### CORRELATION BETWEEN SEEDLING LENGTH AND CANADIAN DOUGLAS-FIR HEIGHT

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Abstract: This paper presents the results of the studies of Douglas-fir seeds from Canada aimed at understanding and controlling the processes of the genetic growth potential of Douglas-fir in Serbia. The research was focused on the early stage of Douglas-fir growth, *i.e.* at the stage of seed germination in the laboratory. We tested the correlation between seed germination, seedling length and the height of plants in the nursery. The seeds from 13 Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) localities were obtained from Canada and compared under laboratory and nursery conditions. The seeds from different altitudes, latitudes and longitudes come from a part of the natural range of Douglas-fir in Canada. Before they are transferred and introduced, seed material must be tested with regard to the potential success of the selected tree species within the shortest possible time. This is necessary for the introduction of a tree species with a widespread natural range. Douglas-fir is a highly productive coniferous tree species with a broad geographical and ecological range. It has a wider natural range than other conifers and greater chances of successful adaptation to new ecosystems. Descriptive statistics, analysis of variance for regression, regression and correlation were used to analyse the data. A strong correlation was established between the height of four-year-old seedlings and seed characteristics (germination rate and seedling length).

Key words: Douglas-fir; seed transfer; introduction; seedling length; growth

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#### **INTRODUCTION**

Good-quality planting material and appropriate adaptive and fast-growing tree species are required both by the timber industry and in the reforestation of degraded sites. From the aspect of forestry, Douglas-fir is considered a promising tree species for the region of Central Europe (Kenk and Ehring, 1995; Kantor et al., 2001; Martiník et al., 2014). It originates from North America, and during the past century, it has become a very important introduced species in Europe, where it has established many hectares of plantations. Movement of seeds from their collection site to other environments within a species range for reforestation or restoration may increase the risk of maladaptation (Campbell and Sugano, 1979).

Introduction cannot be successfully done without testing because the transfer of seeds carries the risk of

low adaptability to new environment and site conditions (Corona et al., 1998; Dunbar et al., 2002; Fontes et al., 2003; Kantor et al., 2001; Petkova, 2011; Podrázský et al., 2013). The program of testing Douglasfir in Serbia started with the introduction of original seed and setting several Douglas-fir provenance tests in Serbia. Initial tests were set with the provenances native to North America. A number of investigations were conducted in the field and different traits were tested (Lavadinović et al., 1996; Lavadinović et al., 1997; Lavadinović and Koprivica, 1999).

Strong genetic control of introduced species should be carried out at the juvenile stage of the tested plant material. Seeds are an excellent system for studying fundamental developmental processes in plant biology (Allen et al., 2007). The present study aimed to determine the potential of seed transfer from natural Douglas-fir populations (from a part of the natural range in Canada) and to confirm the feasibility of its introduction into new site conditions in Serbia. The goal of this research was to define the expected relationship – the correlation between the seed traits in the laboratory and the height of young plants that emerged from these seeds in the nursery. In other words, our attempt was to answer the question whether the genetic potential of Douglas-fir (Silen, 1978) is more clearly expressed in the early stages of its development, i.e. if we study the traits of its seed and young plants.

#### MATERIALS AND METHODS

#### Sample seed collection

This study analyzes seeds from 13 Douglas-fir sources in Canada. The seeds was collected in a part of the native range of Douglas-fir distribution in Canada and obtained through the "Canadian Forest Service" from British Columbia. The geographical characteristics are given in Table 1.

#### Material processing

The basic seed pre-sowing treatment started with three-month seed stratification (Sorensen, 1991). The

seeds were rolled in a moist paper towel, sealed inside a plastic bag and placed in the refrigerator at 5°C. Filter paper was changed every seven days in order to control and prevent seed infection.

#### Laboratory analysis - germination test

In this study, Douglas-fir seed germination and the length of hypocotyls were studied at the Institute for Forestry (in the accredited Laboratory for Seed Testing-Accreditation Body of Serbia - SRPS ISO 17025). This was carried out in the germinator (Copenhagen tank or Jacobsen table), where the temperature of water was controlled by an electronic temperature controller (Kamra, 1968). The temperature program of 25°C for a period of 14 h during day and 20°C for 10 h during night (ISTA, 1985) was applied. The number of germinated seeds was recorded every 2-4 days. A seeds were considered to have germinated when the emerged radicle was at least twice the length of the seed (Doody and O'Reilly, 2005). Germination counts were taken for 21 days. Five replicates of 100 seeds from each provenance were placed on the filter paper of the Jacobsen apparatus, under controlled moisture and temperature regimes. After 21 days, the length of seedlings, i.e. the length of their hypocotyls and radicles, was measured.

Table 1. Geographical characteristics	of Douglas	-fir seeds	from	Canada
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Provenances		Sood zono	Location	Geographical parameters		Altitudo (ma)
No	Code	Seed Zolle	Location	Latitude	Longitude	Attitude (III)
1	03333	East Kootenay	Cranbrook	49° 25'	115° 20'	1050
2	00848	West Kootenay	Inonoaklin	49° 50'	118º 10'	671
3	30667	Shuswap Adams	Mann Creek	51° 35'	120º 10'	600
4	05227	East Kootenay	Gavia Lake TFL 14	50° 56	116° 35'	1070
5	05226	East Kootenay	Nine Bay TFL 14	50° 58 <sup>°</sup>	116° 32'	975
6	03356	Thompson Okanagan	Trout Cr	49° 40 <sup>°</sup>	119° 52'	884
7	03360	Thompson Okanagan	Michell CR	49° 54	119º 37'	1035
8	30460	Shuswap Adams	Mara LK	50° 48'	119°00'	488
9	00278	Thompson Okanagan	Monte Crk	50° 37'	119º 52'	701
10	03383	West Wootenay	Sheep Creek	49º 10 <sup>°</sup>	117° 15'	1000
11	30461	Shuswap Adams	Cooke Creek	50°38 <sup>°</sup>	118° 49'	900
12	03389	West Kootenay	Benton Creek	49° 12 <sup>°</sup>	117° 25'	933
13	05092	East Kootenay	Sun Creek	50° 08'	115° 52'	1000

### Description of the nursery plants

The experiment carried out under nursery conditions used cool stratification of seeds that were sown in containers, where they stayed for two years. Sowing was done in the spring, as indicated as more effective by Sorensen (1978, 1991). The plants were then transplanted into plastic flowerpots where they stayed for another two years. The height of the plants was measured at the age of 4 years.

#### Statistical analysis

Data on the characteristics of seed germination and the height of the plants from Douglas-fir provenances were analyzed using descriptive statistics, analysis of variance for regression, regression and correlation. The methods of simple and multiple regression and correlation analysis were used to test the dependence of the height of plants on seed vigor and the length of seedlings.

#### RESULTS

#### Descriptive statistics of Douglas-fir provenances

The average germination rates of Douglas-fir seeds, the average length of seedlings and the average height of four-year-old Douglas-fir plants are presented in Table 2. Table 3 gives the basic statistics for selected variables ( $X_1$ ,  $X_2$  and  $X_3$ ). Spearman rank correlation coefficients between the studied traits of the investigated provenances are presented in Table 4.

# Dependence of the height of plants of Douglas-fir provenances $(X_3)$ on seed germination rate $(X_1)$

The properties of the linear regression model are as follows:

 $X_3 = 12.9342 + 0.21952X_1$ ;  $S_2 = 4.25$  cm;  $R^2 = 28.55\%$ 

The result of analysis of variance (ANOVA: F = 4.40; P = 0.0600) showed that the observed relationship was not statistically significant at the 5% risk level.

Provenance number	Code	Seed germination in laboratory (%) X <sub>1</sub>	Average length of seedlings(cm) $X_2$	Height of four-year old plants (cm) X <sub>3</sub>
1	03333	71	2.50	28.4
2	00848	96	3.22	37.8
3	30667	98	4.01	38.9
4	05227	95	3.07	27.7
5	05226	91	2.90	29.7
6	03356	70	2.36	30.5
7	03360	66	2.72	29.1
8	30460	98	3.98	40.2
9	00278	98	3.98	34.5
10	03383	86	3.24	35.3
11	30461	97	3.85	32.2
12	03389	81	3.18	28.0
13	05092	88	3.93	25.0

**Table 2.** The characteristics of seeds and height of plants of the studied Douglas-fir

#### Table 3. Summary of statistics

Statistical manamatana	Variables *			
Statistical parameters	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	
Count	13	13	13	
Average	87.31	3.30	32.1	
Standard deviation	11.71	0.59	4.81	
Coefficient of variation	13.42	17.96	14.99	
Minimum	66.0	2.36	25.0	
Maximum	98.0	4.01	40.2	
Range	32.0	1.65	15.2	
Standard skewness	-1.236	-0.175	0.611	
Standard kurtosis	-0.597	-1.036	-0.783	

<sup>\*</sup>X<sub>1</sub> – Germinated seeds in the laboratory (%);

X<sub>2</sub> – Average length of seedlings (cm);

X<sub>3</sub> - Height of four-year-old plants (cm)

Fable 4. Spearma	an Rank	Corre	lations
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Variables	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>
X <sub>1</sub>	-	0.8354	0.5912
		(13)	(13)
		0.0038	0.0406
X <sub>2</sub>		-	0.5117
			(13)
			0.0763
X <sub>3</sub>			-

## Dependence of the height of plants of Douglasfir provenances $(X_3)$ on length of laboratory seedlings $(X_2)$

The properties of the linear regression model are as follows:

 $X_3 = 19.9289 + 3.68479 X_2$ ;  $S_2 = 4.48$  cm;  $R^2 = 20.64\%$ 

The result of ANOVA (F = 2.86; P = 0.1188) showed that the observed relationship was not statistically significant at the 5% level of risk.

Provenance 13 attained an extremely small height of plants. Therefore, the statistical parameters of the linear regression model are presented without this provenance.

 $X_3 = 15.2452 + 5.36677 X_2$ ;  $S_2 = 3.37$  cm;  $R^2 = 49.02\%$ 

The result of ANOVA (F = 9.61; P = 0.0113) showed that the observed relationship was statistically significant at the 5% level of risk.

### Dependence of the height of plants of Douglas-fir provenances $(X_3)$ on the seed germination rate $(X_1)$ and length of laboratory seedlings $(X_2)$

The characteristics of the multiple linear regression model is as follows:

$$X_3 = 13.0743 + 0.193907X_1 + 0.634588X_2;$$
  
 $S_1 = 4.45 \text{ cm}; \text{ } \text{R}^2 = 28.78\%$ 

The result of ANOVA (F = 2.02; P = 0.1813) showed that the observed relationship was not statistically significant at the 5% level of risk.

# Dependence of the laboratory length of seedlings $(X_2)$ on seed germination rate $(X_1)$

The properties of the linear regression model are as follows:

 $X_2 = -0.220878 + 0.0403625X_1;$  $S_e = 0.37 \text{ cm}; \text{ } \text{R}^2 = 63.49\%$ 

The result of ANOVA (F = 19.13; P = 0.0011) showed that the observed relationship was statistically significant at the 5% level of risk.

#### DISCUSSION

The average germination rate of Douglas-fir seeds under laboratory conditions (in a germination bed) was 87.3%, while the average seedling length amounted to 3.3 cm and the plant height to 32.1 cm. The standard deviation was 11.7% for germination rate, 0.59% for seedling length and 4.81% for plant height. Coefficients of variation were 13.42%, 17.96% and 14.99%, respectively. Provenance 7 had the lowest germination rate under laboratory conditions (66%). On the other hand, provenances 3, 8 and 9 achieved the highest rate of 98%. Provenance 6 had the smallest length of seedlings; their average length was 2.36 cm. The seedlings of provenance 3 grew to a maximum average length of 40.1 cm.

The plants of provenance 13 had the smallest height at age of four (25 cm), while the plants of provenance 8 attained the greatest height of 40.2 cm. The investigated provenances showed significant differences in the achieved seed germination rates, as well as in the length of seedlings and height of plants.

The correlation of seed germination rate under laboratory conditions with the length of seedlings and height of plants was statistically significant at the 5% level of risk. The correlation between the length of seedlings and the height of plants was not statistically significant at the 5% level of risk.

This paper presents the results of the first research studies of Douglas-fir seeds from Canada, with the aim of understanding, stimulating and controlling the processes in the genetic potential of Douglas-fir, which should facilitate the selection of provenances for introduction. Based on the obtained results it can be concluded that there is a significant difference in the achieved percentages of seed germination rate, seedling length and height of the four-year-old plants of different provenances of Douglas-fir and the process of future testing and selection of Douglasfir provenances should take into account the results of the laboratory analysis of seeds and the growth of plants in their juvenile phase of development.

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Authors' contributions: Vera Lavadinović set the goal and coordinated the research, controlled the quality of work and tasks and was responsible for research implementation. Vukan Lavadinović participated in defining and organizing information and in the consultation of literature and references; performed statistical analysis of data. Zoran Poduška participated in the experiment program and analysis of research results. Ilija Đorđević carried out tests in the laboratory and nursery, processed research results.

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