The impact of seeds provenance and nursery production method on Austrian pine (*Pinus nigra* Arn.) seedlings quality

V. Ivetić, M. Škorić


Abstract. The influence of seed provenances and seedling production methods on quality of one and two years old seedlings of Austrian pine were investigated. Seeds from three provenances of Austrian pine (Goč, Studenica and Šargan) were used for seedlings production, combined with three production methods: (i) the modified seedbeds (bare-root), (ii) the container type Plantagrah II and (iii) the container type Gočko. Provenance, as well as the combined influence of provenance and production method had minimal influence on the variability of one and two years old Austrian pine seedlings. Nevertheless, the production method had the highest influence. The production system, besides its importance in nursery, will have a high influence on seedlings growth during the first year after planting. Considering the results of this study (e.g. the highest values of the diameter, number of lateral roots, shoot and root dry weight, and quality index and the lowest value of $SQ$ and satisfactory value of $S:R$), we can conclude that the seedlings produced in container type Gočko led to the highest seedlings quality, recommended especially for afforestation on hard sites.

Keywords. Austrian pine, *Pinus nigra*, seedlings, containers, seedling quality.

Authors. Vladan Ivetić (vladan.ivetic@sfb.bg.ac.rs), Mirko Škorić - University of Belgrade – Faculty of Forestry, Kneza Višeslava 1, 11030 Belgrade, Serbia. Manuscript received November 27, 2012; revised June 14, 2013; accepted June 21, 2013; online first July 03, 2013.

Introduction

Austrian pine (*Pinus nigra* Arn.) is one of the most significant tree species used for afforestation on dry sites in Southeastern Europe. Between 1961 and 2007, in Serbia were established 106,389 ha of Austrian pine plantations (Ranković 2009). Production of seedlings with proper quality is important for planting success on poor sites, where Austrian pine is mainly planted. Traditionally, Austrian pine seedlings are produced as bareroot seedlings. Production of containerized seedlings started on mid 70s and, until present day, is characterized by use
of different types of containers.

Production of containerized seedlings has more advantages compared with production of bareroot seedlings. These advantages are connected with increased growth after planting and extended planting season. Also, the containerized seedlings are more likely to develop root deformation, which may jeopardize the planting success and further affect the stability and growth (Zahreddine et al. 2004). Container design has a high impact on root formation and malformation. Additionally, container design affects root development after planting, which can lead to the formation of basal sweep (Rune 2003).

The decision for production of containerized or bareroot seedlings is primarily based on economic inputs and largely depends on the available equipments. However, on this decision, the purpose of the seedlings (for protection forests, timber plantations or planting on dry sites) should have the crucial role. The development of bareroot seedlings is most affected by the nursery cultural practices and properties of substrate growing or soil, while the production of containerized seedlings is most affected by container type. The most important features of the container are cell volume, density of growth, cell height, shape of cross and longitudinal section of the inner cell walls, drain hole and the container material.

There are several published studies on the influence of production method, container type and provenance on quality of Austrian pine seedlings. Containerized seedlings of Austrian pine, compared to bareroot seedlings, have significantly worse deformed root systems (Kolevska 2012). Both shoot and root dry weight of Pinus nigra var. maritima Melv. decreased with increasing growth density, in contrast to seedlings height, which are increased at height densities (Jinks & Mason 1998). Seedling height is greater in larger containers, but shoot and root dry weight and root quality were similar in all containers tested (Khatami-an & Al-Mana 1990). Precultivation of seedlings in deeper mini-plug containers improved seedling morphological attributes and quality (Kostopoulou et al. 2011). Kolevska & Trajkov (2012) found container type and its volume not directly influencing the seedlings quality. The growth analysis, survival and genetic variability of Austrian pine seedlings in response to water deficit, show wide variation among parent trees within each provenance and generally grouping of seedlings from a similar habitat, regardless of provenance (Mataruga et al. 2012).

This paper investigates the influence of production method (production in seedbeds and in two types of containers with different volumes, heights, cross sections, materials, structures, walls and drainage holes, but the same density of growth) and three different seed sources on morphological characteristics of Austrian pine seedlings (height, diameter, the sturdiness coefficient, primary root length, the number of lateral roots, shoot dry weight, root dry weight, the shoot:root ratio and the quality index).

**Materials and methods**

The seedlings were produced in open field, in the nursery of Faculty of Forestry, University of Belgrade at Education and Research Base “Goč” (N 43°33’28.06”; E 20°44’39.25”), at an altitude of 850 m above sea level. The mean annual temperature in nursery is 6.95°C, with annual precipitation of 1009 mm. Seeds of Austrian pine from three provenances in Serbia (Goč, Studenica and Šargan) were used for seedlings production, considering three production methods: the modified seedbeds (bareroot), the container type Plantagrah II and the container type Gočko.

Seedbeds and containers were filled with a substrate mixture of 50% peat and 50% humified bark and sawdust of beech and fir. The substrate was steamed for sterilization for 4 hours at a temperature of 80-90°C.

For production of bareroot seedlings, exist-
ing seedbeds substrates were replaced with prepared mixture to a depth of 15 cm. Sowing was done with 30 g of seed per m². The over-abundant seedlings was removed to a density of 500 seedlings/m².

Container type Plantagration II was made of solid plastic, with dimensions of 32×21.5×18 cm, with 33 cells. Diameter at the top of the cell is 5 cm; a drainage hole at the bottom was 1.5 cm in diameter with three holes on the side. Volume of one cell is 270 cm³. The density of growth is 400 seedlings/m². During cultivation, the seedlings were under standard operations of irrigation, pests and weed control, without fertilization.

The quality of Austrian pine seedlings was examined under a combination of factors - the production method and provenance. At the end of the first and second growing season seedlings from each repetition (189 seedlings derived from 21 seedlings, 3 provenances and 3 production methods) were gently removed without damaging the roots and washed under running tap water. The height was measured as the difference between the cotyledon scar and the base of terminal bud of dormant seedling (Hasse 2007), with an accuracy of 0.1 cm. Diameter is measured at or near the cotyledon scar (Hasse 2007), with an accuracy of 0.1 mm. Furthermore, shoots and roots were separated at the root neck and oven dried in open paper bags for 48 hours, at 80°C. Shoot and root weights were measured on an electronic scale with an accuracy of 0.001 g.

The sturdiness coefficient (equation 1) was calculated according to Roller (1977), and the quality index (equation 2) was calculated using Dickson et al. (1960) method.

\[
SQ = \frac{H}{RCD} \quad (1)
\]

\[
QI = \frac{SLDW}{RCD} + \frac{SDW}{RDW} \quad (2)
\]

where \( H \) – height (cm), \( RCD \) – root collar diameter (mm), \( SLDW \) – seedling dry weight (g), \( SDW \) – shoot dry weight (g), \( RDW \) – root dry weight (g).

One-way ANOVA was used to test differences between mean values of measured characteristics between the provenance and production methods. Factorial ANOVA was used to analyze the interactive effects of higher order multiple categorical dependent variables (factors). Mean values were separated using Tukey’s HSD test, with significance level of \( p < 0.05 \) (\( \alpha = 0.05 \)). The influence of individual factors on the total variability was tested using the Variance Components Analysis. All analyzes were performed in Statistica 7 (StatSoft Inc. USA).

**Results**

The influence of seeds provenance on seedlings height after the first growing season (Table 1), proved to be insignificant, as the height differences were not statistically different (\( p = 0.1922 \)). The other observed features (i.e. \( d \), \( SQ \), \( PRL \), \( LRN \), \( SDW \), \( RDW \), \( S:R \), \( QI \)) had statistically significant differences (\( p = 0.0000 \) to 0.0322). Provenance Šargan stands out, showing the highest values of observed characteristics (\( d \), \( PRL \) and \( S:R \)) or the lowest ones (\( SQ \), \( SDW \), \( RDW \) and \( QI \)).

After the second growing season, the influence of seed provenance on seedlings height was even weaker. There was still no significant difference in height (\( p = 0.6674 \)), number of lateral roots (\( p = 0.0986 \)) and shoot dry weight (\( p = 0.0800 \)), but the differences were significant for the rest of measured parameters (\( p = 0.0019 \) to 0.0324).

After the first growing season (Table 2), the comparison of different production methods
has revealed statistically significant differences for all considered parameters. Nevertheless, bare root seedlings showed the highest values of height, $SQ$, primary root length and $S:R$, and the lowest values for the remaining characteristics.

Bareroot seedlings had the lowest value of quality index (0.08) after the first growing season. However, considering the fact that one-year bareroot seedlings of Austrian pine are not usually used for plantation, this value is not critical. After the second growing season...

### Table 1
Average value of Austrian pine seedlings characteristics from three provenances

<table>
<thead>
<tr>
<th>Parameter</th>
<th>First growing season</th>
<th>Second growing season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gočko</td>
<td>Studenica</td>
</tr>
<tr>
<td>$H$</td>
<td>6.02 (0.79)$^a$</td>
<td>6.27 (1.19)$^a$</td>
</tr>
<tr>
<td>$D$</td>
<td>1.81 (0.32)$^a$</td>
<td>1.84 (0.27)$^a$</td>
</tr>
<tr>
<td>$SQ$</td>
<td>3.44 (0.85)$^a$</td>
<td>3.45 (0.80)$^a$</td>
</tr>
<tr>
<td>$PRL$</td>
<td>18.04(3.07)$^b$</td>
<td>17.75(2.68)$^a$</td>
</tr>
<tr>
<td>$LRN$</td>
<td>7.01 (2.72)$^b$</td>
<td>7.82 (2.44)$^a$</td>
</tr>
<tr>
<td>$SDW$</td>
<td>0.54 (0.20)$^b$</td>
<td>0.50 (0.18)$^b$</td>
</tr>
<tr>
<td>$RDW$</td>
<td>0.30 (0.14)$^b$</td>
<td>0.27 (0.13)$^a$</td>
</tr>
<tr>
<td>$S:R$</td>
<td>1.98 (0.56)$^b$</td>
<td>2.02 (0.70)$^b$</td>
</tr>
<tr>
<td>$QI$</td>
<td>0.17 (0.08)$^b$</td>
<td>0.15 (0.08)$^b$</td>
</tr>
</tbody>
</table>

Note. The analysis is based on the mean values of 9 repetitions with 21 plants in each repetition. Abbreviations: height ($H$), diameter ($D$), the sturdiness coefficient ($SQ$), primary root length ($PRL$), the number of lateral roots ($LRN$), shoot dry weight ($SDW$), root dry weight ($RDW$), the shoot: root weight ratio ($S:R$), the quality index ($QI$) and standard deviation (in parentheses). Mean values in same row followed by different letters are statistically different at $p < 0.05$.

### Table 2
The average value of Austrian pine seedlings characteristics from three provenances

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Production method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First growing season</td>
</tr>
<tr>
<td></td>
<td>Gočko</td>
</tr>
<tr>
<td>$H$</td>
<td>5.84 (0.84)$^a$</td>
</tr>
<tr>
<td>$D$</td>
<td>2.01 (0.32)$^a$</td>
</tr>
<tr>
<td>$SQ$</td>
<td>2.95 (0.46)$^a$</td>
</tr>
<tr>
<td>$PRL$</td>
<td>16.06(0.60)$^a$</td>
</tr>
<tr>
<td>$LRN$</td>
<td>9.31 (1.48)$^a$</td>
</tr>
<tr>
<td>$SDW$</td>
<td>0.57 (0.24)$^a$</td>
</tr>
<tr>
<td>$RDW$</td>
<td>0.30 (0.15)$^b$</td>
</tr>
<tr>
<td>$S:R$</td>
<td>2.04 (0.55)$^b$</td>
</tr>
<tr>
<td>$QI$</td>
<td>0.18 (0.09)$^b$</td>
</tr>
</tbody>
</table>

Note. The analysis is based on the mean values of 9 repetitions with 21 plants in each repetition. Abbreviations: height ($H$), diameter ($D$), the sturdiness coefficient ($SQ$), primary root length ($PRL$), the number of lateral roots ($LRN$), shoot dry weight ($SDW$), root dry weight ($RDW$), the shoot: root weight ratio ($S:R$), the quality index ($QI$) and standard deviation (in parentheses). Mean values in same row followed by different letters are statistically different at $p < 0.05$. 
The impact of seeds provenance and nursery production method ...

son, quality index of bareroot seedlings rises to 0.33. Seedlings produced in container type Gočko, showed the highest values of diameter, number of lateral roots and shoot dry weight, but lower values of shoot height and primary root length. Seedlings produced in containers type Plantagrah II showed the highest values of root dry weight and quality index, but lower values of SQ and S:R. The mean values of all investigated characteristics of the bareroot seedlings are significantly different compared to containerized seedlings (Table 2).

After the second growing season, the situation was similar. The highest values of height, SQ, primary root length and S:R were recorded for bareroot seedlings, which at the same time showed the lowest values for other characteristics. On the other hand, seedlings produced in containers type Gočko showed the highest values of diameter, number of lateral roots, shoot and root dry weight, and quality index, but lowest values of height and SQ. Seedlings produced in containers type Plantagrah II did not show the highest values for any of the observed characteristics, but showed a minimum value of primary root length, shoot dry weight and S:R ratio. Bareroot seedlings were significantly different for height, primary root length and the S:R ratio. The diameter of seedlings produced in containers type Gočko were significantly different compared to other production methods.

Interaction between provenance and production method, after the first growing season (Table 3), was associated with significant differences observed for all traits ($p = 0.0000$ to 0.0257). The SQ showed the highest value for the bareroot seedlings from provenance Studenica, and the lowest for seedlings produced in containers type Plantagrah II from provenance Šargan. The S:R ratio was highest for the bareroot seedlings from provenance Šargan and the lowest for seedlings produced in containers type Plantagrah II, from provenance Studenica. The $QI$ was highest for seedlings produced in containers type Gočko from provenance Studenica and for seedlings produced in container type Plantagrah II from provenance Goč (0.22). On the other hand, the $QI$ was the lowest for the bareroot seedlings from all three provenances (0.08).

After the second growing season, the interaction between provenance and production method (Table 3), showed no significant differences for height ($p = 0.0618$) and primary root length ($p = 0.0693$), but on the edge for the number of lateral roots ($p = 0.0448$). For all other observed characteristics, differences were significant ($p = 0.0000$ to 0.0283).

For all the observed characteristics, the production method had considerably more impact than the provenance and interaction of two factors (Table 4). In the second growing season the influence of production method was highest, but comparing to first growing season declined for all observed characteristics, except for the diameter and shoot dry weight where increased. Effect of production method in the first growing season was the smallest on shoot dry weight (7.2%), and highest on primary root length (77.3%) and SQ (71.4%). In the second growing season, the influence of production method was the smallest on root dry weight (21.3%), and continued to be highest on primary root length (67.4%), while this influence decreased significantly for SQ (55.4%).

The influence of provenance did not exceed 5%, and was the highest for S:R ratio (4.6%) after the first growing season and on primary root length (2%) after the second growing season (Table 4). There was no observed influence of provenance on the height, primary root length, shoot and root dry weight and quality index. In the second growing season, the influence of provenance dropped down for SQ, number of lateral roots and S:R, but rised up for primary root length, root dry weight and quality index.

The combined influence of the two factors on the observed characteristics is higher than the influence of provenance and ranges from 2.2% for SQ to 25.8% for root dry weight after the
Table 3: The average value of Austrian pine seedlings characteristics from three provenances

<table>
<thead>
<tr>
<th>Provenance</th>
<th>Production method</th>
<th>H</th>
<th>D</th>
<th>SQ</th>
<th>PRL</th>
<th>LRN</th>
<th>SDW</th>
<th>RDW</th>
<th>S:R</th>
<th>QI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bare roots</td>
<td>Šargan</td>
<td>6.50</td>
<td>1.49</td>
<td>4.40</td>
<td>21.78</td>
<td>3.81</td>
<td>0.38</td>
<td>0.15</td>
<td>2.46</td>
<td>0.08</td>
</tr>
<tr>
<td>Bare roots</td>
<td>Studenica</td>
<td>7.50</td>
<td>1.69</td>
<td>4.46</td>
<td>20.83</td>
<td>5.28</td>
<td>0.41</td>
<td>0.15</td>
<td>2.82</td>
<td>0.08</td>
</tr>
<tr>
<td>Bare roots</td>
<td>Goč</td>
<td>7.02</td>
<td>1.79</td>
<td>3.95</td>
<td>23.64</td>
<td>5.71</td>
<td>0.46</td>
<td>0.15</td>
<td>3.09</td>
<td>0.08</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>0.0033</td>
<td>0.0154</td>
<td>0.0257</td>
<td>0.0007</td>
<td>0.0090</td>
<td>0.0000</td>
<td>0.0004</td>
<td>0.0000</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

Note. The analysis is based on the mean values of 9 repetitions with 21 plants in each repetition. Abbreviations: height (H), diameter (D), the sturdiness coefficient (SQ), primary root length (PRL), the number of lateral roots (LRN), shoot dry weight (SDW), root dry weight (RDW), the shoot: root weight ratio (S:R), the quality index (QI) and standard deviation (in parentheses). Mean values in same row followed by different letters are statistically different at p < 0.05.

The first growing season (Table 4). After the second growing season, the combined influence of factors ranged from 1.7% for primary root length to 10.2% for SQ. The observed interaction in the second year declined for all characteristics, except for SQ and number of lateral roots with minimal growth where a significant increase was recorded (from 2.2% to 10.2%).
The error, which contain all the same sources of variation except for the variation of the respective effect of interest, represent a large component of total variance. In the first growing season, it ranges from 18.9% for primary root length to 67% for shoot dry weight. The error exceeds the effect of production method on height, diameter and shoot dry weight, as dependent variable. In the second growing season, the share of error in total variance ranges from 28.9% for primary root length to 72.5% for shoot dry weight and exceeds the share of production method on height, diameter, number of lateral roots, shoot dry weight and quality index, as dependent variable.

**Discussion**

Provenance, as well as the combined influence of the two considered factors had a minimal influence on the variability of characteristics of two-year Austrian pine seedlings. Nevertheless, the influence of production system was of the utmost importance. As the influence of provenance is likely to rise with age, the influence of production method usually declines. Varelides et al. (2001) have found a significant site-provenance interaction of 9 year old, while Kolevska & Trajkov (2012) reported that there is no crucial influence of production method on development and vitality of Austrian pine stands at different age. Could be expected that production method, besides its importance in nursery, will have the largest influence within first year after planting.

The highest values of $\text{SQ}$ and height of bare-root seedlings could be explained by the high growth density (500 seedlings/m²), compared to lower density (i.e. 400 seedlings/m²) of container seedlings. Moreover, the bareroot high growth density could explain the lowest values of $D$, and to some extent the $\text{SDW}$ and $\text{RDW}$ in bareroot seedlings. The similar results was reported by Jinks and Mason (1988). Oner and Eren (2008) reported a higher value of both ($H$ and $D$), from containerized, compared to bare-root seedlings. On the other hand, Kolevska & Trajkov (2012) reported higher value of $D$ from bareroot seedlings, but no information on growing density. The maximum value of $\text{PRL}$ could be explained by unhindered growth of primary root and its intergrowth in lower layer of substrate in seedbed, regardless of the fact that depth of the artificial substrate was approximately the same as in the container cell (15 cm).

The significantly decrease of $\text{SQ}$ value in the second growth season for seedlings produced in containers type Gočko and for bare-root seedlings, is normal, as “a high ratio indicates a relatively spindly seedlings while a lower ratio indicates a stouter seedlings” (Haase 2007). For Plantagraph II seedlings, the decreasing of $\text{SQ}$ in the second growing season was minimal. The $S:R$ ratio in the first growing season indicates a good quality of the bareroot seedlings (ratio lower than 3:1) and
the seedlings produced in the container Plantagrah II (ratio lower than 2:1), while this ratio is slightly less favorable for seedling produced in the container type Gočko (the ratio is slightly higher than 2:1). Usually, a S:R of about 2:1 (1.5:1-2.5:1) is viewed as desirable, both for bareroot and containerized seedlings (Bernier et al. 1995). In the second growing season, this ratio is somehow less favorable for seedlings produced in all three production systems. The higher value of S:R in second year is in contrast with the most common situation in woody species – continuous decrease (Wilson 1988). Biel et al. (2004) found a similar change between first and second growing season of Austrian pine seedlings produced in large containers (300 cm³, 387 seedlings/m²). This increase of S:R can be explained with substantial water availability, rather than with ontogeny.

The QI of container seedlings was high in both growing seasons, while it was lower for bareroot seedlings in the first growing season, but significantly higher after the second growing season and much higher than the threshold of 0.09, suggested by Roller (1977). This may be explained by the large increase in D and weight of bareroot seedlings during the second growing season. The QI after the first growing season was highest for seedlings produced in container Plantagrah II (0.20). But during the second year QI increased only by 2.2 times (i.e. to 0.44) compared with quality index of seedlings produced in container Gočko, which increased by 4.05 times, or with bareroot seedlings with an increase of 4.12 times. This may indicate that one growing season would be enough for production of Austrian pine seedlings in containers type Plantagrah II.

Except H and PRL, bare-root seedlings showed lower values of all other observed characteristics. The maximum PRL of bareroot seedlings could be considered as an indicator of good quality, because the total root length proved to be a better indicator of the vitality of the Austrian pine seedlings compared with number of root tips (Chiatante et al. 2002).

Differences in H and D between the seedlings produced in two types of containers were small but consistent confirming the conclusion of Gilman et al. (2010), that the seedlings reach approximately the same H and D in most types of containers, with proper watering and fertilizing.

The superiority of seedlings produced in containers type Gočko may be due to the larger cell volume and bottomless cells. Large drainage holes at the bottom improve root air pruning, drainage and aeration, despite the fact that depth of water saturated layer of growth medium depend on cell height and its physical properties. But small drainage holes present a high risk of hole plugging by the mass of root tips, usually developed at bottom of container cell.

Conclusions

Provenance had a minimal influence on quality of two year old seedlings of Austrian pine, regardless to production method. Production method have a significant influence on quality of two year old seedlings of Austrian pine. Seedlings produced in both types of containers had a better quality compared to bareroot seedling.

Seedlings produced in container type Gočko had the highest values of D, NLR, SDW, RDW and QI and the lowest value of SQ and satisfactory value of S:R. We can consider that the seedlings produced in container type Gočko had the best quality, especially for afforestation on hard sites.

Acknowledgments

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