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MORPHO-ANATOMICAL CHARACTERISTICS AND CONTENT OF NUTRITIVE MACRO ELEMENTS IN NEEDLES OF FIR AND SPRUCE AND THEIR VARIETIES IN SERBIA

M. RATKNIĆ, Z. MILETIĆ and BILJANA NIKOLIĆ

[1] *Institute of Forestry,* 11030 Belgrade, Serbia

Abstract - The aim of the study is to establish, based on the morphological characteristics of shoots and morpho-anatomical characteristics of needles as well as the content of micro-elements in needles, whether there are differences between Pyramidal Fir (*Abies alba* var. *pyramidalis* Carr.) and European Silver Fir (*Abies alba* Mill.), and between Mišić's spruce (*Picea abies* var. *misicii* Matović & Pavlović) and Common Spruce (*Picea abies* (L.) Karst.), and to determine in which characteristics these differences are most pronounced. This study showed that Pyramidal fir (PF) has a two-times higher number of lateral shoots in comparison to European silver fir (ESF), but also shorter and thinner shoots and a lower number of needles, which are slightly less densely distributed on a shoot. In addition, PF terminal shoots are significantly longer and thicker than the lateral, and have a higher number of needles. Two-year-old PF needles are longer, wider and thicker in comparison to ESF needles, and have higher values with respect to diameter of the central cylinder, thickness of cuticle and epidermis, thickness of hypodermis and diameter of resin canals. The most pronounced differences between PF and ESF are related to length of shoots, needle width and diameter of the central cylinder (statistically significant differences). In comparison to ESF, PF has a higher percentage of nitrogen, phosphorus and potassium in needles; however, this is statistically significant only in case of phosphorus. The most apparent morphological specificity of Mišić's spruce (MS) is a rare occurrence of lateral shoots (only once in several years), unlike Common spruce (CS), which has 2-3 new shoots every year. MS has far longer and thicker shoots, while its needles are more numerous, longer, thinner and more densely distributed on the shoot in comparison to CS. Additionally, in comparison to CS, MS needles have a larger diameter of central cylinder, but lower thickness of cuticle with epidermis, lower thickness of hypodermis and smaller diameter of the resin canals. The most pronounced differences between MS and CS are related to length, width and thickness of needles and diameter of central cylinder (statistically significant differences). In comparison to CS, MS has a higher percentage of nitrogen, phosphorus and potassium in needles; however, this is not statistically significant.

Key words: Abies alba var. *pyramidalis*, *Abies alba*, *Picea abies* var. *misicii*, *Picea abies*, morphology, anatomy, nutrient, Serbia

INTRODUCTION

The variability of morphological characteristics of conifer needles has been studied in various habitat conditions, for instance, at different altitudes, (Richardson et al., 2001, Poulos and Berlyn, 2007), different exposures (Lukjanova and Mandre, 2008) or substrates (Bączkiewicz et al., 2005). Significant differences in the morphology of the needles can be manifested during the course of ontogenesis (Apple et al., 2002); however, similarities between needles of different species of the same gender can also occur, particularly in the seedling stadium (Boratyńska et al., 2008). For certain morpho-anatomical characteristics of needles, (length, width and thickness), it has been established that they

are genetically influenced (in *Pinus nigra*, Matziris, 1984). Based on the morphological and anatomical characteristics of needles, it is possible to determine varieties (Dvorak et al., 2001) or hybrids (Jasińska et al., 2010), or even to compare different species (Lakušić and Lakušić, 2011). More recently, these studies have been increasingly frequently combined with chemical (Lauranson - Broyer and Lebreton, 1993) and molecular markers (Dvorak et al., 2001; Jasińska et al., 2010). Some general data on the morphology and habitats of Pyramidal fir and Mišić's spruce have already been published (Pavlović et al., 1995; Matović and Marković, 2000; Rakonjac et al., 2010, etc).

Nutrient uptake from the soil is important for plant development and it largely depends on a complex mechanism of uptake regulation (Tremoileres et al. 1999; Covelo et al. 2008). A correct ratio of certain nutritive elements in assimilation organs is reflected in the growth of forest trees, production of wood volume and resistance to biotic agents associated with dieback. The concentration of nutrient matter in assimilation organs of forest tree species depends on the habitat conditions (Bauer et al., 1997). By adapting to different habitat conditions, forest species adapt to the different amounts of available nutrient matter in the soil. As a result, optimum quantities of nutrient uptake of different provenances, necessary for their proper growth and development, are changing. Differentiation of species is frequently caused by different habitat conditions, which determine the nutrition of forest trees.

The aim of this paper was to establish, based on the morphological characteristics of shoots and morpho-anatomical characteristics of needles, as well as on the content of micro-elements in needles, whether there are differences between Pyramidal fir (*Abies alba* var. *pyramidalis* Carr.) and European silver fir (*Abies alba* Mill.), and between Mišić's spruce (*Picea abies* var. *misicii* Matović and Pavlović) and Common spruce (*Picea abies* (L.) Karst.), and to determine in what characteristics those differences are most pronounced.

MATERIALS AND METHODS

Shoots with needles from five Pyramidal fir (PF) and five European silver fir (ESF) trees were collected at the locality Ogorijevac near the town of Sjenica, Serbia, in which fir, along with beech and spruce, forms a mixed uneven-aged stand. Shoots with needles from one Mišić spruce (MS) and five Common spruce (CS) trees were collected at the locality Paljika, on Mount Kamena Gora, near the town of Prijepolje, Serbia. In both localities, needle samples were taken from the brim of the central part of the crown.

In shoots aged 1-4 years, the number of lateral shoots, length and thickness of shoots, number of needles and average length of needles, were determined. In addition, the number of needles per 1 cm of shoot length was also calculated. The calculation of average needle length was conducted on ca. 432 (PF), 369 (ESF), 240 (MS) and 306 (CS) samples (needles). On the cross section of two-year-old needles, the width and thickness of needles, thickness of cuticle and epidermis, thickness of hypodermis and diameter of the central cylinder, were measured. The average number of resin canals and their diameters was also calculated. Statistical processing involved a total of 108 (PF), 114 (ESF), 24 (MS) and 108 (CS) two-year-old needles, which contained 216 (PF), 228 (ESF), 48 (MS) and 216 (CS) resin canals, respectively. Cross-section measurements through the central part of needles were done with a Leica Galen III microscope. The obtained results were recalculated by means of recalculation coefficients. A range of variation of measured properties (min-max) was determined and the following statistical parameters were processed: mean value, standard error, variance analysis and LSD test. A program Statgraphics Plus (version 5.0; Statistical Graphics Corporation, USA) was used for statistical processing.

In the sampled needles, the content of nitrogen (N), phosphorus (P) and potassium (K) was determined. The content of nitrogen was determined by means of the Kjeldahl method (Džamić et al., 1996). The content of phosphorus and potassium was determined by the analysis of ash, which followed the dry

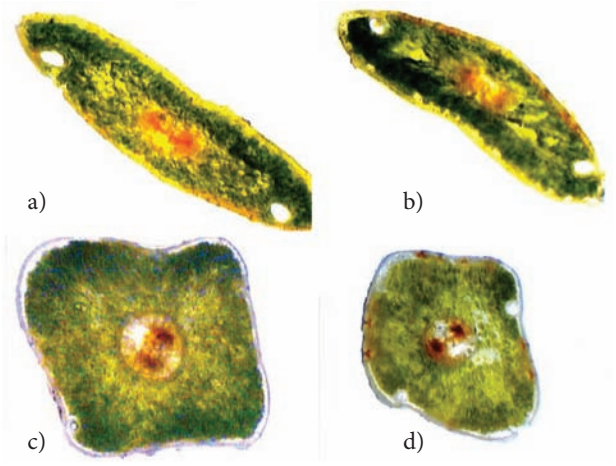

Fig. 1. Needle cross-section of: a) Pyramidal fir (PF); b) European silver fir (ESF); c) Mišić's spruce (MS); d) Common spruce (CS).

burning of samples at a temperature of 450°C and conversion of elements into chlorides. In the above-mentioned analysis, phosphorus was determined by means of the colorimetric method, while potassium was determined by the flame-photometric method. The differences between the percentage-contents of nutritive elements were checked using the LSD test.

RESULTS AND DISCUSSION

Morpho-anatomic properties of Pyramidal fir (PF) and European silver fir (ESF)

PF has a higher range with respect to the number of lateral shoots (1-4) in comparison to ESF (1-3), and their higher average number (1.8 and 1.0, PF and ESF, respectively, results not presented); however, it has less variations with respect to lateral-shoot length (Table 1) and a lower average length of lateral shoots (Tab. 2). These differences are significant in 95% of cases (variance analysis and LSD test, results not presented).

In comparison to ESF, PF has shorter terminal and lateral shoots (Table 2). The differences in length between terminal and lateral shoots are statistically significant in PF (terminal shoots are longer), but not in ESF. In previous studies (Matović et al., 1996), the average length of ESF and PF shoots (aged 1-2 years) was slightly higher than is the case in our studies (Tables 1 and 2).

In terms of shoot thickness, PF is more variable than ESF (Table 1), but on average, it has slightly thinner shoots (1.66 mm and 1.77 mm, respectively, Table 2). However, terminal shoots in both PF and ESF are significantly thicker in comparison to the lateral. In the previous studies, (Matović et al., 1996), PF shoots were thinner.

In comparison to ESF, PF is characterized by lower variations with respect to the number of shoots and their average number (Tables 1 and 2). PF has a significantly higher number of needles in terminal than in lateral shoots. The mean number of PF needles (24.9) is lower in comparison to ESF (29.5) (Table 2), but it is significantly higher in comparison to previous results (Matović et al., 1996).

In terms of the mean length of shoots, there were no significant differences between PF and ESF (Table 2). Furthermore, there were no differences in the needle length of terminal and lateral shoots. The needle length range in PF is lower than in ESF (Table 1). The needle length range in ESF (9-31mm) is higher than it is in literature references (Vidaković, 1982; Matović et al., 1996; Pawlaczyk et al., 2005). The mean length of ESF needles in our studies (18.8 mm) shares similarity with some literature references, while compared to certain specific references, it is even higher (Pawlaczyk et al., 2005). Two-year-old Pyramidal fir needles (19.6 mm) are longer than Common fir needles (18.6 mm), which has already been noted earlier (Matović et al., 1996).

In addition, PF has a slightly lower number of needles per 1cm of shoot length than ESF (7.3 and 7.7, respectively, Table 2). In previous studies (Matović et al., 1996), conducted on a lower number of trees, ESF and PF needles were more densely distributed on the shoots.

PF exhibited lower variability (Table 3), but a higher mean value of width of two-year-old needles

Table 1. Minimal and maximal values of shoot characteristics (PF and ESF).

Species		Pyramidal fir (PF)					European silver fir (ESF)				
Properties	Age	1-year old	2-years old	3-years old	4-years old	Average 1-4	1-year old	2-years old	3-years old	4-years old	Average 1-4
Shoot length (cm)	*Average range*	*1.5 - 5.0*	*1.9 - 5.2*	*2.4 - 4.8*	*2.2 - 5.0*	***1.5 - 5.2***	*1.5 - 6.0*	*2.0 - 5.1*	*2.2 - 6.5*	*3.0 - 6.0*	***1.5 - 6.5***
	Terminal	2.4 - 4.5	2.2 - 4.8	3.0 - 4.8	2.2 - 5.0	**2.2 - 5.0**	1.5 - 5.5	2.0 - 4.5	2.2 - 6.5	3.0 - 6.0	**1.5 - 5.2**
	Lateral	1.5 - 5.0	1.9 - 5.2	2.4 - 4.0	2.7 - 4.5	**1.5 - 5.2**	2.0 - 6.0	2.0 - 5.1	3.0 - 5.5	3.5 - 3.5	**2.0 - 6.0**
Shoot width (mm)	*Average range*	*0.2 - 2.1*	*0.8 - 2.4*	*0.5 - 2.8*	*1.2 - 3.7*	***0.2 - 3.7***	*0.6 - 2.1*	*1.2 - 2.3*	*1.6 - 2.9*	*1.7 - 3.5*	***0.6 - 3.5***
	Terminal	0.8 - 1.7	0.8 - 2.4	1.5 - 2.8	1.2 - 3.7	**0.8 - 3.7**	0.6 - 2.1	1.2 - 2.3	1.6 - 2.9	1.7 - 3.5	**0.6 - 3.5**
	Lateral	0.2 - 2.1	1.2 - 2.3	0.5 - 2.8	1.6 - 2.9	**0.2 - 2.9**	0.9 - 1.7	1.3 - 2.1	1.6 - 2.6	1.7 - 1.7	**0.9 - 2.6**
Number of needles	*Average range*	*14 - 39*	*14 - 46*	*15 - 43*	*14 - 39*	***14 - 46***	*14 - 64*	*12 - 47*	*18 - 49*	*24 - 51*	***12 - 64***
	Terminal	20 - 39	15 - 38	22 - 38	14 - 33	**14 - 39**	14 - 51	14 - 36	18 - 49	24 - 51	**14 - 51**
	Lateral	14 - 39	14 - 46	15 - 43	14 - 39	**14 - 46**	18 - 64	12 - 47	22 - 38	26 - 26	**12 - 64**
Needle length (mm)	*Average range*	*9 - 27*	*12 - 27*	*10 - 26*	*12 - 27*	***9 - 27***	*9 - 31*	*9 - 30*	*11 - 30*	*12 - 30*	***9 - 31***
	Terminal	11 - 27	12 - 27	11 - 26	12 - 27	**11 - 27**	9 - 30	11 - 28	11 - 30	12 - 30	**9 - 30**
	Lateral	9 - 25	12 - 27	10 - 26	12 - 27	**9 - 27**	9 - 31	9 - 30	11 - 29	14 - 28	**9 - 31**

(2.05 mm, Table 4) compared to ESF (1.94 mm) (Figs. 1a, 1b). These differences are significant in 95% of the cases (variance analysis, LSD test, results not presented). However, PF has a lower range of variability and a lower mean value of needle width in comparison to literature references for ESF (Pawlaczyk et al., 2005; Pawlaczyk and Bobowicz, 2008). PF and ESF in our studies had a larger range of variability in comparison to Macedonian ESF (Popnikola, 1974), which has been noted in previous studies (Matović et al., 1996).

In addition, PF has slightly thicker needles in comparison to ESF (502 μm i 501 μm, respectively, Table 4) and a lower range of variability (Table 3). However, these values are lower in comparison to some literature references for ESF (Pawlaczyk et al., 2005; Pawlaczyk and Bobowicz, 2008). Needles from Macedonia exhibited a lower variability in terms of needle thickness (Popnikola, 1974). Our studies have confirmed the view held by Matović et al. (1996) that ESF needles are narrower and flatter than PF needles.

In addition, PF has a statistically significantly larger diameter of central cylinder in comparison to ESF (346 μm and 328 μm, respectively, Table 4) and a higher variability (Table 3). PF also has a greater thickness of cuticle with epidermis, as well as thickness of hypodermis (13.19 μm and 18.98 μm, respectively)

Table 2. Mean values and errors of shoot characteristics (PF and ESF).

Species		Pyramidal fir (PF)					European silver fir (ESF)				
Properties	Age	1-year old	2- years old	3- years old	4- years old	Aver-age 1-4	1-year old	2- years old	3- years old	4- years old	Aver-age 1-4
Shoot length (cm)	*Average*	3.13 ±0.10	*3.31 ±0.09*	*3.60 ±0.49*	*3.74 ±0.09*	***3.42*** *±0.05*	*3.38 ±0.18*	*3.59 ±0.13*	*4.32 ±0.16*	*4.72 ±0.30*	***3.83*** *±0.10*
	Terminal	3.33 ±0.15	3.42 ±0.16	3.77 ±0.12	3.75 ±0.18	**3.56** ±0.08	3.26 ±0.30	3.70 ±0.17	4.33 ±0.23	4.84 ±0.30	**3.95** ±0.14
	Lateral	3.04 ±0.13	3.26 ±0.10	3.51 ±0.07	3.73 ±0.12	**3.33** ±0.06	3.47 ±0.21	3.49 ±0.20	4.29 ±0.24	3.50 ±0.00	**3.68** ±0.13
Shoot width (mm)	*Average*	1.19 ±0.06	1.57 ±0.04	1.90 ±0.06	2.14 ±0.08	***1.66*** *±0.04*	*1.35 ±0.05*	*1.69 ±0.04*	*2.09 ±0.07*	*2.47 ±0.19*	***1.77*** *±0.05*
	Terminal	1.33 ±0.07	1.69 ±0.08	2.08 ±0.08	2.41 ±0.13	**1.86** ±0.07	1.39 ±0.09	1.71 ±0.08	2.16 ±0.10	2.55 ±0.19	**1.89** ±0.07
	Lateral	1.12 ±0.07	1.51 ±0.04	1.81 ±0.07	1.92 ±0.06	**1.54** ±0.04	1.32 ±0.06	1.69 ±0.04	2.01 ±0.09	1.70 ±0.00	**1.63** ±0.05
Number of needles	*Average*	*24.6 ±0.81*	*24.5 ±0.85*	*25.8 ±0.71*	*24.3 ±1.01*	***24.9*** *±0.41*	*31.1 ±2.16*	*25.3 ±1.40*	*31.4 ±1.35*	*34.0 ±2.39*	***29.5*** *±0.93*
	Terminal	26.7 ±1.19	25.7 ±1.59	27.9 ±1.03	24.6 ±1.73	**26.4** ±0.67	29.5 ±3.31	25.4 ±1.67	31.9 ±2.07	34.8 ±2.49	**29.8** ±1.23
	Lateral	23.5 ±1.03	24.0 ±1.00	24.6 ±0.89	24.1 ±1.27	**24.0** ±0.51	32.4 ±2.90	26.7 ±2.30	30.7 ±1.56	26.0 ±0.00	**29.2** ±1.44
Needle length (mm)	*Average*	*18.0 ±0.40*	*19.6 ±0.39*	*18.6 ±0.38*	*18.2 ±0.41*	***18.6*** *±0.20*	*18.4 ±0.48*	*18.6 ±0.45*	*19.2 ±0.51*	*19.3 ±0.70*	***18.8*** *±0.26*
	Terminal	17.8 ±0.57	19.4 ±0.54	18.2 ±0.51	18.3 ±0.59	**18.4** ±0.28	18.6 ±0.66	19.1 ±0.57	19.1 ±0.61	19.3 ±0.74	**19.1** ±0.32
	Lateral	18.2 ±0.55	19.9 ±0.55	19.1 ±0.57	18.1 ±0.57	**18.9** ±0.28	18.1 ±0.70	18.1 ±0.71	19.4 ±0.95	18.7 ±2.23	**18.4** ±0.43
Number of needles per 1 cm of shoot length	*Average*	*7.9*	*7.4*	*7.2*	*6.5*	***7.3***	*9.2*	*7.1*	*7.3*	*7.2*	***7.7***
	Terminal	8.1	*7.5*	*7.4*	*6.6*	***7.4***	*9.0*	*6.9*	*7.4*	*7.2*	***7.6***
	Lateral	*7.7*	*7.4*	*7.0*	*6.5*	***7.2***	*9.3*	*7.6*	*7.2*	*7.4*	***7.9***

than ESF (12.92 μm and 17.97 μm, respectively), but not statistically significantly. These values are higher in Macedonian ESF (Popnikola, 1974) and lower in Polish ESF (Pawlaczyk and Bobowicz, 2008).

In both PF and ESF, a regular occurrence of two resin canals, which mainly touch the hypodermis, was noted. The range of variability and average diameter of resin canals are higher in PF (Tables 3 and 4). In addition, PF has higher values of the above in comparison to literature references for Polish EFS (Pawlaczyk et al., 2005; Pawlaczyk and Bobowicz, 2008), but lower in comparison to firs from Macedonia (Popnikola, 1974).

Content of macro-elements in needles of Pyramidal fir (PF) *and European silver fir* (ESF)

Based on the conducted foliar analyses, it can be concluded that the content of potassium in PF individually varies from 0.257% to 0.491%, while in ESF it varies from 0.270% to 0.403%. The content of phosphorus in PF varies from 0.086% to 0.111%, while in ESF it varies from 0.079% to 0.093%. The content of

Table 3. Minimal and maximal values of anatomic characteristics of needles.

Properties	Species	Pyramidal fir (PF)	European silver fir (ESF)	Mišić's spruce (MS)	Common spruce (CS)
Needle width (μm)	*Average*	1590.31 - 2356.92	983.50 - 2454.36	1300.19 - 1619.36	1093.06 - 2454.36
	Terminal	1590.31 - 2356.22	983.50 - 2209.29	1300.19 - 1619.36	1093.06 - 2209.29
	Lateral	1608.56 - 2318.64	1526.67 - 2209.29		1204.22 - 2454.36
Needle thickness (μm)	*Average*	315.61 - 652.50	373.65 - 971.20	1049.12 - 1511.51	287.60 - 1948.59
	Terminal	315.61 - 640.24	413.31 - 971.20	1049.12 - 1511.51	287.60 - 1948.59
	Lateral	319.42 - 652.50	373.65 - 625.54		392.53 - 1106.77
Central cylinder diameter (μm)	*Average*	204.83 - 481.10	237.83 - 445.15	343.41 - 462.47	141.00 - 431.17
	Terminal	204.83 - 458.94	240.64 - 437.67	343.41 - 462.47	141.00 - 431.17
	Lateral	210.37 - 481.10	237.83 - 445.15		237.12 - 422.97
Cuticle and epidermis thickness (μm)	*Average*	7.00 - 25.08	7.07 - 21.38	8.54 - 18.03	8.06 - 25.50
	Terminal	7.00 - 25.08	7.07 - 21.38	8.54 - 18.03	8.06 - 25.50
	Lateral	8.06 - 19.85	7.81 - 20.62		9.43 - 20.62
Hypoderm thickness (μm)	*Average*	10.82 - 33.14	9.90 - 26.63	10.30 - 20.62	9.90 - 26.63
	Terminal	10.82 - 33.14	9.90 - 24.84	10.30 - 20.62	9.90 - 24.84
	Lateral	11.66 - 25.55	10.00 - 26.63		13.00 - 26.63
Resin canal diameter (μm)	Average	79.62 - 244.17	67.27 - 172.41	33.97 - 90.27	33.02 - 172.41
	Terminal	79.62 - 244.17	72.90 - 172.41	33.97 - 90.27	33.02 - 172.41
	Lateral	86.28 - 198.64	67.27 - 154.95		51.04 - 154.95

Table 4. Mean values and errors of anatomic characteristics of needles.

Properties	Species	Pyramidal fir (PF)	European silver fir (ESF)	Mišić's spruce (MS)	Common spruce (CS)
Needle width (μm)	*Average*	***2052.44*** *± 14.88*	***1939.03*** *± 18.44*	***1507.42*** *± 17.79*	***1800.22*** *± 28.56*
	Terminal	2042.97 ± 20.20	1949.23 ± 23.30	1507.42 ± 17.79	1808.00 ± 37.69
	Lateral	2064.27 ± 19.76	1927.69 ± 28.39		1790.50 ± 44.12
Needle thickness (μm)	*Average*	***502.46*** *± 6.67*	***500.78*** *± 6.22*	***1313.59*** *± 24.58*	***638.05*** *± 27.54*
	Terminal	505.82 ± 9.09	512.63 ± 9.61	1313.59 ± 24.58	677.62 ± 43.11
	Lateral	498.27 ± 8.84	487.60 ± 7.32		588.58 ± 29.61
Central cylinder diameter (μm)	Average	***346.22*** *± 5.64*	***328.49*** *± 4.78*	***394.62*** *± 5.80*	***310.44*** *± 4.77*
	Terminal	349.29 ± 7.58	332.89 ± 6.57	394.62 ± 5.80	312.14 ± 6.62
	Lateral	342.40 ± 7.62	323.60 ± 6.96		308.32 ± 6.92
Cuticle and epidermis thickness (μm)	*Average*	***13.19*** *± 0.28*	***12.92*** *± 0.27*	***12.41*** *± 0.50*	***13.86*** *± 0.29*
	Terminal	13.42 ± 0.42	12.95 ± 0.38	12.41 ± 0.50	13.72 ± 0.41
	Lateral	12.90 ± 0.33	12.89 ± 0.39		14.02 ± 0.39
Hypoderm thickness (μm)	*Average*	***18.98*** *± 0.35*	***17.97*** *± 0.31*	***16.82*** *± 0.53*	***18.09*** *± 0.30*
	Terminal	19.07 ± 0.52	18.05 ± 0.43	16.82 ± 0.53	18.16 ± 0.40
	Lateral	18.88 ± 0.43	17.89 ± 0.45		18.00 ± 0.46
Resin canal diameter (μm)	*Average*	***139.01*** *± 2.50*	***117.51*** *± 1.92*	***54.24*** *± 2.85*	***113.52*** *± 2.34*
	Terminal	140.45 ± 2.44	120.62 ± 2.09	54.24 ± 2.85	116.23 ± 2.74
	Lateral	137.22 ± 2.56	114.06 ± 2.57		110.31 ± 2.22

Table 5. 95.0% LSD test for nutrients in needles ESF and PF.

Nutrient	Fir	Count	Mean	Homogeneous Groups	Difference
K_2O	ESF	5	0.326	X	-0.062
	PF	5	0.388	X	
P_2O_5	ESF	5	0.086	X	*-0.016
	PF	5	0.102	X	
N	ESF	5	1.172	X	-0.072
	PF	5	1.244	X	

* denotes a statistically significant difference

Table 6. Minimal and maximal values of shoot characteristics (MS and CS).

Species		Mišić's spruce (MS)					Common spruce (CS)				
Properties	Age	1-year old	2- years old	3- years old	4- years old	Average 1-4	1-year old	2- years old	3- years old	4- years old	Average 1-4
Shoot length (cm)	*Average*	*6.0*	*6.5*	*6.5*	*7.0*	***6.0***	*3.0*	*3.0*	*3.2*	*4.5*	***3.0***
	range	*-11.5*	*- 8.5*	*- 9.5*	*- 9.5*	***- 11.5***	*-18.0*	*-11.5*	*- 7.5*	*- 5.7*	***- 18.0***
	Terminal	6.0 -11.5	6.5 - 8.5	6.5 - 9.5	7.0 - 9.5	**6.0 - 11.5**	4.5 -18.0	3.0 -11.5	4.0 - 7.0	4.5 - 5.7	**3.0 - 18.0**
	Lateral						3.0 -12.5	3.7 - 8.0	3.2 - 7.5		3.0 - 12.5
Shoot width (mm)	*Average*	5.6	*6.2*	*7.3*	*6.2*	***5.6***	*1.3*	*1.4*	*1.9*	*2.9*	***1.3***
	range	- 8.2	*- 8.6*	*- 8.9*	*- 8.6*	***- 8.9***	*- 7.2*	*- 4.8*	*- 5.9*	*- 3.1*	***- 7.2***
	Terminal	5.6 - 8.2	6.2 - 8.6	7.3 - 8.9	6.2 - 8.6	**5.6 - 8.9**	1.4 - 7.2	1.4 - 4.8	2.0 - 5.9	2.9 - 3.1	1.4 - 7.2
	Lateral						1.3 - 5.4	1.8 - 3.6	1.9 - 4.6		**1.3 - 5.4**
Number of needles	*Average*	*220*	*171*	*117*	*99*	***99***	*41*	*16*	*22*	*28*	***16***
	range	*- 402*	*- 234*	*- 243*	*- 279*	***- 402***	*- 383*	*- 211*	*- 115*	*- 38*	***- 383***
	Terminal	220 - 402	171 - 234	117 - 243	99 - 279	**99 - 402**	42 - 383	16 - 211	22 - 115	28 - 38	**16 - 383**
	Lateral						41 - 291	45 - 170	33 - 109		33 - 291
Needle length (mm)	*Average*	*12*	*9*	*10*	*13*	***9***	*9*	*11*	*13*	*12*	***9***
	range	*- 26*	*- 24*	*- 23*	*- 25*	***- 26***	*- 23*	*- 23*	*- 24*	*- 20*	***- 24***
	Terminal	12 - 26	9 - 24	10 - 23	13 - 25	**9 - 26**	9 - 23	11 - 23	13 - 24	12 - 20	**9 - 24**
	Lateral						9 - 22	12 - 20	13 - 21	15 - 18	**9 - 22**

nitrogen individually varies from 0.976% to 1.355% (PF) and 1.107% to 1.263% (ESF), respectively. The average content of nitrogen, phosphorus and potassium is higher in PF than in ESF (Table 5), but the differences between PF and ESF are statistically significant only with respect to phosphorus. It should be emphasized that both examined fir species are located in the same stand, where they are spatially mixed. The habitat conditions, both climatic and edaphic, under which both species grow and develop, are identical. Therefore, nutrition conditions of the examined macro-elements are also the same for both species. This means that the established difference in the amount of phosphorus in the needles of the examined species is most probably genetically conditioned.

Morpho-anatomic properties of Mišić's spruce (MS) and Common spruce (CS)

The specificity of Mišić's spruce is the absence of lateral branching of shoots during the course of several years, while the number of lateral shoots in CS varies from 1 to 3 (2.6 in average, results not presented).

The length of MS shoots (of average age 1-4 years) varies less (5.5 cm) than the length of CS shoots (16 cm) (Table 6), while the shoot length is higher (8.09 cm and 7.07 cm, respectively, Table 7). The differences between the length of terminal and lateral shoots were statistically significant in CS, while the differences between the terminal shoot lengths of MS and CS were low and insignificant.

The range of limit values for shoot thickness is higher in CS than in MS (Tab. 6), but MS shoots are thicker (7.3 mm and 2.8 mm, respectively, Table 7).

The variation in the number of needles is slightly lower in MS in comparison to CS (Table 6), but the average number of needles in an MS shoot is nearly two times higher (220 and 115, respectively, Table 7).

In addition, MS has a larger number of needles per 1 cm of shoot length in comparison to CS (27.2 and 16.2, respectively, Table 7), but lower in comparison to previous results (Matović and Pavlović, 1994).

MS has statistically significantly longer needles than CS (18mm and 16.9 mm, respectively, Table 7). CS has significantly shorter needles in the lateral shoots (16.0 mm) than in terminal shoots (17.4 mm). The needle length range in both MS and CS (17 mm and 15 mm, respectively, Table 6) is significantly higher in comparison to literature values (Matović and Pavlović, 1994).

MS exhibited a significantly lower mean value of needle width than CS (1507 μm and 1800 μm, respectively, Table 4) and a lower range of variation (Table 3, Figs. 1c, 1d), even in comparison to literature references (Matović and Pavlović, 1994).

In addition, MS has significantly thicker needles in comparison to CS (1313 μm and 638 μm, respectively, Table 4) and a lower range of variation (Table 3). MS also has a statistically significantly larger diameter of central cylinder in comparison to CS (394 μm and 310 μm, respectively) and a lower variation. MS has a lower thickness of cuticle with epidermis and a lower thickness of hypodermis in comparison to CS, as well as a lower variation of these properties, but the differences are not statistically significant.

In a needle cross-section, MS and CS have 0-2 resin canals. Their range of variability (Table 3) and mean diameter are lower in MS than in CS (54 μm and 113 μm, respectively, Table 4).

Content of macro-elements in needles of Mišić's spruce (MS) and Common spruce (CS)

By means of foliar analysis, it was established that the content of potassium in MS is 0.529%, while in CS it varies individually from 0.444% to 0.732%. The content of phosphorus in MS is 0.145%, while in CS it varies from 0.099% to 0.180%. The content of nitrogen in MS is 1.194%, while in CS it is from 1.009% to 1.240%. Although MS has higher mean values of these nutrition elements, the differences between MS and CS are not statistically significant (Table 8).

Table 7. Mean values and errors of shoot characteristics (MS and CS).

Species		Mišić's spruce (MS)					Common spruce (CS)				
Properties	Age	1-year old	2-years old	3-years old	4-years old	Aver-age 1-4	1-year old	2- years old	3- years old	4- years old	Aver-age 1-4
Shoot length (cm)	*Average*	*8.38 ±1.22*	*7.50 ±0.46*	*8.00 ±0.74*	*8.50 ±0.54*	***8.09*** *±0.37*	*8.14 ±0.34*	*6.51 ±0.25*	*5.11 ±0.25*	*5.10 ±0.60*	***7.07*** *±0.21*
	Terminal	8.38 ±1.22	7.50 ±0.46	8.00 ±0.74	8.50 ±0.54	**8.09** ±0.37	9.76 ±0.73	7.59 ±0.50	5.59 ±0.36	5.10 *±0.60*	**7.97** ±0.42
	Lateral						7.50 ±0.33	5.90 ±0.21	4.75 ±0.31		**6.61** ±0.22
Shoot width (mm)	*Average*	*6.48 ±0.59*	*7.35 ±0.53*	*8.03 ±0.38*	*7.28 ±0.63*	***7.28*** *±0.28*	*2.56 ±0.13*	*2.81 ±0.12*	*3.33 ±0.22*	*3.00 ±0.10*	***2.77*** *±0.09*
	Terminal	6.48 ±0.59	7.35 ±0.53	8.03 ±0.38	7.28 ±0.63	**7.28** ±0.28	3.15 ±0.29	3.42 ±0.24	3.84 ±0.36	3.00 ±0.10	**3.38** ±0.16
	Lateral						2.34 ±0.12	2.46 ±0.08	2.94 ±0.25		**2.46** ±0.08
Number of needles	*Average*	289.5 ±39.71	203.8 ±12.99	*185.8 ±25.96*	*202.8 ±37.79*	***220.4*** *±69.48*	*140.5 ±7.67*	*102.7 ±5.87*	*67.3 ±5.21*	*33.0 ±5.00*	***115.3*** *±5.02*
	Terminal	289.5 ±39.71	203.8 ±12.99	185.8 ±25.96	202.8 ±37.79	**220.4** ±69.48	163.4 ±17.50	118.6 ±12.55	71.1 ±10.98	33.0 ±5.00	**125.0** ±10.45
	Lateral						131.6 ±7.95	93.9 ±5.45	64.7 ±4.83		**110.5** ±5.37
Needle length (mm)	*Average*	*18.7 ±2.86*	*17.5 ±0.39*	*18.8 ±0.27*	*17.1 ±0.35*	***18.0*** *±0.18*	*16.4 ±0.29*	*17.2 ±0.24*	*17.0 ±0.32*	*16.9 ±0.44*	***16.9*** *±0.15*
	Terminal	18.7 ±2.86	17.5 ±0.39	18.8 ±0.27	17.1 ±0.35	**18.0** ±0.18	17.0 ±0.41	18.0 ±0.32	17.4 ±0.39	16.9 ±0.48	**17.4** ±0.20
	Lateral						15.7 ±0.38	16.1 ±0.32	16.2 ±0.48	16.3 ±0.88	**16.0** ±0.22
Number of needles per 1 cm of shoot length	*Average*	*34.6*	*27.2*	*23.2*	*23.8*	***27.2***	*17.3*	*15.8*	*13.2*	*6.5*	***16.2***
	Terminal	34.6	27.2	23.2	23.8	**27.2**	16.7	15.6	12.7	6.5	**15.6**
	Lateral						17.5	15.9	13.6		16.7

Table 8. 95.0% LSD test for nutrient in needles CS and MS.

Nutrient	Spruce	Count	Mean	Homogeneous Groups	Difference
K_2O	CS	5	0.616	X	-0.086
	MS	1	0.530	X	
P_2O_5	CS	5	0.134	X	0.006
	MS	1	0.145	X	
N	CS	5	1.106	X	0.084
	MS	1	1.194	X	

CONCLUSIONS

In comparison to ESF, PF has a nearly two-times higher number of lateral shoots, but shorter and thinner shoots and a lower number of needles, which are slightly less densely distributed on the shoot. Additionally, PF terminal shoots are significantly longer and thicker than the lateral, and have a higher number of needles. PF and ESF needles have a similar length, but two-year-old PF needles are longer, wider and thicker than ESF needles. As a result, PF needles have significantly higher values with respect to the diameter of the central cylinder, thickness of hypodermis and diameter of the resin canals. The most pronounced differences between PF and ESF are related to the length of shoots, width of needles and diameter of central cylinder. In comparison to literature results, PF and EF in our studies have a lower shoot length, lower density of needles on a shoot, higher length and lower width and thickness of needles. In addition, they have a higher thickness of cuticle and epidermis, higher thickness of hypodermis and a larger diameter of resin canals in comparison to firs from Poland, but lower values of the same properties than firs from Macedonia. PF needles contain a higher percentage of nitrogen, phosphorus and potassium than ESF needles, but the differences are statistically significant only with respect to the content of phosphorus.

The most apparent MS morphological specificity is a rare occurrence of lateral shoots (only once in several years), unlike CS, which has 2-3 new lateral shoots every year. MS has significantly longer and thicker shoots than CS. In addition, MS needles are more numerous, longer, thinner and more densely distributed on the shoot than is the case with CS needles. MS needles also have a larger diameter of central cylinder, but lower thickness of cuticle with epidermis, lower thickness of hypodermis and lower diameter of resin canals than CS needles. The most pronounced differences between MS and CS are related to the length of needles, width and thickness of needles and diameter of central cylinder. In comparison to literature results, MS needles are less densely distributed on the shoot and have a higher range of needle length, but not of width. MS needles contain a higher percentage of nitrogen, phosphorous and potassium than CS needles, but the differences are not statistically significant.

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