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Original Article

Correlation between the phenology of leafing and growth characteristics of Douglas-fir provenances in Serbia

Sırbistan'da Douglas göknarının gelişim özellikleri ile yapraklanma fenolojisi arasındaki korelasyon

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ABSTRACT

The study of periodic plant life cycle events, or phenology, is of great importance both in the process of species introduction and in the period of its adaptation to new environmental conditions. We assumed that the leaf-out process correlates with the elements of plant growth. The research we focused on these dependencies in order to select the provenances which would be the most suitable for introduction in Serbia. The research was aimed at investigate the dependencies of leafing on the height, diameter, and height increment of Douglas-fir provenances within the experiment in Serbia. The experiment was established using native seed material originating from different provenances from a part of the natural range of Douglas-fir distribution in North America. Therefore, it has a significant effect on the elements of plant growth. The obtained data were analyzed using descriptive statistics, analysis of variance, regression and correlation. It was concluded that the correlation between the time of leaf-out (bud break) and morphological characters of Douglas-fir trees of different provenances was statistically weak. The correlation was the strongest between the time of leaf-out and the height of plants.

Keywords: *Pseudotsuga menziesii*, introduction, leaf-out, height, height increment

ÖZ

Periyodik bitki hayat döngüsü olaylarını inceleyen fenoloji, türlerin başlangıç süreci ve de yeni çevre koşullarına adaptasyon sürecinde önemlidir. Yapraklanma sürecinin bitkinin büyüme unsurlarıyla ilişkili olduğunu düşündük. Bu çalışmada, Sırbistan'da Douglas göknar ağacının yetiştirilmesi için en uygun olan kaynakları seçmek amacıyla bu faktörlere odaklandık. Yapraklanmanın yükseklik, çap ve yükseklik artışına olan bağımlılığının incelenmesi amaçlandı. Deney Kuzey Amerika'da doğal Douglas göknarı dağılımı gösteren bölgeden farklı kaynaklardan alınan doğal tohum materyali kullanılarak yapıldı. Bu nedenle, bitkinin gelişimi unsurları üzerinde anlamlı etkiler gözlendi. Elde edilen veriler tanımlayıcı istatikler, varyans analizi, regresyon ve korelasyon kullanılarak değerlendirildi. Yapraklanma (tomurcukların patlaması) zamanı ile farklı kaynaklardan alınan Douglas göknarının morfolojik özellikleri arasındaki korelasyon istatiksel olarak zayıftı. Diğer yandan, yapraklanma zamanı ile bitkilerin yükseklikleri arasında daha güçlü bir ilişki izlendi.

Anahtar Kelimeler: *Pseudotsuga menziesii*, başlangıç, yapraklanma, yükseklik, yükseklik artışı

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INTRODUCTION

Phenology studies the timing of bud dormancy breaking and leafing or opening of dormant winter buds. It has emerged from the shadows to become a major component of climate change studies. In part this is because the phenological responses of species to temperature, particularly in plants, are very strong. Leafing-out of woody plants marks the beginning of a growing season in forests and it is one of the most important drivers of ecosystem processes. There is substantial variation in the timing of leaf-out, both within and among species, but the leaf development of almost all tree and shrub species is highly sensitive to temperature (Polgar and Primack, 2011).

Carolus Linnaeus is looked upon as the father of modern phenological networks. The first known phenological network was installed by him in Sweden in the middle of the 18th century. In his work `*Philosophia Botanica*`, he outlined methods for compiling annual plant calendars of leaf opening, flowering, fruiting and leaf fall, together with climatological observations "... so as to show how areas differ..." (Linnaeus, 1751).

Phenological change is relatively easy to identify, especially in comparison with changes in distribution, fecundity, population size, morphology, etc. Even with the relatively modest levels of global warming experienced so far, phenological change has become very evident (Sparks et al., 2009a; Sparks et al., 2009b).

Genetic regulation of the leaf-out process has been well-documented (Campbell and Sugano, 1975; White et al., 1979; Li and Adams, 1993;Myking and Heide, 1995). Furthermore, many authors (Campbell and Sugano, 1979 ; White et al., 1979; Li and Adams, 1993; Myking and Heide, 1995; Partanen et al., 1998; Vesk and Westoby, 2004; Ziello, et al. 2009; Caroline and Primack, 2011) have investigated phenological p*rocesses* by which *bud burst* is *synchronized* to *seasonal cycles*.

Phenological recording of plants in Europe has a long history and is currently concentrated in the hands of state meteorological agencies and focused on species of commercial importance (agricultural and horticultural crops, forest trees) or amenity species associated with human settlements (Nekovar et al., 2008).

Conifer growth is driven by genetic potential, physiological and morphological responses to environmental conditions (Harrington and Chain, 1994), which gives special importance to phenological studies of introduced species.

Douglas-fir (*Pseudotsuga menziesii*, Mirb/Franco) is a conifer species of high commercial importance in Canada and North America (Arno et al.,1977). In Europe, it has been recognized as a productive fast-growing species with a high degree of adaptability. Adapted to a moist, mild climate, it grows bigger and more rapidly than the in land variety. These trees commonly live more than 500 years and occasionally more than1,000 years (Franklin et al., 1981; Hermann et al.,1990).

Provenance test is the best way to test seed transfer, genetic identity and potential growth of Douglas-fir trees. Otherwise, introduction may be risky and time-consuming, the choice of provenance wrong and its adaptation inadequate. The model of provenance test stresses the significance of genetic differences and reveals significant differences and dependencies that provenances show in the given period of phenological research. Provenance tests are often used to determine genetic responses of seed sources to transfer to different climates (Schmidtling 1994).

The phenology of bud burst is fundamental to tree survival and growth in temperate and boreal regions of the world (Sakai and Larcher, 1987; Bailey and Harrington, 2006). Plants have evolved mechanisms to use photoperiod and temperature cues to balance the benefits from early bud burst with the probability of significant damage from spring frost (Sakai and Larcher, 1987; Hannerz, 1999).

Early expansion of vegetative tissue is advantageous for producing biomass and maintaining site dominance; however, early tissue expansion also increases the risk of frost damage from late-spring freezing temperatures (Heide, 2003). In species with preformed growth, buds are the plant investment for the crown growth in the next year (Heide, 2003).

The task of plant phenology is to observe and record the periodically recurring growth stages and to study the regularities and dependency of the yearly cycles of development on environmental conditions (Koch and Scheifinger, 2004; Koch et al., 2009).

Since provenance test is the best way to test seed transfer, genetic identity and potential growth of Douglas-fir trees, the Institute of Forestry in Belgrade established the first tests of Douglas-fir in Serbia with native seed material originating from Canada and North America (Lavadinović and Koprivica, 1996; Lavadinović and Koprivica, 1999; Lavadinović et al., 2001).

MATERIALS AND METHODS

Seed Material

The collection of native Douglas-fir seeds, which was obtained from the part of the natural range of Douglas-fir in North America (Table 1), was used to produce seedlings. Having been given the appropriate treatment, 2+2 transplants were fit to be planted on the mountain of Juhor (central Serbia).

Table 1. Geographical features of Douglas-fir provenances in Juhor experiment

	Provenance Code	Code number	Latitude (º)	Longitude (º)	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	205-37	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

The average latitude of Douglas-fir provenances in the sample is 44.0 degrees, the longitude is 120 degrees and the altitude amounts to 1010 m above sea level. The coefficients of variation are 8.7%, 4.2% and 67.1% respectively.

Juhor Provenance Test

Douglas-fir provenance test on the mountain of Juhor (43°47'N, 18°58'E and 650 m. a.s.l.) was established on the site of mountain beech forest (*Fagetum moesiaca montanum* Jovanović 1976). It was set in a random block system. The test included 20 provenances with 15 repetitions (blocks). The blocks were arranged in the direction of contour lines. The plants were spaced 2 x 2 m. The same spacing was between the provenances and the blocks were spaced 4 m apart. Each block included twelve plants of each provenance, which means that each block had 240 plants, and the whole test 3600 plants, covering an area of about 2 ha. Parent rock contains only shales and soil is brown forest soil. This area is under the influence of humid continental climate, which is characterized by warm and dry summers, with the temperature ranging from 20 to 35°C and harsh and cold winters with the temperatures between 0 to -15°C, with sharp frosts and a deep snow cover. This area is typically characterized by uneven distributions of rainfall during the year.

Method of Data Collecting and Processing

Field research included measurements of tree dendrometric characters (height, diameter at breast height and height increment) and recording of *bud flushing phenology* in different provenances (number of days from the beginning of the calendar year to the date of leaf-out). A caliper was used to measure tree diameters at 1.3 meters above ground, with an accuracy of 1 mm. The height and height increment were measured using a tree measuring stick, with an accuracy of 1 cm. Phenological characteristics (changes in the development of buds) were recorded twice a week and the observed changes were recorded in the Manual for the collection of field data. The results of the measurements of the morphological characters of Douglas-fir trees and monitoring of phenological characteristics are shown in Table 2.

Several statistical methods were used for the purpose of processing data on leaf-out phenology and the elements of tree growth in different Douglas-fir provenances. They are: descriptive statistics, analysis of variance, regression and correlation.

The correlation between the timing of leaf-out and morphological characters (height, diameter and height increment) of the trees belonging to the studied Douglas-fir provenances were studied using the methods of simple and multiple regression and correlation.

RESULTS AND DISCUSSION

Descriptive Statistics of the Observed Provenance Characteristics

Table 3 presents the most important statistical indicators of leaf-out timing (Y) and height (X1), diameter (X_2) and height increment (X_3) of Douglas- fir provenances.

Table 2. Leaf-out timing and morphological characters of Douglas-fir trees in Juhor provenance test

Provenance number	Leaf-out (day)	Bud break (date)	Height (m)	Diameter (cm)	Height Increment (cm)
1	145.6	25.05.	5.16	11.5	89
2	143.3	23.05.	5.17	11.3	76
3	146.8	26.05.	5.58	12.0	88
4	146.5	26.05.	4.99	10.8	84
9	146.6	26.05.	2.82	6.5	43
10	147.7	27.05.	5.06	10.9	83
11	142.8	22.05.	5.24	11.8	85
12	144.8	24.05.	4.72	11.0	80
15	149.2	29.05.	5.16	11.3	83
16	148.7	28.05.	3.53	8.2	56
17	136.6	16.05.	3.48	8.2	57
18	147.0	27.05.	5.37	11.6	84
20	150.0	30.05.	5.10	11.6	86
22	141.9	21.05.	3.94	9.0	64
23	139.7	19.05.	3.70	8.1	62
24	147.0	27.05.	2.86	6.7	45
26	143.3	23.05.	4.86	11.2	82
27	144.6	24.05.	4.63	10.4	78
30	147.3	27.05.	4.69	10.3	77
31	155.1	04.06.	5.29	10.6	77

Table 3. Basic statistical indicators of the observed variables

	Variables			
Statistical indices	Y_1	X_1	X_2	X_3
Count	20	20	20	20
Average	145.72	4.56	10.15	73.95
Standard deviation	3.95	0.85	1.72	14.22
Coeff. of variation (%)	2.71	18.74	16.95	19.24
Minimum	136.6	2.82	6.50	43.0
Maximum	155.1	5.58	12.0	89.0
Range	18.5	2.76	5.5	46.0
Standard skewness	-0.18	-1.77	-1.88	-1.99
Standard kurtosis	1.37	-0.33	-0.19	-0.01

In the following analyses the time of bud break is taken as the dependent variable and the independent variables are: height, diameter, and height increment.

Table 4. The coefficients of linear correlation of the observed variables

Variables	Y	X_1	X_2	X_3
Y	-	0.3280	0.2342	0.2329
		(20)	(20)	(20)
		0.1580	0.3202	0.3231
X1		-	0.9808	0.9669
			(20)	(20)
			0.0000	0.0000
X2			-	0.9802
				(20)
				0.0000
X3				-

Table 5a. The parabolic regression model

Parameter	Estimate	Error	Statistic	p
Constant	181.721	24.8326	7.31784	0.0000
X_1	-19.9618	12.2606	-1.62812	0.1219
X_1^2	2.55959	1.45678	1.75702	0.0969

Table 5b. Analysis of variance for regression

Source	Sum of Squares	Df	Mean Square	F-Ratio	p
Model	72.4194	2	36.2097	2.75	0.0920
Residual	223.478	17	13.1458		
Total	295.897	19			

Regression equation is $Y=181.721–19.9618\ X_1+2.55959\ X_1^2$.
Coefficient of determination is 24.47%.
Standard error of regression is 3.63 days.

Table 6a. The parabolic regression model

Parameter	Estimate	Error	Statistic t	p
Constant	164.44	33.4568	4.915	0.0001
X_2	-4.8425	7.36632	-0.657384	0.5197
X_2^2	0.287578	0.39275	0.732218	0.4740

Table 6b. Analysis of variance for regression

Source	Sum of Squares	Df	Mean Square	F-Ratio	p
Model	24.7854	2	12.3927	0.78	0.4754
Residual	271.112	17	15.9478		
Total	295.897	19			

Regression equation is $Y=164.44–4.8425\ X_2+0.287578\ X_2^2$
Coefficient of determination is 8.37%.
Standard error of regression is 3.99 days.

Table 7a. The parabolic regression model

Parameter	Estimate	Error	Statistic t	p
Constant	169.3	23.4379	7.22331	0.0000
X_3	-0.829983	0.727106	-1.14149	0.2695
X_3^2	0.0066779	0.00540745	1.23494	0.2336

Table 7b. Analysis of variance for regression

Source	Sum of Squares	Df	Mean Square	F-Ratio	p
Model	39.0895	2	19.5447	1.29	0.2999
Residual	256.808	17	15.1064		
Total	295.897	19			

Regression equation is $Y=169.3–0.829983\ X_3+0.0066779\ X_3^2$
Coefficient of determination is 13.21%.
Standard error of regression is 3.88 days.

The Correlation between the Observed Characteristics of Provenances

Table 4 gives the coefficients of simple linear correlation (with the sample size and statistical significance) between the observed characteristics of Douglas-fir provenances.

We are most interested in the coefficient of correlation between the budbreak timing (the number of days from the beginning of the year) and the tree height, diameter, and height increment.

Regression between the Observed Provenance Characteristics

Linear and parabolic dependence of the bud break (leaf-out) on the morphological characters of different provenances of Douglas fir trees was preliminary tested. Based on the obtained values of statistical indicators of regression and correlation, it was concluded that in all cases the curvilinear relationship was slightly better than the linear one. Therefore, we present only the results of parabolic regression and correlation.

Dependence of the Leaf-Out Timing (Y) on the Provenance Height (X_1)

Tables 5a and 5b present statistical characteristics of the observed parabolic regression model.

Dependence of the Leaf-Out Timing (Y) on the Provenance Diameter (X_2)

Tables 6a and 6b present statistical characteristics of the observed parabolic regression model.

Dependence of the Leaf-Out Timing (Y) on the Provenance Height Increment (X_3)

Tables 7a and 7b present statistical characteristics of the observed parabolic regression model.

Dependence of the Leaf-Out Timing (Y) on the Provenance Height (X_1), Diameter (X_2) and Height Increment (X_3)

Tables 8a and 8b present statistical indicators of the multiple curvilinear regression model.

Table 8a. Multiple curvilinear regression model

Parameter	Estimate	Error	Statistic t	p
Constant	110.374	36.8723	2.99341	0.0104
X_1	105.136	73.8815	1.42304	0.1783
X_1^2	-9.17071	7.43524	-1.23341	0.2393
X_2	13.4992	26.6501	0.506533	0.6210
X_2^2	-0.922698	1.25712	-0.733977	0.4760
X_3	-7.46076	2.87993	-2.59061	0.0224
X_3^2	0.0468486	0.0177518	2.63909	0.0204

Table 8b. Analysis of variance for multiple curvilinear regression

Source	Sum of Squares	Df	Mean Square	F-Ratio	p
Model	174.251	6	29.0419	3.10	0.0411
Residual	121.646	13	9.35739		
Total	295.897	19			

Regression equation is $Y=110.374+105.136\ X_1-9.17071\ X_1^2+13.4992\ X_2-0.922698\ X_2^2-7.46076\ X_3+0.0468486\ X_3^2$
Coefficient of determination is 58.89%.
Standard error of regression is 3.05 days.

Table 8b shows that the observed multiple regression is statistically significant ($p<0.05$) on the whole. However, the impact of many variables in Table 8a is not statistically significant ($p> 0.05$). If we apply the stepwise regression, only the constant remains in the model.

Table 3 shows that the coefficient variation in the number of days (from the beginning of the year) to the bud break is very small (2.71%), which means that the studied Douglas-fir provenances do not differ much in this respect. The minimum number of days is 137 and the maximum 155. It means that all provenances open their buds within 18 days. Regarding height, diameter and height increment the provenances vary in a narrow range of 17% for diameters to 19% for height and height increment. The obtained results suggest that the correlation between the budbreak timing and the height, diameter, and height increment is small, which was later confirmed in the analysis performed by means of regression and correlation.

Table 4 shows that the none of the coefficients of correlation are statistically significant ($p<0.05$). The highest correlation coefficient is between the number of days to the bud break and height (0.328) followed by diameter (0.234) and height increment (0.233). In all cases, the relationship is linear and positive, which means that an increase in the height, diameter or height increment of Douglas-fir provenances prolong the budbreak timing (increases the number of days). Regression and correlation will provide a better insight into these results. The relationship between the elements of growth (height, diameter, and height increment) is naturally statistically significant ($p<0.05$).

Provenance height accounts for approximately 24.4% of the total provenance variance in the number of days to the leaf-out. The rest is due to some other factors that haven`t been considered by this research. Standard error of regression is 3.63 days.

Provenance diameter accounts for only 8.37% of the total provenance variance in the number of days to the leaf-out. Standard error of regression is 3.99 days.

Provenance height increment accounts for only 13.21% of the total provenance variance in the number of days to the leaf-out. Standard error of regression is 3.88 days.

It follows that the combined changes in the height, diameter, and height increment of provenances account for 58.89% of changes in the leaf-out timing of Douglas-fir provenances in the Juhor test. The standard error of regression is 3.05 days.

CONCLUSION

The research of different Douglas-fir provenances originating from North America was aimed at determining the dependences of plant leaf-out timing on the elements of growth. The following conclusions are:

Provenances don`t differ much in the date of leaf-out onset. The coefficient of variation is 2.71%. However, all the provenances that were included in the Juhor experiment leafed out within 18 days. This number of days can bring a lot of risks to plants, since spring is a season with sharp fluctuations in air temperatures. The plants of provenance 31 were the last to leaf out (on the 155^{th} day from the beginning of the year), while the plants of provenance 17 had the earliest leaf -out onset (on the 137^{th} day from the beginning of the year).

Since the provenances vary in height, diameter and height increment in a narrow range of 17% for diameter up to 19% for height and height increment, the dependence of the budbreak timing on height, diameter, and height increment is in the simple regression models small and statistically insignificant. However, the relationship obtained in the fixed model of a multiple curvilinear regression is statistically significant ($p<0.05$). The combined variation in the height, diameter, and height increment of provenances accounts for 58.89% of the variation in the leaf-out timing (bud break).

In all cases, there is a simple positive linear correlation, which means that with an increase in the height, diameter and height increment of Douglas-fir provenances, they increase the number of days to the bud break. More successful provenance (measured by the dimensions and height increments of their trees) broke buds later than the less successful ones. However, the parabolic relationship presented in this paper showed a bit more reliable values of statistical indicators.

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