Initial Responses of Containerized Black Pine (*Pinus nigra* Arnold) Seedlings to Leaf Removal Prior to Out-planting

FERHAT KARA[1*] AND OSMAN TOPACOGLU[1]
[1]*Kastamonu University, Faculty of Forestry, Department of Forest Engineering, 37100, Kastamonu, Turkey*
**Corresponding author: email: fkara@kastamonu.edu.tr; phone: +90-533-7757755*

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Abstract

The initial survival and growth of seedlings following out-planting affect the success of plantation activities. Due to transplanting shock, mortality is usually higher and the growth rate is lower in the first growing season following out-planting. In this study, the influence of leaf removal on the initial survival and growth of containerized black pine (*Pinus nigra* Arnold) seedlings was observed. Thirty seedlings were randomly assigned to one of four treatments: 0%, 25%, 50%, and 75% removal of the existing needles. Height growth, root-collar diameter (RCD) growth, increase in dry stem mass and dry root mass, and mortality were observed during the first growing season following out-planting. All seedlings survived the first growing season. Removal of needles did not affect the height growth, RCD growth, or dry stem mass of the seedlings. However, there was a statistically significant relationship between the removal of needles and the dry root mass; the dry root mass increased with increasing removal of needles. The initial results show that better root development is associated with needle defoliation. However, long-term observations of the seedling responses are recommended.

Keywords: Black pine, defoliation, dry mass, growth, survival.

Introduction

Black pine (*Pinus nigra* Arnold) is one of the main native tree species in Turkey (Ertekin and Özel 2010) and is an important tree species of the Mediterranean regions providing high-quality timber to the region (Bogunic et al. 2007). This species has a wide distribution area in Turkey and mostly occurs at elevations between 400 m and 2100 m above sea level. In Turkey, black pine can be found as pure and mixed stands, and covers an area of approximately 4,700,000 hectares which is 22% of the total forested land (General Directorate of Forestry 2014). Black pine is one of the most commonly used pine species in large plantations throughout Turkey as well as in several other countries within the Mediterranean region (Ertekin and Özel 2010).

Due to its tolerance to drought, black pine is a highly preferred tree species for planting within arid and semi-arid areas in Turkey (Temel et al. 2011). Due to problems with the natural regeneration of black pine, artificial regeneration has been used to increase regeneration success in Turkey (Çalışkan et al. 2014). It has been suggested that nearly half of the black pine forests are degraded, and that those degraded stands should be restored via artificial reforestation (General Directorate of Forestry 2014). However, during artificial regeneration activities, a higher rate of seedling survival and growth is necessary for restoration success because transplanted seedlings are usually exposed to stress (i.e. transplant shocks) during the acclimation process in the first year of planting (Yamashita et al. 2016). Accordingly, higher mortality and lower growth are usually observed during the first year following out-planting (Brockway et al. 2006). Therefore, maximizing seedling survival and growth in the first year of planting is important for the success of reforestation and afforestation (Saramäki and Hytönen 2004, Janson and Baumanis 2005).

Abiotic and biotic stresses negatively affect the growth and quality of trees in plantation areas (Barry and Pinkard 2013). Some of the transplant shocks (i.e. stresses) caused by sudden changes in environmental conditions may include photo-damage following high-intensity light, drought, and high or low temperatures (Close et al. 2005, Struve 2009). Slower growth of seedlings is commonly caused by transplant shock, which can lead to the deterioration of the healthy status of seedlings or even to their death. Determining the transplant shock would increase plantation success and reduce the cost of reforestation. While some studies have revealed that leaf removal reduces growth due to decreasing carbon gain (Harris and Bassuk 1995, Goheen et al. 2007), other studies have suggested that a reduction in the amount of leaf area for evergreen species may minimize transplant shock and increase survival and growth (Yamashita et al. 2016) or have no influence (Solomon 1983). However, there has not been such a study conducted for black pine seedlings. Therefore, the main objective in this study was to determine the influence of needle removal on the initial survival and growth of containerized black pine seedlings in Turkey.

Materials and Methods

Study area

This study was conducted at the main campus of Kastamonu University located in Kastamonu city, northern Turkey (Figure 1). The study area is located within the Euro-Siberian phytogeographic region (Çolak et al. 2009), and within the natural range of black pine. The study area shows the typical characteristics of a terrestrial Black Sea climate with cold winters and rainy summers with an average annual precipitation of approximately 580 mm. The average temperature of the study area is 9.7 °C. Within the study area, the soil is loamy clay, and the topography is nearly flat. The elevation varies from 700 m to 800 m a.s.l.

Figure 1. Location of the study area

Study design

Three-year old containerized seedlings were obtained from the nursery of the Daday Forest Planning Directorate, which is approximately 25 km from the study area. The nursery is located at an elevation of 800 m a.s.l. The seedlings were 18-cm deep-plug containerized with a rooting volume of 450 cm^3 (5 cm × 5 cm × 18 cm) (Figure 2). Seedlings with dark green foliage and larger root-collar diameters were selected (120 seedlings in total); the main criteria for the selection of the seedlings in this study were their identical height and physical appearance.

A total of 120 seedlings were transported from the nursery on the day of planting in March 2016. Seedlings were randomly assigned to one of four treatments: 0%, 25%, 50% and 75% removal of the existing needles (i.e. 30 seedlings for each treatment). Needle removal was mass-based and conducted beginning from the stem base toward the shoot tip. The needle mass was visually determined for each seedling. For example, for the 50% removal, 50% of the existing needles from the stem base to the shoot tip were removed as suggested by Yamashita et al. (2016). A total of 20 seedlings for each treatment (80 seedlings total) were randomly planted in the field, where the previous vegetation cover had been removed. Seedlings were hand-planted on the same day in March 2016 approximately 1 m from each other to minimize competition. Planting was completed following a rainy period, and no further watering was applied. Weeding was performed 3 months following planting. Planted seedlings were tagged and numbered after planting to monitor their growth and survival.

Figure 2. A containerized black pine seedling

Field measurements

As stated above, 120 black pine seedlings were transported from the nursery and 80 were planted in the field. The remaining 40 seedlings (i.e. 10 seedlings from each treatment) were removed from their containers and growing media, and their roots were thoroughly washed in March 2016. Those 40 seedlings were separated into leaves, main stem and roots to determine the initial dry mass of the stems and roots. All sections were dried in an oven (70 °C for 72 hours) before measuring the initial dry masses as suggested by Yamashita et al. (2016). Then, all sections were weighed using a precision balance. These initial measurements were used to determine the increase in the average dry stem mass and dry root mass of the seedlings at the end of the first growing season.

The initial root-collar diameter (RCD) of the planted black pine seedlings was recorded soon after the planting in the field (i.e. March 2016). The RCD was measured by a digital calibre with the millimetre scale. In addition, the initial height of each black pine seedling was measured using a ruler. The height and RCD measurements were repeated at the end of the first growing season (March 2017) to calculate the height and RCD growth. The mortality of the planted black pine seedlings was also recorded during the first growing season.

At the end of the first growing season (March 2017), the black pine seedlings were uprooted and brought to the lab. They were separated into leaves, main stem, and roots after their roots were thoroughly washed. The dry mass of the stem and root were measured after they were dried in an oven (70 °C for 72 hours) using the same procedure as explained above.

The influence of the removal of needles on the height growth, RCD growth, mortality, and dry mass of the stem and root of black pine seedlings were determined using a one-way analysis of variance (ANOVA) statistical model with the R software package (R Development Core Team 2011). The one-way ANOVA model is recommended to test if there are any statistically significant differences among the means of three or more independent groups (Walpole and Myers 1993). In addition to ANOVA test, comparisons among the means were conducted using the *t*-test (Student test). Multiple comparisons of means of needle removal were performed using Tukey's method with the aid of the R software package (R Development Core Team 2011).

Results

The initial height and RCD of the black pine seedlings did not significantly change for the differing amounts of needle removal ($P = 0.08$ and 0.47, respectively). The average initial height of the black pine seedlings was 35.42 (± 0.6) cm. There were no significant relationships between the amount of needle removal and the initial dry stem mass ($P = 0.34$) and initial dry root mass ($P = 0.16$). The average initial dry root mass was 1.1 (± 0.13) g; while the initial dry stem mass was 4.76 (± 0.33) g. At the end of the first growing season (March 2017), there was no statistically significant relationship between the amount of needle removal and the seedling mortality ($P = 0.9$). Survival was high (99.7 %) despite needle removal (Table 1); only one seedling died across all planted black pine seedlings.

Table 1. Survival rate of the planted black pine seedlings for varying amounts of needle removal

Amount of needle removal	Survival, %
No removal (0%)	100
25%	95
50%	100
75%	100

At the end of the first growing season (March 2017), there was no statistically significant effect of needle removal on the height growth of the black pine seedlings ($P = 0.299$) (Table 2) (Figure 3). Height growth ranged from 1 cm to 12.7 cm with an average of 5.69 (± 0.3) cm across all amounts of needle removal at the end of the first growing season.

Table 2. ANOVA tables for the differences among the means of the amount of needle removal for height growth, RCD growth, dry stem mass, and dry root mass of black pine seedlings

Dependent Var.	Source of Variation	*Df*	Su *Sq.*	Mean *Sq.*	*F*-value	*P*-value
Height Growth	Amount of Removal	3	28.3	9.438	1.245	0.299
	Residuals	76	575.9	7.578		
RCD Growth	Amount of Removal	3	2.76	0.92	2.55	0.062
	Residuals	76	27.42	0.36		
Dry Stem Mass	Amount of Removal	3	5.21	1.738	0.265	0.850
	Residuals	76	249.1	6.55		
Dry Root Mass	Amount of Removal	3	7.32	2.44	5.93	0.002
	Residuals	76	14.81	0.41		

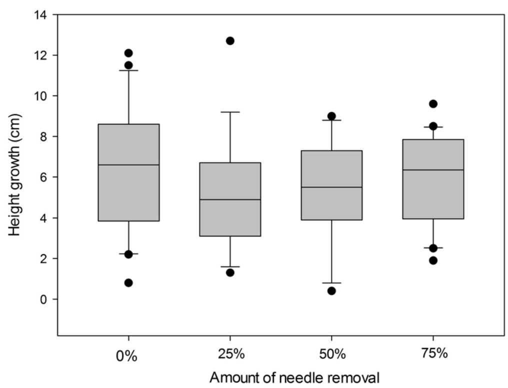

Figure 3. The means of the height growth of black pine seedlings under varying intensity of needle removal. Bars represent the standard deviations for the amounts of needle removal while the dots indicate the potential outliers

There was no statistically significant relationship between the amount of needle removal and the RCD growth of black pine seedlings ($P = 0.062$) (Table 2) (Figure 4). The RCD growth of the planted black pine seedlings ranged from 0.01 mm to 3.03 mm with an average of 0.85 (± 0.05) mm across all amounts of needle removal at the end of the first growing season.

The dry stem mass of the black pine seedlings was not significantly affected by the amount of needle removal ($P = 0.85$) (Table 2) (Figure 5). The dry stem mass of the planted black pine seedlings ranged from 3.21 g to 12.41 g with an average of 7.86 (± 0.38) g across all amounts of needle removal at the end of the first growing season. The increase in the average dry stem mass was 3.1 (± 0.19) g across all amounts of needle removal (Figure 5).

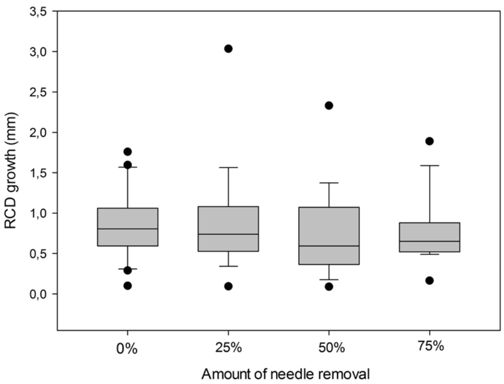

Figure 4. The means of the RCD growth of black pine seedlings under varying intensity of needle removal. Bars represent the standard deviations for the amounts of needle removal while the dots indicate the potential outliers

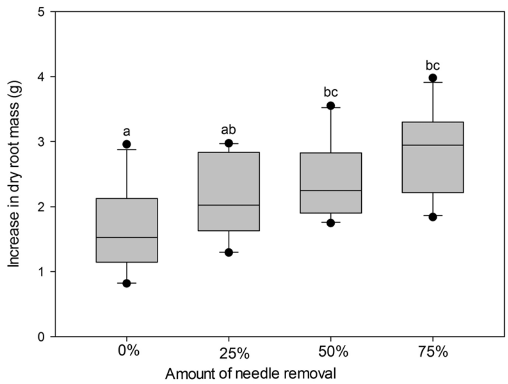

Figure 6. The means of the dry root mass of black pine seedlings under varying intensity of needle removal. Bars represent the standard deviations for the amounts of needle removal while the dots indicate the potential outliers. Means sharing same letters are not significantly different

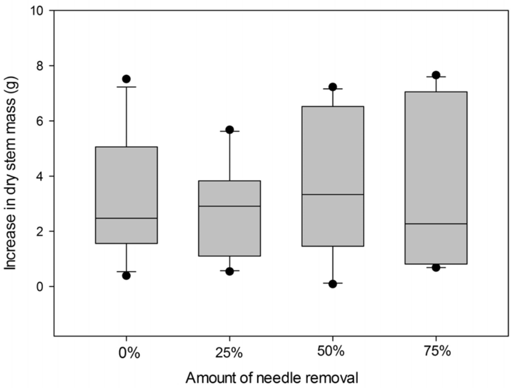

Figure 5. The means of the dry stem mass of black pine seedlings under varying intensity of needle removal. Bars represent the standard deviations for the amounts of needle removal while the dots indicate the potential outliers

The dry root mass of the planted black pine seedlings was significantly affected by the amount of needle removal ($P = 0.002$) (Table 2) (Figure 6). The dry root mass of the black pine seedlings increased with increasing amounts of needle removal. The highest increase in the dry root mass was observed for the seedlings with 75% needle removal (Figure 6). The dry root mass of the planted black pine seedlings ranged from 1.63 g to 3.98 g with an average of 2.25 (± 0.12) g across all amounts of needle removal at the end of the first growing season (Figure 6). Multiple comparisons of means using Tukey's method showed that the differences between means of the dry root mass were significant between 0% removal and 50% removal ($P = 0.04$), and between 0% removal and 75% removal ($P = 0.001$). No significant effect of needle removal was observed between 0% removal and 25% removal ($P = 0.29$), between 25% removal and 50% removal ($P = 0.82$), or between 50% removal and 75% removal ($P = 0.47$).

Discussion and Conclusions

Higher survival is usually observed following the planting of containerized seedlings in comparison to bare-root seedlings (Barnett et al. 1996, Rodriguez-Trejo et al. 2003, Kara et al. 2015). The high survival rate of containerized seedlings can be associated with the availability of nutrients in the containers (Yamashita et al. 2016). Another reason for the high survival rate of such planted seedlings may be the size of the seedlings; three-year-old black pine seedlings likely acclimatize well to field environments. Gülcü and Ēelik (2016) observed the mortality of containerized black pine seedlings planted in an arid area and stated that the average survival was high (83%) at the end of the first growing season despite the negative influence of dry environments on seedling survival. Ertekin and Özel (2010) monitored the survival of bare-root black pine seedlings in a plantation area, and found a lower survival rate (i.e., 67%). Given the studies in the literature, the survival of the containerized black pine seedlings in this study appears consistent.

The planted seedlings were subject to both transplant shock and needle removal. However, because they were planted under the same environmental conditions, the growth differences between seedlings could be associated with the needle removal. Lower seedling growth rates may be expected as a result of needle removal because the decreasing number of needles may result in reduced photosynthetic activities, and consequently, lower carbon gains (Ida et al. 2012). Barry and Pinkard (2013) monitored the effects of defoliation on the RCD and height growth of eucalypts seedlings and found that defoliated seedlings had shorter heights and smaller RCDs. Conversely, some studies have revealed that needle removal does not have an influence on the RCD growth of seedlings (Solomon 1983), which appears consistent with our findings. Yamashita et al. (2016) suggested that leaves on the lower part of the stem are less functional; therefore, it is likely that the removal of these needles (i.e., needles on the lower part of the stem) did not influence the height growth and RCD growth of the containerized black pine seedlings. Our findings regarding the influence of needle removal on the height growth and RCD growth of planted seedlings substantiate these statements.

We observed that the dry root mass increased with increasing amounts of needle removal. Seedlings of some species spend most of their initial energy on their root growth (Brockway et al. 2006), and carbohydrates reserved in the root system support their height growth afterwards (Keeley and Zedler 1998). Therefore, it is possible that the containerized black pine seedlings used most of their energy to develop their root system following out-planting. It has been suggested that seedlings exposed to leaf removal may develop better stomatal conductance to maintain higher photosynthesis and generate more photosynthetic products (Thompson et al. 2004). Therefore, it is likely that the containerized black pine seedlings with higher needle removal generated more photosynthetic products and used them for their initial root growth. Because leaves on the lower part of the stem are considered less functional (Yamashita et al. 2016), the removal of these needles may reduce the influence of transplant shock. Compared to the findings given in previous studies, our findings regarding the influence of needle removal on the root biomass appear acceptable.

Leaf removal may reduce plant growth due to decreasing carbon gains (Harris and Bassuk 1995, Goheen et al. 2007). However, some studies suggest that the reduction in the amount of leaf area for evergreen species may have positive influences, such as increasing survival and growth (Solomon 1983, Yamashita et al. 2016). Our findings confirm that needle removal for black pine seedlings may provide better root development following transplanting. However, long-term observations of seedling responses should be conducted to examine further responses of seedlings to the amount of needle removal. The economic feasibility of needle removal should also be examined in the long term. If the positive effects of needle removal are confirmed by longer observations, the method should be implemented in practice.

Black pine is one of the most commonly used pine species in large plantations throughout Turkey. Plantation success may decrease and the cost of reforestation may increase due to the high mortality and slower growth of seedlings soon after out-planting. The results of our study indicate that a reduction in the amount of leaf area for evergreen species may minimize transplant shock and increase survival and growth. Higher dry root masses were observed when a higher number of needles was removed. The initial results suggest that better root development can be achieved following needle defoliation. However, long-term observations of responses of seedlings with varying sizes and ages to needle removal are recommended. Future observations are required to improve our understanding of the applicability of needle removal in favour of the survival and growth of out-planting seedlings.

Black pine is one of the main important tree species in the Mediterranean region. However, this species is also tolerant to cold temperatures, and can occur at higher elevations of up to 1800 m a.s.l. Atalay (2012) stated that black pine occurs high in mountains where temperatures are as low as -30°C. Even though the natural distribution of black pine primarily consists of the Mediterranean region, this species has been introduced to northern Europe. For example, Kochanowski and Bednarz (2007) observed tree ring chronologies in black pine growing in Słowinski National Park in northern Poland. Słowinski National Park is located very close to the Baltic coast and is situated within the Baltic region (Kochanowski and Bednarz 2007). According to Parzych and Sobisz (2012), Słowiski National Park in northern Poland is under the direct influence of the Baltic Sea. In another study, Cedro (2006) observed the influence of temperature and rainfall on black pine in northwestern Poland. Note that black pine is an exotic tree species in Poland, and was introduced to Europe (Cedro 2006). Moreover, Hanso and Drenkhan (2009) monitored the impacts of a pathogen (*Diplodia pinea*) in black pine forests in southeastern Estonia. Black pine has been introduced to countries within and near the Baltic region, and the species has found favourable soil and climatic conditions in this region (Parzych and Sobisz 2012); therefore, the findings of this study may interest people who study black pine in those regions.

This study highlights the initial growth of planted black pine seedlings following needle removal. However, note that transplanting shock, which influences the initial growth and survival of seedlings following out-planting, is of global concern in forestry. Therefore, the data in this study will not only benefit foresters, who work with black pine, but may also inform additional studies aiming to mitigate the effects of transplanting shock for other species worldwide including in the Baltic region. Our study may encourage similar studies for native tree species in the Baltic region such as the Scots pine (*Pinus sylvestris* L.).

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