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VARIABILITY OF EPITHELIAL CELLS IN THE RESIN DUCT OF DOUGLAS-FIR NEEDLES

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Abstract: *The research on the interactions between the genetic potential of introduced provenances and the environmental features of the locations in which the plantations were established was carried out in Douglas-fir plantations in Serbia. The two-way ANOVA was conducted in order to study the effects of the site conditions in the localities of Douglas-fir provenance tests in Serbia on the anatomical properties of needles. These analyses look into the effects of two factors (locality and provenance) on the number of epithelial cells in the resin duct of Douglas-fir needles.*

Keywords: Douglas-fir, provenance, introduction, resin ducts, epithelial cells

VARIJABILNOST BROJA EPITELNIH ĆELIJA U SMOLNOM KANALU ČETINA DUGLAZIJE

Abstract: *U kulturama duglazije u Srbiji obavljaju su istraživanja interakcija genetskog potencijala introdukovanih provenijencija sa ekološkim odlikama lokacija gde su kulture podignute. U cilju bližeg upoznavanja efekta interakcije stanišnih uslova lokaliteta, gde su osnovani provenijenični testove duglazije u Srbiji, na anatomska svojstva četina, obavljena je dvofaktorijalna analiza varijanse. U ovim analizama ispitivan je uticaj dva faktora (lokalitet i provenijencija) na broj epitelnih ćelija u smolnom kanalu četina duglazije.*

Ključne reči: Duglazija, provenijencija, introdukcija,smolni kanali, epitelne ćelije

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1.INTRODUCTION

Douglas-fir (*Pseudotsuga menziesii*/Mirb. /Franco) is a very common tree species and accounts for 67% of 2 238 samples taken from all site types (Pfister *et al.*, 1977). It has the widest ecological amplitude of all western conifers and a notable diversity of genetic ecotypes (Moserud and Rehfeldt, 1990). The transfer of exotic tree species (Larsen and Syrack, 1946; Schober, 1959; Spurr, 1961; Schober, 1963; Kriek, 1974; Namkoog, 1979) entails the risk incurred by the lack of knowledge about the productivity and adaptability of introduced species to the environmental conditions of the sites which are outside its range of distribution.

Forest tree production is determined by the expression of physiological processes within specific environmental-genetic regimes. Temperature and soil are major environmental factors that affect the physiological processes of a plant and combined with the genetic variation within a seedling determine the type of organism that will be produced (Jensen and Gatherum, 1965; Dykstra and Gatherum, 1967; Schultz, 1970).

Douglas-fir can be described as a productive coniferous tree species with highly-valued wood, wide ecological amplitude and high-quality essential oils.
In chemotaxonomic terms, Douglas-fir belongs to a large group of aromatic medicinal plants that synthesize numerous and diverse biochemical metabolites. The mixtures of volatile products of Douglas-fir metabolism are the source of its distinctive scent and taste. They are contained in essential oils which can be found in all parts of the plant. Essential oils derived from Douglas-fir needles make significant raw materials both for the chemical industry and for its related industries, mainly because a great number of synthetic preparations that have the same effects as these oils have been found to have adverse side effects. Nowadays, essential oils have found the widest application in the pharmaceutical and cosmetic industry, where they are used as antiseptics, insecticides, deodorants and for masking the odor of synthetic products (Lavadinović, 2008).

The significance of the morphological and anatomical structure of needles, as well as the function and structure of resin ducts, have been analyzed by numerous authors (Matović and Lavadinović, 1999; Gerling *et al*., 2015;). Due to the high quality of its essential oils and their wide application in the cosmetic and pharmaceutical industry, Douglas-fir has also been the subject of numerous studies in Serbia (Tešević *et al*., 2002; Tešević and Lavadinović, 2009).

In order to investigate the genetic potential of Douglas-fir in its new ecosystems of Serbia, the Institute of Forestry in Belgrade has established several experimental plots of Douglas-fir of different provenances originating in North America.

The primary goal of the experiments (on the mountain of Juhor near Jagodina and in the village of Tanda near Bor) was to determine the effects of the geographical parameters of the original localities of Douglas-fir provenances – their geographical latitude, geographical longitude and altitude on the growth of trees with the aim of selecting the most adaptable provenances to be used in the cultivation on similar sites.

2. MATERIAL AND METHODS

The study area covered Douglas-fir provenance tests established in Central Serbia on the mountain of Juhor and in the village of Tanda located at the foot of the mountain massif of Deli Jovan in Eastern Serbia. Douglas-fir seedlings were raised in the nursery of the Institute of Forestry in Belgrade from the seeds native to North America. The seeds originate from a part of the natural range of Douglas-fir distribution with 20 provenances that differ in the latitude, longitude and altitude (Table 1, Lavadinović,V., Koprivica, M. 1996)).

The experiment on the mountain of Juhor was established on a beech site (*Fagetum moesiaca montanum* Jov. 1976) on acid brown soil (dystric cambisol) over gneiss. `Tanda` sample plot is located in FMU `Stol` in `Bor` Forest Administration on the site of oak, Hungarian oak and Turkey oak (*Querceto conferte cerris* Rud.) on brown acid soil and sierozem (Lavadinović, 2008).

Table 1. *Geographical coordinates of the tested Douglas-fir provenances (Lavadinović,V., Koprivica, M. 1996)*

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
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 205-08	27	42.7	122.5	1050
Oregon 204-04	30	45.0	121.5	900
Washington 205-17	31	47.7	123.0	300

A two-way analysis of variance of the number of epithelial cells in the resin ducts was carried out in order to identify the provenances whose genetic potential is most suited to the environmental conditions of the forest communities on whose sites the experiments were established.

Fresh needles were fixed in 50% ethyl alcohol and transported to the laboratory, where permanent anatomical cross-sections of 30 randomly selected needles were made. Permanent anatomical preparations of 17 μm thickness were cut in the middle of the needle using a microtome. They were then dyed by safranin red and toluidine blue and washed with water. This was followed by dehydration with ethyl alcohol, increasing the alcohol concentration from 50% to 96%. The

cross-sections were eventually fixed with xylol for several hours, after which the needles were glued to the slides using Canada balsam, covered with cover glass and dried in a dryer at a temperature of 60ºC. Three weeks later, the number of epithelial cells in the resin duct was counted (Paraffin processing method).

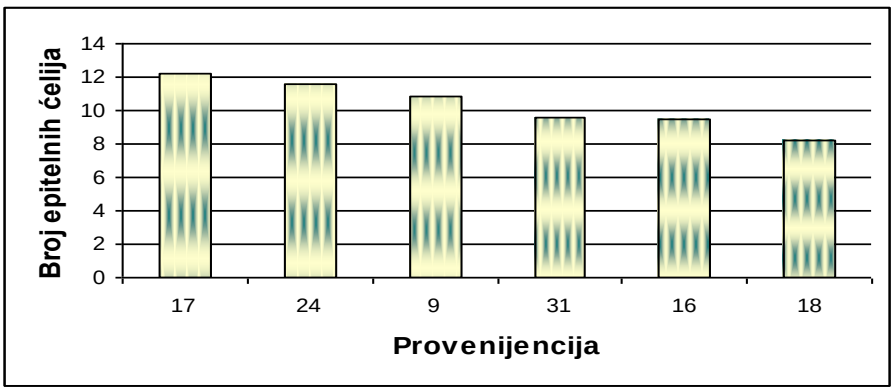
3. RESULTS AND DISCUSSION

Table 2 and Graphs 1 and 2 show the results of the two-way analysis of variance (locality x provenance) for the number of epithelial cells in the resin ducts of Douglas-fir needles at both localities.

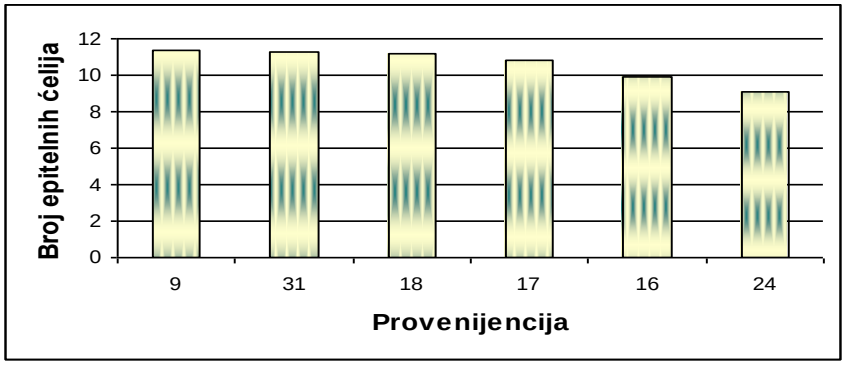
Table 2. *Two-way (locality x provenance) ANOVA for the number of epithelial cells in the resin ducts*

Source of variation	Sum of squares	Degree of freedom	Variance	F-ratio	p-value
A: Locality	8.14	1	8.1	10.57	0.013
B: Provenance	156.856	5	31.3711	40.94	0.000
Interaction AB	311.833	5	62.3667	81.39	0.000
Errors	266.667	348	0.766284		
Total	**743.456**		**359**		

The results of the analysis of variance (Table 2) show that:
a) there are statistically significant differences in the mean values of the number of epithelial cells between the site of Juhor and the site of Tanda;
b) there are statistically significant differences in the mean values of the number of epithelial cells between provenances;
c) in certain provenances, the interaction between the `locality` factor and `the provenace` factor affects the mean value of the number of epithelial cells.



Graph 1. *Interprovenance variation of the number of epithelial cells in the resin duct at Juhor*



Graph 2. *Interprovenance variation of the number of epithelial cells in the resin duct at Tanda*

The effects of locality on the number of epithelial cells

Table 3. *MSD test of the effects of locality on the number of epithelial cells*

Locality	Sample size	Mean value	Difference error mean	Homogeneous groups
Juhor	180	10.3111	0.0652467	X
Tanda	180	10.6111	0.0652467	X
Comparison			Differences	+/– Limits
Juhor–Tanda			*–0.3	0.181483

* statistically significant difference

Table 1. *MSD test of the effects of the locality on the number of epithelial cells*

Locality	Sample size	Mean value	Difference error mean	Homogeneous groups
16	60	9.7	0.113011	X
18	60	9.7	0.113011	X
24	60	10.3667	0.113011	X
31	60	10.4333	0.113011	X
9	60	11.0667	0.113011	X
17	60	11.5	0.113011	X
Comparison			**Differences**	**+/– Limits**
9–16			* 1.36667	0.314337
9–17			*–0.433333	0.314337
9–18			* 1.36667	0.314337
9–24			* 0.7	0.314337
9–31			* 0.633333	0.314337
16–17			*–1.8	0.314337
16–18			0.0	0.314337
16–24			*–0.666667	0.314337
16–31			*–0.733333	0.314337
17–18			* 1.8	0.314337
17–24			* 1.13333	0.314337
17–31			* 1.06667	0.314337
18–24			*–0.666667	0.314337
18–31			*–0.733333	0.314337
24–31			–0.0666667	0.314337

* statistically significant difference

The results shown in Table 3 point to statistically significant differences in the mean number of epithelial cells of Douglas-fir needles obtained from Juhor and Tanda localities. The average number of epithelial cells in the Douglas-fir needles

from Tanda locality (10.61) is significantly higher than the number of these cells in the needles of Douglas-fir trees from Juhor locality (10.31). The range of variation of this property for the analyzed provenances is shown Graphs 1 and 2. Using the MSD test, we got a deeper insight into the effects of the characteristics of the locality where the provenance tests were conducted on the number of epithelial cells of Douglas-fir needles.

The results presented in Table 4 show that there are statistically significant differences in the number of epithelial cells between provenances. However, the results of the MSD test indicate that provenances 16 and 18, 24 and 31 are homogeneous since there are no statistically significant differences in the mean values of the number of epithelial cells.

4. CONCLUSIONS

On the basis of the examinations carried out through Douglas-fir provenance tests on the (*Fagetum moesiaca montanum* Jov. 1976) site on acid brown soil (dystric cambisol) over gneiss and on the site of oak, Hungarian oak and Turkey oak *(Querceto conferte cerris* Rud.) on brown acid soil and sierozem, we can draw the following conclusions:

- there are statistically significant differences in the mean values of the number of epithelial cells between the localities of Juhor and Tanda;
- there are statistically significant differences in the mean values of the number of epithelial cells between provenances;
- in certain provenances, the interaction between the `locality` factor and `the provenace` factor affects the mean value of the number of epithelial cells.
- the average number of epithelial cells in the needles of Tanda locality is significantly higher than the number of these cells in the needles of Douglas-fir trees of Juhor locality.
- there are statistically significant differences in the number of epithelial cells between provenances. However, the results of the MSD test indicate that the provenances 16 and 18, 24 and 31 are homogeneous because they don`t show statistically significant differences in the mean values of the number of epithelial cells and there is an interaction between the variability factors (locality and provenance), i.e., a change in one variability factor affects the change in the treatment of another factor.

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Summary

Coniferous tree species are rich in essential oils, which have a protective role for the plant itself and a wide use in the pharmaceutical, cosmetic and food industry.

The resin is an organic liquid containing terpenes, resin acids and other compounds found in the resin ducts of all plant parts. Resin ducts are surrounded by epithelial cells. Aromatic effects of essential Douglas-fir oils make this type of conifer very popular in urban greening and green area establishment. The study deals with the effects of two factors (locality and provenance) on the number of epithelial cells in the resin duct.

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Rezime

Četinaske vrste drveća su bogate etarskim uljima, koje imaju zaštitnu ulogu za samu biljku i široku upotrebnu u farmaceutskoj, kozmetičkoj i prehrambenoj industriji.

Smola je organska tečnost koja sadrži terpene, smolne kiseline i druga jedinjenja koje se nalaze u svim delovima biljke u smolnim kanalima. Smolni kanali su obloženi epitelnim ćelijama. Etarska ulja iz četina duglazije zbog aromatičnog efekta čine ovu vrstu četinara vrlo popularnom za urbano ozelenjavanje i formiranje zelenih masiva. U ovim analizama ispitivan je uticaj dva faktora (lokalitet i provenijencija) na broj epitelnih ćelija u smolnom kanalu.