

## **Influence of high environmental temperature ability of seeds from the genus of oaks (*Quercus*)**

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### **Abstract**

*This work describes the effects of high temperatures and fire simulations in nature (100°C for 5min) on the viability of oak genus seeds. In this work, germination of five oak seed genotypes has been studied by the standard method. The result is presented with the help of quantitative indicators of germination (technical germination, vigor).*

*To study the relationship of analyzed characteristics, Two Factorial Analysis of Variance was used as well as the Fisher's Least Significant Difference Test for the significance level  $\alpha=0,05$ .*

*The aim of the research is to investigate the tolerance of analyzed oak seed genotypes on fires in the environment, which is important both for natural regeneration and for seedling production. Both analyzes show differences between the two genotypes in germination of acorns, and the significant influence of the applied treatment. Also, differences between the genotypes in terms of reaction on the high-temperature treatment are very clear, emphasizing the genotype 2 (close to 50% of technical germination after treatment). Comparison of thermally untreated and treated seed shows differences between genotypes. Generally speaking, oak acorns showed sensitivity on the heat treatment (100°C for 5min) regarding germination. Low germination of oak seed exposed to stressful conditions reduces its potential for natural spreading in the forest environment. These results are important for further research of oak seed tolerance on fires in the environment.*

**Keywords:** fire, seed germination, oak (*Quercus*)

### **Introduction**

In nature, in a short period of time, fires in can completely destroy a whole forest complexes with habitats of high biological diversity with many species (S. ŽIVANOVIĆ [46]). The reactions on fire are different: some species in areas affected by fire will appear only after the fire, some, which existed before the fire, will be developed in accordance with the newly created terms and some will perish after the fire (C. CHANDLER & al. [4], M.P. MALANSON and L. TRABAUD [12]).

Initial stages of plant growth is sprouting of seeds. Seed germination is an important stage in the life cycle of seeds (JOVIČIĆ & al [19]). Seed germination is the most important feature because only germinated seeds can give a new plant (STILINOVIĆ [38], MILOŠEVIĆ & al. [28]). Adaptation to the environmental conditions required quality for reproductive success of seed germination.

In relation to natural regeneration, ability of seed to preserve capability for germination after the fire in nature is particularly important. Different plant species and even tissues have different requirements according to the environmental temperature. GUTTERMAN [13], believes that the seeds, compared to other parts of the plant, are the most resistant to extreme environmental conditions. Beginning of germination and intensity of

enzymatic processes that occur in the seed that sprouts are significantly influenced by temperature. The average temperatures during the growing season, for individual plant species are very different, and so to say, the minimum temperature at mezophite, which belongs to the oak genotype, that has been the subject of research, is 0-10°C, 15-25°C optimum, and maximum 35-45°C

High soil temperatures during the fire, break seed dormancy of some plant species in the soil. Different plant species and different organs of the same plant and even tissues have specific requirements to temperature (M.B. STEVANOVIĆ and M.M. JANKOVIĆ [39]). Depending of the period of growth and development, different are demands for temperature. Generally, plant tissue does not tolerate temperatures above 45°C. During evolution, certain plant species, have developed the so-called CAM (Crassulacean acid metabolism) of photosynthesis type, thus being adapted on high temperature conditions up to 65°C. The question is the highest temperature a seed is able to endure, maintaining the needed capability for germination. Some authors (C. CHANDLER & al. [4], LEKIĆ [24]) reported that dry seed can bear temperatures up to 120°C. Temperature is a significant opportunity for the start of germination (J.G. PAUSAS and J.E. KEELEY [31], J.E. KEELEY & al. [22], M.E. HANLEY & al. [14]). Germination occurs within a certain temperature range. Minimum temperature for the growth and development of plants is relatively high (except for the polar sea algae) and for tropical species it is between 10-15°C. Maximum temperature for most plants is between 45-55°C (E.E.L. BORGES and A.B. RENA [3], COCHRANE [6]).

A particular type of risk from high temperatures and overheating of plants and vegetation is a fire, (COCHRANE [5]). According to Martin [25], mortality of plants and seeds from fire depends on the duration of exposure to lethal temperatures. Mortality can occur after short exposure to high temperatures while mortality at low temperatures is often caused by long-term exposure. Vulnerability of the seeds on fire exposure is caused by the type and intensity of fire and frequency fire passes (S. GÓMEZ-GONZÁLEZ & al. [40]).

National Forest Inventory of Serbia revealed 49 species of trees, where the dominant are broadleaf species (40) in relation to the coniferous (9), (S. BANKOVIĆ & al. [1], [2]). Oaks in Serbia are presented by ten species and some of them are the most important forest species, covering about 25% of the total growing stock of Republic of Serbia.

Oak flourishes from April till May, while the acorn (nut), matures in September or October. Seeds of *Quercus* species germinate underground (hypogeal) when specific microclimate conditions are met.

Seeds of oak recalcitrant, sensitive to drying and low temperature during storage (W.E. FINCH-SAVAGE & al. [9]). In this sense, the high temperature treatment can provoke cracking of the pericarp, which is favorable for germination, but also has influence on drying and necrosis of acorns, which reduces its chances for survival and germination. There are many studies that analyze the impact of heat on the germination of different types of wood (J.M KANE & al. [20], KEELEY [21], R.M. NUNEZ and L. CALVO [29], PAUSAS [30], O. REYES & al. [32] and [33], O. REYES and M. CASAL [34], [35] and [36], L. VALBUENA and R.TARREGA [48]).

The aim of the research presented in this work, was to determine the effects of high temperature, caused by fire simulation, on acorns and on the viability of seeds from oak genus.

## Materials and Methods

The germination of oak seeds genus was analyzed (*Quercus*). Germination testing was performed according to the regulations of the International Seed Testing Association (International Seed Testing Association-ISTA, [17] and [18]). Material was collected on 13.10.2012. in General Nature reserve Bukovo in Negotin municipality, in the mountain oak forest on the altitude of 90m above the sea level. Exposure of parent trees in the forest is so different that the selection was done in all parts of the forest. Also, the attention was paid that no old trees or those whose fruit and leaves were damaged by parasites could have been chosen. Mature acorns were collected from the ground. For each genotype, 100 seeds were collected, and then exposed to high temperature of 100°C (fire simulation in the laboratory) for 5 minutes. After this treatment, germination ability of the seed was analyzed.

Seed germination testing was performed in laboratory by conventional methods. The tests were performed in the laboratory of the Institute of Lowland Forestry, University of Novi Sad. Before placing seeds in optimal conditions, such as in a standard laboratory test, the seeds has been exposed to a temperature of 42°C and humidity of 100% for a period of 96 hours (J.G. HAMPTON and D.M. TEKRONY [16]). During the experimental period, oak seeds were kept in complete darkness, simulating conditions in the ground, and they were only illuminated by weak green light (25W).

After seven days of incubation period, germination energy (GE) was estimated, and on the 14-th day, total germination (TG) ability was determined. Untreated seeds served as control. The technique of the sprouting in pots with Perlite in a dark chamber at 15°C, was used.

The data were processed by STATISTICA StatSoft version 10 program [41]. The following statistical tests were used: Two Factorial Analysis of Variance and Fisher's Least Significant Difference Test for significance level  $\alpha$ 0,05. For the purpose of statistical analysis of variance, data were transformed (arc sin/percentage), so that their distribution frequency was normal, as a condition for application of Analysis of Variance and Least Significant Difference (LSD) Test.

After the LSD test was done, transformed values were shown in Table 4.

To see the results in the tables below, following codes were used:

Genotype G1 - sample taken from the periphery of the forest on the east side,

Genotype G2 - sample taken from the periphery of the forest on the north side, 3m distance from the stream,

Genotype G3 - sample taken from the periphery of the forest on the west side,

Genotype G4 - sample taken from the periphery of the forest on the south side,

Genotype G5 - sample taken from the central part of the forest.

In addition, the observed genotypes show significant variety in the seed size, Table 1.

**Table 1.** Characteristics of Oak crop genotype

Genotype	Yield length [mm]	Yield diameter [mm]	Mass 1000 yield [gr]
G1	od 26,4 do 37,2	od 13,8 do 19,0	5708
G2	od 27,0 do 43,2	od 15,2 do 22,5	6224
G3	od 20,2 do 28,0	od 11,8 do 18,6	3112
G4	od 22,6 do 33,6	od 15,0 do 19,1	4233
G5	od 27,2 do 39,6	od 14,1 do 18,8	4386

*Environmental conditions*

Negotin is located in the northeastern part of Serbia. Its geographical location is determined by the 44° 13' North latitude and 22° 31' East longitude. Negotin ( $\varphi$  44° 13' N,  $\lambda$  22° 31' E, H = 42m) is located in the plane, surrounded by mountains Miroč, Crni vrh and Deli Jovan on the western and northern side and by of the Danube and Timok on the eastern and southern part. This causes a very specific climate of the region as the steppe-continental with average annual temperature of 11.1°C and rainfall of 646mm (period 1961 ÷ 1990), [50]

Many authors (KOLIĆ [23], MILOSAVLJEVIĆ [27], ŽIVANOVIĆ [47]) indicate that this region has an arid climate. Based on the classification of geographic regions in Serbia, Negotin area is classified as Eastern (Balkan) Serbia. Lower Danube area, situated along the rivers Danube and Timok, is the most western part of the Wallachia plain.

Mountainous areas of the Negotin territory, where there are trees, are predominantly with the land of bad characteristics (Rendzina, brown acid soils, Sirozem) (CVJETIČANIN & al. [8]). Land Bukovo sites with good water permeability. Negotin has low forest coverage (25.28%).

*Climate conditions*

Negotin has an average annual temperature of 11.1°C. The hottest period is June-August with an average daily temperature of 20.2 to 22.1°C, while the coldest months are January-February with an average daily temperature of - 1.1 to 1.1°C. In Table 2, the values of the average monthly and annual temperatures in the different period are given [50].

**Table 2.** Average monthly and annual average air temperature in Negotin, °C

Period	Month												year
	1	2	3	4	5	6	7	8	9	10	11	12	
1961/1990	-1,1	1,1	5,5	11,8	16,9	20,2	22,1	21,2	17,3	11,0	5,7	1,3	11,1
2012.	0,9	-4,2	9,7	14,4	18,1	24,4	27,2	25,7	20,7	13,7	7,9	-0,7	13,1

The year in which observations were made and data collected in terms of air temperature can be considered as different from the multi-year average temperature variations. Table 2 shows that the average monthly values of air temperature during the growing season were above long-term average values. During the growing season in 2012, the average air temperature was 21.8°C, which is 3.5°C higher than the annual average. July will be remembered as the month with the highest maximum temperatures since measurements are made in this area

Based on long-term averages, during the growing season averaged values of rainfall are 287.4mm, being favorable for the development of forestry. Maximum average values of falls are maximal in May (68.1mm), and minimal (40.7mm) in August, Table 3.

Total quantity of summer precipitation for 2012 in Negotin was below the limit values compared to the reference period 1961-1991, Table 3 Data on rainfall during the 2012th year are far below long-term averages. The lack of rainfall has caused the drying of topsoil and a significant deterioration of the moisture in the deeper soil layers.

**Table 3.** Precipitation in Negotin for various periods, mm

Period	Month												year
	1	2	3	4	5	6	7	8	9	10	11	12	
1961÷1990	41.0	51.8	56.8	62.6	68.1	67.1	48.8	40.7	40.8	47.8	64.7	55.8	646.0
2012.	63.3	63.7	0.5	64.9	108.0	31.0	27.0	1.2	6.8	45.3	40,5	89.7	541.9

## Results and Discussion

The results of factorial analysis of variance indicated that both controlled factors (genotype and treatment of high temperature) and their interaction had a significant impact on the variation of the three observed characteristics. This influence in all cases was highly significant (Table 4). Especially interesting is the significant effect of genotype  $\times$  treatment interaction, because it points to significant differences in the response of acorn genotypes on high temperature treatment. According to F values and assess the significance of possible impacts of certain controlled sources of variation, it was concluded that regarding the seed moisture, differences among genotypes were strongly dominating, while the technical germination was caused by the influence of the treatment. These results indicate that the effect of treatment did not have impact on seed moisture, but high temperature probably had a direct inhibitory effect on metabolic processes in seeds.

**Table 4.** Analysis of variance for the analyzed characteristics of acorns

	The sum of squares	Number of degrees of freedom	Middle of the square	F-test <sup>1)</sup>
Seed humidity				
Genotype (A)	1737.272	4	434.318	104.958**
Treatment (B)	34.804	1	34.804	8.411**
Interaction A $\times$ B	77.305	4	19.326	4.670**
Error	82.760	20	4.138	
Germination energy				
Genotype (A)	4966.263	4	1241.566	61.701**
Treatment (B)	1579.030	1	1579.030	78.472**
Interaction A $\times$ B	4005.519	4	1001.380	49.765**
Error	744.522	37	20.122	
Technical germination of seed				
Genotype (A)	6906.493	4	1726.623	32.234**
Treatment (B)	15460.514	1	15460.514	288.627**
Interaction A $\times$ B	6350.270	4	1587.568	29.638**
Error	1981.933	37	53.566	

Treated seeds had a slightly higher humidity than not treated. The reason for this may be the fact that the additional amount of moisture was created by the process of necrosis, created under the influence of high temperature. This was not the case in all genotypes.

High moisture content was characteristic of the genotypes G2 and G3, as well as the occurrence of germs after 7 days (Table 5). The technical control showed that their viability was the highest, especially the case with genotype G3 (90.53%).

Genotype G2 had the best tolerance to treatment. G2 also had lower technical viability than genotype G3, but it has achieved almost 50% of technical germination of treated acorns. The nature of this acorn genotype resistance certainly deserves further attention. The characteristics of genotype G2 were certainly caused by higher moisture content in the soil during the growing season. A similar result about the causality between the seed germination and moisture are presented by LEKIĆ [24] and JOVIĆIĆ & al [19]. Other genotypes showed a very low tolerance to high temperature treatment, suggesting that fires certainly are not favorable for the germination of this species. The same results have been presented by L.

VALBUENA and R.TARREGA [48], and O. REYES and M. CASAL [34] in their research works.

The results obtained in this study indicate that there are significant differences in the tolerance of the genotypes of acorns under the treatment of high temperatures. Also, the method could be further improved by varying the temperature and length of exposure in order to obtain a more precise differentiation between the genotypes. A similar result about the effects of different fire temperatures on germination were reported (L. VALBUENA and R.TARREGA [48], O. REYES and M. CASAL [34]).

It should be noted that the results of this study are related to the genetic potential of the seed in a given area with microclimatic conditions of the environment. This means that there is a possibility of other results and conclusions if the tests are set up in other environmental conditions.

**Table 5.** Least significant difference test for significance level  $\alpha 0,05$

Genotype	Treatment	Seed humidity (%)		Germination energy (%)		Technical germination (%)	
G1	control	24.86	e *	0.00	d	10.49	d
	treated	24.00	e	0.00	d	3.83	de
G2	control	43.57	ab	13.79	b	68.64	b
	treated	41.79	b	4.50	c	47.06	c
G3	control	40.52	b	56.47	a	90.53	a
	treated	45.97	a	0.00	d	0.18	ef
G4	control	29.85	d	0.00	d	52.23	bc
	treated	36.01	c	0.00	d	0.00	e
G5	control	27.07	de	0.00	d	64.15	bc
	treated	28.88	d	0.00	d	1.50	ef
G1		24.43	d	0.00	c	6.78	d
G2		42.68	a	8.57	b	58.05	a
G3		43.24	a	13.61	a	30.30	b
G4		32.93	b	0.00	c	12.34	cd
G5		27.98	c	0.00	c	21.40	bc
	control	33.17	b	5.70	a	55.58	a
	treated	35.33	a	0.18	b	4.92	b

+ Values with the same letter are not significantly different

This study found no correlation between seed size and percentage of technical germination. In fact, it can not be concluded that bigger seeds are more resistant to high temperatures, as it was presented in research works of TRABAUD [44], D.A. COOMES, and P.J. GRUBB [7], and COCHRANE [5].

## Conclusions

On the basis of the results of seed quality test done on five oak genotypes we conclude:

All the traits in controlling germination ranged from 10.49% in genotype G1 to 90.53% genotype G3.

Technical germination of the treated seeds was very low, except in genotype G2, where it was close to 50%.

Level of germination energy and total germination was higher in untreated samples compared to the treated ones. Lower percentage of seed germination are characteristic of treated oak genotypes.

The results confirmed the fact that the fires in nature have a significant impact on the survival of rare and endangered plant species in a given area. The results show that the temperature of the fire in nature have a statistically significant effect on the seed germination of oak genus. High temperatures reduce seed germination in nature. However, it should be noted that in some years, environmental factors (particularly temperature and precipitation) may significantly affect on reducing of germination. This indicates the importance of taking the necessary measures to protect forests from fire. It can be concluded that this type of oak will feature infertility which would cause negative effects on the process of natural regeneration. Further researches in this area would be of importance in assessing the damage from the fire, the selection of parent trees in the seed stands and nursery production.

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