UDK 630*232.12:582.475 *Pseudotsuga menziesii* (Mirb.) Franco (497.11)=111
Original scientific paper

THE DIFFERENCES OF NEEDLE LENGTH OF DOUGLAS-FIR PROVENANCES AT TWO SITES IN TEST PLANTATIONS

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Abstract: *Douglas-fir* (*Pseudotsuga menziesii*/Mirb.Franco) is the most successful introduced and ecologically adapted species in Europe since 1827. Its natural range extends from British Columbia in the north, as far as New Mexico in the south. Douglas-fir has the most extensive latitudinal range of any North American commercial conifer, from 19° to 55° North latitude (New Mexico – British Columbia). Genetic and ecological diversity of Douglas-fir results from its wide natural range of species distribution. Such a wide distribution of a species requires the testing of its characteristics.

One of the models for testing the genetic diversity, variability and adaptability of introduced species is the provenance test.

This study was conducted to evaluate the effects of site environmental factors upon morphological characteristics of Douglas-fir needles.

Key words: Douglas-fir, provenances, needle length, sites, Serbia

RAZLIČITOST DUŽINE ČETINA PROVENIJECIJA DUGLAZIJE NA DVA STANIŠTA U OGLEDIMA

Izvod: *Duglazija* (*Pinus menziesii* / Mirb.Franco) je najuspešnija introdukovana vrsta i ekološki prilagođena vrsta četinaru u Evropi od 1827. Prirodni areal proteže se od Britanske Kolumbije na severu, pa do Novog Meksika na jugu. Duglazija ima najširi raspon geografske širine od bilo kog severnoameričkog komercijalnog četinaru, od 19 ° do 55 ° severne geografske širine (Novi Meksiko – Britanska Kolumbija). Genetička i ekološka raznovrsnost Duglazije je rezultat njenog širokog spektra distribucije vrsta. Takva široka distribucija vrsta zahteva testiranje karakteristika. Jedan od modela za ispitivanje genetske

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različitosti, varijabilnost i prilagodljivost introdukovanih vrsta je provenijencijski test. Ova istraživanja su sprovedena da se procene efekti staništa ekoloških faktora na morfološke karakteristike četina duglazije u test kulturama na dva lokaliteta u Srbiji.

Ključne reči: Duglazija, provenijencija, dužina četina, staništa, Srbija

1. INTRODUCTION

Climate conditions and environment may have an impact on the productivity of conifer trees by influencing the morphology (size and surface characteristics) and function (capacity for gas exchange) of conifer needles. Needle characteristics, including length, width, stomatal density, needle function are important for water vapour and transpiration. Needle length increases significantly with increased temperature (Martha E. Apple *et al.*, 2000).

More specifically, the objective was to determine whether site environment conditions affect the morphology and function of Douglas-fir needles of different provenances at two sites in Serbia.

In order to be able to forecast and estimate the state and dynamics of forest ecosystems, it is necessary to understand the mechanisms and character of response reactions of trees to the different complexes of environmental factors. Balanced metabolism, in which an optimum supply of nutrients is of prime importance, is indispensable to preserve functional and structural integrity of a tree (Malle Mandre and Aljona Lukjanova, 2011).

Institute of Forestry Belgrade established two experimental plots, in Central (Juhor) and east Serbia (Tanda) of Douglas-fir provenances, with original seeds from North America.

Douglas- fir, as exotic species need to be provenance tested before introduction.

Genetics and ecological adaptability can be confirmed, by morphological and anatomical variable characteristics which is the aim of this research. For that reason, two experimental plots with twenty Douglas fir provenances were established in Serbia .

A considerable number of quantitative and qualitative properties were analyzed in Douglas-fir test plantations in Serbia (Lavadinović. V., Isajev,V., Koprivica, M. 1998, Lavadinović, V., Isajev,V., Koprivica, M.2001, Lavadinović, V., Isajev,V., (2003)., Lavadinović,V. (2008)

2. MATERIAL AND METHOD

Two experimental fields of Douglas fir provenances in Serbia were the basis for the sampling and analysis.

The samples were taken from the selected provenances for the analysis of the existence and effects of the impact of site conditions on the variability of morphometric characters of Douglas-fir needles at both locations. The selection criteria in choosing the provenances for sampling in this study were the results of the studies of variability of inventory variables. Two provenances with the

smallest, two with the average and two with the largest mean values of inventory variables were taken for this study.

Fresh needles were fixed in 50% ethyl alcohol and transported to the laboratory, where they made a permanent anatomical sections with 30 needles, randomly chosen. Permanent anatomic preparations thickness 17 μm were made in the middle of the needle microtome, stained with safranin red and toluidine blue and rinsed with water, followed by dehydration was carried out by increasing the ethyl alcohol concentration of alcohol of 50% to 96%. Fully fixing section was performed in xylene for several hours, after which the needles were glued on glass slides Canada balm, covered with the covering glass and dried in an oven at 60 ° C. After three weeks, the morph metric traits were measured: width, length, thickness of the needle.

Geographical characteristics of the original seeds of Douglas-fir, which were collected from a part of the natural range of species is shown in Table 1.

Table 1. *Geographical co-ordinates of the tested Douglas-fir provenances*

Provenance Number	Our mark	Latitude (° N)	Longitude (°E)	Altitude (m)
Oregon 205–15	1	43,7	123,0	750
Oregon 205–14	2	43,8	122,5	1200
Oregon 202–27	3	45,0	122,4	450
Oregon 205–38	4	45,0	121,0	600
Washington 204–07	9	49,0	119,0	1200
Oregon 205–13	10	43,8	122,5	1050
Oregon 205–18	11	44,2	122,2	600
Oregon 202–22	12	42,5	122,5	1200
Washington 202–17	15	47,6	121,7	600
Oregon 201–10	16	44,5	119,0	1350
Washington 201–06	17	49,0	120,0	750
Oregon 202–19	18	45,3	123,8	300
Washington 204–09	19	49,0	119,3	900
Oregon 205–11	20	45,0	123,0	150
New Mexico 202–04	22	32,9	105,7	2682
New Mexico 202–10	23	36,0	106,0	2667
Oregon 202–31	24	44,3	118,8	1500
Oregon 205–29	26	42,6	122,8	900
Oregon 204–04	30	45,0	121,5	900
Washington 205–17	31	47,7	123,0	300

3. RESULTS

According to Table 2, it can be concluded that:

- There are statistically significant differences between the mean values of needle length at the localities Juhor and Tanda;
- There are statistically significant differences between the mean values of needle length of individual provenances;
- There is an interaction between the factors “locality” and “provenance”. Mean value of needle length in individual provenances is affected by the changes in locality, and the opposite.

Table 2. *A two-way analysis of variance (locality x provenances) for needle length*

Source of variation	Sum of squares	Degree of freedom	Variance	F	p-value
A: Locality	306.178	1	306.178	87.93	0.000
B: Provenance	6221.76	5	1244.35	357.37	0.000
Interaction AB	538.989	5	107.798	30.96	0.000
Error	1211.73	348	3.48199		
Total	8278.66	359			

Difference of needle lengths on sites

The least significant difference (LSD) test examined the difference in mean values of needle lengths for two factors (Locality and Provenance)

Table 3. *LSD test for localities*

Locality	Sample size	Mean value	Difference	Homogeneous groups
Juhor	180	31.7389	0.139084	X
Tanda	180	33.5833	0.139084	X
			Difference	+/- Limits
Juhor-Tanda			*-1.84444	0.386861

- indicates a statistically significant difference
-

Table 3 shows that there is a statistically significant difference in the lengths of Douglas fir needles for the sites Juhor and Tanda. The average value of needle length at Tanda (33.58 mm) was significantly higher than at Juhor (31.74 mm), and the test shows that there is an influence of environmental factors on these two sites on needle lengths (Figures 1 and 2).

Difference of needle lengths in provenances

LSD test checked the difference in needle lengths mean values in different provenances (geographical origin)

Table 4. *LSD test for provenances*

Provenance	Sample size	Mean value	Difference	Homogeneous groups
31	60	27.9333	0.240901	X
18	60	28.3667	0.240901	X
9	60	29.85	0.240901	X
24	60	34.25	0.240901	X
16	60	37.7667	0.240901	X
17	60	37.8	0.240901	X
Comparison		Difference		+/-Limit
9-16		*-7.91667		0.670062
9-17		*-7.95		0.670062
9-18		* 1.48333		0.670062
9-24		*-4.4		0.670062
9-31		* 1.91667		0.670062
16-17		-0.0333333		0.670062
16-18		* 9.4		0.670062
16-24		* 3.51667		0.670062
16-31		* 9.83333		0.670062
17-18		* 9.43333		0.670062
17-24		* 3.55		0.670062
17-31		* 9.86667		0.670062
18-24		*-5.88333		0.670062
18-31		0.433333		0.670062
24-31		* 6.31667		0.670062

* indicates the statistically significant difference

According to the results of analysis of variance (Table 4), it can be concluded that there are statistically significant differences between the mean values of needle length in some provenances. LSD test demonstrates that provenances 31 and 18 make one homogenous group, and provenances 16 and 17 the other. Smaller range of variation of needle length of these provenances in relation to the other can be tentatively considered as a consequence of their genetic closeness which caused similar phenotypic expression of the properties of the interaction with the external factors of the location where the tests were established.

The differences are shown on Diagram 1 for locality Juhor, and on Diagram 2 for locality Tanda.

Graph 1. *Variability in needle length among the provenances on the locality of Juhor*

Graph 2. *Variability in needle length among the provenances on the locality of Tanda*

4. CONCLUSION

The investigation of the influence of two factors (site and provenance), the two-way ANOVA was carried out on the Douglas-fir provenance trail at two sites in Serbia.

The major conclusion is that care must be exercised in describing the trait variability, so the particular environmental gradient must be specified.

The LSD test shows that the provenances 31 (Washington 205–17) and 18 (Oregon 202–19) make one homogenous group, and provenances 16 (Oregon 201–10) and 17 (Washington 201–06) the other, so the differences in their mean values are not statistically significant. The lower range of character variation in these provenances, compared to others, can be conditionally considered as a consequence of their genetic similarity which conditioned the similar phenotype expressions in the interaction with the external factors of the sites where the tests were established.

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