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STUDY ON SEED GERMINATION OF THIRTEEN DOUGLAS-FIR PROVENANCES

Vera Lavadinović[1*], Vukan Lavadinović[2], and Zoran Poduška[1]

[1]Institute of Forestry, Kneza Viseslava 3, Belgrade, Serbia. [*]E-mail: veralava@eunet.rs.
[2]Faculty of Forestry, Kneza Viseslava 1, Belgrade, Serbia. E-mail: vukan.lavadinovic@sfb.ac.rs

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Abstract

Rehabilitation of degraded forest ecosystems requires introduction of fast-growing productive and adaptive tree species, such as Douglas-fir. However, introduction of new species always carries an element of risk. This risk can be decreased by appropriate testing. Therefore, it is necessary to investigate the origin and success of species and to select the most suitable provenances. A very important stage in the process of provenance testing is the early control of seed material, which can improve and increase the growth of good-quality seedlings and provide better results in afforestation and establishment of new vegetation. Therefore, the Institute of Forestry in Belgrade set the germination test of Douglas-fir seed under different test conditions in order to identify and select the best seed sources for afforestation. Original Douglas-fir seed of different provenances in Canada was taken as seed material. The test included collection of seeds originating from thirteen different provenances in natural forests of Canada. The aim of the research was to determine the potentials and germination differences among the seeds of common provenances in different conditions – in laboratory and in nursery – and to select the best provenances. The relationship of seed germination and the geographic origin could be another important indicator for selection of Douglas-fir provenances. Regression and correlation analyses were used to determine the statistical dependence of the seed germination in different condition on the geographical origin of Douglas-fir seeds.

Key words: introduction, selection, seed transfer, seed quality, geographic origin,

Introduction

Introduction of adaptive allochthonous tree species has become increasingly important in forestry due to the modified growing conditions caused by the global climate change. Testing of new species with greater growth potentials and better chances to survive in the changed climate conditions will soon become a pressing need (Kjaer and Hansen 2013). The main objective of introducing exotic species into the existing and new forest ecosystems is to come up with the best solution that meets economic, ecological and aesthetic needs of the vegetation region of Serbia.

Introduction, as a model in forestry, entails a risk of selecting a bad provenance of the tree species of interest, which in turn causes long-term environmental effect on the vegetation structure of the region. Populations of species originating from different geographical locations (provenances) often show differential success due to the effect of genotype. Furthermore, provenances show significant differences in the characteristics of seedlings and the seed germination test reveals differences in their growth performance (Khurana and Singh 2001b).

Human beings have shaped forest ecosystems in Central Europe since prehistorical times, thus reducing the extent of forests to gain space for agriculture, thereby changing tree communities by cultivating the most useful tree species (Carnus et al. 2005).

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is a tree species with considerable growth potential, which makes it one of the most productive tree species of Canada and USA. As a fast-growing and highly productive species, it has become one of the most commonly introduced species in Europe. Douglas-fir was first introduced to Europe from USA more than 150 years ago. It was then planted on a large scale and it has now become economically most important exotic tree species in European forests (Schmid et al. 2014).

Knowledge on germination and seedling establishment is of pivotal importance for understanding such community processes as plant recruitment and succession and for the management of plant populations (Khurana and Singh 2001a). Douglas-fir, as an introduced species, has found an important place in the gene pool of forest tree species in Serbia (Šijačić-Nikolić and Milovanović 2007). Several sample plots of Douglas-fir trees of different provenances originating from USA and Canada were established with the purpose of testing characteristics of this species (Lavadinović and Koprivica 1999, 2000; Lavadinović et al. 1996; Radonja et al. 2003).

Seeds are one of the main sources of plants for restoration (Commander et al. 2010). Our aim was to get a deeper insight into the ecophysiology of seed germination of introduced species in different conditions. Understanding the seed germination and dormancy is important for many areas of plant science and technology, such as agriculture, restoration ecology and forestry (Baskin and Baskin 1998).

Germination under nursery or field conditions is often limited by unpredictable environmental factors. Epigeal germination is by far the most common in woody plants. All gymnosperms, and the major families of angiosperms have epigeal germination (Schmidt 2000). Knowledge on the ecology of germination and seedling growth is vital, not only for understanding the community processes of plant recruitment and succession, but also for developing strategies for the conservation of biodiversity and restoration of forests. In essence, seed is a miniature tree because it is responsible for its regeneration and ultimately for its reproductive success (Khurana and Singh 2001a). The objective of the present study was to determine the compatibility of seed germination of different Douglas-fir provenances under controlled laboratory and outdoor nursery conditions.

Material and Methods

In order to determine the characteristics of germination and differences between Douglas-fir provenances, the Institute of Forestry in Belgrade set a test of seed germination under different conditions. The seed from thirteen Douglas-fir provenances was used for the purpose of experimental analyses.

The seed was obtained through The National Tree Seed Centre of`Canadian Forest Service` in British Columbia, from the native range of Douglas-fir distribution in Canada. Spatial distribution of the provenances is shown in Figure 1, and their geographical characteristics are given in Table 1.

The germination of Douglas-fir seeds in laboratory were tested on the germination table (“Copenhagen table” or “Jakobson table”) where the water temperature was controlled by electronic digital temperature controller (Kamra 1968), temperature program of 25 °C for 10 hours (during day) and 20 °C for 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 emerged radicle was at least twice the length of the seed (Doody and O’Reilly 2005).

The basic seed pretreatment for nursery sowing included three-month seed stratification in a refrigerator at 5 °C. The seeds were rolled into a moist paper towel, sealed inside a plastic bag and placed in the refrigerator. The filter paper was changed every seven days in order to control and prevent seed infection.

In spring, the stratified seeds were sowed into containers in the nursery of the Institute of Forestry. Seed germination capacity was tested by applying the standard method of container seed

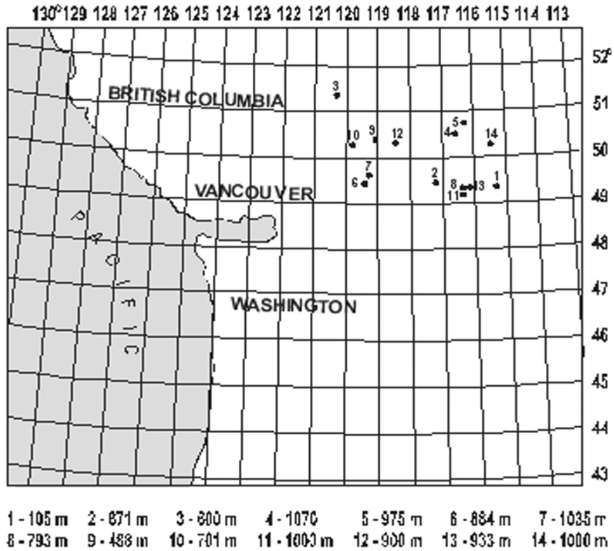

Fig. 1. Map of geographical distribution of the tested provenances.

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

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

sowing. We used 217 containers with 53 cells. Two seeds were sown in each cell, which made 21,412 seeds altogether. The containers were placed in the outdor under natural conditions of heat and light.

Several statistical methods, such as descriptive statistics, regression, correlation and testing, were used to process data of the germination rate of the seeds from different provenances. The methods of simple and multiple regression and correlation analysis were used to test the relationship of the seed germination rate with latitude, longitude and altitude of the seed source locations.

Results

Germination rates of Douglas-fir seeds from different provenances are presented in Table 2.

The average germination rate of Douglas-fir seeds under laboratory conditions (laboratory climate chamber) amounted to 87 %, while it was 34 % under nursery conditions. Standard deviations were 11.71 % and 9.30 %, respectively, and coefficients of variation were 13.42 % and 27.34 % (Table 3). Provenance 7 (Michell CR – 03360) had the lowest germination rate under laboratory conditions 66 %, while provenances 3 (Mann Creek – 30667), 8 (Mara LK – 30460) and 9 (Monte Crk – 00278) had the highest germination rate of 98 %. The lowest rate of germination under nursery conditions (21 %) was achieved by provenance 13 (Sun Creek – 05092). Provenance 3 (Mann Creek – 30667) had the highest germination percentage (50.7 %) under the same conditions. There was a significant difference in the germination rate of the seeds from different Douglas-fir provenances.

Table 2. Germination of Douglas-fir provenances under different conditions.

Provenance		Percentage of germinated seeds in the laboratory, %	Planted seeds in the nursery, number	Seedlings in the nursery, number	Seedlings in the nursery, %
No	Code				
1	03333	71	1 484	374	25.2
2	00848	96	1 802	808	44.8
3	30667	98	1 696	860	50.7
4	05227	95	2 014	632	31.4
5	05226	91	1 908	595	31.2
6	03356	70	1 696	568	33.5
7	03360	66	1 484	463	31.2
8	30460	98	1 590	731	46.0
9	00278	98	1 484	606	40.8
10	03383	86	1 590	586	36.8
11	30461	97	1 802	461	25.6
12	03389	81	1 484	354	23.8
13	05092	88	1 378	289	21.0
Average		**87**			**34.0**
Total			**21 412**	**7 327**	

Table 3 presents the main statistical parameters for the selected dependent (Y_1 and Y_2) and independent variables (X_1, X_2 and X_3).

Table 4 shows the coefficient matrix of the simple linear regression (r) between all variables.

The simple correlation coefficient of the germination of seeds in the laboratory with the latitude and altitude is statistically significant. However, the correlation coefficient between the seed germination in the nursery is statistically significant with the longitude and altitude, but not with the latitude.

Table 5 presents the coefficient matrix of the partial linear correlation (r) between all variables.

There is a statistically significant net correlation only between the seed germination under laboratory conditions and latitude at a confidence interval of 95 %. Other partial correlation coefficients are not statistically significant, suggesting that latitude, longitude and altitude have a common influence on seed germination. The statistical tests in laboratory and nursery are shown in Table 6.

(A) Laboratory Dependence of Douglas-fir seed germination in the laboratory (Y_1) on latitude (X_1)

The main parameters of parabolic regression are shown in Table 6, as the model number 1.

Model suggests that with increasing latitude provenance increases the parabolic their seed germination in the laboratory.

Dependence of Douglas-fir seed germination in the laboratory (Y_1) on longitude (X_2)

The main parameters of parabolic regression are shown in Table 6, as the model number 2.

The observed correlation is extremely weak. Only 7.68 % of the variance of seed germination under laboratory conditions can be explained by longitude. The correlation is not statistically significant (p = 0.67).

Table 3. Summary statistics.

Variables	Y_1	Y_2	X_1	X_2	X_3
Average	87.31	34.0	49.98	117.84	869.8
Standard deviation	11.71	9.30	0.70	1.65	190.7
Coefficient of variation	13.41	27.34	1.41	1.40	21.93
Minimum	66.00	21.00	49.10	115.20	488.00
Maximum	98.00	50.70	51.35	120.10	1070.00

Legend: Y_1 – percentage of germinated seeds in the laboratory; Y_2 – percentage of seedlings in the nursery; X_1 – latitude; X_2 – longitude; X_3 – altitude.

Table 4. Correlations.

Indicator	Y_1	Y_2	X_1	X_2	X_3
Y_1	-	**0.478**	**0.692**	**0.131**	**–0.577**
(p)		(0.098)	(0.0088)	(0.669)	(0.0391)
Y_2		-	**0.417**	**0.657**	**–0.821**
(p)			(0.156)	(0.015)	(0.0006)
X_1			-	**0.2764**	**–0.4392**
(p)				(0.3606)	(0.1332)
X_2				-	**–0.6504**
(p)					(0.0161)
X_3					-

Note: In all cases where the probability (p) is smaller than 0.05, the coefficient of correlation is statistically significant at a 95 % confidence interval, and if (p) is smaller than 0.01, the confidence interval is 99 %.

Table 5. Partial correlations.

Indicator	Y_1	Y_2	X_1	X_2	X_3
Y_1	-	**0.0812**	**0.6385**	**–0.4816**	**–0.4633**
(p)		(0.8234)	(0.0469)	(0.1587)	(0.1775)
Y_2		-	**0.0387**	**0.2898**	**–0.5346**
(p)			(0.9155)	(0.4166)	(0.1114)
X_1			-	**0.2759**	**0.1646**
(p)				(0.4403)	(0.6495)
X_2				-	**–0.4259**
(p)					(0.2197)
X_3					-

Dependence of Douglas-fir seed germination in the laboratory (Y_1) on altitude (X_3)

The main parameters of parabolic regression are shown in Table 6, as the model number 3.

We tested whether the seed germination rate under laboratory conditions shows a parabolic decrease with the increased provenance altitude, but this relationship was not statistically significant.

Dependence of Douglas-fir seed germination in the laboratory (Y_1) on latitude (X_1), longitude (X_2) and altitude (X_3)

Several regression models (additive and substitutive) were applied and additive models produced better results.

The additive model with fixed independent variables are shown in Table 6, as the model number 4. In model (4) some regression coefficients are not statistically significant. However, multiple regression, as a whole, was statistically significant. Latitude, longitude and altitude accounted for 89.02 % of the variance of the seed germination under laboratory conditions.

When using stepwise multiple regression, altitude emerged as the only significant variable. The results are shown in Table 6, as the model number 5.

Substitutive model (with the multiplication of independent variables) together with the method of stepwise multiple regressions are shown in Table 6, as the model number 6.

We can see that model (6), which includes the product of latitude with altitude and the product of longitude with altitude, produces better results than model (5), which includes only altitude.

(B) Nursery
Dependence of Douglas-fir seed germination in the nursery (Y_2) on latitude (X_1)

Dependence of Douglas-fir seed germination in the nursery on latitude was determined by the parameters in Table 6, model number 7.

The model point out that the nursery seed germination parabolically increases with the increased provenance latitude. This dependence is, statistically speaking, expressed more clearly.

Dependence of Douglas-fir seed germination in the nursery (Y_2) on longitude (X_2)

Dependence of Douglas-fir provenance germination in the nursery on longitude was determined by the parameters in Table 6, model number 8.

The model shows that the nursery seed germination parabolically increases with the increased provenance longitude.

Dependence of Douglas-fir seed germination in the nursery (Y_2) on altitude (X_3)

Dependence of the provenance seed germination in the nursery on altitude was determined by the parameters in Table 6, model number 9.

The model point out that the nursery seed germination parabolically decreases with the increased provenance altitude.

Table 6. Statistical tests in laboratory and nursery.

(A) Laboratory			(B) Nursery		
Observed correlation	**Model No**	**Regression equation**	**Observed correlation**	**Model No**	**Regression equation**
Dependence of Douglas-fir seed germination in the laboratory (Y_1) on latitude (X_1)	(1)	$Y_1 = -6045.75 + 233.525 \cdot X_1 - 2.21678 \cdot X_1^2$ $R^2 = 48.64$ %, $S_t = 9.20$ %, $F = 4.74$ (0.0357)	Dependence of Douglas-fir seed germination in the nursery (Y_2) on latitude (X_1)	(7)	$Y_2 = 16790.1 - 674.828 \cdot X_1 + 6.7929 \cdot X_1^2$ $R^2 = 28.78$ %, $S_t = 8.59$ %, $F = 2.02$ (0.1833)
Dependence of Douglas-fir seed germination in the laboratory (Y_1) on longitude (X_2)	(2)	$Y_1 = -19123.4 + 325.669 \cdot X_2 - 1.37997 \cdot X_2^2$ $R^2 = 7.68$ %, $S_t = 12.33$ %, $F = 0.42$ (0.6707)	Dependence of Douglas-fir seed germination in the nursery (Y_2) on longitude (X_2)	(8)	$Y_2 = 1599.81 - 30.3381 \cdot X_2 + 0.144667 \cdot X_2^2$ $R^2 = 43.29\%$, $S_t = 7.67\%$, $F = 3.82$ (0.0586)
Dependence of Douglas-fir seed germination in the laboratory (Y_1) on altitude (X_3)	(3)	$Y_1 = 113.95 - 0.0242805 \cdot X_3 - 0.00000699164 \cdot X_3^2$ $R^2 = 33.29$ %, $S_t = 10.48$ %, $F = 2.49$ (0.1321)	Dependence of Douglas-fir seed germination in the nursery (Y_2) on altitude (X_3)	(9)	$Y_2 = 89.5408 - 0.0954227 \cdot X_3 + 0.0000347496 \cdot X_3^2$ $R^2 = 68.53$ %, $S_t = 5.71\%$, $F = 10.89$ (0.0031)
Dependence of Douglas-fir seed germination in the laboratory (Y_1) on latitude (X_1), longitude (X_2) and altitude (X_3)	(4)	$Y_1 = -33209.4 - 37.8105 \cdot X_1 + 580.332\, X_2 - 0.0927229 \cdot X_3 + 0.499367 \cdot X_1^2 - 2.48145 \cdot X_2^2 + 0.0000809411 \cdot X_3^2$ $R^2 = 89.02$ %, $S_t = 5.49$ %, $F = 8.11$ (0.0112)	Dependence of Douglas-fir seed germination in the nursery (Y_2) on latitude (X_1), longitude (X_2) and altitude (X_3)	(10)	$Y_2 = 6146.55 - 484.514 \cdot X_1 + 101.413 \cdot X_2 - 0.125137 \cdot X_3 + 4.84896 \cdot X_1^2 - 0.424873 \cdot X_2^2 + 0.0000605416 \cdot X_3^2$ $R^2 = 78.19$ %, $S_t = 6.14$ %, $F = 3.59$ (0.0728)
	(5)	$Y_1 = -487.889 + 11.5091 \cdot X_1$ $R^2 = 47.89$ %, $St = 8.83$ %, $F = 10.11$ (0.0088)		(11)	$Y_2 = 68.8286 - 0.0400435 \cdot X_3$ $R^2 = 67.47$ %, $S_t = 5.54$ %, $F = 22.82$ (0.0006)
	(6)	$Y_1 = 124.403 + 0.00816858 \cdot X_1 \cdot X_3 - 0.00382914 \cdot X_2 \cdot X_3$ $R2 = 67.17$ %, $St = 7.35$ %, $F = 10.23$ (0.0038)		(12)	$Y_2 = 69.3253 - 0.000813673 \cdot X_1 \cdot X_3$ $R^2 = 66.84$ %, $S_t = 5.92$ %, $F = 22.17$ (0.0006)

Note: *p*-values are shown in the parentheses.

Statistically speaking, this dependence is so far the clearest one.

Dependence of Douglas-fir seed germination in the nursery (Y_2) on latitude (X_1), longitude (X_2) and altitude (X_3)

Several regression models (additive and substitutive) were applied. It is interesting to note that additive models produced better results.

The additive model with fixed independent are present in Table 6, model number 10.

Although 78.19 % of the variance of the provenance germination rate under nursery conditions was explained, some regression coefficients in model (10) were not statistically significant, which also applied to the regression as a whole. Therefore, we applied the method of (stepwise) multiple regression.

The results are present in Table 6, as model number 11.

Model (11) included only altitude, which accounted for 67.47 % of the observed correlation. The regression was statistically significant at a 99 % confidence.

Substitutive model (with the multiplication of independent variables) together with the method of stepwise multiple regressions are present in Table 6, as model number 12.

It is obvious that model (12), which included the product of latitude with altitude, didn't produce better results than model (11), which included only altitude.

Testing the difference in seed germination between Douglas-fir provenances under different conditions Comparison of medians

The difference between the medians of two samples (a provenance under laboratory and a provenance under nursery conditions) was tested Mann-Whitney *W*-test.

The result of the test is: $W = 169.0$, $P = 0.00002$

It was concluded that the difference between the seed germination medians in the laboratory and in the nursery was statistically significant at a 99 % confidence interval.

Comparison of proportions

The difference between the proportions of two samples (a provenance under laboratory and a provenance under nursery conditions) was tested by z-statistic test.

The result of the test is: $z = –40.0913$, $P = 0.0000$

It was concluded that the difference between the proportions of seed germination under laboratory and under nursery conditions was statistically significant at a 99 % confidence interval.

Both tests showed that the difference between the achieved seed germination rates under laboratory and under nursery conditions had a high statistical significance. Seed germination rate was much higher under laboratory conditions than in the nursery. The germination ratio was approximately 3:1.

Conclusions

The most important conclusion that can be drawn from this study is that the justification for performing the seed germination test of Douglas-fir provenances was proved. There are significant differences between the seed germination rates of Douglas-fir provenances under laboratory and nursery conditions. The compatibility of seed germination of thirteen common provenances under different conditions is

r = 0.478 (p = 0.098). The percentage of seed germination was higher in the laboratory and ranged from 66 to 98 %, while it was 21–51 % under nursery conditions. Provenances 3, 8, and 9 had the highest germination percentage.

The dependence of seed germination rate of Douglas-fir provenances on latitude, longitude and altitude was statistically determined under laboratory and under nursery conditions. We concluded that:

– The seed germination rate increases parabolically with the increased latitude of Douglas-fir provenances both in the laboratory and nursery. The relationship is stronger in the laboratory.

– The seed germination rate increases parabolically with the increased longitude of Douglas-fir provenances both under laboratory and under nursery conditions. The relationship is stronger under nursery conditions.

– The seed germination rate decreases parabolically with the increased altitude of Douglas-fir provenances both under laboratory conditions and in nursery. Dependence is stronger in the nursery. This dependence is statistically most pronounced.

Comparisons of medians and comparisons of proportions of two samples (thirteen provenances under laboratory and under nursery conditions) showed that the difference in the achieved seed germination rate of Douglas-fir provenances is statistically significant at a 99 % confidence interval. Considerably higher germination rate was achieved under laboratory conditions than in the nursery. The ratio was approximately 3:1.

Our future research studies will include the analysis of some additional aspects of Douglas-fir seed germination, all with the aim of getting a deeper insight into the problem of reliable selection of the provenances to be introduced into the forest sites of Serbia.

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