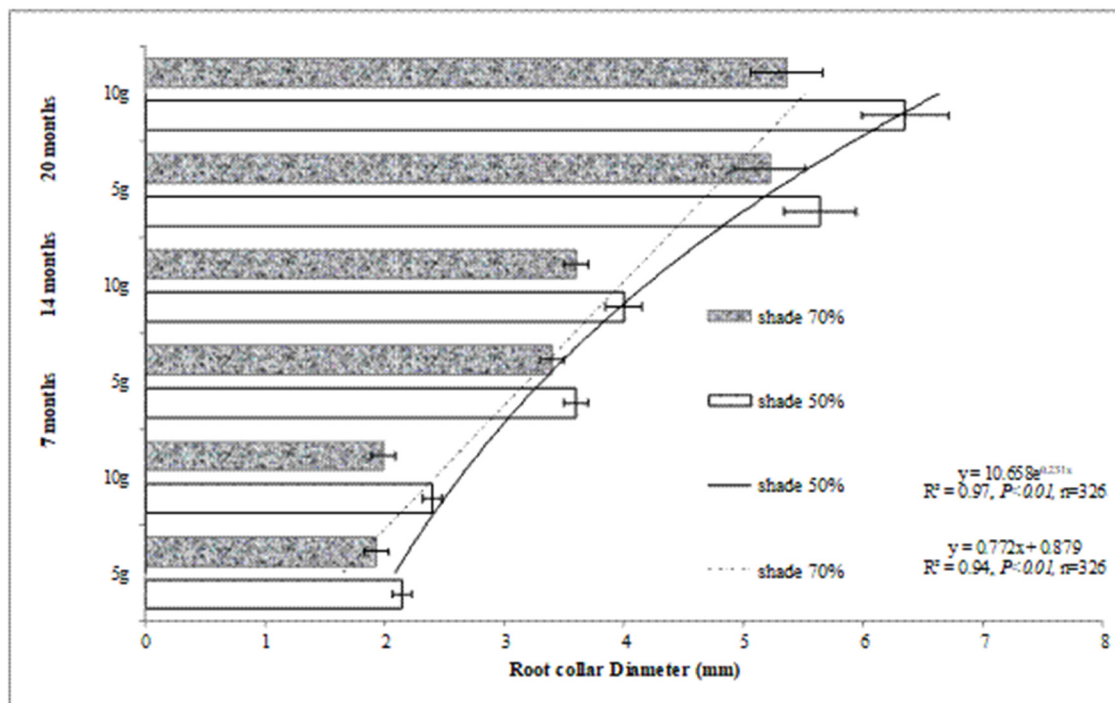


**Figure 3.** Seedlings' RCD growth trend (means  $\pm$  standard error) during the 20 months after sowing in the nursery, in the two shade regimes (50% and 70%). Continuous line: 10 g fertilization dose; dashed line: 5 g fertilization dose.





**Figure 4.** Seedlings' SH growth trend during the 20 months after sowing in the nursery, in the two doses of fertilization (5 g and 10 g) and the two shade regimes (50% and 70%). The error bars represent the std error of the mean.



**Figure 5.** Seedlings' RCD growth trend during the 20 months after sowing in the nursery, in the two doses of fertilization (5 g and 10 g) and the two shade regimes (50% and 70%). The error bars represent the std error of the mean.

#### 4. Conclusions

In the open nursery environment, at the end of the 2nd growth period, the containerized *P. nigra* seedlings overcame the target morphology (>25 cm in SH and >5 mm in

RCD), with dimensions that have not been reported by other studies so far. The treatments applied—fertilization with high N participation, and shade—both had a significant effect on the seedlings' morphological characteristics. The higher the N fertilization dose the larger the seedlings produced. The 50% shade exponentially increased the seedlings' SH and RCD with the time, regardless of the fertilization. The results of this study suggest that the combination of a high fertilization dose (10 g water-soluble fertilizer per litre of substrate) and intermediate shade (50%) accelerate the seedlings' growth and result in more robust and large seedlings in terms of their above- and below-ground morphological characteristics, which are considered to be indicators for a successful seedling field performance. However, taking into consideration the threat of climate change and the expected increase of wildfires, the need for species conservation is imperative. The results of this research contribute to an efficient production method of black pine seedlings of suitable size and quality, which is recommended for the restoration of burnt species' ecosystems.

**Author Contributions:** Conceptualization, P.G. (Petros Ganatsas); methodology, P.G. (Petros Ganatsas) and M.T.; software, M.T.; validation, M.T. and V.I.; formal analysis, P.G. (Petros Ganatsas), M.T.; investigation, P.G. (Petros Ganatsas), M.T., P.G. (Panagiota Giannaki), N.K.; data curation, M.T., P.G. (Panagiota Giannaki), N.K.; writing—original draft preparation, P.G. (Petros Ganatsas), M.T., V.I.; writing—review and editing, M.T., V.I.; supervision, P.G. (Petros Ganatsas). All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable. The study did not require ethical approval.

**Informed Consent Statement:** Not applicable.

**Acknowledgments:** We would like to thank the three anonymous referees whose comments contributed to improve this manuscript. Also, we would like to thank Despina Paitaridou (Ministry of Environment and Energy, General Directorate of Forest, Greece) for seeds supply. The work of Vladan Ivetić in this study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia according to the agreement number 451-03-9/2021-14/200169.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Pérez-Sánchez, J.; Jimeno-Sáez, P.; Senent-Aparicio, J.; Díaz-Palmero, J.M.; Cabezas-Cerezo, J.D.D. Evolution of burned area in forest fires under climate change conditions in southern Spain using ANN. *Appl. Sci.* **2019**, *9*, 4155. [\[CrossRef\]](#)
2. Singleton, M.P.; Thode, A.E.; Meador, A.J.S.; Iniguez, J.M. Increasing trends in high-severity fire in the southwestern USA from 1984 to 2015. *For. Ecol. Manag.* **2019**, *433*, 709–719. [\[CrossRef\]](#)
3. Espelta, J.M.; Retana, J.; Habrouk, A. An economic and ecological multi-criteria evaluation of reforestation methods to recover burned *Pinus nigra* forests in NE Spain. *For. Ecol. Manag.* **2003**, *180*, 185–198. [\[CrossRef\]](#)
4. Ganatsas, P.; Daskalakou, E.; Paitaridou, D. First results on early postfire succession in an *Abies cephalonica* forest (Parnitha National Park, Greece). *iForest* **2012**, *5*, 6–12. [\[CrossRef\]](#)
5. Tiscar, P.A.; Lucas-Borja, M.E.; Candel-Pérez, D. Lack of local adaptation to the establishment conditions limits assisted migration to adapt drought-prone *Pinus nigra* populations to climate change. *For. Ecol. Manag.* **2018**, *409*, 719–728. [\[CrossRef\]](#)
6. Ganatsas, P. Forest characteristics of Black pine ecosystems and restoration of burned stands. In *New Approaches to the Restoration of Black Pine Forests*; Kakouros, P., Chrysopolitou, V., Eds.; Management Body of Mount Parnonas and Moustos Wetland: Sparta, Greece, 2010; p. 7.
7. Ivetić, V.; Škorić, M. The impact of seeds provenance and nursery production method on Austrian pine (*Pinus nigra* Arn.) seedlings quality. *Ann. For. Res.* **2013**, *56*, 297–305.
8. Ivetić, V.; Grossnickle, S.; Škorić, M. Forecasting the field performance of Austrian pine seedlings using morphological attributes. *iForest Biogeosciences For.* **2016**, *10*, 99–107. [\[CrossRef\]](#)
9. Ocak, A.; Kurt, L.; Oz, M.; Tug, G.N. Floristical and ecological studies on burned black pine (*Pinus nigra* Arn. subsp. *Pallasiana* (Lamb) Holmboe) forest area at central Anatolia. *Asian J. Plant Sci.* **2007**, *6*, 892–905. [\[CrossRef\]](#)
10. Retana, J.; Espelta, J.M.; Habrouk, A.; Ordóñez, J.L.; de la Sola-Morales, F. Regeneration patterns of three Mediterranean pines and forest changes after a large wildfire in northeastern Spain. *Ecoscience* **2002**, *9*, 89–97. [\[CrossRef\]](#)
11. Fyllas, N.M.; Dimitrakopoulos, P.G.; Troumbis, A.Y. Regeneration dynamics of a mixed Mediterranean pine forest in the absence of fire. *For. Ecol. Manag.* **2008**, *256*, 1552–1559. [\[CrossRef\]](#)

12. Lucas-Borja, M.E.; Candel-Pérez, D.; Onkelinx, T.; Fule, P.Z.; Moya, D.; de las Heras, J.; Tiscar, P.A. Seed origin and protection are important factors affecting post-fire initial recruitment in pine forest areas. *Forests* **2017**, *8*, 185. [\[CrossRef\]](#)
13. Ordóñez, J.L.; Retana, J.; Espelta, J.M. Effects of tree size, crown damage and tree location on post-fire survival and cone production of *Pinus nigra* trees. *For. Ecol. Manag.* **2005**, *206*, 109–117. [\[CrossRef\]](#)
14. Ordóñez, J.L.; Molowny-Horas, R.; Retana, J. A model of the recruitment of *Pinus nigra* from unburned edges after large wildfires. *Ecol. Model.* **2006**, *197*, 405–417. [\[CrossRef\]](#)
15. González-Olabarria, J.R.; García-Gonzalo, J.; Mola-Yudego, B.; Pukkala, T. Adaptive management rules for *Pinus nigra* Arnold ssp. *salzmannii* stands under risk of fire. *Ann. For. Sci.* **2017**, *74*, 52. [\[CrossRef\]](#)
16. Van Haverbeke, D.F. *Pinus nigra*. European black pine. In *Silvics of North America. Conifers. Agriculture Handbook 654*; U.S. Department of Agriculture, Forest Service: Washington, DC, USA, 1990; Volume 1, 877p.
17. Isajev, V.; Fady, B.; Semerci, H.; Andonovski, V. *EUFORGEN Technical Guidelines for Genetic Conservation and Use for European Black Pine (Pinus nigra)*; International Plant Genetic Resources Institute: Rome, Italy, 2004; 6p.
18. Ivetić, V.; Maksimović, Z.; Kerkez, I.; Devetaković, J. Seedling quality in Serbia—Results from a three-year survey. *Reforest* **2017**, *4*, 27–53. [\[CrossRef\]](#)
19. Yıldız, O.; Altundağ, E.; Çetin, B.; Teoman Güner, Ş.; Sarginci, M.; Toprak, B. Experimental arid land afforestation in Central Anatolia, Turkey. *Environ. Monit. Assess.* **2018**, *190*, 355. [\[CrossRef\]](#)
20. Oliet, J.A.; Planelles, R.; Artero, F.; Valverde, R.; Jacobs, D.F.; Segura, M.L. Field performance of *Pinus halepensis* planted in Mediterranean arid conditions: Relative influence of seedling morphology and mineral nutrition. *New For.* **2009**, *37*, 313–331. [\[CrossRef\]](#)
21. Devetaković, J.; Maksimović, Z.; Ivanović, B.; Baković, Z.; Ivetić, V. Stocktype effect on field performance of Austrian pine seedlings. *Reforest* **2017**, *4*, 21–26. [\[CrossRef\]](#)
22. Pinto, J.R.; Marshall, J.D.; Dumroese, R.K.; Davis, A.S.; Cobos, D.R. Establishment and growth of container seedlings for reforestation: A function of stocktype and edaphic conditions. *For. Ecol. Manag.* **2011**, *261*, 1876–1884. [\[CrossRef\]](#)
23. Villar-Salvador, P.; Puertolas, J.; Cuesta, B.; Penuelas, J.L.; Uscola, M.; Heredia-Guerrero, N.; Rey-Benayas, J.M. Increase in size and nitrogen concentration enhances seedling survival in Mediterranean plantations. Insights from an ecophysiological conceptual model of plant survival. *New For.* **2012**, *43*, 755–770. [\[CrossRef\]](#)
24. Landis, T.D.; Tinus, R.W.; McDonald, S.E.; Barnett, J.P. Chapter 1—Mineral nutrients and fertilization. In *The Container Tree Nursery Manual. Seedling Nutrition and Irrigation*; No. 674; US Department of Agriculture, Forest Service: Washington, DC, USA, 1989; Volume 4.
25. Jacobs, D.F.; Davis, A.S.; Dumroese, R.K.; Owen, T.; Burney, O.T. Nursery cultural techniques facilitate restoration of *Acacia koa* competing with invasive grass in a dry tropical forest. *Forests* **2020**, *11*, 1124. [\[CrossRef\]](#)
26. Luis, V.C.; Lorca, M.; Chirino, E.; Hernandez, E.I.; Vilagrosa, A. Differences in morphology, gas exchange and root hydraulic conductance before planting in *Pinus canariensis* seedlings growing under different fertilization and light regimes. *Trees* **2010**, *24*, 1143–1150. [\[CrossRef\]](#)
27. González, O.M.M.; José, Á.P.R.; Arnulfo, A.; Ciro, J.H.D.; Armando, J.C.S.; Rodríguez, R.L. Raw sawdust substrates and fertilization in the plant quality of *Pinus cooperi* Blanco seedlings grown at the nursery. *Rev. Mex. Cienc. For.* **2018**, *9*, 203–225. [\[CrossRef\]](#)
28. Shi, W.; Grossnickle, S.C.; Li, G.; Su, S.; Liu, Y. Fertilization and irrigation regimes influence on seedling attributes and field performance of *Pinus tabulaeformis* Carr. *Forestry* **2019**, *92*, 97–107. [\[CrossRef\]](#)
29. Toca, A.; Villar-Salvador, P.; Oliet, J.A.; Jacobs, D.F. Normalization criteria determine the interpretation of nitrogen effects on the root hydraulics of pine seedlings. *Tree Physiol.* **2020**, *40*, 1381–1391. [\[CrossRef\]](#)
30. Toca, A.; Oliet, J.A.; Villar-Salvador, P.; Catalán, R.A.M.; Jacobs, D.F. Ecologically distinct pine species show differential root development after outplanting in response to nursery nutrient cultivation. *For. Ecol. Manag.* **2019**, *451*, 117562. [\[CrossRef\]](#)
31. Ordóñez, J.L.; Franco, S.; Retana, J. Limitation of the recruitment of *Pinus nigra* in a gradient of post-fire environmental conditions. *Écoscience* **2004**, *11*, 296–304. [\[CrossRef\]](#)
32. Heiskanen, J. Effects of pre- and post-planting shading on growth of container Norway spruce seedlings. *New For.* **2004**, *27*, 101–114. [\[CrossRef\]](#)
33. Villar-Salvador, P.; Planelles, R.; Enriquez, E.; Rubira, J.P. Nursery cultivation regimes, plant functional attributes, and field performance relationships in the Mediterranean oak *Quercus ilex* L. *For. Ecol. Manag.* **2004**, *196*, 257–266. [\[CrossRef\]](#)
34. Puértolas, J.; Benito, L.F.; Peñuelas, J.L. Effects of nursery shading on seedling quality and post-planting performance in two Mediterranean species with contrasting shade tolerance. *New For.* **2009**, *38*, 295–308. [\[CrossRef\]](#)
35. Leão, N.V.M.; Shimizu, E.S.C.; Felipe, S.H.S. Shading improves initial growth and quality of *Parkia multijugabenth.* seedlings. *Aust. J. Crop Sci.* **2019**, *13*, 1908–1913. [\[CrossRef\]](#)
36. Santelices, R.; Espinoza, S.; Cabrera, A.M. Effects of shading and slow release fertilizer on early growth of *Nothofagus leonii* seedlings from its northernmost distribution in Central Chile. *Bosque* **2015**, *36*, 179–185. [\[CrossRef\]](#)
37. Tsakalidimi, M.; Ganatsas, P.; Jacobs, D.F. Prediction of planted seedling survival of five Mediterranean species based on initial seedling morphology. *New For.* **2013**, *44*, 327–339. [\[CrossRef\]](#)
38. Kolevska, D.D.; Dimitrova, A.; Cokoski, K.; Basova, M. Growth and quality of *Pinus nigra* (Arn.), *Pinus sylvestris* (L.) and *Pinus pinaster* (Aiton) seedlings in two container types. *Reforest* **2020**, *9*, 21–36. [\[CrossRef\]](#)

39. Grossnickle, S.C. Importance of root growth in overcoming planting stress. *New For.* **2005**, *30*, 273–294. [\[CrossRef\]](#)
40. Puértolas, J.; Jacobs, D.F.; Benito, L.F.; Peñuelas, J.L. Cost-benefit analysis of different container capacities and fertilization regimes in *Pinus* stock-type production for forest restoration in dry Mediterranean areas. *Ecol. Eng.* **2012**, *44*, 210–215. [\[CrossRef\]](#)
41. Mariotti, B.; Maltoni, A.; Jacobs, D.F.; Tani, A. Container effects on growth and biomass allocation in *Quercus robur* and *Juglans regia* seedlings. *Scand. J. For. Res.* **2015**, *30*, 401–415.
42. South, D.B. Planting morphologically improved pine seedlings to increase survival and growth. In *Forestry and Wildlife Research Series*; No. 1; Waters, L., Jr., Ed.; Alabama Agricultural Experiment Station: Alabama City, AL, USA, 2000; p. 12.
43. Deligoz, A.; Bayar, E.; Gur, M.; Genc, M. An assessment of the important seedling properties for reforestation in *Pinus nigra* J. F. Arnold subsp. *nigra* var. *caramanica* (Loudon) Rehder from Three Provenances. In *Proceedings of the International Caucasian Forest Symposium*, Artvin, Turkey, 24–26 October 2013; pp. 13–17.
44. Chiatante, D.; Di Iorio, G.S.; Scippa, A.; Schirone, B. Root architectural and morphological response of *Pinus nigra* Arn. and *Quercus robur* L. to nutrient supply and root density in the soil. *Ann. Bot. Nuovaserie* **2004**, *IV*, 159–171.
45. Tsakalimi, M.; Tsitsoni, T.; Ganatsas, P.; Zagas, T. A comparison of root architecture and shoot morphology between naturally regenerated and container-grown seedlings of *Quercus ilex*. *Plant Soil* **2009**, *324*, 103–113. [\[CrossRef\]](#)
46. Haase, D.L. Seedling root targets. In *National Proceedings: Forest and Conservation Nursery Associations 2010*; RMRS-P-65; Riley, L.E., Haase, D.L., Pinto, J.R., Eds.; USDA Forest Service, Rocky Mountain Research Station: Fort Collins, CO, USA, 2011; Volume 65, pp. 80–82.
47. Dafis, S.; Chatzistathis, A. *Reforestations—Forest Nurseries*; Giahoudis Editions: Thessaloniki, Greece, 1989. (In Greek)
48. Genç, M.; Güner, T.; Şahan, A. Morphological research on 2 + 0-year-old Black pine seedlings in Eskişehir, Eğirdir and Seydişehir forest nurseries. *Turk. J. Agric. For.* **1999**, *23*, 2.
49. Jinks, R.L.; Kerr, G. Establishment and early growth of different plant types of Corsican pine (*Pinus nigra* var. *maritima*) on four sites in Thetford Forest. *Forestry* **1999**, *72*, 293–304. [\[CrossRef\]](#)
50. Eken, O.; Öner, N. An assessment of the important morphological properties of anatolian black pine seedlings in semiarid forest nursery. *Fresenius Environ. Bull.* **2017**, *26*, 4158–4162.
51. Collet, C.; Löf, M.; Pages, L. Root system development of oak seedlings analysed using an architectural model. Effects of competition with grass. *Plant. Soil* **2006**, *279*, 367–383. [\[CrossRef\]](#)
52. Grossnickle, S.C.; MacDonald, J.E. Why seedlings grow: Influence of plant attributes. *New For.* **2018**, *49*, 1–34. [\[CrossRef\]](#)
53. Tsakalimi, M.; Zagas, T.; Tsitsoni, T.; Ganatsas, P. Root morphology, stem growth and field performance of seedlings of two Mediterranean evergreen oak species raised in different container types. *Plant Soil* **2005**, *278*, 85–93. [\[CrossRef\]](#)
54. Grossnickle, S.C. *Ecophysiology of Northern Spruce Species: The Performance of Planted Seedlings*; NRC Research Press: Ottawa, QC, Canada, 2000.
55. Wang, J.; Li, G.; Pinto, J.R.; Liu, J.; Shi, W.; Liu, Y. Both nursery and field performance determine suitable nitrogen supply of nursery-grown, exponentially fertilized Chinese pine. *Silva. Fenn.* **2015**, *49*, 1–13. [\[CrossRef\]](#)