UDC 575:630
DOI: 10.2298/GENSR1503079L
Original scientific paper

VARIABILITY OF SEEDLINGS OF DOUGLAS-FIR PROVENANCES INTRODUCED FROM CANADA

Vera LAVADINOVIĆ [1], Vukan LAVADINOVIĆ [2], Ilija DJORDJEVIĆ[1]

1- Institute of Forestry, Belgrade, Serbia
2- Faculty of Forestry, Belgrade

Lavadinović V., V. Lavadinović, I. DJordjević (2015): *Variability of seedlings of douglas-fir provenances introduced from Canada*.- Genetika, Vol 47, No. 3, 1079-1090.

Introduction of Canadian provenances of Douglas-fir (*Pseudotsuga menziesii* Mir / Franco) in Serbia started with the first phase of testing their genetic potential by studying the effects of geographic characteristics of the locations from which the provenances originated (latitude, longitude and altitude) on the variability of the measured seedling properties. In the laboratory of the Institute for Forestry in Belgrade, germinability of Douglas-fir seeds was tested on the germination table ("Copenhagen table" or "Jakobson table") by the standards of ISTA. The analysis of variance and the regression and correlation analysis were applied in the study of the effects of geographic parameters of Canadian provenance locations on the variability of seedlings. The results show that there is a statistically significant effect of the provenance latitude on the length of seedlings. The effect of altitude is slightly smaller, while the longitude of the provenance location has the smallest effect on the studied property. The study of the variability of Douglas-fir provenances in their juvenile development, as seedlings, is essential for reliable planning and implementation of further tests within pilot projects on allochthonous sites in Serbia.

Key words: introduction, Douglas-fir, provenances, variability, length of seedlings

INTRODUCTION

The term \`genecology\` was first applied by TURESSON (1923) to the study of genetic variation of plants in relation to source environments. It was followed by active research studies by AITKEN and HANNERZ (2001), HOWE *et al*. (2003), JOHNSON (1997), ROCHE (1969) etc.

Seed transfer distances are expressed in geographic distances (latitude, longitude and elevation) by the following authors: CAMPBELL (1986), CARTER (1996), KRAKOWSKI, STOEHR

Corresponding author: Vera Lavadinović, Institute of Forestry, Kneza Višeslava 3, Belgrade, 11030, Serbia. Phone: + 381-11-355-33-55. Email: veralava@eunet.rs

(2009), SCHMIDTLING (1994). The introduction program implies the introduction of alien species into new environments (JORGE *et al*., 2011). Introduced species should be better or equal quality when compared to native species. The quality of the introduction process should be justified by economic and environmental characteristics of the newly-introduced tree species.

In order to eliminate the effects of long-lasting errors, provenance test is used as a reliable method of forest tree selection (CHING and HINZ, 1978; GRIFFIN, 1978; MONSERUD and REHFELDT, 1990). The selection of provenances is the most important task for tree breeders.The strong genetic control of introduced species should be started in the juvenile period of the tested plant material.

The main growth characteristics are related to height, diameter, and volume. Douglas-fir is among the most important and productive tree species, fast growing, and the most common forest tree of Canada and Northwest America (ALLEN and OWENS, 1972). The geographic variation in growth can be determined and the selection of provenances done at early stages of plants.

Its wide ecological amplitude and spectrum of genetic variability, high adaptability to different sites, high quantity and good quality of wood and its decorative value have made Douglas-fir (*Pseudotsuga menziesii* Mir / Franco) one of the most commonly grown allochthonous conifer species in western and central Europe. Its biological attributes and genetic constitution in the mosaic of ecological factors on allochthonous sites are the main indicators of the success of its introduction. Disjunctive range of Douglas-fir distribution in North America has brought about certain differences in the gene pool of spatially isolated populations, whose adaptability and phenotypic expression are readily observed when they are grown on the sites where the species is non-native (SILEN, 1978).

Several decades of research within Douglas-fir provenance tests in Serbia, on Juhor near Jagodina and in Tanda near Bor, were aimed at studying the effects of geographic parameters of the source locations of Douglas-fir provenances - latitude, longitude and altitude on the adaptability and the growth and development of the trees on allochthonous sites in Serbia (LAVADINOVIĆ *et al*., 1996, 2001; 2011, 2013).
Based on the results of previous studies, three provenances of Douglas-fir from Oregon and one from Washington were recognized as suitable for further cultivation in Serbia. Given that these provenances come from the north-most part of Douglas-fir range of distribution in the USA, subsequent research included thirteen provenances of the species from the territory of Canada. These are the first research studies of the potentials of Canadian Douglas-fir provenances for its successful introduction in the Western Balkans. The paper presents the results of studying the effects of geographic parameters of the source locations of Canadian provenances - latitude, longitude and altitude on the variability of the measured seedling properties.

MATERIALS AND METHODS

Sample seed collection

The conducted laboratory analysis used original seeds of thirteen Douglas-fir provenances from a part of its natural range of distribution in Canada. The spatial distribution of the provenances is shown in Figure 1.

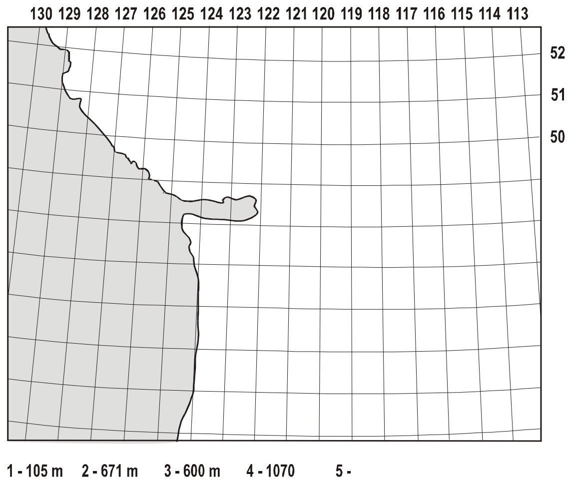


Figure 1: Map of geographical distribution of the tested provenances

Seeds were collected and distributed by the "Canadian Forest Service" from British Columbia. Geographic characteristics of the tested provenances are given in Table 1.

Table 1. Geographic characteristics of the tested provenances of Douglas-fir

Provenance		Location	Longitude	Latitude	Altitude (m)
No	Code				
1	03333	Cranbrook	49^{o} 25'	115^{o}20'	1050
2	00848	Inonoaklin	49^{o} 50'	118^{o}10'	671
3	30667	Mann Creek	51^{o} 35'	120^{o}10'	600
4	05227	Gavia Lake	50^{o} 56'	116^{o}35'	1070
5	05226	Nine Bay	50^{o} 58'	116^{o}32'	975
6	03356	Trout Cr	49^{o} 40'	119^{o}52'	884
7	03360	Michell CR	49^{o} 54'	119^{o}37'	1035
8	30460	Mara LK	50^{o} 48'	119^{o}00'	488
9	00278	Monte Crk	50^{o} 37'	119^{o}52'	701
10	03383	Sheep creek	49^{o} 10'	117^{o}15'	1000
11	30461	Cooke creek	50^{o}38'	118^{o}49'	900
12	03389	Benton creek	49^{o} 12'	117^{o}25'	933
13	05092	Sun Creek	50^{o} 08'	115^{o}52'	1000

Material processing

The properties of seeds were studied in laboratory conditions. The test included one hundred seeds of each provenance, placed on a germination table with five repetitions. Seeds were previously stratified for three months. They were rolled into moist paper towels, sealed inside plastic bags and placed in the refrigerator at 5°C). The paper towels were changed every seven days, in order to control and prevent the occurrence of seed infection.

Laboratory analysis- Germination test

The germination rate of Douglas-fir seeds in the laboratory was tested on the germination table ("Copenhagen table" or "Jakobson table") by the standards of ISTA. Water temperature of the germination table was controlled by an electronic digital temperature controller (KAMRA, 1968), with the temperature regime of 25°C for a period of 10 hours (during day) and 20°C for a period of 14 hours (during night). The number of seeds that germinated during the test was recorded every two to four days. A seed was considered to have germinated when the radicle emerged was at least twice the length of the seed (DOODY and O'REILLY, 2005).

Twenty-one days after the seeds had been put to germinate, we measured the height of hypocotyls, *i.e.* the length of seedlings of each provenance using a caliper square with an accuracy of 0.1 *mm*.

Statistical analysis

The data on the length of seedlings of different provenances were studied applying descriptive statistics and correlation and regression analysis. Simple and multiple regression and correlation models were applied to examine the dependence of the length of seedlings on latitude, longitude and altitude of the source locations of the provenances in Canada.

RESULTS AND DISCUSSION

A comparative study of Douglas-fir seedlings originating from thirteen provenances in Canada was conducted with the aim of determining the traits of genetic, morphological and physiological variability, which is an important basis for its successful introduction into allochthonous sites in Serbia. The results of the research on the spontaneous variability of Douglas-fir seedlings are presented in both tables and graphs. The conducted comparative study of the variability of Douglas-fir seedlings from Canadian provenances at juvenile stages, *i.e.* twenty-one days after they have germinated from seed, are of both theoretical and practical importance for the future introduction of this species in Serbia.

At early stages of juvenile development of trees, seedlings and annual plants are the most abundant both in natural spontaneous and in artificially-established populations of newly-established forests or in experimental tests. Since the number of seedlings and juvenile plants is the largest, they comprise the greatest share of the gene pool of the populations they originate from. The first spontaneous selection is done when the seeds are at the stage of germination and at cotyledon stage.

Research studies dealing with other coniferous species found a correlation between morphometric characteristics of young seedlings and better properties of developed seedlings and trees (TUCOVIC and ISAJEV, 1985). Differences in the coloration of cotyledons and hypocotyls always and mostly without errors indicate the coloration of micro and macro strobes or flower and fruiting inflorescences (TUCOVIC and ISAJEV, 1985). Therefore, the data presented in tables and graphs are important for further and more intensive assessment of the genetic potentials of Canadian provenances for their introduction in Serbia.

Geographic characteristics of the locations of Canadian provenances (independent variables) and the mean length of seedlings are shown in Table 2.

Table 2. Geographic coordinates and the length of seedlings

Provenance number	Geographic coordinates			Length of seedlings (Y) (cm)
	Latitude (X_1)*	Longitude (X_2)	Altitude (X_3)	
1	49.25	115.20	1050	2.50
2	49.50	118.10	671	3.22
3	51.35	120.10	600	4.01
4	50.56	116.35	1070	3.07
5	50.58	116.32	975	2.90
6	49.40	119.52	884	2.36
7	49.54	119.37	1035	2.72
8	50.48	119.00	488	3.98
9	50.37	119.52	701	3.98
10	49.10	117.15	1000	3.24
11	50.38	118.49	900	3.85
12	49.12	117.25	933	3.18
13	50.08	115.52	1000	3.93

X_1 – latitude (^{0}); X_2 – longitude (^{0}) ; X_3 – altitude (m); Y – length of seedlings (cm)

Table 3. The main statistical parameters of the original provenances and the length of seedlings

Variables	X_1	X_2	X_3	Y
Count	13	13	13	13
Average	49.98	117.84	869.77	3.30
Standard deviation	0.70	1.65	190.72	0.59
Coeff. of variation (%)	1.41	1.40	21.93	17.96
Minimum	49.1	115.2	488.0	2.36
Maximum	51.35	120.1	1070.0	4.01
Range	2.25	4.9	582.0	1.65
Stnd. skewness	0.488	-0.373	-1.330	-0.175
Stnd. kurtosis	-0.613	-1.00	-0.357	-1.036

Table 4. The coefficients of simple and Table 5 coefficients of partial correlation

Variables	X_1	X_2	X_3	Y
X_1	-	**0.2764**	**-0.4392**	**0.6114**
(p)		0.3606	0.1332	0.0264
X_2		-	**-0.6504**	**0.2656**
(p)			0.0161	0.3804
X_3			-	**-0.5952**
(p)				0.0319
Y				-

The results in Table 4 show that there is a statistically significant correlation between the length of seedlings and the latitude and between the length of seedlings and the altitude ($p < 0.05$). However, the correlation between the length of seedlings and the longitude is not statistically significant.

Table 5. Partial Correlations

Variables	X_1	X_2	X_3	Y
X_1	-	**0.2625**	**-0. 142**	**0.1808**
(p)		0.5300	0.9733	0.6683
X_2		-	**-0.2132**	**0.2287**
(p)			0.6121	0.5860
X_3			-	**-0.3467**
(p)				0.4001
Y				-

However, Table 5 shows that the partial (net) correlation between the length of seedlings of the studied provenances and their geographic characteristics is not statistically significant. This means that besides the direct, there is a joint effect of geographic characteristics of the provenances on the length of their seedlings. A deeper insight into these correlations is given further on.

1. Dependence of the length of seedlings of provenances (Y) on latitude (X_1)

The results of this correlation are shown in Tables 6a and 6b and in Graph 1.

Table 6a. Parameters of the linear regression between the length of seedlings and the latitude

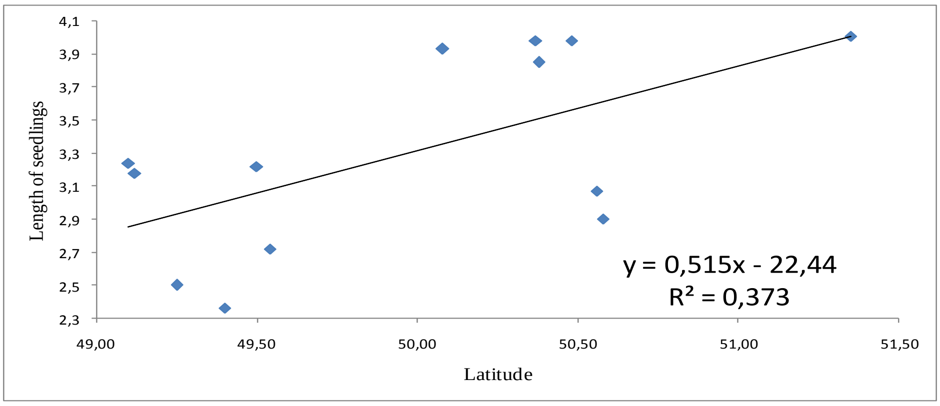
Parameter	*Estimate*	*Standard error*	*T - Statistic*	*P-Value*
CONSTANT	-22.442	10.0473	-2.23364	0.0472
X_1	0.515132	0.201018	2.56262	0.0264

Table 6b. Analysis of Variance

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-ratio*	*P-Value*
Model	1.5796	1	1.5796	6.57	0.0264
Residual	2.64588	11	0.240534		
Total	4.22548	12			

Both parameters in the linear regression model are statistically significant, as well as the whole regression. The coefficient of determination is 37.38%, and the standard error of regression 0.49 cm.

Graph 1. Dependence of the length of seedlings of different provenances on latitude



Graph 1 shows that the length of seedlings linearly increases with the latitude, which means that the higher latitude provenances are the better ones. The relationship is statistically significant ($p < 0.05$).

2. Dependence of the length of seedlings of provenances (Y) on longitude (X_2)
The results of this correlation are shown in Tables 7a and 7b and in Graph 2.

Table 7a. Parameters of the linear regression between the length of seedlings and longitude

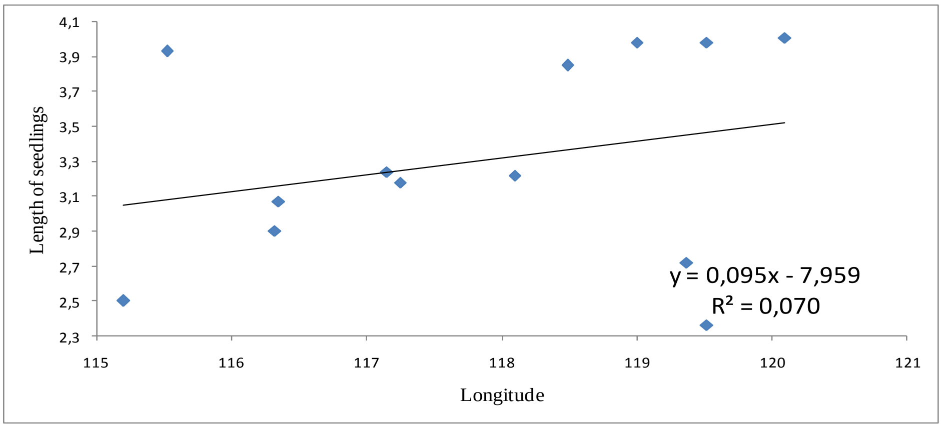
Parameter	*Estimate*	*Standard error*	*T - Statistic*	*P-Value*
CONSTANT	-7.95977	12.3262	-0.645759	0.5317
X_2	0.095579	0.10459	0.913813	0.3804

Table 7b. Analysis of Variance

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-ratio*	*P-Value*
Model	0.29814	1	0.29814	0.84	0.3804
Residual	3.9734	11	0.357031		
Total	4.22548	12			

Both parameters in the linear regression model are statistically significant, as well as the whole regression. The coefficient of determination is 7%, and the standard error of regression 0.60 cm.

Graph 2. Dependence of the length of seedlings of different provenances on longitude



Graph 2 shows that the length of seedlings linearly increases with longitude, which means that the higher longitude provenances are expected to be more prospective for future introduction. However, the relationship is not statistically significant ($p > 0.05$) and has no practical significance.

3. Dependence of the length of seedlings of provenances (Y) on altitude (X_3)

The results of this correlation are shown in Tables 8a and 8b and in Graph 3.

Table 8a. Parameters of the linear regression between the length of seedlings and altitude

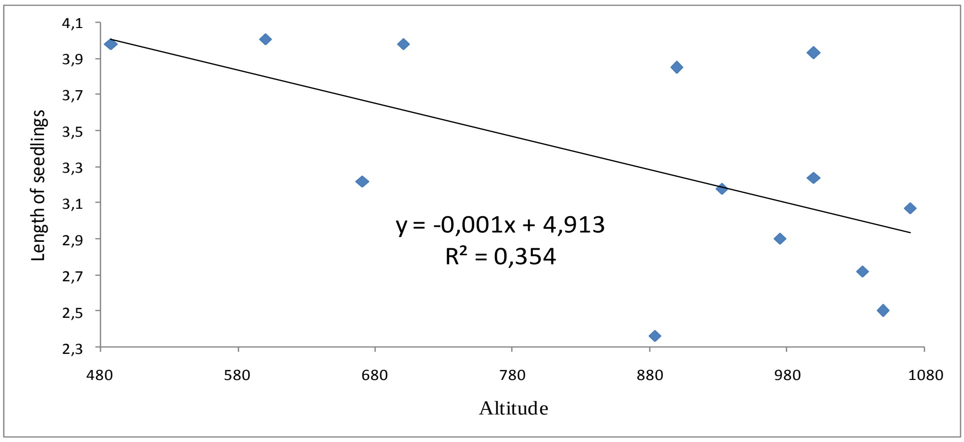
Parameter	*Estimate*	*Standard error*	*T- Statistic*	*P-Value*
CONSTANT	4.91363	0.670087	7.33283	0.0000
X_3	-0.00185	0.000754	-2.45627	0.0319

Table 8b. Analysis of Variance

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-ratio*	*P-Value*
Model	1.49668	1	1.49668	6.03	0.0319
Residual	2.7288	11	0.248072		
Total	4.22548	12			

Both parameters in the linear regression model are statistically significant, as well as the whole regression ($p < 0.05$). The coefficient of determination is 35.42%, and the standard error of regression 0.50 cm.

Graph 3. Dependence of the length of seedlings of different provenances on altitude



Graph 3 shows that the length of seedlings linearly decreases with altitude, which means that the lower altitude provenances are the better ones. This relationship is statistically significant ($p < 0.05$).

4. Dependence of the length of seedlings of different provenances (Y) on latitude (X_1) longitude (X_2) and altitude (X_3)

This correlation was determined using the model of multiple nonlinear regressions, considering independent variables. The impact of the variables is expressed as a parabola of the second order. Since many of the parameters in the model were not statistically significant, the method of stepwise multiple regression was further applied. Latitude was selected as the statistically significant variable, but only its linear member. The results were equal to the results of the simple linear regression, as presented in Tables 6a and 6b, and in Graph 1.

The analyzed properties of Douglas-fir seedlings are indicators of the future development of seedlings, which justifies a more comprehensive study of seedlings of different provenances. The research of ROHMEDER and SCHÖNBACH, (1959) was the first to observe that morphological characteristics of Douglas-fir seedlings and primary needles correlated with the physiological capacity of mature trees to resist spring or winter frosts. Spirally twisted cotyledons of Douglas-fir seedlings indicate greater sensitivity to spring frosts, which is of particular importance in the selection of provenances for introduction (ROHMEDER and SCHÖNBACH, 1959). It may be useful to introduce the determination of seedling variability properties into the routine controls of seed transfer properties, especially in the process of introduction, because their morphological and physiological traits can provide reliable data necessary for further activities in field tests and in afforestation.

CONCLUSIONS

The study of the variability in the length of Douglas-fir seedlings from thirteen Canadian provenances, with the accent on their dependence on geographical characteristics of the source locations concluded the following:

- Simple correlation (Table 4) between the length of seedlings of the studied provenances and their latitude as well as altitude was statistically significant (p <0.05). However, the simple correlation between the length of seedlings and longitude was not statistically significant (p> 0.05).
- Partial or net correlation (Table 5) between the length of seedlings of the studied provenances and their geographic characteristics didn`t find statistically significant differences in any of the studied cases (p> 0.05).
- The increasing latitude of the provenances linearly increases the length of seedlings (Graph 1), which means that the greater latitude provenances are better for introduction. The relationship is statistically significant (p <0.05).
- The increasing longitude of the provenances linearly increases the length of seedlings (Graph 2), which means that the higher longitude provenances can be expected to be better for introduction. However, this relationship isn't statistically significant (p> 0.05) and there is no practical significance.
- The increasing altitude of the provenances linearly decreases the length of seedlings (Graph 3), which makes lower altitude provenances better for introduction. This relationship is statistically significant (p <0.05).
- The study of the growth of Douglas-fir provenances by studying the length of seedlings shows that the provenances listed in Table 2 under 3, 8, 9, 13 and 11 can be considered as more promising for introduction. These provenances originate from higher latitudes and lower altitudes in Canada. Therefore, the program of Douglas-fir introduction should take this property into consideration when selecting the most promising provenance genotypes originating from Canada.

ACKNOWLEDGMENT

This paper was realized as a part of the project "Studying climate change and its influence on the environment: impacts, adaptation and mitigation" (43007) financed by the Ministry of Education and Science of the Republic of Serbia within the framework of integrated and interdisciplinary research for the period 2011-2015.

Received July27th, 2015
Accepted October 20th, 2015

REFERENCES

AITKEN, S.N. and M.HANNERZ (2001): Genecology and gene resources management strategies for conifer cold hardiness. P.23-53.In:F.J.Bigras and S.J.Columbo (eds.), Conifer cold hardiness:kluwer,Dordrecht,The Netherlands.

ALLEN, G.S. and J.N. OWENS (1972): The life history of Douglas-fir. Inf. Can., Can. For. Serv., Ottawa, 139p.

CAMPBELL, R.K. (1986): Mapped genetic variation of Douglas-fir to guide seed transfer in southwest Oregon. Silvae Genet., *35*:85-96.

CARTER, K.K. (1996): Provenance tests as indicators of growth response to climate change in 10 north temperate tree species. Can. J. For. Res., *26*:1089-1095.

CHING, K.K. and P.N. HINZ (1978): Provenance study of Douglas-fir in the Pacific Northwest region. Silvae Genet., *27*:229-233.

CRUZ-NICOLÁS, J., J. JESÚS VARGAS-HERNÁNDEZ, P. RAMÍREZ-VALLEJO and J. LÓPEZ-UPTON (2011): Genetic diversity and differentiation of Pseudotsuga menziesii (Mirb.) Franco populations in Mexico. Rev. Fitotec. Mex., Vol. *34 (4)*: 233 – 240.

DOODY P. and C. O'REILLY (2005): Effect of moist chilling and priming treatments on the germination of Douglas-fir and Noble fir seeds. Seed Science& Technology, *33*: 63–76

GRIFFIN, A.R. (1978): Geographic variation in Douglas-fir from the coastal ranges of California. II. Predictive value of a regression model for seedling growth variation. Silvae Genet., *27*:96-101.

HOWE, G.T., S.N. AITKEN, D.B. NEALE, K.D. JERMSTARD, N.C. WHEELER and T.H.H. CHEN (2003): From genotype to phenotype: Unraveling the complexities of cold adaptation in forest trees.Can. J. Bot., *81*:1247-1266

KAMRA, S.K. (1968): Effect of different distances between water level and seed bed on Jacobsen apparatus on the germination of Pinus silvestrisL.seed. Technical Report.Stockholm: Sverigeslantbruks universitet. Studia forestalia Suecica 65, Skogshögskolan.ISSN, 0039-3150, 17p.

JOHNSON, G.R. (1997): Site-to-site genetic correlations and their implication on breeding zone size and optimum number of progeny test sites for coastal Douglas-fir. Silvae Genet., *46*: 280-285.

KRAKOWSKI, J., M. STOEHR (2009): Coastal Douglas-fir provenance variation: patterns and predictions for British Columbia seed transfer. Annals of Forest Science, Vol. *66*, Issue *8*: p 811.

LAVADINOVIĆ, V., V., ISAJEV, M. KOPRIVICA (2001): Influence of provenances gene pool of Douglas-fir on height increment of trees in experimental test in East Serbia. Genetika, Vol. *33 (1-2)*:11-17.

LAVADINOVIĆ, V., V., ISAJEV, M. KOPRIVICA (1996): Effect of Seed Germination on Survival and Height of Two-year Douglas fir (*Pseudotsuga taxifolia* Britt.) of Different Provenances. Genetika, Vol. *28 (2)*: 103-114.

LAVADINOVIĆ,V., Z., MILETIĆ, V., ISAJEV, M. KRSTIĆ (2011): Variability of nitrogen content in the needles of Douglas-fir provenance (*Pseudotsuga Menziesii* Mir / Franco). Genetika, Vol. 43 *(2)*: 407-417

LAVADINOVIĆ, S.,V., MILETIĆ, Z., LAVADINOVIĆ, V., ISAJEV (2011): Variability In Magnesium Concetration In Needles Of Different Douglas-Fir Provenances. Forestry Ideas, Vol. 17 (1): 74-79. Sofia, Bulgaria.

LAVADINOVIĆ, V., V., POPOVIĆ, E., POPOV, M.V., LAVADINOVIĆ (2013): Testing of Canadian Douglas-fir height in juvenile phase, Sustainable Forestry Tom 67-68, Institute of Forestry, p. 23-31, Belgrade, ISSN 1821-1046.

MONSERUD, R.A. and G.E. REHFELDT (1990): Genetic and environmental components of variation of site index in inland Douglas-fir. For. Sci., *36*:1-9.

ROCHE, L. (1969): A genecological study of the genus *Picea* in British Columbia. New Phytologist., *68*: 505-554.

ROHMEDER E., H., SCHÖNBACH (1959): Genetik und Züchtung der Waldbäume. Hamburg: 58-90.

SCHMIDTLING, R.C. (1994): Use of provenance tests to predict response to climatic change: loblolly pine and Norway spruce. Tree Physiol., *14*:805-817.

SILEN, R.R. (1978): Genetics of Douglas-fir. USDA Forest Service Research Paper, 34. Washington DC.

TURESSON, G. (1923): The scope and import of genecology. Hereditas, *4*:171-176.

TUCOVIĆ A., V. ISAJEV (1985): Spontaneous and induced variability of Serbian spruce at "Bela Zemlja" seed source in Titovo Uzice region. Bulletin of the Faculty of Forestry 67, University of Belgrade - Faculty of Forestry, Belgrade (95-116).

VARIJABILNOST KLIJAVACA PROVENIJENCIJA DUGLAZIJE INTRODUKOVANIH IZ KANADE

Vera LAVADINOVIĆ [1], Vukan LAVADINOVIĆ [2], Ilija DJORDJEVIĆ[1].

1- Institutza šumarstvo, Bograd
2- Šumarksai fakultet, Bolgrad

Izvod

Jedan od metoda ispitivanja gen-ekološke distance transfera semena introdukovanih vsrta je putem provenijeničnog testa. Transfer semena introdukovanih vrsta nosi sa sobom rizika da li će vrsta u novim stanišnim uslovima potvrditi svoju produktivnost kao i u prirodnim populacijama porekla semena, tj svoju genetičku stabilnost u ekološkoj interakciji. Transfer semena introdukovanih vrsta podrazumeva testiranje svih karakteritika duglazije. U radu je ispitivana zavisnost dužine klijavaca duglazije, poreklom iz Kanade, različitih provenijencija od geografskih karakteristika porekla semena. Za analizu podataka korišćen je metod analize varijanse i metod regresione i korelacione analize. Zaključeno je da je statistički značajan uticaj geografske širine na dužinu klijavaca provenijencije, dok je nešto manji uticaj nadmorske visine a najmanji uticaj geografske dužine, odnosno da se uticaji geografskih karakteristika provenijencija ispoljavaju i kod ispitivanja semena u laboratorijskim uslovima. U programu introdukcije duglazije, ovo svojsvo, treba uzeti u obzir pri izboru najperspektivnijih genotipova provenijencija poreklom iz Kanade

Primljeno 27.VII 2015.
Odobreno 20. X. 2015.