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VARIABILITY OF RESIN CANAL DIAMETER IN THE NEEDLES OF DIFFERENT DOUGLAS-FIR PROVENANCES

Vera LAVADINOVIĆ[1], Dragica OBRATOV-PETKOVIĆ[2], Ljubinko RAKONJAC[1], Zoran MILETIĆ[1], Filip JOVANOVIĆ[1], Milan KABILJO[1]

Abstract: *Douglas-fir is the most commonly grown allochthonous species in the forests of Europe. Growth speed, adaptive power, favorable wood properties, as well as low sensibility to pests are the attributes that have contributed the most to the success and occurrence of Douglas-fir plantations in Europe. Introduction program of an exotic species includes testing of its provenances in new ecosystems and localities. In Serbia, testing of the genetic potentials of Douglas-fir, native to North America, has begun by seting up provenance trials on several locations. Afforestation with exotic tree species must be justified and it should fulfill the aims of the introduction. Therefore, it is neccessary to test all the traits of the introduced species to show the effects of ecological factors. The aim of this research was to determine the ecological adaptations of Douglas-fir in Serbia by studying its anatomic characteristics. In the paper, the influence of locality conditions on the diameter of resin canals in the needles of different Douglas-fir provenances was investigated on the sites where the provenance tests were set up. A two-way ANOVA was performed, by investigating the effects of site and provenance factors on the diameter of resin canals. Resin canals serve as an important diagnostic taxonomic characteristic in conifers, while resin itself has important physiological and protective role in conifers.*

[1]Dr Vera Lavadinović, Dr Ljubinko Rakonjac, Dr Zoran Miletić, Dr Filip Jovanović, MSc Milan Kabiljo, Institute of Forestry, 3 Kneza Višeslava, 11030 Belgrade, Serbia.
[2]Dr Dragica Obratov-Petković, Faculty of Forestry, University of Belgrade, 1 Kneza Višeslava, 11030 Belgrade, Serbia.
Author for correspondence: Dr Vera Lavadinović, Institute of Forestry, 3 Kneza Višeslava, 11030 Belgrade, Serbia, +381628838010, e-mail: veralava@outlook.com
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Key words: Douglas-fir, provenance, introduction, resin canals, Serbia

VARIJABILNOST PREČNIKA SMOLNIH KANALA U ČETINAMA RAZLIČITIH PROVENIJENCIJA DUGLAZIJE

Izvod: *U šumama Evrope najzastupljenija introdukovana vrsta četinara je duglazija. Veliki kapacitet produktivnosti i adaptivnosti, kao i kvalitet drveta i mali broj štetočina, prednosti su koje su doprinele da se ova vrsta uspešno primenjuje u šumskim kulturama Evrope. Program introdukcije egzotične vrste podrazumeva testiranje njenih različitih provenijencija u novim ekosistemima i lokalitetima. U Srbiji, program testiranja genetskog potencijala duglazije, poreklom iz Severne Amerike, započet je osnivanjem provenijeničnih testova na više lokacija. Koncept pošumljavanja sa egzotičnim vrstama drveća mora biti opravdan i da ispuni cilj introdukcije. Iz tog razloga, potrebno je testirati sve karakteristike introdukovane vrste, koje će prikazati uticaj ekoloških faktora. Cilj ovog istraživanja je da se utvrde ekološke adaptacije duglazije u Srbiji proučavanjem njenih anatomskih karakteristika. U radu su istraživani efekti stanišnih uslova lokaliteta, gde su osnovani provenijenični testovi duglazije u Srbiji, na prečnik smolnih kanala u četinama duglazije različitih provenijencija. Obavljena je dvofaktorijalna analiza varijanse, ispitivani su faktori lokaliteta i provenijencija, kao i njihov uticaj na prečnik smolnih kanala. Smolni kanali su važna taksonomska karakteristika četinara, dok sadržaj smole ima važnu fiziološku i zaštitnu ulogu u njima.*

Ključne reči: duglazija, provenijencije, introdukcija, smolni kanali, Srbija

1. INTRODUCTION

Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco, fam. Pinaceae] occurs in all ecosystems of Europe, providing with fast and high-quality wood production, landscape diversity, as well as lucrative materials for medicinal and industrial use. Its natural range of distribution is in the northern part of Canada and the Pacific coast, up to 3,000 m a. s. l. on Rocky Mts. Its wide geographical range in terms of latitude, longitude (from New Mexico to Vancouver) and altitude has produced a number of provenances.

In the publication "*Douglas-fir – an option for Europe*" (Spiecker *et al.*, 2019), the authors emphasise the fact that this species has highly adaptive genetic variability, and it is very suitable for introduction. The first seeds were introduced in Europe by David Douglas in 1827 and then planted at Dropmore Park (Buckinghamshire, UK), where there is a tree which is usually considered the oldest Douglas-fir of Europe (Da Ronch *et al.*, 2016; Elwes and Henry, 1969).

Douglas-fir is an up-and-coming, highly productive introduced species, not only in Europe but also in the world; hence, it was a subject of numerous studies and a topic of many scientific conferences (Chen *et al.*, 1986; Hermann and Lavender, 1987, 1990).

In Serbia, the study and the testing of Douglas-fir provenances from Canada and the United States of America has begun in 1978 at the Institute of Forestry, Belgrade, by setting up provenance trials on different sites (Lavadinović

and Koprivica, 1996, 1996a, 1999, 2000; Lavadinović and Isajev, 2005; Lavadinović, 2005; Lavadinović *et al.*, 2010, 2011, 2015, 2017, 2018).

Resin canals are a common, distinguishing feature in conifers (Fahn, 1979; Page, 1989). Resin canals are relatively large intercellular spaces surrounded by an epithelium of secretory parenchyma cells (Fahn, 1979) that occur in many organs of coniferophytes. In Pinaceae, they are found in xylem, bark, needles and seeds (Lin *et al.*, 1995; Farjon, 1998; Wu and Hu, 1997). In addition to pines, a number of other conifer species, including Douglas-fir, exhibit resin canals in their needles. Apart from the needles, the resin ducts are distributed in the cortex, primary xylem and secondary xylem of stems. In the needles, two lateral, abaxial ducts occur in the mesophyll (Wu and Hu, 1997). The two adaxial resin ducts of *Pseudotsuga menziesii* needles are located in direct contact with epidermis. They present a central midvein with variable diameter, surrounded by a thin endodermis. Each canal is sometimes partially surrounded by sclerenchyma fibers with lignified walls (Pădure *et al.*, 2008).

Resin canals exude a complex of secondary metabolites (resin or pitch) as a result of injury from wind, fire or attack by wood-boring insects. There are many commercial purposes for resin, including lacquers, varnishes and turpentine (Kramer and Kozlowski, 1979). The resin itself is an organic liquid containing terpenes, resin acids and other compounds. Needle resin serves as defense against insects and other animals, which confront the distasteful substance when boring into the conifer's leaves. Resin is antiseptic and aromatic and prevents the development of fungi and deters insects. The resins may disrupt the feeding, digestion or metabolic function of the attacker (https://www.hunker.com).

Resin canals also serve as an important diagnostic taxonomic characteristic in conifers (Lin *et al.*, 2000; Frankis, 1989). Specifically, the cross-sectional number of resin canals in conifer needles can be used to distinguish genetic variation among families and subspecies (Helmers, 1943; Keng and Little, 1962) and is thus commonly used as an index in forest breeding programs (Richardson, 1998., Lin *et al.*, 2001).

The aim of this research was to determine the ecological adaptations of Douglas-fir in Serbia by studying its anatomic characteristics. The influence of locality conditions on the diameter of resin canals in the needles of different Douglas-fir provenances was investigated on the sites where the provenance tests were set up.

2. MATERIAL AND METHODS

Provenance trial plantations in Serbia were established as a starting material for the provenance tests. The experimental plots on Mt. Juhor (near the city of Jagodina) and Tanda (near Bor) were set in order to begin with the testing program of introduced species.

The plantations were established using seedlings produced in the nursery of the Institute of Forestry, Belgrade. The seedlings were raised from seed which has been collected in one part of the native range of Douglas-fir’s distribution in North America. The origin of the provenances is shown in Table 1.

The experiment on Mt. Juhor was set on the site of mountain beech forest (*Fagetum moesiacae montanum* Jov. 1976) on acid brown soil (dystric cambisol) over gneiss. The "Tanda" sample plot is located in FMU "Stol" (FAS "Bor") on the site of Hungarian and Turkey oak forest (*Quercetum frainetto-cerris* Rud. 1949) on acid brown soil and sierozem (Lavadinović, 2009).

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

Provenance code	Mark	Latitude (ºN)	Longitude (ºE)	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	300
Washington 205-17	31	47.7	123.0	300

Source: Lavadinović and Koprivica (1996)

For the purposes of the analysis of site influence on the variability of anatomical structure of Douglas-fir needles collected on both sites where the provenance tests were set, samples were taken only from selected provenances. The selection of provenances was based on previous research of the variability of mensurational parameters in Douglas-fir (Lavadinović, 2009). In each locality three groups of provenances were formed: 1 – the best, 2 – medium and 3 – the worst. The paper analyzes two provenances from each category – the best (18 and 31), medium (16 and 17) and the worst (9 and 24).

Fresh needles were fixed in 50% ethyl alcohol to make permanent anatomical cross-sections, which contained 30 randomly chosen needles. The median anatomical sections were cut to a thickness of 17 μm, using a microtome. After that, they were stained with Safranin red and Toluidine blue and washed with water. Ethyl-alcohol dehydration was then performed by increasing the concentration of alcohol from 50% to 96%. Post-fixation of the sections was performed with xylene, which required several hours to complete. Finally, the needles were glued to the cover glass using Canadian balsam, covered with the glass and dried in an oven at 60°C (Lavadinović *et al.*, 2017). Morpho-anatomic features were measured three weeks later. Taking into account the sample sizes of

the groups being compared, all the obtained numerical data were analyzed using the two-way ANOVA and LSD test.

3. RESULTS AND DISCUSSION

The results of the two-way analysis of variance (site x provenance) for the property diameter of the needle resin canals of Douglas-fir are given in Table 2. Based on the results presented, it can be concluded that there are:

a) statistically significant differences between the mean values of resin canal diameters in the needles collected on Mt. Juhor and Tanda sites;
b) statistically significant differences between the mean values of resin canal diameters in the needles of some provenances analyzed;
c) significant influence of the interaction of "site" and "provenance" factors on the mean values of resin canal diameters.

Table 2. *A two-way analysis of variance (site x provenance) for the property diameter of the needle resin canals of Douglas-fir*

Source of variation	Sum of squares	The degree of freedom	Variance	F-ratio	*p-value*
A: Site	13634.6	1	13634.6	87.16	0.000
B: Provenance	52465.9	5	10493.2	67.08	0.000
Interaction AB	42525.4	5	8505.08	54.37	0.000
Errors	54435.8	348	156.425		
Total	163062.0	359			

3.1. The effect of site on the diameter of resin canals

Based on to the results presented in Table 3, it can be concluded that there are statistically significant differences between the mean values of resin canal diameters of the needles collected on Mt. Juhor and Tanda. The mean resin canal diameter determined for the provenances at Tanda site (91.1 μm) is significantly greater than that determined for the needles collected on Mt. Juhor (78.79 μm). In addition, variability of the resin canal diameters of the analyzed provenances was detected on both sites, as shown in Graphs 1 and 2.

Table 3. *An LSD test of site influence on the diameter of resin canals*

Site	Sample size	Mean values	Standard error of the difference between the means	Homogeneous groups
Juhor	180	78.79	0.932216	X
Tanda	180	91.1	0.932216	X
Comparison			Differences	+/– Limit
Juhor–Tanda			*–12.3083	2.59295

*statistically significant difference

3.2. The effect of provenance on the diameter of resin canals

The LSD test was used to determine whether there is a relation between the genefond of the introduced provenances and the variability of resin canal diameter in the needles of Douglas-fir. The results are presented in Table 4.

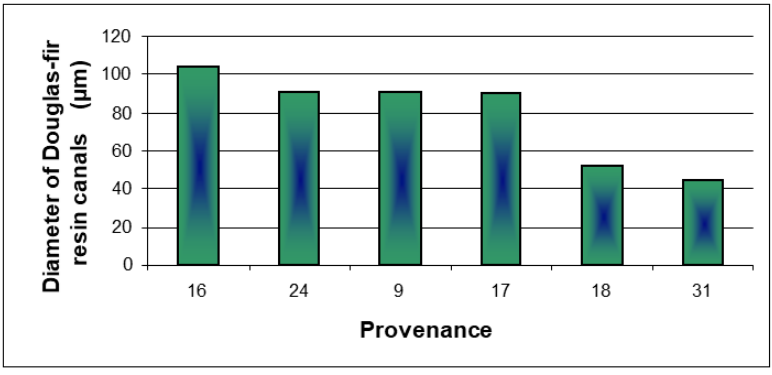
The results presented in Table 4 indicate that there are statistically significant differences between the resin canal diameters of Douglas-fir; however, the results of the LSD test show that some of the provenances analyzed (31, 18, 24, 16, 9 and 17) were homogeneous, i.e. the mean values of the resin canal diameters were not statistically significant for the same provenances.

Table 4. *An LSD test of provenance influence on the diameter of resin canals*

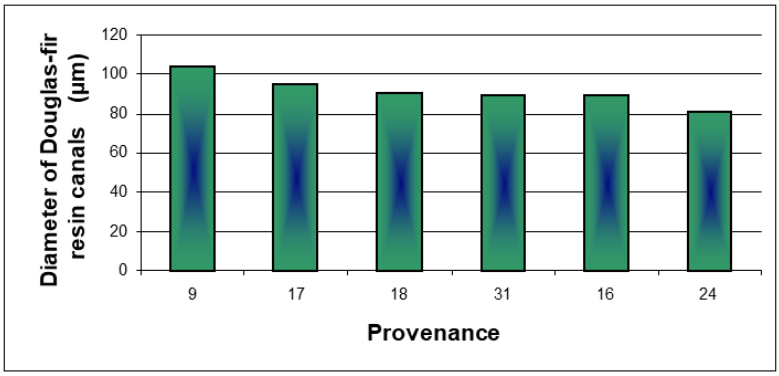
Provenance	Sample size	Mean values	Standard error of the difference between the means	Homogeneous groups
31	60	67.250	1.61465	X
18	60	70.925	1.61465	X
24	60	85.875	1.61465	X
16	60	89.625	1.61465	X
9	60	97.250	1.61465	X
17	60	98.750	1.61465	X

Comparison	Differences	+/– Limit
9–16	* 7.625	4.49111
9–17	–1.5	4.49111
9–18	* 26.325	4.49111
9–24	* 11.375	4.49111
9–31	* 30.0	4.49111
16–17	* –9.125	4.49111
16–18	* 18.7	4.49111
16–24	3.75	4.49111
16–31	* 22.375	4.49111
17–18	* 27.825	4.49111
17–24	* 12.875	4.49111
17–31	* 31.5	4.49111
18–24	*–14.95	4.49111
18–31	3.675	4.49111
24–31	* 18.625	4.49111

*statistically significant difference



Graph 1. *Variability of resin canal diameter between different provenances on Mt. Juhor*



Graph 2. *Variability of resin canal diameter between different provenances on Tanda site*

The results are comparable with those published by Pădure *et al.* (2008). In the study of the relationships of *Pseudotsuga menziesii* populations in Romania, several anatomical characters were found to be significant, including resin duct diameters in the needles. Resin ducts varied in diameter from 31,25 to 68,70 µm (Pădure *et al.*, 2008). Therefore, it can be concluded that allmost all provenances analyzed in our study (except for 31) had greater average resin duct diameters than those presented in the literature.

4. CONCLUSIONS

Douglas-fir is an introduced conifer species that should take a significant place in the ecosystems of Serbia. As it comes from a wide natural range of distribution, the proper provenance selection is a necessary step in the confirmation of its genetic potentials in new ecological conditions. The genetic control of its anatomical and morphological traits could be tested by provenance trials. In the context of genetic improvement programmes of introduced tree species, Institute of Forestry, Belgrade, has assessed different Douglas-fir provenances in Serbia.

In the present study, the hypothesis that there are significant differences between the mean values of resin canal diameters of the needles of Douglas-fir on different experimental plots in Serbia has been confirmed. In some provenances, it was determined that there are significant differences between the mean values of resin canal diameters and that the interaction of "site" and "provenance" factors has significant influence on the mean values.

The results of this study will contibute to better selection of suitable provenances for the introduction of Douglas-fir in Serbia. In addition, the strategy of the introduction of exotic tree species will contribute to better afforestation programs and will provide high-quality raw materials for the wood industry.

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Summary

Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco, fam. Pinaceae] occurs in all ecosystems of Europe, providing with fast and high-quality wood production, landscape diversity, as well as lucrative materials for medicinal and industrial use. Its natural range of distribution is in the northern part of Canada and the Pacific coast, up to 3,000 m a. s. l. on Rocky Mts. Its wide geographical range in terms of latitude and longitude (from New Mexico to Vancouver), as well as altitude, has produced a number of provenances.

The successful introduction of Douglas-fir depends on the selection of the most suitable provenances. In Serbia, two provenance trial plantations of the species has been established in order to test its adaptability to the climatic and environmental conditions of new habitats.

In this paper, the analysis of variability of resin canal diameter in the needles of different Douglas-fir provenances was conducted on two sites in Serbia. It was determined that there are significant differences between the mean values of resin canal diameters of some provenances and that the influence of the interaction of "site" and "provenance" factors on the mean values was also significant. Resin has a protective role in conifers.

The results of the present study will contribute to better selection of suitable provenances for the introduction of Douglas-fir in Serba.

VARIJABILNOST PREČNIKA SMOLNIH KANALA U ČETINAMA RAZLIČITIH PROVENIJENCIJA DUGLAZIJE

Vera LAVADINOVIĆ, Dragica OBRATOV-PETKOVIĆ, Ljubinko RAKONJAC, Zoran MILETIĆ, Filip JOVANOVIĆ, Milan KABILJO

Rezime

Duglazija [*Pseudotsuga menziesii* (Mirb.) Franco, fam. Pinaceae] je zastupljena u svim ekosistemima Evrope, obezbeđujući kvalitetan i brz prirast drvne mase, pejzažnu raznolikost, kao i unosan materijal za medicinsku i hemijsku industriju. Prirodno je rasprostranjena u severnom delu Kanade i priobalnom delu Pacifika, sve do 3000 m nadmorske visine Stenovitih planina. Ovako širok vertikalni i horizontalni areal (od Novog Meksika do Vankuvera) raspolaže velikim brojem provenijencija.

Za introdukciju duglazije potreban je pravilan izbor provenijencije. U Srbiji su postavljeni provenijenični ogledi s ciljem testiranja adaptivnosti ove vrste na klimatske i stanišne uslove novih lokaliteta.

U radu su izvedena istraživanja prečnika smolnih kanala u četinama duglazije različitih provenijencija na dva lokaliteta u Srbiji. Konstatovano je da postoje razlike u srednjim vrednostima prečnika smolnih kanala kod pojedinih provenijencija, kao i to da kod pojedinih provenijencija interakcija između faktora „lokalitet" i „provenijencija" utiče na srednju vrednost prečnika smolnih kanala. Uloga smole u četinarima je zaštitna.

Ovim istraživanjem će se doprineti većoj pouzdanosti pri izboru odgovarajućih provenijencija u programu introdukcije duglazije u Srbiji.