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# Seedling quality in Serbia - Results from a threeyear survey

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#### Abstract

During a three-year survey in 14 forest nurseries in Serbia we measured seedlings of 19 species and 96 stocktypes of both conifers and broadleaves. Seedlings were measured in the nurseries for height and diameter, and subsamples was taken for measurements of shoot and root dry weight, and presence of mycorrhiza. Results of mean values and variation of measured morphological attributes are presented and compared to Serbian standard for seedlings quality. We found mycorrhiza on seedlings root from almost every nursery, seedbed and tray. Our results show that current nursery cultural practice need to be improved and that seedlings quality standards should be updated.

#### Keywords

Seedling quality; Morphological attributes; Forest nursery; Cultural practice

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## **1** Introduction

According to the Law on forest reproductive material, tree seedlings need to meet requirements by a grading standard. The current standards are SRPS D.Z2.111:1968 for conifers, and SRPS D.Z2.112:1968 for broadleaved species. These standards applies to seedlings produced from seed, and only to conifer species listed in standard JUS D.Z1.100 and broadleaved species listed in standard JUS D.Z1.130. All other species not listed in these two standards do not need to meet a requirements on the size. However, the size requirements for grading of seedlings are not given for all species listed in these two standards. Both standards (SRPS D.Z2.111:1968 and SRPS D.Z2.112:1968), together with a general terms (JUS D.Z2.110) are adopted in year 1967, and become operational in 1968. From that time, many new technologies were introduced (e.g. production of seedlings in containers is introduced in mid-1970) and lots of cultural operations are significantly improved (e.g. seedbed preparation, fertilization, irrigation, weed, pests, and disease control).

There is a variety of technologies and equipment used for seedling production in Serbia – from manually sowing and growing of seedlings at the open field to mechanized sowing in containers and growing of seedlings in the greenhouse. Approximately 75% of all seedlings produced in Serbian nurseries are bareroot. Two main ways of bareroot seedlings production are in seedbeds prepared on the open field and in the modified Duneman seedbeds. For production of seedlings in containers a total of six container types are used, four are domestic and two are imported. A small quantity of seedlings is produced in Nisula rolls.

Grading and quality testing of seedlings at operational level are mostly based on morphology (Mohammed 1997), with seedlings height and root collar diameter as the most used attributes (Stilinović 1960). Initial height and diameter are equal in forecasting seedlings field performance (Ivetić et al 2016a). Measurement of both height and diameter is nondestructive, fast, and easy (Ivetić 2013). Shoot height can have both positive and negative effect on seedling field performance, while root collar diameter have only positive effect (as reviewed by Ivetić and Devetaković 2016). This is why seedlings are graded for height on both minimum and maximum requirements, while for diameter seedlings are only graded to a minimum standard (Landis et al. 1994).

Ratios like height:diameter (HD), shoot:root dry weight (SR), and Dickson quality index (DQI) are not used at operational level in Serbian nurseries for seedlings grading. HD ratio is generally similar to the diameter in forecasting seedlings growth on the field

(Thompson 1985), and relative potential of shoot height and diameter to forecast seedlings field performance is improved by combining them in the HD (Ivetić et al. 2016b). The low value of HD indicate the higher potential of seedlings to survive and grow in stressful environment (Johnson and Cline 1991; Škorić 2014; Ivetić et al. 2016b) although there are opposite reports (see Li et al. 2011; Tsakaldimi et al. 2012; Devetaković et al. 2017). The lower SR ratio (McTague and Tinus 1996; Grossnickle 2005a, 2012; Ivetić et al. 2016b) and larger root system (Burdett 1990; Grossnickle 2005b) results in higher rate of seedlings field survival, mostly because the reduced water stress (Rose et al. 1993; Stewart and Bernier 1995). As one of the most comprehensive quality index, which combine seedling biomass with height and diameter (Dickson et al. 1960), DQI is positively correlated to both survival and growth of seedlings after field planting (Tsakaldimi et al. 2012; Škorić 2014; Ivetić et al. 2016b).

In this paper we present the results of a three-year survey of seedlings quality in Serbian nurseries and compare them to the official grading standard. Considering that development of seedling quality standard represent the attempt to express a various effects and variables by a single number, in this paper we present only a descriptive statistics for different species at the same age. Comparisons are made for these species listed in the official Serbian standard for seedling quality.

# 2 Material and methods

#### 2.1 Nursery and species

The seedlings were measured in total of 14 nurseries in Serbia (Table 1 and Figure 1) at the altitude ranging from 110 to 1,302 m a.s.l. During a three-year survey, a total of 19 species and 96 different stocktypes were found (Table 2).



Figure 1. The Position of nurseries under a three-year survey.

	Table 1.	Geographical coordinates and altitudes of nurse	eries under a three	-year survey.	
Nr	Name	Owner	Longitude	Latitude	
1	Belgrade	University of Belgrade – Faculty of Forestry	20°25'32.59"	44°46'56.69"	110
2	Selište	Public Enterprise Srbijašume	22° 5'40.99"	43°55'33.81"	180
3	Vlasotince	Public Enterprise Srbijašume	22° 8'13.53"	42°58'8.34"	268
4	Kamena gora	Public Enterprise Srbijašume	19°33'37.02"	43°17'7.57"	1302
5	Lazićev salaš	Public Enterprise Srbijašume	21°22'55.24"	43°56'30.18"	125
6	Lučka reka	Public Enterprise Srbijašume	20°16'1.92"	43°33'53.30"	510
7	Mišljenovac	Public Enterprise Srbijašume	21°34'18.93"	44°32'25.11"	131
8	Nupare	Public Enterprise Srbijašume	21°18'47.21"	43°28'16.38"	252
9	Pirot	Public Enterprise Srbijašume	22°34'54.09"	43° 8'34.71"	369
10	Požega	Public Enterprise Srbijašume	20° 2'56.08"	43°50'28.79"	304
11	Ribnica	Public Enterprise Srbijašume	20°41'31.97"	43°40'29.17"	247
12	Rogot	Public Enterprise Srbijašume	21° 2'39.31"	44° 8'48.38"	120
13	Uvac	Public Enterprise Srbijašume	19°57'45.36"	43°16'48.86"	1027
14	Tara	National Park Tara	19°33'8.27"	43°57'47.36"	306

#### 2.2 Field measurements

From each seedbed in a single nursery, a minimum of 40 seedlings were measured for height and diameter. A rigid metal frame  $(0.5 \times 1 \text{ m})$  was set on four spots along the seedbed, at approximately 12.5%, 37.5%, 62.5%, and 87.5% of seedbeds length. In each frame all seedlings were counted and 10 randomly selected seedlings were measured for height and diameter. For conifers, a needle color is graded by a color palette sample. At the same spot a soil/substrate sample is taken for measurement of pH, electric conductivity, and total dissolved salts in water solution. Effect of growing density, pH, electric conductivity, total dissolved salts, and the relation of needle color with seedling size is not part of this paper and will be presented in a following papers. From the first three frames in a seedbed, one randomly selected seedling was carefully lifted, packed and transported to laboratory. From the fourth frame in the seedbed, two randomly selected seedlings were taken. For container seedlings similar procedure was followed, but without use of metal frame. Measurements were taken at four spots along the container stock in a growing area and samples war taken for laboratory measurements on the same way as for bareroot seedlings. From measured heights and diameters, the HD was calculated as height (cm)/diameter (mm).

Table 2. Tree species and stocktypes of measured seedlings (SB – seedbed, DB – Duneman beds, NR – Nisula rolls, C<sub>H120</sub> – Container Hiko SS 120, C<sub>H220</sub> – Container Hiko 220, C<sub>PL1</sub> – Container Plantagrah 1, C<sub>PL2</sub> – Container Plantagrah 2, C<sub>Pl</sub> – Container Pirosad, C<sub>JU</sub> – Container Jukosad).

Tree	e species	Stocktype	Tree	species	Stocktype			
1	Abies alba	3+2 SB	12	Pinus nigra	1+0 SB			
		1+0 DB			1+0 DB			
		2+0 DB			2+0 SB			
2	Acer platanoides	1+0 SB			2+0 DB			
		2+0 SB			3+0 SB			
		3+0 SB			3+0 DB			
		1+1 SB			2+2SB			
3	Acer pseudoplatanus	1+0 SB			2+0 DB			
		2+0 SB			1+0 C <sub>PL1</sub>			
		1+0 C <sub>H220</sub>			1+0 C <sub>JU</sub>			
		1+0 DB			2+0 C <sub>PL1</sub>			
4	Castanea sativa	1+0 SB			1+0 C <sub>PL2</sub>			
5	Corylus colurna	1+0 SB			2+0 C <sub>PL2</sub>			
6	Fagus sylvatica	1+0 SB			1+0 C <sub>PI</sub>			
	5 ,	2+0 SB			2+0 C <sub>PI</sub>			
		1+0 DB			1+0 C <sub>H120</sub>			
		1+0 C <sub>H220</sub>			2+0 C <sub>H120</sub>			
7	Fraxinus angustifolia	2+0 SB			3+0 C <sub>H120</sub>			
8	Fraxinus excelsior	1+0 SB			3+0 CPI 2			
-		2+0 SB	13	Pinus sylvestris	1+0 SB			
		3+0 SB			2+0 SB			
		2+0 DB			3+0 SB			
		1+1 SB			1+0 Cmp			
		1+0 Cuasa			1+0 Cp12			
a	lualans niara	1+0 SB						
,	Sugiuns nigiu	2+0 SB			2+0 Curazo			
10	lualans reaia	2+0 SB			2+0 CH120			
11	Pirea ahies	1+0 SB						
		2±0 SB			2+0 CH120			
		2+0 38	1/	Brunus avium				
		3+0 3B	14					
		4+0 3B						
		1+1 SB	10	Decudatoura manziacii	1+1 SB			
		2+1 3D	15	Pseudotsugu menziesii	1+0.56			
			16	Quarque patraca				
			10	Quercus petrueu				
		3+1 SB						
		2+2 SB	4 -	Oursease in the second	3+U 2B			
		3+2 SB	1/	Quercus robur	1+0 2R			
		1+0 DB			2+0 SB			
		2+0 DB			3+0 SB			
		2+1 DB			4+0 SB			
		3+0 DB	18	Quercus rubra	1+0 SB			
		2+2 NR	-		2+0 SB			
		3+1 NR	19	Robinia pseudoacacia	1+0 SB			
		1+0 C <sub>H120</sub>			2+0 SB			
		2+0 C <sub>H120</sub>						
		2+0 C <sub>PL1</sub>						
		2+2 C <sub>PL1</sub>						
		3+0 C <sub>PL1</sub>						
		3+0 C <sub>H120</sub>						
		2+1 NR						

#### 2.3 Laboratory measurements

Seedlings taken from the seedbeds and containers was measured for height and diameter, and then their roots were carefully washed under the running tap water. Roots were immediately examined under the 100x-500x microscope for the presence of mycorrhiza. Than the seedlings were cut off at root collar and shoots and roots were placed in an open paper bags for drying. Seedlings were dried at temperature of 65-68°C, for 48 hours, and then measured for the weight on an electronic scale with precision of 0.01 g. From measured heights and diameters, the HD was calculated as height (cm)/diameter (mm). From measured shoot (SDW) and root (RDW) dry weight, the SR was calculated as SDW/RDW. Dickson quality index is calculated by the formula:

 $DQI = \frac{\text{Total seedling weight } (g)}{(HD) + (SR)}$ 

#### 2.4 Statistical analysis

A mean values and standard deviations were calculated for seedlings (1+0, 2+0, 3+0, and 4+0) and transplants (1+1, 2+1, 2+2, 3+1, and 3+2) of the same age of each species. The range, lower (LQ) and upper (UQ) quartiles, as well as the lower (L10%) and upper (U10%) ten percent were calculated for all species, but shown only for species listed in the official quality standards. The normality tests (Kolmogorov-Smirnov and Liliefors) were run for all species and ages, but not shown due to large number of results. All statistical analysis were done in Statistica 7 software.

# 3 Results

#### 3.1 Seedlings morphological attributes

#### 3.1.1 Seedlings 1+0

During a three-year survey in Serbian nurseries, we have found a one-year old seedlings (1+0) of 18 species, four conifers and 14 broadleaves (Table 3). In general, a broadleaved seedlings reach larger values and variability of heights and diameters, smaller SR ratio and larger DQI value.

#### 3.1.2 Seedlings 2+0

During a three-year survey in Serbian nurseries, we have found a two-year old seedlings (2+0) of 14 species, four conifers and 10 broadleaves (Table 4). In general, again a broadleaved seedlings reach larger values and variability of heights and diameters, smaller SR ratio and larger DQI value. Two-year old seedlings of *Acer platanoides* and *Quercus petraea* have a smaller mean value of height and diameter than a one-year seedlings of the same species (Table 3 and 4). For most of the species, HD and SR shows smaller and DQI show larger values in second than in the first year.

Table 3. Descriptive statistics (mean values – MV and standard deviation – SD) of 1+0 seedlings height (H, cm), root collar diameter (D, mm), height:diameter ratio (HD), shoot to root dry weight ratio (SR), Dickson quality index (DQI).

		ł	1	[	)	Н	D	S	R	D	QI
	Ν	SV	SD	SV	SD	SV	SD	SV	SD	SV	SD
Abies alba	48	3.83	0.93	0.92	0.26	4.5	1.75	2.31	1.01	0.007	0.002
Acer platanoides	138	32.63	12.25	7.05	2.43	5.49	6.11	0.28	0.13	0.90	0.21
Acer pseudoplatanus	533	39.32	21.42	7.16	2.39	5.40	2.10	0.72	0.46	1.47	1.22
Castanea sativa	40	19.4	4.84	6.99	1.96	2.94	1.03				
Corylus colurna	150	41.40	13.00	7.51	1.83	5.62	1.58	0.49	0.14	1.89	0.58
Fagus sylvatica	360	26.06	11.93	4.26	1.54	6.31	2.24	0.66	0.19	0.72	0.38
Fraxinus excelsior	397	28.96	26.05	6.48	3.05	3.49	1.63	0.62	0.50	7.87	11.21
Juglans nigra	155	40.69	18.17	7.64	1.68	5.53	2.69	0.60	0.37	1.85	0.96
Juglans regia	75	37.24	14.38	12.9	3.40	2.83	0.70				
Picea abies	592	5.43	3.28	1.11	0.62	5.36	2.90	2.06	2.05	0.03	0.03
Pinus nigra	1067	7.25	3.47	1.92	0.72	3.97	1.68	1.92	0.93	0.29	0.26
Pinus sylvestris	496	6.40	2.95	1.79	0.82	3.78	1.39	2.04	2.30	0.11	0.12
Prunus avium	151	75.08	36.94	8.98	4.50	8.60	2.04	1.22	0.10	2.10	0.48
Pseudotsuga menziesii	80	8.07	2.28	0.80	0.35	12.6	7.65				
Quercus petraea	131	30.6	20.0	7.11	2.89	4.20	2.08	1.39	0.42	2.52	1.37
Quercus robur	76	11.0	5.38	3.61	1.64	3.20	1.20	0.88	0.93	1.41	0.97
Quercus rubra	354	26.89	23.02	4.49	3.32	6.01	1.73	0.66	0.55	0.36	0.60
Robinia pseudoacacia	252	91.44	43.09	8.95	3.57	10.35	3.49	1.09	0.28	0.99	0.42

Table 4. Descriptive statistics (mean values – MV and standard deviation – SD) of 2+0 seedlings height (H, cm), root collar diameter (D, mm), height:diameter ratio (HD), shoot to root dry weight ratio (SR), Dickson quality index (DQI).

		Н		D		HD		SR		DQI	
	Ν	SV	SD	SV	SD	SV	SD	SV	SD	SV	SD
Abies alba	117	7.20	1.47	1.75	0.37	4.17	0.71	1.08	0.37	0.12	0.06
Acer platanoides	135	18.5	6.99	6.78	1.82	2.80	1.01	0.43	0.11	2.38	1.78
Acer pseudoplatanus	189	83.92	34.29	13.4	4.57	6.65	3.12	0.95	0.23	2.07	1.46
Fagus sylvatica	93	27.95	12.05	5.83	1.96	4.83	1.56	0.84	0.31	1.72	2.05
Fraxinus angustifolia	75	61.84	15.60	12.6	3.23	5.00	0.89				
Fraxinus excelsior	240	70.83	24.88	14.60	6.67	5.25	1.92				
Juglans nigra	66	83.47	40.16	11.9	3.40	7.15	3.26				
Picea abies	761	15.2	8.31	2.76	1.33	5.68	2.79	1.68	1.04	0.11	0.11
Pinus nigra	798	14.8	5.25	4.49	1.99	3.64	1.36	3.02	1.06	0.69	1.02
Pinus sylvestris	361	16.38	10.21	4.75	3.48	4.07	2.04	1.93	1.28	0.36	0.89
Quercus petraea	160	22.97	11.11	5.87	1.94	4.11	1.98				
Quercus robur	66	29.58	22.77	6.54	3.24	4.12	1.78				
Quercus rubra	45	32.9	9.51	4.64	1.27	7.23	1.90	0.38	0.22	1.60	0.92
Robinia pseudoacacia	45	132.9	62.90	12.39	6.57	11.25	3.36	1.26	0.26	0.45	0.15

#### 3.1.3 Seedlings 3+0

During a three-year survey in Serbian nurseries, we have found a three-year old seedlings (3+0) of 7 species, three conifers and four broadleaves (Table 5). Three-year old seedlings of *Acer platanoides* have a smaller mean values of height and diameter than one-year old seedlings (Table 3 and 5).

Table 5. Descriptive statistics (mean values – MV and standard deviation – SD) of 3+0 seedlings height (H, cm), root collar diameter (D, mm), height:diameter ratio (HD), shoot to root dry weight ratio (SR), Dickson quality index (DQI).

		н		D		HD		SR		DQI	
	Ν	SV	SD	SV	SD	SV	SD	SV	SD	SV	SD
Acer platanoides	90	30.7	7.25	7.01	1.53	4.44	0.90	0.31	0.07	1.69	0.64
Fraxinus excelsior	71	105.0	37.25	14.1	8.06	9.06	5.16	1.00	0.32	2.92	2.03
Picea abies	578	27.63	12.65	4.82	2.68	6.15	2.13	2.93	2.57	0.28	0.31
Pinus nigra	506	19.71	11.68	4.76	2.57	4.24	1.39				
Pinus sylvestris	128	34.99	10.61	8.15	4.22	5.26	2.34	3.42	2.06	0.53	0.46
Quercus petraea	45	64.62	19.47	8.26	2.57	8.02	1.75	0.75	0.12	1.87	1.20
Quercus robur	138	84.92	26.56	18.69	17.38	9.33	18.77				

#### 3.1.4 Seedlings 4+0

During a three-year survey in Serbian nurseries, we have found a four-year old seedlings (4+0) of two species, *Quercus robur* and *Picea abies* (Table 6). Diameter of four-year old *Quercus robur* seedlings is smaller than of three-year old (Table 5 and 6).

Table 6. Descriptive statistics (mean values – MV and standard deviation – SD) of 4+0 seedlings height (H, cm), root collar diameter (D, mm), height:diameter ratio (HD), shoot to root dry weight ratio (SR), Dickson quality index (DQI).

		н		[	)	н	D	SR		DQI	
	Ν	SV	SD	SV	SD	SV	SD	SV	SD	SV	SD
Picea abies	96	51.53	15.48	7.62	3.51	7.45	2.03	1.60	0.52	0.72	0.63
Quercus robur	58	104.7	24.94	17.4	4.07	6.1	1.21				

#### 3.1.5 Seedlings 1+1

During a three-year survey in Serbian nurseries, we have found a two-year old seedlings (1+1) of three broadleaved species (Table 7). While a transplanting had a positive effect on *Acer platanoides* seedlings, it was an opposite for *Fraxinus excelsior*. Compared to seedlings of the same age (2+0), transplants of *A. platanoides* have all morphological attributes improved.

Table 7. Descriptive statistics (mean values – MV and standard deviation – SD) of 1+1 seedlings height (H, cm), root collar diameter (D, mm), height:diameter ratio (HD), shoot to root dry weight ratio (SR), Dickson quality index (DQI).

		Н		I	D		HD		SR		QI
	N	SV	SV SD		SD	SV	SD	SV	SD	SV	SD
Acer platanoides	46	69.94	18.09	10.1	2.73	7.02	1.33	0.69	0.12	1.79	0.40
Fraxinus excelsior	130	34.54	13.95	8.48	1.97	4.00	1.23	0.46	0.11	3.87	2.05
Prunus avium	45	100.6	30.67	11.4	3.06	8.82	1.60	0.64	0.21	2.54	1.04

#### 3.1.6 Seedlings 2+1

During a three-year survey in Serbian nurseries, we have found a three-year old seedlings (2+1) only of *Picea abies* (Table 8). Three-year old transplants (2+1) of *P. abies* have a smaller mean values of height and diameter compared to the seedlings of the same age (3+0), but values of HD, SR, and DQI are much better (Table 5 and 8).

Table 8. Descriptive statistics (mean values – MV and standard deviation – SD) of 2+1 seedlings height (H, cm), root collar diameter (D, mm), height:diameter ratio (HD), shoot to root dry weight ratio (SR), Dickson quality index (DQI).

		Н		D		HD		SR		DQI	
	Ν	SV	SD								
Picea abies	197	20.9	7.85	4.07	1.76	5.30	1.16	1.74	1.16	0.42	0.44

#### 3.1.7 Seedlings 2+2

During a three-year survey in Serbian nurseries, we have found a four-year old seedlings (2+2) of *Picea abies* and *Pinus nigra* (Table 9). Transplants of *P. abies* have a smaller mean values of height and diameter and larger HD and SR than seedlings of the same age (4+0).

Table 9. Descriptive statistics (mean values – MV and standard deviation – SD) of 2+2 seedlings height (H, cm), root collar diameter (D, mm), height:diameter ratio (HD), shoot to root dry weight ratio (SR), Dickson quality index (DQI).

		Н		D		HD		SR		DQI	
	Ν	SV	SD	SV	SD	SV	SD	SV	SD	SV	SD
Picea abies	218	32.2	9.20	6.20	2.02	5.55	1.93	2.00	0.87	0.73	0.42
Pinus nigra	75	84.52	16.58	26.2	5.25	3.36	0.98				

#### 3.1.8 Seedlings 3+1

During a three-year survey in Serbian nurseries, we have found a four-year old seedlings (3+1) only of *Picea abies* (Table 10). Again, the transplants of *P. abies* have a smaller mean values of height and diameter and larger HD and SR than seedlings of the same age (2+2 and 4+0).

Table 10. Descriptive statistics (mean values – MV and standard deviation – SD) of 3+1 seedlings height (H, cm), root collar diameter (D, mm), height:diameter ratio (HD), shoot to root dry weight ratio (SR), Dickson quality index (DQI).

		н		D		HD		SR		DQI	
	Ν	SV	SD								
Picea abies	90	20.0	6.61	3.79	1.37	5.52	1.38	1.23	0.36	1.03	0.81

#### 3.1.9 Seedlings 3+2

During a three-year survey in Serbian nurseries, we have found a five-year old seedlings (3+2) of *Abies alba* and *Picea abies* (Table 11). Five-year old transplants of *P. abies* are smaller than four-year old seedlings (4+0).

Table 11. Descriptive statistics (mean values – MV and standard deviation – SD) of 3+2 seedlings height (H, cm), root collar diameter (D, mm), height:diameter ratio (HD), shoot to root dry weight ratio (SR), Dickson quality index (DQI).

		H D				HD		SR		DQI	
	Ν	SV	SD	SV	SD	SV	SD	SV	SD	SV	SD
Abies alba	40	19.1	4.84	7.62	1.52	2.53	0.54				
Picea abies	50	39.12	8.30	8.03	2.25	5.07	1.16	1.58	0.88	1.09	0.56

# 3.2 Comparison to the grading standard

# 3.2.1 Abies alba



Figure 2. Comparison of measured height (left) and diameters (right) of 2+0 *Abies alba* seedlings: MV – mean value, SD – standard deviation, L10% - lower 10% of values, U10% - upper 10% of values, LQ – lower quartile, UQ – upper quartile. Blue – the second class, green – the first class by Serbian seedlings quality.

> There are no rejects of a measured two-year old *Abies alba* seedlings based on a height, and more than a half seedlings are in the first class (Figure 2, left). Quite opposite, measured two-year seedlings have a very small values of diameter with almost 70% of seedlings which do not meet the minimal requirement for diameter (Figure 2, right).



Figure 3. Comparison of measured height (left) and diameters (right) of 3+2 *Abies alba* seedlings: MV – mean value, SD – standard deviation, L10% - lower 10% of values, U10% - upper 10% of values, LQ – lower quartile, UQ – upper quartile. Blue – the second class, green – the first class by Serbian seedlings quality.

Opposite to the two-year old seedlings, a transplanted 3+2 *Abies alba* seedlings have more developed diameters than heights. A total of 75% of measured seedlings do not meet the minimal requirement for the height (Figure 3, left), but all seedlings meet the minimal requirement for the diameter (Figure 3, right) with over 95% in the first class.

# 3.2.2 Acer platanoides

One-year old seedlings of *Acer platanoides* are of good quality. All measured seedlings meet the minimal request based on height (Figure 4, left) with over 90% in the first class. Although a small number of measured seedlings should be rejected based on diameter, over 95% seedlings are in the first class (Figure 4, right).



Figure 4. Comparison of measured height (left) and diameters (right) of 1+0 *Acer platanoides* seedlings: MV – mean value, SD – standard deviation, L10% - lower 10% of values, U10% - upper 10% of values, LQ – lower quartile, UQ – upper quartile. Blue – the second class, green – the first class by Serbian seedlings quality.

Unlike one-year old seedlings, a two-year old reach very small values of measured heights (Table 4), much smaller than those of one-year old seedlings (Table 3), resulting with over 90% of rejects (Figure 5, left). Like for the heights, a two-year old seedlings have smaller mean value of diameter (Table 4) than one-year old seedlings (Table 3), but still, all seedlings have meet the minimal requirement (Figure 5, right).





#### 3.2.3 Acer pseudoplatanus

One-year old Acer pseudoplatanus seedlings shows better results for the diameters, with less than 5% of measured seedlings below a required minimum (Figure 6, right), than for height, with over 15% below the minimum requirement (Figure 6, left). Opposite to one-year old seedlings, all measured two-year old Acer pseudoplatanus seedlings meet the minimal requirement for the height (Figure 7, left). Only a few percent of measured seedlings do not meet the minimal requirement for the diameter (Figure 7, right).













Figure 8. Comparison of measured height (left) and diameters (right) of 1+0 *Castanea sativa* seedlings: MV – mean value, SD – standard deviation, L10% - lower 10% of values, U10% - upper 10% of values, LQ – lower quartile, UQ – upper quartile. Blue – the second class, green – the first class by Serbian seedlings quality. One-year old Castanea sativa seedlings are of excellent quality. All measured seedlings meet the minimal requests for the both height and diameter, with 95% of seedlings in the first class based on height (Figure 8, left) and all seedlings in the first class based on diameter (Figure 8, right).





Figure 9. Comparison of measured height (left) and diameters (right) of 1+0 *Fagus sylvatica* seedlings: MV – mean value, SD – standard deviation, L10% - lower 10% of values, U10% - upper 10% of values, LQ – lower quartile, UQ – upper quartile. Blue – the second class, green – the first class by Serbian seedlings quality standard.

One-year old seedlings of *Fagus sylvatica* are of poor quality. Almost 20% of measured seedling do not meet the minimal requirement for height (Figure 9, left), and almost 40% of seedlings do not meet the minimal requirement for diameter (Figure 9, right).



Figure 10. Comparison of measured height (left) and diameters (right) of 2+0 *Fagus sylvatica* seedlings: MV – mean value, SD – standard deviation, L10% - lower 10% of values, U10% - upper 10% of values, LQ – lower quartile, UQ – upper quartile. Blue – the second class, green – the first class by Serbian seedlings quality standard. Two-year old seedlings of *Fagus sylvatica* shows a small values of measured heights, and their mean value is close to that of one-year old seedlings (Figure 10 and Tables 3 and 4). Over 10% of measured seedlings do not meet the minimal requirement for height (Figure 10, left), and 23% of measured seedlings do not meet the minimal requirement for diameter (Figure 10, right).

#### 3.2.6 Fraxinus angustifolia



Figure 11. Comparison of measured height (left) and diameters (right) of 2+0 *Fraxinus angustifolia* seedlings: MV – mean value, SD – standard deviation, L10% - lower 10% of values, U10% - upper 10% of values, LQ – lower quartile, UQ – upper quartile. Blue – the second class, green – the first class by Serbian seedlings quality.

Two-year old *Fraxinus angustifolia* seedlings are of very good quality. All measured seedlings have meet the minimal requirements for both height and diameter, with 75% of seedlings in the first class based on the height (Figure 11, left) and 95% of seedlings in the first class based on the diameter.

#### 3.2.7 Fraxinus excelsior

One-year old *Fraxinus excelsior* seedlings shows a large variability of height, with 10% of seedlings with extremely large values on the one side, and with more of 25% of measured seedlings below the minimal requirement on the other side (Figure 12, left). Distribution of measured heights was not normal, resulting with SD almost equal to mean value (Table 3). The same seedlings shows much better results for diameters, with just a few seedling below the minimal requirement and over 90% of measured seedlings in the first class (Figure 12, right).

Opposite to one-year old *Fraxinus excelsior* seedlings, a two-year old seedlings shows very good results of measured heights, with just a few seedlings which do not meet a minimal requirement, and with over 80% of seedlings in the first class (Figure 13, left). The results of measured diameters are even better, with all seedlings over the minimal requirement and around 90% of seedlings in the first class (Figure 13, right).



Figure 12. Comparison of measured height (left) and diameters (right) of 1+0 *Fraxinus excelsior* seedlings: MV – mean value, SD – standard deviation, L10% - lower 10% of values, U10% - upper 10% of values, LQ – lower quartile, UQ – upper quartile. Blue – the second class, green – the first class by Serbian seedlings quality standard.



Figure 13. Comparison of measured height (left) and diameters (right) of 2+0 *Fraxinus excelsior* seedlings: MV – mean value, SD – standard deviation, L10% - lower 10% of values, U10% - upper 10% of values, LQ – lower quartile, UQ – upper quartile. Blue – the second class, green – the first class by Serbian seedlings quality standard.

#### 3.2.8 Picea abies

Over 50% of measured one-year old *Picea abies* seedlings have not meet the minimum request for the height (Figure 14, left). The results for the diameter are a slightly better, but still one third of seedlings should be rejected based on the diameter (Figure 14, right).

Two-year old seedlings of *Picea abies* are of poor quality. Over 70% of measured seedlings are in the first class based on the height, but still over 15% seedlings should be rejected (Figure 15, left). Based on the diameter, over 25% of measured seedlings should be rejected (Figure 15, right).

Over 10% of measured four-year old transplants (2+2) of *Picea abies* should be rejected based on both height and diameter (Figure 17). Still, these results are much better than those for four-year old transplants (3+1). The mean values of four-year old



transplants (3+1) of *Picea abies* are at the level of minimal requirement for the height (Figure 17, left) or below this level for diameter (Figure 17, right).

Figure 14. Comparison of measured height (left) and diameters (right) of 1+0 *Picea abies* seedlings: MV – mean value, SD – standard deviation, L10% - lower 10% of values, U10% - upper 10% of values, LQ – lower quartile, UQ – upper quartile. Blue – the second class, green – the first class by Serbian seedlings quality.







Figure 16. Comparison of measured height (left) and diameters (right) of 3+0 *Picea abies* seedlings: MV – mean value, SD – standard deviation, L10% - lower 10% of values, U10% - upper 10% of values, LQ – lower quartile, UQ – upper quartile. Blue – the second class, green – the first class by Serbian seedlings quality.





The heights of measured five-year old *Picea abies* seedlings are relatively good, with the mean value at the level of the first class and with 10% of seedlings which have not meet the minimal requirements (Figure 18, left). Less of 10% of measured seedlings should be rejected based on diameter and 80% of measured seedlings are in the first class (Figure 18, right).



Figure 18. Comparison of measured height (left) and diameters (right) of 3+1 *Picea abies* seedlings: MV – mean value, SD – standard deviation, L10% - lower 10% of values, U10% - upper 10% of values, LQ – lower quartile, UQ – upper quartile. Blue – the second class, green – the first class by Serbian seedlings quality.



Figure 19. Comparison of measured height (left) and diameters (right) of 3+2 *Picea abies* seedlings: MV – mean value, SD – standard deviation, L10% - lower 10% of values, U10% - upper 10% of values, LQ – lower quartile, UQ – upper quartile. Blue – the second class, green – the first class by Serbian seedlings quality.

#### 3.2.9 Pinus nigra



Figure 20. Comparison of measured height (left) and diameters (right) of 1+0 *Pinus nigra* seedlings: MV – mean value, SD – standard deviation, L10% - lower 10% of values, U10% - upper 10% of values, LQ – lower quartile, UQ – upper quartile. Blue – the second class, green – the first class by Serbian seedlings quality.

Over 20% of one-year old *Pinus nigra* seedlings have not meet the minimal requirement for the height (Figure 20, left), and 29,6% of measured seedlings did not reach the height of 5 cm. Around 10% of measured seedlings have not meet the minimal requirement for diameter (Figure 20, right).



Figure 21. Comparison of measured height (left) and diameters (right) of 2+0 *Pinus nigra* seedlings: MV – mean value, SD – standard deviation, L10% - lower 10% of values, U10% - upper 10% of values, LQ – lower quartile, UQ – upper quartile. Blue – the second class, green – the first class by Serbian seedlings quality.

Over 10% of measured two-year seedlings of *Pinus nigra* seedlings should be rejected based on the height, but 70% of seedlings are in the first class (Figure 21, left). Less than 5% of measured two-year seedlings of *Pinus nigra* seedlings should be rejected based on the diameter, and 80% of seedlings are in the first class (Figure 21, right).

Four-year old transplants (2+2) of *Pinus nigra* are of very good quality. There are no rejects based on both height and diameter. Except a few seedlings based on height, all measured seedlings are in the first class (Figure 22).

Four-year old transplants (2+2) of *Pinus nigra* are of very good quality. There are no rejects based on both height and diameter. Except a few seedlings based on height, all measured seedlings are in the first class (Figure 22).



Figure 22. Comparison of measured height (left) and diameters (right) of 2+2 *Pinus nigra* seedlings: MV – mean value, SD – standard deviation, L10% - lower 10% of values, U10% - upper 10% of values, LQ – lower quartile, UQ – upper quartile. Blue – the second class, green – the first class by Serbian seedlings quality.



## 3.2.10 Pinus sylvestris

Figure 23. Comparison of measured height (left) and diameters (right) of 1+0 *Pinus sylvestris* seedlings: MV – mean value,
SD – standard deviation, L10% - lower 10% of values, U10% - upper 10% of values, LQ – lower quartile, UQ – upper quartile. Blue – the second class, green – the first class by Serbian seedlings quality standard.

One-year old *Pinus sylvestris* seedlings are of poor quality. Almost 25% of measured seedlings do not meet the minimal requirements for the height, and only 30% of seedlings are in the first class (Figure 23, left). The same is with diameter, with almost 25% of measured seedlings below the minimal requirements for the diameter, and only 37% of seedlings are in the first class (Figure 23, right).

Two-year old seedlings of *Pinus sylvestris*, like the one-year-old, are of poor quality. More than 25% of measured seedlings do not meet the minimum requirements for height and diameter (Figure 24), and almost 10% of measures two-year old seedlings of *Pinus sylvestris* have a diameter smaller than 1 mm.



Figure 24. Comparison of measured height (left) and diameters (right) of 2+0 *Pinus sylvestris* seedlings: MV – mean value, SD – standard deviation, L10% - lower 10% of values, U10% - upper 10% of values, LQ – lower quartile, UQ – upper quartile. Blue – the second class, green – the first class by Serbian seedlings quality standard.

#### 3.2.11 Pseudotsuga menziesii

One-year old *Pseudotsuga menziesii* seedlings are of poor quality, with 10% of measured seedlings do not meet the minimal requirement for height (Figure 25, left), and 77,5% of measured seedlings with diameters smaller than 1 mm, which do not meet the minimal requirement for diameter (Figure 25, right).



Figure 25. Comparison of measured height (left) and diameters (right) of 1+0 *Pseudotsuga menziesii* seedlings: MV – mean value, SD – standard deviation, L10% - lower 10% of values, U10% - upper 10% of values, LQ – lower quartile, UQ – upper quartile. Blue – the second class, green – the first class by Serbian seedlings quality standard.

#### 3.2.12 Robinia pseudoacacia

Almost all measured seedlings meet the minimal height set by the standard and almost 90% are in the first class (Figure 26, left). Like for the heights, more than 90% of measured seedlings meet a diameter limit for the first class (Figure 26, right).

Two-year old seedlings of *Robinia pseudoacacia* shows a very good growth in both height and diameter. According to height, more of 10% of measured seedlings do not meet the minimal height, but more that 75% of seedlings are in the first class (Figure 27, left). According to diameter, there is a less rejects, but also a much less seedlings in the first class (56%) (Figure 27, right).









#### 3.3 The presence of mycorrhiza

Mycorrhization by artificial inoculation is not an operational practice in Serbian forest nurseries. However, the results of a three-year survey shows the presence of mycorrhizal fungi in all nurseries, seedbeds and containers, and for all species (Figure 28).



Figure 28. Mycorrhizal fungi on seedlings of *Quercus petraea* (the upper row), *Pinus nigra* (the middle row), and *Picea abies* (the lower row).

# 4 Discusion

#### 4.1 Seedling size and quality standard

Seedlings produced in Serbian nurseries during a three-year survey are comparable to the seedlings produced at operational scale and for the research reported by a number of authors. Seedlings of *Abies alba* (1+0 and 2+0) are in the range reported by Petrović (1952). Seedlings of *Acer platanoides* are larger than previously reported for seedlings 1+0 (Aldhous 1972). Seedlings of *Acer pseudoplatanus* are in the range (Aldhous 1972) or larger than previously reported for 1+0 (Radoglou and Raftoyanis 2002) and 2+0 (Popović et al. 2017). However, 2+0 seedlings of *A. pseudoplatanus* are smaller from those reported by lvetić et al. (2016a). Seedlings of *Castanea sativa* 1+0 reached only a half of targeted height suggested by Petrović (1952).

Seedlings of Corylus colurna are larger than previously reported for seedlings 1+0 (Aldhous 1972; Ninić-Todorović et al. 2012). Seedlings of Fagus sylvatica are larger than previously reported for seedlings 1+0 (Aldhous 1972; Konnert and Ruetz 2003; Ivetić et al. 2016a), but smaller than those reported by Wrzesinski (2015). Seedlings of Fraxinus angustifolia 2+0 are larger than previously reported by Petrović (1952). Seedlings of Fraxinus excelsior 1+0 are larger than previously reported by Salaš (2002), are shorter but thicker than reported by (Maltoni et al. 2010) and 2+0 are larger than previously reported by Ivetić et al. (2016a). Seedlings of Juglans nigra 1+0 are larger than previously reported by Aldhous (1972). Seedlings of Juglans regia 1+0 are larger than previously reported by Aldhous (1972) and Ćirković-Mitrović et al. (2012) and comparable to those reported by Tani et al. (2007). Seedlings of Picea abies of all ages are smaller than previously reported by Petrović (1952), but seedlings 2+0 are in the range reported by Repač et al. (2014). Seedlings of Pinus nigra 1+0 are in the range reported by (Jinks and Mason 1998) but larger than those reported by Ivetić and Škorić (2013), and 2+0 are larger than previously reported by Petrović (1952) and Ivetić and Škorić (2013). Seedlings of *Pinus sylvestris* 1+0 are smaller, but 2+0 are in the range previously reported by Petrović (1952). Seedlings of Prunus avium 1+0 are much larger than previously reported by Aldhous (1972) and double of size reported by Stjepanović and Ivetić (2013). Seedlings of Quercus petraea 1+0 are much larger than previously reported by Aldhous (1972). Seedlings of Quercus robur 1+0 are in the range suggested by Aldhous (1972), but much smaller than previously reported by Petrović (1952). Additionally, seedlings of Quercus robur 2+0 are five time smaller than seedlings of the same age produced in a large containers (Mariotti et al. 2015). Seedlings of Quercus rubra 1+0 are larger than previously reported by Aldhous (1972) and Popović et al. (2015), but smaller than those reported by Ivetić et al. (2016a). Seedlings of Q. rubra 1+0 are taller but with smaller diameter compared to results reported by Day and Parker (1997) and Salifu and Jacobs (2006). Seedlings of Robinia pseudoacacia are larger than previously reported by Kolevska et al. (2015) and Ivetić et al. (2016a).

Our results, however, indicate the need for improvement of current cultural practices in Serbian nurseries, as well as need for update of official quality standard. The testing of seedlings quality must be an obligatory and regular practice in forest nurseries, especially in those operating in large systems like public enterprises. Monitoring the seedlings quality is important in forest nurseries, because better understanding and application of cultural practices which improve seedling quality promote the reforestation success (Duryea 1984). For the most of nursery cultural practices, the effects on seedling quality and planting success are well known and described (as reviewed by Ivetić and Devetaković 2016), and their application should be additionally monitored and controlled in nurseries which operate in the same system as the final user. In most public enterprises for forest management, the nurseries and silvicultural service responsible for reforestation are parts of the same system, resulting in low quality control. The current quality standard should be improved by extending the list of tree species and by adapting the minimal requirements for H, D, and general condition of seedlings. The list of species should be extended to economically important tree species, which seedlings are produced and used in artificial forest regeneration. The minimal requirements should be adjusted to nursery technologies as well as to the purpose of seedlings use. Seedlings grading is effective only when it is based on understanding that different nurseries and different parts of the same nursery represents a different entities, and when substrate/soil fertility, growing density in the seedbeds, and irrigation are standardized and controlled (Kormanik 1989). This statement is supported by the results of our study. As seedlings quality should be defined on the outplanting site and not in the nursery (Dumroese et al. 2016), the minimal requirements regarding seedlings H, D, and general condition need to be supported by a site or purpose specific requirements. These specific requirements should be defined on results of field trials.

Finally, the control of seedlings quality before outplanting must be obligatory for all reforestation programs. In addition to reduced costs of reforestation by improved survival and lack of need for re-planting on the site (Sampson et al. 1997), this practice will improve all aspects of seedlings production. This quality control should be conducted by experts outside the system which manage the seedlings production in forest nurseries.

#### 4.2 The presence of mycorrhizae

The rate and diversity of mycorrhizae naturally established in the nursery depends on environmental – nursery conditions, cultural practices and host species (Croghan 1984; Molina and Trappe 1984). It is known that high fertilization rates (Cram and Dumroese 2012), especially with nitrogen and phosphorus (Flykt et al. 2008) can suppress mycorrhizae development, and low level of fertilization rates in Serbian nurseries can be one of the reasons for colonization success.

The presence of mycorrhiza in all samples is not unusual. In six forest nurseries in South Tyrol, on conifer seedlings, the rate of mycorrhization was 100% in autumn and about 97% in spring in all nurseries and on all plant species (Bacher et al. 2010). Pietras et al. (2013) founded that artificial inoculation of beech seedlings in the nurseries is not necessary due to abundant natural colonization. The fact that the mycorrhization in the container is not erratic indicate the abundance of fungus spores landing and washing in the growing media (Castellano and Molina 1989).

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