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The influence of crown defoliation on the variability of some physiological and morphological properties of silver fir (*Abies alba*) seeds in the seed zone of Dinaric beech-fir forests in Croatia

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Abbreviations

TSW – 1000 seed weight
SR_GKiVK – Seed region of Gorski Kotar and Lika (3.3.1.)
SR_V – Seed region of Velebit (3.3.2.)
SR_B – Seed region of Biokovo (3.3.3.)
ISTA – International Seed Testing Association

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Abstract

Background and Purpose: *Negative anthropogenic influence on the environment became visible in the 1980s and the result was the emergence of forest degradation. Silver fir (*Abies alba*) is highly sensitive to atmospheric and pedospheric pollution and it is one of the most endangered tree species in the entire Europe. The aim of this research was to determine the influence of different levels of crown defoliation on the phenotypic variability between and within the populations of silver fir in the zone of Dinaric beech and fir forests*

Material and Methods: *Within the area of silver fir forests in the Forest Administrations of Delnice, Senj and Split, permanent experimental plots were established in order to monitor the influence of different levels of crown defoliation on some properties of seeds: germination capacity, the percentage of fresh ungerminated seeds, rotten and decayed seeds, empty seeds and 1000 seed weight. Statistical analysis was carried out by parametric and non-parametric methods. Various models of variance analysis were carried out in the analysis of the variability of traits.*

Results: *The average germination capacity of silver fir seeds for the investigated area was 27.9 %, and ranged from 16.2 to 39.3 %. CV was high and indicated a great heterogeneity between the provenances. Average values of fresh ungerminated seeds were 12.6 %, and ranged from 9.1% to 19.6%. Average values of dead seeds were 16.1 % and ranged from 8.2 to 28.5%. The provenances from the area of Gorski kotar showed the largest portion of dead seeds, which was the area with the highest index of forest decline. The average value of empty seeds in the researched area was 43.4% and it ranged from 29.9% to 63.0%. CV ranged from 33.9% to 74.0% indicating a heterogeneity within the provenances. The share of empty seeds was influenced by the air pollution and the presence of insects on generative organs, and had a negative influence on the germination capacity. The average TSW in the researched area was 57.4 g and ranged from 49.4 g to 73.6 g. Duncan's test confirmed the variability between the provenances for all investigated seed properties.*

Conclusion: *A statistically significant intrapopulation and interpopulation variability of physiological seed properties was determined in all studied provenances and the influence of different levels of crown defoliation on their variability was not determined. Considering the morphological properties of seeds, a statistically significant variability between the provenances and the levels of crown defoliation was determined as well as for the interaction of these two factors.*

INTRODUCTION

Silver fir (*Abies alba* Mill.) grows in the forests of Croatia in the area of approximately 200 000 hectares, and it is ecologically and economically one of the most significant tree species in the area of selection forests (1, 2). It forms pure or mixed forest stands with beech and spruce on limestone and silicate rocks, forming four defined and clearly described forest communities. It is naturally distributed in the Dinarides, and it also forms an isolated enclave on the northern slopes of Biokovo. It grows on a few isolated mountains in the area between Sava and Drava rivers. Altitudinal distribution of silver fir ranges between 400 and 1500 meters above sea level (3). According to the current forest management plan (2006–2015), the percentage of silver fir is 8% in the growing stock and 6% in the allowable annual cut (4).

Harmful consequences of the negative anthropogenic influence on the environment became visible in the 1980s. Atmospheric and pedospheric pollution results in the appearance of forest decline and it is reflected in the weakened vitality of many tree species. Forest decline was perceived simultaneously through almost the entire Europe and in the United States of America. Silver fir as a species of narrow ecological amplitude is particularly sensitive to pollution and it is one of the most endangered species in Croatia and Europe (5). Significant damage to silver fir trees was 72.4% in Croatia in 2009 (6).

Knowing the modification of the variability of some properties of a specific species is very significant in improving and conserving forest's genetic resources and the species itself. The emergence of the variability is reflected both in the entire area of distribution of a specific species and within one forest stand (7). The variability of an individual unit is affected by internal and external factors, such as genotype and environment. Consequently, the changes in an individual unit stemming from the changes in the genetic material are hereditary (mutations or genetic recombination) and the changes resulting from the consequences of environmental effects are non-hereditary (modifications). In fact, the rapidly changing environment effects, especially climate changes and pollutions, have a significant impact on the genetic variability of individual trees. Based on the aforementioned facts, the systematic research of the variability of silver fir is necessary to continue and to intensify (8).

It has long been assumed that silver fir is a species of very small variability (9). The results of the experiments and research on the provenance trials established in the first half of the 20th century proved the existence of both geographical and physiological variability between the provenances of silver fir (10-12). Recent research on the variability of silver fir, in the provenance trials and in the research on genetic markers, confirmed the existence of physiological variability and climatic races (13).

The aim of this study was to determine the influence of different levels of crown defoliation on the phenotypic variability of seeds between and within the populations of silver fir in the zone of Dinaric beech and fir forests. The set aims resulted in the task of determining the influence of crown defoliation classes on some physiological and morphological properties of the seeds.

MATERIALS AND METHODS

The description of the permanent experimental plots and operating modes

Permanent experimental plots have been established within the area of silver fir forests in the zone of Dinaric beech and fir forests in the Forest Administrations (FA) of Delnice, Senj and Split in order to monitor the influence of different levels of crown defoliation classes on some physiological and morphological seed properties. Table 1 shows basic data concerning permanent experimental plots.

Trees of different levels of crown defoliation on each experimental plot were selected according to the methods of the ICP Forests program (14).

The degree of defoliation defines three categories of vitality:

– Vitality 1 (VIT 1 1-3 SO) represents older damaged trees with the levels of crown defoliation „1", „2", „3",

– Vitality 2 (VIT 2 0 SN) represents older undamaged trees with the level of crown defoliation „0",

– Vitality 3 (VIT 3 0 MN) represents younger undamaged trees with the level of crown defoliation „0".

Minimum distance between trees from which cone samples were taken was one to two tree heights. The selected trees were marked and the estimation parameters were measured. Permanent experimental plots were established in FA Delnice and FA Split in 1990 and in FA Senj in 1994. Cone samples were collected during crop years. Depending on the amount of crops, the minimum of 10 and the maximum of 30 cones were collected from each tree. Cone samples were gathered by professional climbers. Cone samples were labelled, separately packaged and transported to a laboratory for further testing.

Investigated properties and methods for testing the quality of silver fir seeds

This research included some physiological and morphological seed properties: germination capacity, the percentages of fresh ungerminated, dead and empty seeds, and 1000 seed weight (TSW). ISTA methods were used for testing seed quality. Germination of the seeds was determined by the method „The Germination Test", Chapter 5, ISTA Rules, and TSW was determined by the

Table 1 *Basic data about permanent experimental plots*

Ordinal number	Forest office	Management unit/ compartment	Provenance	Ecological- managerial forest type	Parent rock	Altitude (m)	Longitude	Latitude
1	Fužine	Brloško 5,6,7	Brloško	I-C-10	limestone	812-975	45.18 N	14.43 E
2	Skrad	Jasle 59	Jasle	I-C-40	silicates	630	45.26 N	14.55 E
3	Ravna gora	Višnjevica 78	Višnjevica	I-C-10	limestone	770	45.30 N	14.56 E
4	Skrad	Rudač 40	Rudač	I-C-40	silicates	770-794	44.55 N	14.56 E
5	Vrbovsko	Miletka 17	Miletka	I-C-40	silicates	660-700	45.20 N	15.05 E
6	Vrbovsko	Gluhe drage 25	Gluhe drage	I-C-10	limestone	680-750	44.55 N	15.01 E
7	Gerovo	Lividraga 73,79	Lividraga	I-C-10	limestone	940-1022	45.35 N	14.32 E
8	Gerovo	Vršice 51	Vršice	I-C-10	limestone	820-900	45.35 N	14.32 E
9	Tršće	Crni lazi 49	Crni lazi	I-C-10	limestone	900-928	44.55 N	14.59 E
10	Tršće	Rudnik 13	Rudnik	I-C-40	silicates	840-930	44.55 N	14.38 E
11	Prezid	Milanov vrh 32,39	Milanov vrh	I-C-10	limestone	920-1067	44.55 N	14.33 E
12	N.P. Risnjak	Risnjak 2	Risnjak 2	I-C-40	silicates	685	45.25 N	14.44 E
13	N.P. Risnjak	Risnjak 1	Risnjak 1	I-C-11	limestone, dolomites	680	45.25 N	14.44 E
14	Krasno	Nađak bilo	Nađak bilo	I-C-10	limestone	885	44.50 N	15.00 E
15	Krasno	Štirovača 24,34	Štirovača	I-C-10	limestone	1083	44.40 N	15.03 E
16	N. Vinodolski	Duliba 1	Duliba	I-C-10	limestone	718	45.09 N	14.56 E
17	Imotski	Kaoci	Kaoci	III-C-20	limestone	1050	43.925 N	17.15 E

„Weight Determination", Chapter 10; ISTA Rules (15). The investigated properties were examined on the working samples of prescribed size.

Seed germination

Silver fir's seeds are dormant and are unable to germinate immediately. Initially, the seeds were prechilled using cold-moist method at a temperature from 1 to 5°C for 21 days. Afterwards, the seeds were placed for germination. Seed germination was carried out on a working sample size of 4 x 100 seeds, in thermostat germination apparatus at an alternating temperature from 20° to 30°C, on top of the filter paper during 28 days. The temperature of 20°C was maintained during 16 hours and of 30°C during 8 hours. During the germination process the seeds were lighted with cool white florescent lamps (750-1250 lux). Germination energy was counted on the 14th day and the germination capacity at the end of test period on the 28th day. At the end of a germination test, the classifications of fresh ungerminated, dead and empty seeds were determined. The results of the germination test were expressed as percentage by a number of normal seedlings, abnormal seedlings, fresh ungerminated seeds, dead seeds and empty seeds. The sum of all percentage was 100. The percentage of the normal seedlings represented germination capacity.

According to ISTA, germination of a seed is defined as the emergence and development of the seedling to a stage where the aspect of its essential structure indicates whether or not it is able to develop further into satisfactory plant under favourable conditions in the field. In order to clarify these terms, the definitions of the specific categories are stated below.

Normal seedlings show the potential for a continued development into satisfactory plants with well-developed essential structures such as root system, shoot axis, cotyledons, primary leaves and terminal bud or shoot apex. The percentage of normal seedlings represented germination capacity.

Abnormal seedlings do not show the potential to develop into a normal plant. Seedlings can be damaged, deformed, unbalanced or decayed as a result of primary infection.

Fresh ungerminated seeds have failed to germinate because of dormancy, but remain clean and firm, and have a potential to develop into a normal seedling.

Dead seeds (*rotten and decayed seeds)* are the seeds which at the end of the test period are neither hard nor fresh nor have produced any part of the seedling.

Empty seeds are the seeds which are completely empty or contain some residual tissue.

1000 seed weight

The weight of 1000 seeds was determined on a working sample size of 8 x 100 seeds which was taken from the

Table 2 *Descriptive statistic parameters for physiological and morphological properties of Silver fir seeds*

Traits	N	$\bar{x}$			s	CV (%)		
		Mean	Min	Max		Mean	Min	Max
Germination capacity (%)	351	27.9	16.2	39.3	20.9	74.9	50.3	91.1
Ungerminated seeds (%)	351	12.6	9.1	19.6	11.8	93.7	50.2	128.0
Dead seeds (%)	351	16.1	8.2	28.5	17.20	106.8	68.4	148.5
Empty seeds (%)	351	43.4	29.9	63.0	24.1	55.5	33.9	74.0
1000 seed weight (g)	4248	57.4	49.4	73.6	20.90	36.4	28.0	45.0

pure seeds component. Each group was weighed in grams with an accuracy of one decimal place. The data obtained were statistically analyzed; the variance, standard deviation, arithmetic mean and the coefficient of variation (CV) were calculated. The average mass value of 100 seeds for forest tree species was calculated in the case when CV was not exceeding 4.0. By multiplying the value with 10 the mass of 1000 seeds was obtained.

Statistical procedures in the processing of data

Statistical analyses were carried out by parametric methods, since the distribution of continuous properties (such as the indicators of cones and seed properties) follows a normal distribution. Certain variables (such as provenances and vitality) which are discreet and do not follow normal distribution were analysed by nonparametric methods (Kruskal-Wallis test).

In all variants of the analysis (in individual provenances, in parallel multiple provenances) a detailed description of all the properties was carried out (arithmetic mean, standard deviation, a range of data and the coefficient of variation and the frequency or relative frequency) according to the year when the data was collected and according to the provenance and vitality.

Variability analysis of individual properties was carried out on different models of variance analysis with physiological and morphological properties of seeds as a dependent variable and with provenances, vitality and repetitions as factors. Homogeneous subsets of these properties in provenances were determined by Duncan's test.

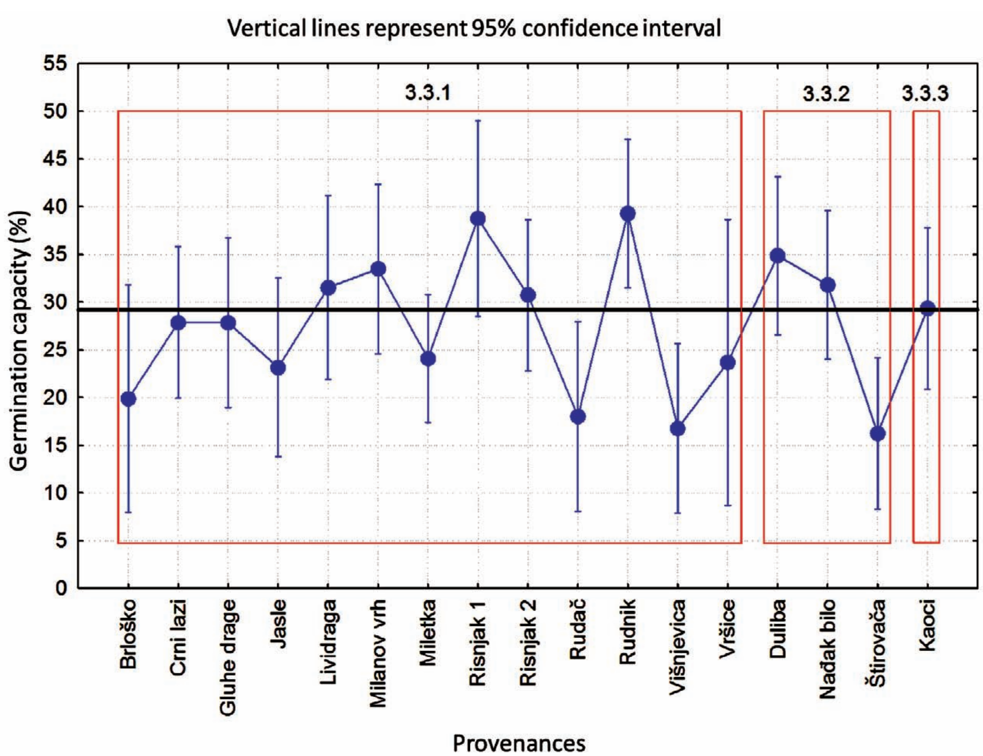

Figure 1. *The average values of germination capacity (%) for provenances according to grand total average*

Applied statistical methods were based on theoretical and practical images in several standard textbooks on statistics and forest biometrics (16, 17). The data were analysed with STATISTICA 8.0 (18).

RESULTS AND DISCUSSION

Results of two-factor variance analysis (ANOVA) for testing of physiological seed properties with factors „Provenance" and „Vitality" and „Provenance" and „Level of crown defoliation" showed that different levels of crown defoliation and different vitality did not affect the physiological seed properties, neither directly nor in the interaction with the provenance. According to Gradečki-Poštenjak (19), the influence of the level of crown defoliation in different provenances was not confirmed Since visible influence was not observed, further analysis of physiological seed properties was conducted by one-factor variance analysis with „Provenance" as a factor. Hence, only the differences between the provenances were shown in relation to the tested properties. The differences between the provenances were also confirmed by a non-parametric test (Kruskal-Wallis test) on multiple independent samples.

Physiological properties of silver fir's seeds

Germination capacity

The results of the basic statistical parameters of distribution of germination capacity are shown in Table 2 and Figure 1. The lowest germination capacity was noticed in provenance Štirovača (16.2%) and the highest in provenance Rudnik (39.9%). CV ranged from 50.3% in the provenance Milanov vrh to 91.1% in provenance Štirovača. A high percentage of CV indicates a strong heterogeneity within provenances, as seen in Figure 1.

High germination capacity was noticed in provenances Risnjak 1 and Rudnik from the seed region of Gorski Kotar and Velika Kapela (SR_GKiVK) and the prove-

Table 3 *The results of the analysis of variance for physiological and morphological properties of silver fir seeds between and within provenances*

Source of variation	Sum of squares	Degrees of freedom	Mean square	F-value	*p*
		Germination capacity			
Between provenances	16946.6	16	1059.2	2.601	*<0.001*
Within provenances	135990.6	334	407.2		
Total	152937.1	350			
		Fresh ungerminated seeds			
Between provenances	3812.0	16	238.2	1.785	*<0.032*
Within provenances	44589.6	334	133.5		
Total	48401.5	350			
		Dead seeds			
Between provenances	13444.5	16	840.3	3.116	*<0.001*
Within provenances	90063.1	334	269.7		
Total	103507.7	350			
		Empty seeds			
Between provenances	30314.2	16	1894.6	3.657	*<0.001*
Within provenances	173037.9	334	518.1		
Total	203352.1	350			
		1000 seed weight			
Provenance (PRO)	167768.5	16	10485.5	27.049	*< 0.001*
Vitality (VIT)	4508.3	2	2254.1	5.815	*0.003*
Interaction: PRO * VIT	47966.7	24	1998.6	5.156	*< 0.001*
Model	220243.5	42	5243.9	13.527	*< 0.001*
Residual	1598659.2	4124	387.6		
Total	1818902.7	4166	436.6		

nances Duliba and Nađak bilo from the seed region of Velebit (SR_V). The provenance Višnjevica from the SR_GKiVK and the provenance Štirovača from SR_V had the lowest value of seed germination. The provenance Kaoci from the seed region Biokovo (SR_B) had the average value of germination slightly higher than the average of the investigated provenances. The average values of seed germination showed the same trend as the average values of seed germination energy. For investigated properties, three provenances from the SR_GKiVK – Brloško, Rudač, Višnjevica and the provenance Štirovača from the SR_V were distinguished by low average values.

The existence of significant variability ($p<0.001$) between the provenances was established by ANOVA (Table 3) and confirmed by a nonparametric test (Kruskal-Wallis test) on multiple independent samples (data not shown).

Duncan's test (Table 4) confirmed the existence of variability between the provenances. Homogeneous provenances were grouped together. Provenance Višnjevica form the SR_GKiVK and the provenance Štirovača from the SR_V were marked as the worst with the lowest values of average germination. Considering the seed regions, most of the provenances from the SR_GKiVK showed lower values of the germination capacity. The exceptions were the provenances from the SR_GKiVK, Risnjak 1 and Rudnik, which standed out as the provenances with the highest average germination. Both provenances were situated on a silicate geological substrate, which may be an indication of the influence of geomorphological factors on the tested property. According Gradečki-Poštenjak (19), the provenances placed on silicate rocs in Gorski Kotar show higher values of seed germination. Provenance Kaoci from the SR_B was with average values of germination between both seed regions.

The average value results of silver fir germination capacity in the researched area corresponded with the references (20 – 25).

In the years of good seed crop, the value of germination capacity was higher (26.22). In the investigated area, 1997 was the year of full crop for silver fir. According to Gradečki-Poštenjak (19), the average value of seed germination in 1997 was 37.0%. Provenance Štirovača from the SR_V had the lowest average value of germination (25.09%) and in the provenance Kaoci from the SR_B, the average value of seed germination was 40.59%.

In the period from 1959 to 1995, silver fir seed germination in Gorski Kotar (FA Delnice) gradually decreased from an average of 41.6% to an average of 33.58% (25). The situation was slightly better in FA Senj and FA Split.

Table 4 *Homogeneous subset of provenances (α = 0.05) with physiological and morphological properties of silver fir seeds acquired by Duncan's test*

Germination capacity (%)							Ungerminated seed (%)				Dead seeds (%)							Empty seed (%)					
Provenance	$\bar{x}$	1	2	3	4	5	Provenance	$\bar{x}$	1	2	Provenance	$\bar{x}$	1	2	3	4	5	Provenance	$\bar{x}$	1	2	3	4
Štirovača	16.2						Štirovača	9.1			Miletka	8.2						Lividraga	29.9				
Višnjevica	16.8						Vršice	9.1			Risnjak 1	9.2						Milanov vrh	32.9				
Rudač	18						Nađak bilo	9.6			Kaoci	10						Brloško	33.4				
Brloško	19.9						Duliba	10			Duliba	10.8						Rudnik	33.7				
Jasle	23.2						Miletka	10.1			Nađak bilo	11						Risnjak 2	36.3				
Vršice	23.7						Risnjak 1	10.2			Štirovača	11.7						Gluhe drage	36.7				
Miletka	24.1						Crni lazi	10.8			Risnjak 2	13.4						Jasle	37.3				
Gluhe drage	27.9						Višnjevica	11			Rudnik	15.8						Crni lazi	41.6				
Crni lazi	27.9						Rudnik	11.2			Milanov vrh	17						Risnjak 1	41.8				
Kaoci	29.4						Rudač	11.8			Crni lazi	19.7						Rudač	42				
Risnjak 2	30.8						Gluhe drage	13.7			Višnjevica	20.6						Kaoci	44.2				
Lividraga	31.5						Lividraga	14.2			Vršice	21.6						Duliba	44.3				
Nađak bilo	31.8						Jasle	16.2			Gluhe drage	21.8						Vršice	45.6				
Milanov vrh	33.5						Kaoci	16.4			Jasle	23.4						Nađak bilo	47.6				
Duliba	34.9						Milanov vrh	16.6			Lividraga	24.4						Višnjevica	51.6				
Risnjak 1	38.8						Brloško	18.3			Rudač	28.2						Miletka	57.5				
Rudnik	39.3						Risnjak 2	19.6			Brloško	28.4						Štirovača	63				

Legend: Different letter colour denote different seed regions: Gorski Kotar i Velika Kapela (3.3.1.); Velebit (3.3.2.); Biokovo (3.3.3.)

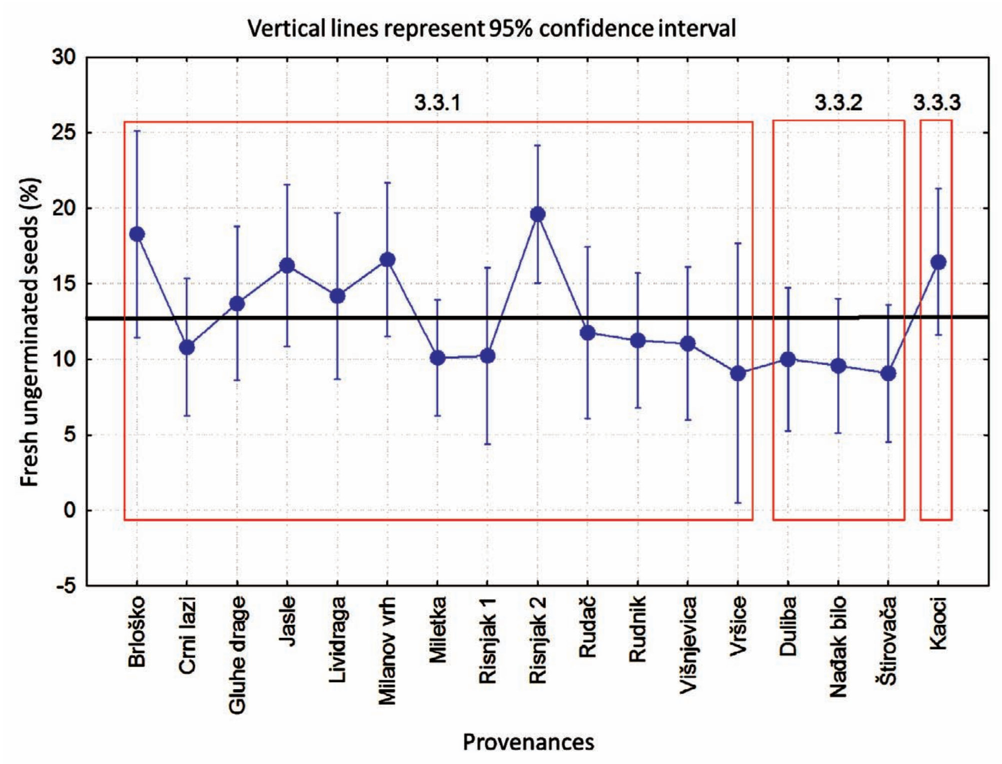

Figure 2. *The average values of fresh ungerminated seeds (%) for provenances according to grand total average of provenances*

Gradečki-Poštenjak (19) also stated that for the germination capacity she found a statistically significant variability between subpopulations, and for different levels of crown defoliation as well as for their interaction she did not establish a statistically significant difference.

Germination capacity represents a measure for assessing the seed quality (27-29). High germinated seeds are an important factor in ensuring the success of work in forest regeneration and reforestation (30, 31).

Fresh ungerminated seeds

The results of the statistical analysis of the basic parameters of distribution for the fresh ungerminated seeds are shown in Table 2 and Figure 2.

CV was high, the highest for Vršice (128.0%) and the lowest for Milanov vrh (50.2%), which indicated a heterogeneity in the provenances.

A high share of fresh ungerminated seeds was found in the provenances Risnjak 2 (19.6%) and Brloško (18.3%) from the SR_GKiVK, which was higher than the overall average in the researched provenances.

All three provenances from the SR_V had equal shares of fresh ungerminated seeds which were lower than the overall average in the researched provenances. The provenance Kaoci (SR_B) had a higher share of fresh ungerminated seeds than the overall average in the researched provenances.

By variance analysis (Table 3), the existence of statistically significant variability ($p<0.032$) between provenances was established, which was also confirmed by nonparametric test (Kruskal-Wallis test) of multiple independent samples (data not shown).

The results of Duncan's test (Table 4) showed that the provenances from the SR_V had the lowest share of fresh ungerminated seeds. Provenance Risnjak 2 was singled out with the highest average value of fresh ungerminated seeds. Most provenances from the SR_GK_VK are situated in the same homogeneous subsets. Provenance Kaoci from the SR_B showed higher average values of fresh ungerminated seeds in relation to the average value in the researched provenances.

The share of fresh ungerminated seeds indicates seed dormancy of silver fir seeds. Dormant seed is the seed which does not germinate at favourable temperature, and in favourable water and air conditions. This kind of seed is blocked, which means that it cannot germinate immediately. It has to go through physical and physiological changes before germination. The process of breaking seed dormancy is called a pretreatment. Seed dormancy is under great genetic control and it is characteristic for each individual kind of seed. Environmental conditions during

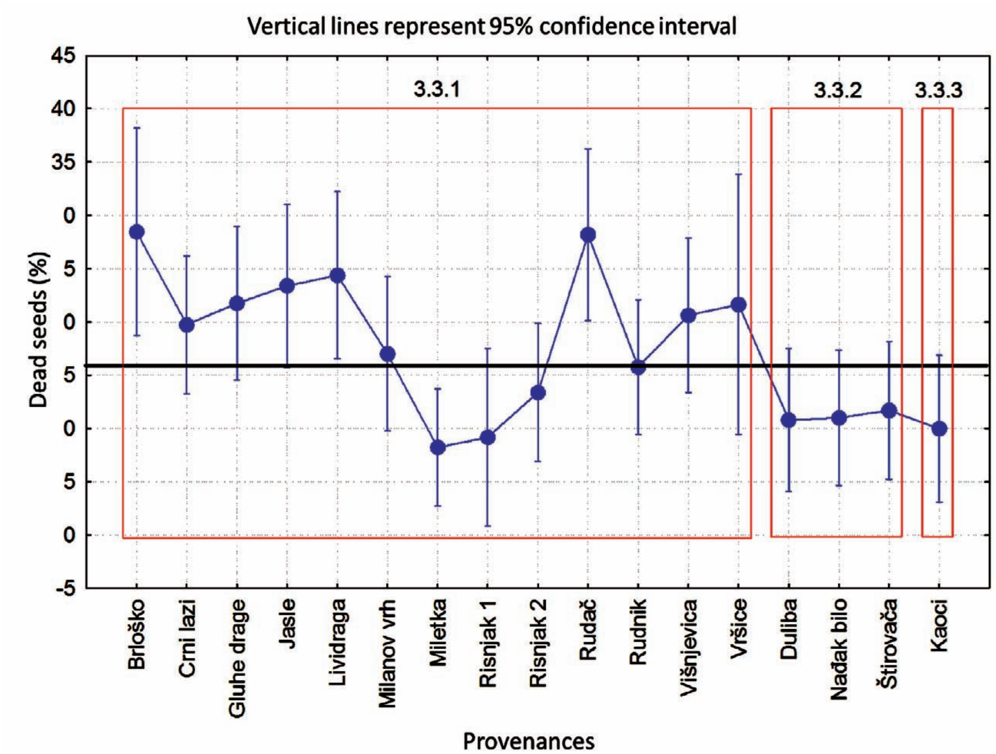

Figure 3. *The average values of dead seeds' percentage for provenances according to grand total average*

seed ripening can influence the level of dormancy and secondary dormancy can be caused by the manipulation of seed and environmental conditions after the collection (32-34). It is necessary to know the mechanism and the type of dormancy in order to be able to apply appropriate methods for breaking seed dormancy. Silver fir seeds have the embryo dormancy. It can be removed, completely or partially, by pretreatment, depending on the level of seed dormancy.

In the observed period of the research, the highest share of fresh ungerminated seeds was noticed in the provenances Risnjak 2 and Brloško from the SR_GKiVK. In both provenances, a secondary dormancy was seen, which was created under the influence of tree age and environmental conditions. In the provenance Risnjak 2, in forest stands where the samples were collected, mature and over-mature trees prevailed. Consequently, we assume that the age influences slower seed germination. In addition, in both provenances, the negative effects of polluted environment and a high level of air pollution influenced slower seed germination. Selective forests in Gorski Kotar were under great influence of polluted atmosphere. Due to the location of pollutants (industrial zone in Lombardy and the industry in Rijeka), cyclone movements and geographical locations, the burdens on southern and south-western sides of Gorski Kotar are immense, which corresponds to the location of both provenances (35).

Dead seeds

The assessments of the basic statistical parameters of distribution of the dead seeds are shown in Table 2 and Figure 3. CV were high and it ranged from 86.4% in the provenance Milanov vrh to 148.5% in the provenance Miletka. Heterogeneity between the provenances was high, which was also confirmed by ANOVA (Table 3.)

Figure 3 shows the average values of the distribution of dead seeds in the researched provenances. High distribution of dead seeds was noticed in provenances Brloško (28.5%) and Rudač (28.2%), (SR_GKVK), which was almost twice as high in comparison to the overall average. All three provenances from the SR_V had a low percentage of dead seeds (from 10.8% to 11.7%) and in the provenance Kaoci from the SR_B the share of dead seeds was 10.0%. In both seed regions, the share of dead seeds was lower than the grand total average in the researched provenances.

ANOVA (Table 3.) confirmed the existence of a statistically significant variability between the provenances ($p<0.001$) which was also confirmed by nonparametric test (Kruskal-Wallis test) on multiple independent samples (data not shown).

The results of Duncan's test (Table 4) showed the grouping of homogeneous populations considering the average values of dead seeds (rotten and decayed).

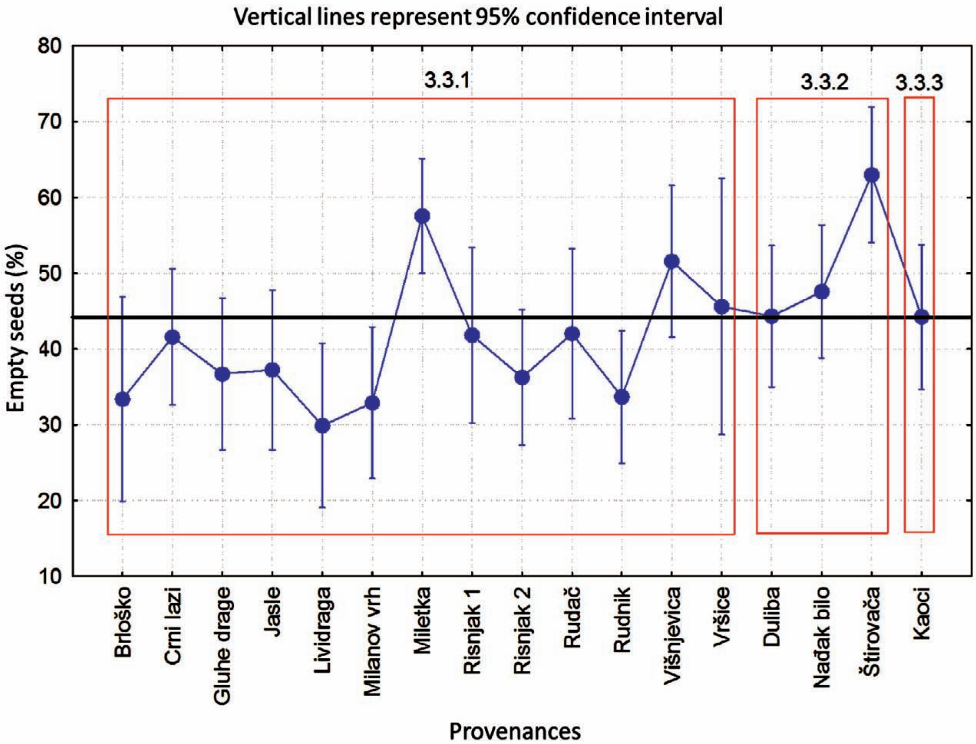

Figure 4. *The average values of empty seeds (%) for provenances according to grand total average*

The provenances Miletka and Risnjak 1 stood out as the provenances with the lowest average values of dead seeds and they were a part of a special homogeneous group. The provenance Rudač and Brloško showed the highest average values and they were a part of a special homogeneous group. The provenances from the SR_GK I VK showed the largest share of dead seeds in relation to the provenances from the SR_B and SR_V. Provenances Lividraga, Rudač and Brloško are located in FA Delnice, which have been affected by a severe forest dieback caused by increased air pollution. These results corresponded to the research of the state of forest damage conducted in FA Delnice, which has been the area with the highest index of forest dieback from the beginning of monitoring since nowadays (26, 36–41).

According to Regent (26), the share of dead seeds is higher in the provenances which are situated on limestone parent material. In addition, the share of dead seeds is significantly higher in the first year after an insect attack and it decreases gradually in the following years. The results of this research did not confirm the results of the previous research done by Regent. Gradečki-Poštenjak (19) confirmed a statistically significant interaction between subpopulations and the level of crown defoliation.

Empty seeds

By statistical analysis of the basic parameters of distribution, the share of empty seeds is shown in Table 2. The share of empty seeds according to provenances and seed regions in relation to the grand total average is shown in Figure 4. The lowest value was for Lividraga (29.9%) and the highest value was for Štirovača (63.0%). CV ranged from 33.9% (Kaoci) to 74.0% (Duliba) and it was high, which points to a heterogeneity within the provenances.

The average value of the share of empty seeds for the researched area was 43.4%. High average values of empty seeds were found in the provenance Višnjevica (51.6%) from the SR_GKiVK. The values were higher than the overall average. The provenance Štirovača (63.0%) from the seed region Velebit had the highest share of empty seeds in relation to the researched area and in relation to the seed region to which it belongs. The average values of empty seed in the provenance Kaoci (44.2%) from the SR_B were almost the same as the average of the researched population.

ANOVA (Table 3) confirmed the existence of statistically significant variability (*p<0.001*) between the provenances which was also confirmed by a nonparametric test (Kruskal-Wallis test) on multiple independent samples (data not shown).

The results of Duncan's test (Table 4) showed the grouping of provenances according to the homogeneous subsets. The lowest average values of the empty seed were noticed in the provenances Lividraga and Milanov vrh and the highest in the provenance Višnjevica from the SR_ GKiVK. The provenances Duliba, Nađak bilo and

Štirovača from the SR_V had higher average values of empty seeds than the overall average for the researched provenance.

The provenance Štirovača (63.0%) showed the highest average values of the share of empty seeds on a regional level as well as in relation to all the researched provenances. As with other physiological properties, the provenance Kaoci SR_B was positioned between the SR_GKiVK and the SR_V.

The share of empty seeds in the researched provenances was significant and had a negative influence on seed germination. According to Gradečki-Poštenjak (19), the share of empty seeds in natural populations of silver fir, which are negatively influenced by the polluted atmosphere, is high and it ranges from 38.13% to 65.5%. Schroeder (42) has researched the share of empty seeds in damaged natural populations of silver fir, which was 29.0%. The presence of insects on generative organs and the decreased pollen germination influence the increased share of empty seeds. Two species of insects from the group of spermatophagous insects- the wasp (*Megastigmus suspectus* Borr.) and the gal midge (*Resseliella piceae* Seitn.) -are particularly harmful because their larvae feed on the seed endosperm during their development. Wasps lay eggs in the developing cones and larvae develop in the seed and feed on the endosperm. One larva can be found per seed. According to Harapin, Hrašovec (43), the seed loss due to the negative effect of these pests can be up to 10%, and according to Skrzypczynska (44), the seed loss can be significantly higher. The populations of seminiphagous use the strategy of entering the diapauses through which they ensure a sufficient number of eclosing units in order to prolong the periods when the seed crop is low. Therefore, in the years of lower crop amounts, the proportion of insects is greater. Insects present on generative organs have a negative influence on natural reforestation because they further reduce the amount of full seeds per cone, which is especially emphasized in the years of low seed crop.

Reduced pollen germination occurs because of a negative influence of harmful pollutants in the atmosphere (45, 46). The influence of pollution on pollen germination is unquestionable. Pollen is more sensitive to air pollution than the leaf mass (47). Kormuťák (45) found that the pollen germination decreases by 20% in moderately polluted areas, and from 76% to 84% in heavily polluted areas. The negative influence of air pollution on vegetative parts of the plant is a well-known and a well-studied phenomenon (48, 49).

According to Gradečki-Poštenjak (19), with a level of significance of 1%, a statistically significant variability was established between subpopulations in the share of empty seeds.

At the level of significance of 5%, the interaction between the subpopulations and the level of crown defoliation was established. No difference was established for different levels of crown defoliation.

Figure 5 shows the average values of physiological properties of seeds sorted by seed regions. The average value of germination energy ranged from 15.2% (SR_B) to 17.8%

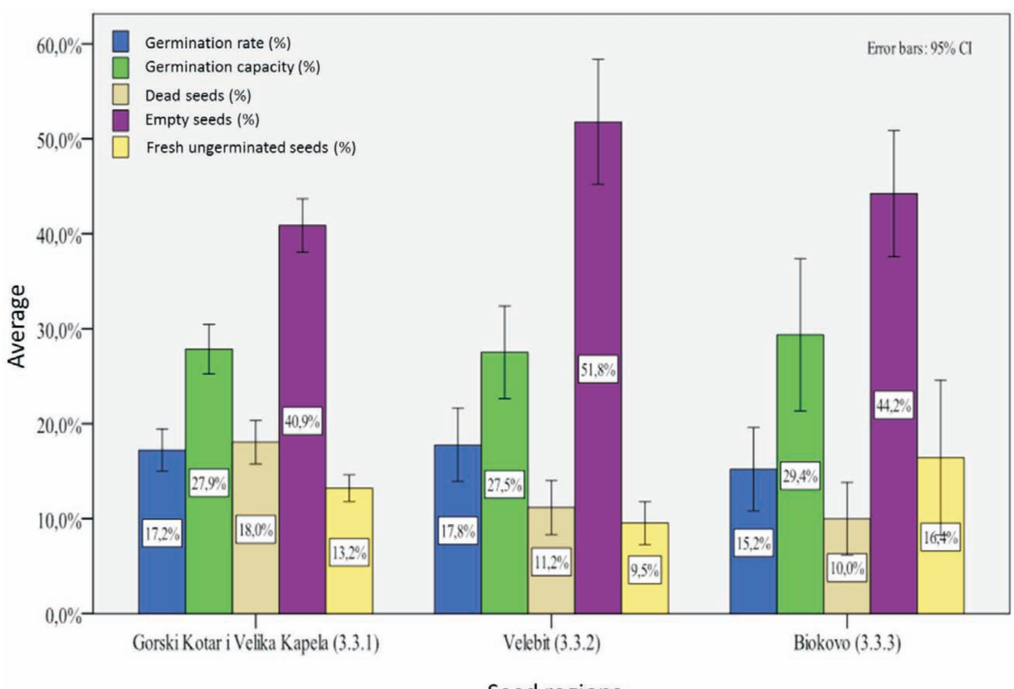

Figure 5. *The average values of physiological properties of seeds according to seed regions*

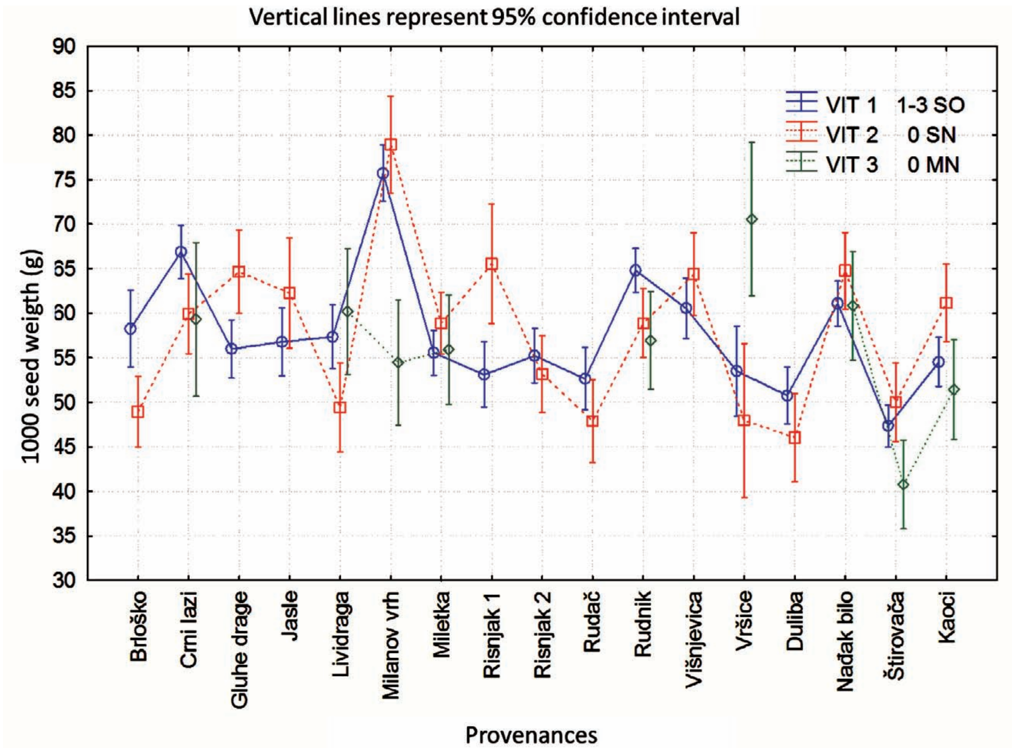

Figure 6. *The average values of TSW (g) according to provenances and vitality*

(SR_V). The average value of seed germination ranged from 27.9% (SR_GKiVK) to 29.4% (SR_B). The average values of rotten and decayed seed ranged from 10.5% (SR_B) to 18.0% (SR_GKiVK). The average values of the share of empty seeds ranged from 44.2% (SR_B) to 51.8% (SR_V). The share of fresh ungerminated seed ranged from 9.5% (SR_V) to 13.2% (SR_GKiVK). In relation to the other two seed regions, provenance Kaoci from the SR_B had the lowest average values for the properties of germination energy and rotten and decayed seeds, and had the highest average values for the germination capacity. According to the average values of fresh ungerminated and empty seeds, the seed region Biokovo was placed in the middle of the investigated seed regions.

The conducted research of physiological properties of seed showed a statistically significant variability between the provenances, but not in relation to different levels of crown defoliation in the period from 1991 to 2001. It also showed that the investigated physiological properties of seeds are under a great influence of environmental factors.

The morphological properties of silver fir's seeds

Morphological properties of cones were based on two different tree vitalities researched in the period from 1991 to 1996. Vitality 1 (VIT 1) represented older damaged silver fir trees with the level of crown defoliation from „1" to „3". Vitality 2 (VIT 2) represented older undamaged silver fir trees with the level of crown defoliation „0". In the period from 1997 to 2001, a third vitality was introduced (VIT 3) and represented younger undamaged trees with the level of crown defoliation „0".

1000 seed weight (TSW)

The parameters of the distribution of TSW per provenances and vitality are shown in Table 2.

The seeds sampled in Duliba show the lowest value (49.4 g) and those from Milanov vrh show the highest value (73.6 g).

According to ANOVA for TSW, statistically significant differences ($p<0.001$) per provenances and vitality were confirmed. Significant interactions ($p<0.001$) between the factors were also confirmed (Provenance x Vitality) (Table 3).

Figure 6. shows the average values of TSW per provenances and tree vitality. Provenances Brloško, Crni lazi, Lividraga, Risnjak 2, Rudač, Rudnik and Vršice from the SR_GKiVK had higher TSW in the older damaged trees category (VIT 1). Other provenances from the same seed region had higher TSW in the older undamaged trees category (VIT 2).

Provenances from the SR_V (Nađak bilo and Štirovača) had lower TSW in the older damaged trees category (VIT 1), while the provenance Duliba had a

Table 5 *Spearman's correlation coefficients for morphological properties of cones, seeds and vitality*

	VIT	DC	SC	TSC	BSD	TSW
VIT	–	0.052**	0.059**	0.005	0.053**	0.009
DC	0.052**	–	0.530**	0.725**	0.417**	0.386**
SC	0.059**	0.530**	–	0.641**	0.275**	0.312**
TSC	0.005	0.725**	0.641**	–	0.446**	0.529**
BSD	0.053**	0.417**	0.275**	0.446**	–	0.076**
TSW	0.009	0.386**	0.312**	0.529**	0.076**	–

Legend:	** p < 0,01
	VIT – vitality
	DC – length of cones (mm)
	SC – width of cones (mm)
	TSC – weight of dried seeds (g)
	BSD – number of filled seeds
	TSW – 1000 seed weight (g)

higher TSW in the same tree category. The provenance Kaoci from the SR_B had a lower TSW in the older damaged trees category (VIT 1).

In the younger undamaged trees category (VIT 3), provenance Vršice from the SR_GKiVK had the highest value of TSW. Provenances Crni lazi, Milanov vrh and Rudnik had the lowest.

In the SR_V, the provenances showed different results. Nađak bilo had the average values of TSW as well as in the older damaged trees category (VIT 1), and the provenances Štirovača had the lowest TSW in the younger undamaged trees category and in the researched area. The provenance Kaoci had the lowest average values of TSW in the younger undamaged trees category (VIT 3).

The average values of TSW in the researched area amounted to 57.5 g on limestone and 57.3 g on silicate. According to Regent (26), larger cones had larger seeds, regardless of the geological parent rock. Negative consequences of crown damage, as well as of harmful insects, were reflected in the generally reduced size and weight of the seeds. Gradečki and Poštenjak (25) stated the average TSW as 57.2 g in the range from 46.13 g to 68.52 g. Ballian (24) found that the average values of TSW range from 51.50 g to 58.15 g. By researching the variability of the morphological seed properties of different subpopulations in the area of Gorski Kotar and Lika, a significant variability was established between the researched subpopulations, the levels of crown defoliation as well as their interaction. The average TSW was 50 to 55 g (19).

Table 5 shows the values of correlation coefficient for morphological properties of cones, seeds and vitality.

In relation to the vitality, a positive correlation was found in the length and width of the cones and in the number of full seed per cone. The younger undamaged trees (VIT 3) have the largest cones and the largest number of full seeds per cone.

Based on the conducted research of morphological seed properties, a statistically significant variability between the provenances, the level of crown defoliation and the interaction of Provenance x Vitality was established.

CONCLUSIONS

By researching the influence of a different level of crown defoliation on the variability of certain physiological and morphological properties of silver fir seeds in the area of Dinaric beech and fir forests, a statistically significant intrapopulation and interpopulation variability of the physiological seed properties was established until an influence of a different level of crown defoliation was confirmed to have influence on their variability. Regarding morphological seed properties, a statistically significant variability was confirmed between the provenances and the levels of crown defoliation, as well as for the interaction of these two factors.

Considering the seed regions, most provenances from Gorski Kotar and Velika Kapela showed lower values of germination energy and germination capacity. A positive correlation was established between germination energy and germination capacity. The provenances growing on silicate geological substrate showed higher values of germination energy and germination capacity, which indicates an influence of geomorphological factors on the quality of the seeds.

The provenances from the seed region Gorski Kotar and Velika Kapela had higher shares of rotten and decayed seeds in relation to those from the seed regions Velebit and Biokovo. The received results correspond to the research on the state of forest damage because the area of FA Delnice has had the highest index of forest damage since the beginning of monitoring of the state of forest ecosystems in Croatia.

The share of empty seed in natural populations of silver fir which are placed under a negative influence of harmful pollutants in the atmosphere is high, indicating the negative consequences of anthropogenic influence on the seed quality. The presence of pests on generative organs and the reduced pollen germination results in a higher share of empty seed, which also reduces seed germination.

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