

**VARIABILITY IN GERMINATION AND GERMINATION DYNAMICS OF
DIFFERENTLY TREATED SEEDS OF SERBIAN SPRUCE (*PICEA OMORIKA*
PANČIĆ/PURKYNĚ)**

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Cvetkovic B., M. Mataruga, V. Isajev, J. Levic, A. Lucic, V. Trkulja,
and Z. Kremenovic (2013): *Variability in germination and germination
dynamics of differently treated seeds of Serbian spruce (Picea omorika
Pančić/Purkyně)*. Genetika, Vol 45, No. 1, 109-119.

Genetic-physiological approach was used in the study of the quality of
Serbian spruce seed (*Picea omorika* /Pančić/Purkyně), collected in the
populations on the left bank of the river Drina. The seed originated from the
three populations that represent the overall ecological and productive conditions
of Serbian spruce populations in Bosnia and Herzegovina. Two natural
populations were selected: Veliki Stolac and Gostilj, and one planted forest
population in Srebrenica.

The cones were collected in late autumn 2009 and early spring 2010.
They were collected from five trees from Srebrenica and Gostilj, and seven trees
from Veliki Stolac. Immediately after they were collected, the cones and seeds
were processed and germination tests were done. Germination, germination
viability and germination dynamics were studied in three categories of seed
treatment: 1) control unit (just processed seed), 2) seed stored for six months at

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0-4 °C, 3) seed stored for seven months at 0-4° C and treated with fungicide after five months of storing. The number of germinated seed was observed and noted on the third, fourth, fifth, seventh, tenth, fourteenth, twenty-first and twenty-eighth day.

There were significant differences in germination dynamics at the population level and the level of treatment during the first couple of days of germination test. Fungicide (*captan*) acted as an inhibitor on seed germination process. The seeds originating from the largest population of Veliki Stolac showed the best response to storing treatment with fungicide related to germination dynamics.

The seed originating from Srebrenica and kept at low temperatures 0-4 °C without treatment with fungicides showed the best result in germination. Regardless of the presence of differences in the dynamics of germination, significant differences in germination regarding different treatments on the last day of the test, were not recorded.

Key words: Serbian spruce, seed treatment, germination, germination dynamics

INTRODUCTION

A closer study of Serbian spruce variability in natural sites and especially in a plantation, where tree size and layout make the research easier, provides the base for a quicker study of genetic and physiological base for seed production, as well as germination ability and germination dynamics of differently treated seeds of this species.

Serbian spruce (*Picea omorika* Pančić/Purkyně) is a tertiary relict and endemic species. It's areal occupies the mid-course of the river Drina in Bosnia and Herzegovina (B&H) and Serbia, and inhabits rocky and hardly accessible terrains, in the form of small isolated populations. It occurs spontaneously in just a few small populations and it is estimated that all populations are formed from about 10,000 individuals. Since 1998 it has been on the IUCN list as an endangered plant species (vulnerable) (IUCN, 2012). It is considered a species with the some features of depauperate species (NASRI *et al.*, 2008). The species is characterized by reduced genetic variation in natural habitats in B&H (BALLIAN *et al.*, 2006). To protect the gene pool and improve the production of planting material, *in-situ* conservation was carried out. Preservation of populations was conducted according to the best phenotype features and they serve only as a source of generative reproductive material. There are six populations protected in this way, four of them are seed stands (natural populations) and two are planted forests (MATARUGA *et al.*, 2005). Two natural populations in Višegrad area, and a planted forest from Srebrenica area, are the bases for seed production in nurseries in the Republic of Srpska (entity in B&H).

There have not been detailed studies of the influence of different treatments and storing methods on seed germination, nor of the response to different treatments, except for a few mid-twentieth century ones which referred to the seed germination originating from the populations which are more accessible. The research conducted on natural renewal in natural populations (DINIĆ, 1989a, 1989b, 1991a, 1991b; DINIĆ and OSTOJIĆ, 2009) and the influence of storing (BATOS and NIKOLIĆ, 2004) did not include seed treatment with fungicides or potential impact of such treatment on the dynamics of its germination. Fungicides are known to reduce the germination of spruce and pine and Douglas fir; (LITTKE, 1990, LITTKE, 1996), cause the slow

germination of pines and inhibit the germination of some other seeds such as Austrian pine, Norway spruce etc., but we do not know their impact on Serbian spruce seed. Recently, the dynamics of germination was studied for some other species in B&H, such as Austrian pine (MATARUGA *et al.* 2010; MATARUGA *et al.* 2011)

Serbian spruce seed is categorized as one of the most expensive seeds of woody plants in Europe. It is necessary to study and directly use the parameters of genetic-physiological characteristics of seeds as the basis for sustainable seedlings production in forestry, in natural populations in B&H and Serbia. Also, there is a great need to preserve and develop a new plant from each seed, especially in natural habitats that exist in B&H. Based on these facts, our aim was to collect Serbian spruce seed samples from the less accessible habitats and to examine the possibility of its prolonged storage after various seed treatments.

MATERIALS AND METHODS

Based on literature data for Serbian spruce sites in B&H, and the data from the Registry of forestry seed objects in the Republic of Srpska (Republic of Srpska - one of the entities in Bosnia and Herzegovina) (MATARUGA *et al.*, 2005) three populations that represent the overall genetic, environmental and production characteristics, were selected for the research. Two natural populations were selected in Višegrad area and those are the populations: Veliki Stolac and Gostilj, and one population of anthropogenic origin that has the same purpose as the two above-mentioned populations. The selected objects vary in ecological and production characteristics, and the basic data are given in table 1.

Table 1. Basic ecological data of studied populations

Population	Coordinates	Altitude [m]	Exposition	Parental rock	Soil	Plant community
Srebrenica	E 44°00'42,4" N 19°26'13,6"	860	North East	Andenzit	District cambisol	<i>Fagetum montanum</i>
Veliki Stolac	E 43°55'18,2" N 19°17'18,4"	1100-1600	North East	Limestone	Black soil	<i>Piceo-Abieti-Fagetum</i>
Gostilj	E 43°51'26,5" N 19°20'10,9"	1040-1304	North East	Limestone	Rendzine	<i>Pinetum nigrae</i>

We selected five representative individual from Srebrenica and Gostilj, and seven from Veliki Stolac (17 trees in total) and we collected cones (figure 1).

Cones were collected during December 2009 in population Srebrenica and in April 2010 in natural populations Veliki Stolac and Gostilj during a cold period. The collection was carried out from the tops of standing trees, and also from fallen trees during the winter 2009/2010. Climbing a tree was technically impracticable due to the characteristic type of a very narrow crown. Collected cones were packed in paper bags with labels of origin (population and individual) and transported to the laboratory.

Cone processing was carried out in laboratory conditions in a drying oven at a temperature of 40 °C. At this temperature, cone opening was fast, and the cones released seeds.

The obtained amounts of seed were separated into three equal parts that were planned for different treatments.



Figure 1. Populations where cones were collected

1 – Srebrenica, 2 – Veliki Stolac, 3 – Gostilj

The first part of the seed was only processed and serves as control seed. The second part of the seed was treated by being placed in storage for six months in fridge at a temperature from 0 to 4 °C (hereinafter referred to as processed and stored seed - PSS). The third part of the seed was placed in storage for five months at a temperature from 0 to 4 °C and then treated with fungicide Captan P50 and returned to the store for two more months (hereinafter referred to as processed, stored and fungicide treated seed - PSFS).

Germination was conducted according to ISTA standards. We studied the germination, germination viability for an extended period of 28 days at a variable temperature from 20 to 30 °C, and the dynamics of germination was monitored on the third, fourth, fifth, seventh, tenth, fourteenth, twenty-first and twenty-eighth day. Seed inactivity parameter was used as an indicator of the dynamics of seed germination.

$$SI = \frac{n_1 \cdot t_1 + n_2 \cdot t_2 + n_3 \cdot t_3 + \dots + n_n \cdot t_n}{n_1 + n_2 + n_3 + \dots + n_n}$$

Where the following are : SI – seed inactivity

$n_1, n_2, n_3 \dots n_n$ - number of germinated seeds

$t_1, t_2, t_3, \dots t_n$ - day number

Analysis of variance and Duncan's test were conducted following the instructions for the use of statistical analysis in agriculture (HADŽIVUKOVIĆ, 1991).

RESULTS AND DISCUSSION

Serbian spruce seed originating from different populations showed significant differences in germination viability, germination and germination dynamics. Significant differences exist between the different treatments that the seed had undergone. All tested seed, collected from three populations - two natural forests and a planted forest, showed different results for the dynamics of germination of three treatments.

Germination

There are differences in the germination of the seed originating from different populations, where the seed originating from the trees from planted forest Srebrenica attained the best results. The best germination viability (84.49%) and germination (87.60%) had the seed from Srebrenica population, which was kept at a low temperature for six months (table 2). Average germination variability between populations is approximately $\pm 5\%$ in comparison to the control unit, except in the case of PSFS seed originating from Gostilj population.

Table 2. Quality parameters for Serbian spruce seed

Population	Parameter	Control		PSS		PSFS	
		Average	C _v [%]	Average	C _v [%]	Average	C _v [%]
Srebrenica	Germination viability	68,80	15,33	84,49	14,12	52,80	33,15
	SI	7,31	10,67	6,44	7,42	8,98	17,68
	Germination	84,00	12,04	87,60	10,57	85,40	7,29
Veliki Stolac	Germination viability	38,46	45,48	53,14	43,16	31,29	54,53
	SI	8,68	19,93	7,07	18,89	8,98	15,44
Gostilj	Germination	58,57	29,24	61,86	33,37	55,50	39,45
	Germination viability	33,57	45,48	55,00	32,17	21,50	70,51
	SI	9,72	9,16	6,99	10,73	10,74	18,67
Average	Germination	65,15	17,70	65,40	23,84	55,50	31,65
	Germination viability	46,24	48,42	62,29	36,28	34,74	59,27
	SI	8,58	18,41	6,86	14,72	9,50	19,05
	Germination	67,99	25,51	70,47	28,07	64,65	33,58

Legend: SI- Seed inactivity in days, C_v - Coefficient of variation

Dynamics of seed germination at the population level

The results indicate that the process of seed germination from the control until ended after 14 days, when 99% of seed germinated, compared to the total number of germinated seeds at the end of the test (figure 2). In the first 4 days there is no germination and on the fifth day, the seed begins to germinate. The highest percentage of seed germination was recorded on the seventh day.

PSS seed showed the best results in reducing seed inactivity in the process of germination. The treatment of seed storage, without the use of fungicides, proved to be most favorable for Serbian spruce seed because it reduced the variation in seed inactivity of germination and accelerated the process of germination. At the population level, the seed from Srebrenica population attained the best results, while the seed from natural populations Gostilj had the lowest values (figure 3). Planted forests are typically grown at smaller altitudes and

sunny exposures. This has a direct impact on increasing the quality of parameters of seed germination.

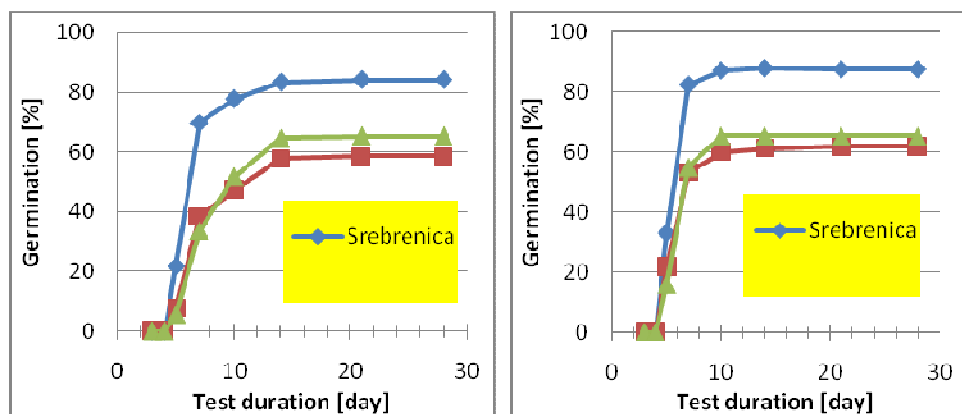


Figure 2 and 3. Serbian spruce germination dynamic of control unit and PSS seed

Till 10th day of germination test, PSS seed achieved 98.4% germination of the total number of germinated seeds. The results obtained from this test are similar to the results obtained from BERJAK and WANG (2000), who studied the characteristics of seed species *Picea mariana*, where they proved that during the cooling treatments in the seed reparations the mechanism that increases the percentage of germination was activated. It can be assumed that the treatment of storing seed at a low temperature, as in our case, affected the dynamics of seed germination by accelerating the process of germination, and also reducing the variability. After 14 days, there was no germination (figure 4).

PSFS seed germinate throughout all test period (figure 4). Due to the influence of fungicides, there is a delay in the process of seed germination. In addition, the process of germination has two peaks that occur on the 7th and 21st day (figure 5), which was not the case in the previous two treatments. The appearance of germination postponing can be interpreted as the influence of fungicides on some half-sib lines, where one part of the seed showed tolerance to inhibitory influence of fungicides, and the other part of the seed was susceptible to the effects of fungicides.

Dynamics of seed germination at the level of treatment

At the level of treatment, PSS seed reached the least seed inactivity with an average value of 6.86 days, while PSFS seed attained the maximum seed inactivity of germination - 9.50 days.

Although there are significant differences in the germination viability and seed inactivity mean value, in favor of the PSS seed, germination tests showed very small differences in germination at the end of the test (figure 6).

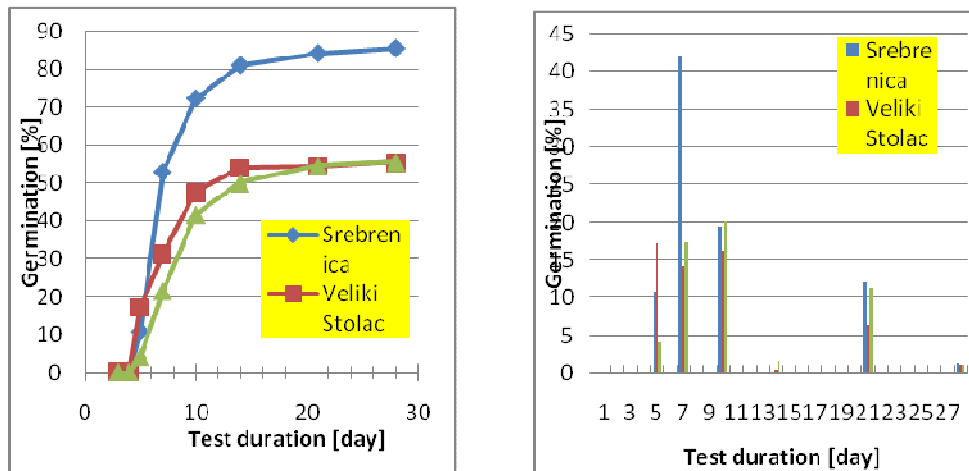


Figure 4 and 5. Serbian spruce germination dynamic and germination per days for PSFS seed

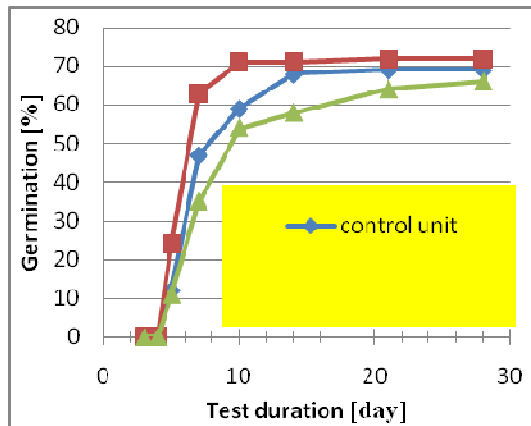


Figure 6. Serbian spruce germination dynamics of: control, PSS and PSFS

One of the reasons could be the controlled environment where the test was conducted. There was no adverse influence during the test which is not the case in non-controlled conditions. In case we did it in nursery conditions or in a natural habitat, we could expect different results due to variable environmental conditions.

Multi-factor analysis of variance of the seed inactivity indicates that there are significant differences in the interaction between treatments and populations. Seed from different populations responds differently to treatment (table 3).

Table 3. Interaction between treatments and populations for SI

Intercept	Deg. of freedom	Sum of squares	Medium of squares	F	P
Treatment	2	251.83	125.92	69.897***	0.0000
Population	2	70.21	35.11	19.488***	0.0000
Treatment x Population	4	33.30	8.33	4.622***	0.0014
Error	195	351.29	1.80		
Total	203	699.19			

Seed from all populations best responded to the treatment of seed storage at a low temperature without fungicide treatment, achieving the lowest average seed inactivity. Duncan's test indicates that the seeds from the area Gostilj best respond to the process of storage, significantly reducing seed inactivity (table 4).

Fungicide (*captan*) acted as an inhibitor of the germination process, by slowing down the process. The seed from the population Veliki Stolac had the best response to fungicide treatment, almost equalizing seed inactivity with the seed from the planted forest Srebrenica, which showed the best results in other two treatments.

Table 4. Duncan test of interaction between treatments and populations for SI

Treatment	Population	SI	1	2	3	4	5
PSS	Srebrenica	6.44	****				
PSS	Gostilj	6.99	****	****			
PSS	Veliki Stolac	7.07	****	****			
Control	Srebrenica	7.31		****			
Control	Veliki Stolac	8.68			****		
PSFS	Veliki Stolac	8.98			****	****	
PSFS	Srebrenica	8.98			****	****	
Control	Gostilj	9.72				****	
PSFS	Gostilj	10.74					****

Based on these results, the conclusion is that Serbian spruce seed storage at low temperatures should be used as a seed treatment before sowing, in order to speed up the germination process and equalize the dynamics of seed germination among the seed from different populations. This is particularly important for nurseries in order to get reliable information about Serbian spruce seed behavior.

Although there are some differences in seed inactivity between different treatments, they do not reflect the overall germination. The differences in germination observed at the end of the test, for all populations together, are not significant.

The seed from natural populations has approximately the same germination viability and germination during the storage treatment at low temperatures. The results can be used in the production of seedlings in nursery production in the open field. The delay in germination may be fatal for seed due to adverse climatic factors of the environment, leading to the reduction in the number of seedlings produced by this species and thus reducing the economic gains in production.

CONCLUSION

The results illustrate the adaptation of Serbian spruce conditioned by the genetic ability of the species to produce a stable and high yield on diverse sites.

The differences between plantations and individual trees in the germination ability and germination dynamics of differently treated seeds of Serbian spruce are major indicators of genetic variability in its reproductive cycle. The interaction of environmental characteristics and genotypes of extreme and average trees illustrate the reproductive ability of Serbian spruce on different sites and indicates that this species achieves the cenological and not ecological optimum on its natural sites.

The seed from natural populations has approximately the same germination viability and germination during the storage treatment at low temperatures. The results can be used in the production of seedlings in nursery production in the open field. The delay in germination may be fatal for seed due to adverse climatic factors of the environment, leading to the reduction in the number of seedlings produced by this species and thus reducing the economic gains in production.

Seed trees, i.e. bearers of genetically high-quality seed, are especially significant for modern nursery production. In further research, these trees, disregarding the different half-sib lines, should be constantly recorded and the quality of yield should be examined.

Application of genetic selection programs can lead to the production of planting stock of desired and defined properties, which could survive the stress of environmental factors, thanks to its genetic, physiological and morphological properties.

Received July 2nd, 2012

Accepted November 07th, 2012

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UTICAJ RAZLIČITIH TRETMANA ČUVANJA SEMENA OMORIKE (*PICEA OMORIKA PANČIĆ/PURKYNE*) NA KLIJAVOST I DINAMIKU KLIJANJA SEMENA

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Izvod

Kvalitet semena omorike, (*Picea omorika* /*Panč/ Purkyne*), iz tri populacije, na levoj obali reke Drine, u Bosni i Hercegovini analiziran je primenom genetičko-fizioloških metoda.

Za analize je korišćeno seme, iz tri populacije omorike koje reprezentuju ukupne ekološko-proizvodne uslove njenih populacija u Bosni i Hercegovini. Dvije populacije su u delu njenog spontanog javljanja: Veliki Stolac i Gostilj, a jedna populacija je antropogenog porekla u okolini Srebrenice. Šišarice su sakupljene krajem 2009. i početkom 2010. godine sa pet stabala iz Srebrenice, sedam sa Velikog Stolca i pet sa Gostilja. Neposredno nakon sakupljanja šišarice i seme su doradeni. Energija klijanja, klijavost i dinamika klijanja ispitivana je kod tri kategorije semena: 1) sveže sjeme (kontrola); 2) seme skladišteno šest meseci na 0-4°C; 3) seme skladišteno sedam meseci na 0-4°C, sa tim što je bilo tretirano fungicidom nakon petog meseca čuvanja. Dinamika klijanja sjemena praćena je trećeg, četvrtog, petog, sedmog, desetog, četrnaestog, dvadestprvog i dvadesetmog dana.

Dobijeni rezultati ukazuju na adaptacioni potencijal omorike za proizvodnju kvalitetnog semena na ekološki različitim staništima. Interakcija uslova staništa i genotipova stabala ukazuju na to da omorika na svojim prirodni staništima postiže cenološki optimum, a ne i ekološki.

U sprovedenim analizama nisu evidentirane značajne razlike u dinamici klijavosti na nivou populacija i na nivou tretmana tokom prvih nekoliko dana testa klijavosti. Fungicid (Captan) delovao je kao inhibitor procesa klijanja semena. Seme poreklom iz najveće populacije Veliki Stolac, najbolje je ragovalo na tretman skladištenja sa fungicidom sa obzirom na dinamiku klijanja.

Seme poreklom iz Srebrenice koje je čuvano na niskim temperaturama 0-4 °C, bez tretmana fungicidom, pokazalo je najbolji rezultat što se tiče klijavosti. Bez obzira na prisustvo razlika u dinamici klijanja, značajne razlike u klijavosti, u odnosu na različite tretmane, poslednjeg dana testa nisu zabeležene.

Primljeno 02. VII.2012.

Odobreno 07. XI. 2012.