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MORPHOLOGICAL-BIOLOGICAL PROPERTIES OF FRUIT AND SEED OF BEECH *(Fagus sylvatica L.)* GROWING AT DIFFERENT ALTITUDES

MORFOLOŠKO-BIOLOŠKE ZNAČAJKE PLODOVA I SJEMENA BUKVE (Fagus sylvatica L.) S RAZLIČITIH NADMORSKIH VISINA

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Abstract

The paper examines morphological-biological characteristics of beech fruit and seed growing at different altitudes. Research was conducted in seven localities in the area of Velebit at altitudes ranging from 521 to 1,535 m a.s.l. Fruits were collected and yield rates were assessed during autumn 2007. Seed analyses were performed in the seed and nursery production laboratory of the Department of Forest Ecology and Silviculture of the Faculty of Forestry of Zagreb University. All the elements of seed quality were tested in accordance with the ISTA rules (International Rules for Seed Testing, 2006). The average beechnut viability obtained with the tetrazolium method was 81.4%. A positive and significant (r= 0.69452) correlation between the altitude and empty (non-vital) beech seed was confirmed. The average weight of 1,000 seeds was 118.9 g. A correlation between altitude and weight of 1,000 seeds was negative and significant (r= -0.6315). The average beechnut weight from all the seven localities was 0.24 g. There were statistically significant differences in beechnut weight in relation to the localities under study. A statistically significant difference in beechnut length was established with regard to yield rate described as average and very good, or good and very good. The average beechnut length at average yield amounted to 14.72 mm, at good yield it was 15.05 mm, and at very good yield it was 16.03 mm. The overall laboratory beech seed germination after 17 weeks of testing in sand at a constant temperature of 5°C was the highest in the Krecelj locality (39.3%) and the lowest in the Velika Stražbenica locality (34.8%), whereas average germination was 37.3%. A correlation between altitude and laboratory seed germination was positive and significant (r= 0.48350). With a rise in the altitude of provenances, laboratory germination of beechnut increases significantly.

Key words: *Fagus sylvatica* L., altitude, seed yield, seed viability, 1,000 seed weight, laboratory germination

Sažetak

U radu se istražuju morfološko-biološke značajke plodova i sjemena bukve sa različitih nadmorskih visina. Istraživanja su provedena na sedam lokaliteta na području Velebita sa rasponom nadmorskih visina od 521-1535 m n.v. Tijekom jeseni 2007. godine sakupljeni su plodovi i obavljena je procjena stupnja uroda. Analize sjemenena obavljene su u laboratoriju za sjemenarstvo i rasadničarstvo Zavoda za ekologiju i uzgajanje šuma Šumarskog fakulteta Sveučilišta u Zagrebu. Svi elementi kvalitete sjemena

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ispitivani su u skladu sa pravilima ISTA (International Rules for Seed Testing, 2006). Prosječni vitalitet bukvice dobiven metodom tetrazola iznosio je 81,4%. Utvrđena je pozitivna i značajna (r= 0,69452) korelacija između nadmorske visine i šturog (nevitalnog) sjemena bukve. Prosječna težina 1000 sjemenki iznosila je 118,9 g. Korelacija između nadmorske visine i težine 1000 sjemenki je negativna i značajna (r= -0,6315). Prosječna težina bukvice sa svih sedam lokaliteta iznosila je 0,24 g. Težina bukvice statistički se značajno razlikovala s obzirom na istraživane lokalitete. Dobivena je statistički značajna razlika u duljini bukvice s obzirom na stupanj uroda osrednji i vrlo dobar odnosno dobar i vrlo dobar. Prosječna duljina bukvice kod osrednjeg uroda iznosila je 14,72 mm, kod dobrog uroda 15,05 mm odnosno kod vrlo dobrog 16,03 mm. Ukupna laboratorijska klijavost bukvice nakon 17 tjedana ispitivanja u pijesku na konstantnoj temperaturi od 5°C bila je najveća na lokalitetu Krecelj (39,3%) a najmanja na lokalitetu Velika stražbenica (34,8%) dok je prosječna klijavost iznosila 37,3%. Korelacija između nadmorske visine i laboratorijske klijavosti sjemena je pozitivna i značajna (r= 0,48350). S porastom nadmorske visine provenijencije značajno se povećava laboratorijska klijavost bukvice.

Ključne riječi: *Fagus sylvatica L., nadmorska visina, urod sjemena, vitalitet sjemena, težina 1000 sjemenki, laboratorijska klijavost*

INTRODUCTION
UVOD

Common beech (*Fagus sylvatica* L.) is the most widespread tree species in the forest fund of Croatia. It occurs in different communities and in the sites with distinct vertical and horizontal distribution (Matić et al. 2003b). In Croatia, it inhabits a variety of sites and altitudes from 100 m a.s.l. in lowland Croatia to 1,500 m a.s.l. in the Dinaric range, where beech trees assume the stunted appearance (Seletković and Tikvić 2003).

Common beech is a monoecious species that is pollinated by wind. It is characterized by the possibility of self-fertilisation, but generally speaking, there is a high degree of heterosis (Merzeau et al. 1994, Müller-Starck 1996, Rossi et al. 1996).

The importance of beech seed to be used for interventions in the existing stands in the regeneration stage, whether it is the introduction of seeds or of nursery-raised seedlings, is increasing daily. Seeds or seedlings of common beech are also frequently used in selection beech-fir stands, in which intensive dieback of fir creates the space for the beech (Matić et al. 2003a).

As seen from the data on the planned and needed number of seedlings of common beech (Žgela 2002), there is an increasing need for beech seedlings, which requires the collection of sufficient quantities of good quality seed.

Knowing the morphological and biological properties of beech seed from different localities allows us to improve regeneration and viability of beech stands (Gradečki et al. 2003).

The effect of site parameters on the quantitative properties of seed is of particular importance. Altitude is one of the site features that affect morphological and biological seed properties (Farmer and Barnett 1972, Dorne 1972, Cavieres and Arroyo 2000, Oršanić et al. 2006, Oršanić et al. 2009).

There is a strong correlation between full yield of beech seed and improved biological characteristics of beechnuts (Crnković 2009). Full seed yield occurs at different time intervals, which may be 4, 6, 8 or 10 years (Šmelkova 1996). The interaction between climate and ample seed yields has been confirmed by numerous investigations (Hilton and Packham 1995, Hilton and Packham 2002, Övergaard et al. 2006).

A dormant seed is a seed which is incapable of germination under favourable conditions of temperature, moisture and air. Such a seed is blocked and is unable to germinate immediately, but must first undergo physical and psychological changes (Gradečki et al. 2003). It requires a certain period of time to pass from the stage of morphological maturity to the stage of physiological maturity. This period is overcome by the length of seed stratification (Matić et al. 2003a). Germination tests have demonstrated that, compared to seed germination at lower altitudes, unfavourable climatic conditions at higher altitudes result in a higher percentage of dormancy frequency or in special temperature requirements for germination (McDonough 1970).

Seed germination is the most important qualitative seed characteristic. It depends on several factors, such as seed maturity, health status, collection method, time of collection and seed storage. According to research by Šijačić-Nikolić et al. (2007), germination of beechnuts from different provenances in Serbia ranged between 58.33% and 88.88%. Nursery beechnut germination from 79 provenances in the Republic of Croatia in full mast year (2001) was between 1% and 37% (Gradečki et al. 2006).

The objective of this research was to (1) identify the effect of altitude on beech tree yield in 2007, (2) examine the elements of beech seed quality (absolute weight, laboratory germination or viability) in relation to altitude, (3) check if there is a time difference in overcoming beechnut seed dormancy in the laboratory with regard to altitude, and (4), establish a correlation between viability and laboratory germination of beech seed.

MATERIAL AND METHODS
MATERIJALI I METODE

In the course of autumn 2007, beechnuts (*Fagus sylvatica* L.) were collected from several localities situated at different altitudes in the area of North Velebit. The trees in the localities were selected randomly. The altitudes were determined with a GPSmap 60CSx device, and exposure with a compass.

A six-point yield classification system (none, very bad, poor, average, good and very good) was conducted according to Kapper, who is cited by Vincent (1965). The fruits were collected from different parts of the crowns of standing trees using shears with a telescopic handle or were picked manually from freshly felled trees. In the locality Pod Pogledalcem, beechnuts were collected from a nylon cover previously spread under the standing trees.

All the analyses were performed in the Seed and Nursery Production laboratory of the Department of Forest Ecology and Silviculture of the Faculty of Forestry of the University of Zagreb. A random sample of 30 fruits per tree was taken for morphometric analysis. Fruit length and width were measured with a digital calliper (0.01 mm), and fruit was weighted on the laboratory scale "Sartorius" (0.01 g). The number of beechnuts in 1 kg was counted during data processing. The absolute seed weight was determined according to the ISTA Rules (International Rules for Seed Testing 2006). Beechnut viability was examined with the tetrazolium method, and the assessment was made according to the ISTA Rules (ISTA Working Sheets on Tetrazolium Testing, Volume II, Tree and Shrub Species 2003). As set down by a standard procedure for testing laboratory germination of seeds of the genus *Fagus* L. species (ISTA, International Rules for Seed Testing, Chapter 5: The Germination Test 2006), stratification was required for the duration of 16 (12-20) weeks at a temperature of 3-5 °C. Laboratory seed germination was tested in a refrigerator at a constant temperature of 5°C. Sterile alluvial sand was used as a medium. The germinated seeds were checked and classified every week in accordance with the ISTA Rules (ISTA, International Rules for Seed Testing, Chapter 5: The Germination Test 2006). Data were statistically processed using Statistica (StatSoft, Inc. 2003) software.

RESEARCH RESULTS
REZULTATI ISTRAŽIVANJA

Table 1 provides some basic data on the investigated beech trees growing at altitudes between 393 and 1.535 m a.s.l. (an altitudinal difference of 1,142 m). With regard to the social position of the trees in the stand, all the trees were registered as boundary ones. The trees mostly grew in the NE expositions and at inclinations of 6-24°. Tree yields varied from none in the MU Senjska Draga to very good in the MU Bršljun, Lom and Senjsko Bilo.

Table 1 Data on the investigated beech trees (*Fagus sylvatica* L.) from different altitudes in the area of Velebit in 2007
Tablica 1 Podaci o istraživanim stablima bukve (Fagus sylvatica L.) sa različitih nadmorskih visina na području Velebita u 2007. godini

Forest Management Unit *Gospodarska jedinica*	Senjska Draga	Bršljun	Senjsko Bilo	Nadžak Bilo	Lom	Senjsko Bilo	Nadžak Bilo	NP Sjeverni Velebit
Forest District, section *Odjel, odsjek*	30a	19c	29b	98a	22a	48a	86a	-
Forest site *Šumski predjel*	Pizdulinova Jaruga	Pod Pogledalcem	Mala Snježnica	Velika Stražbenica	Santinova Ložnica	Snižnica	Krecelj	-
Social position of tree *Socijalni položaj stabla*	edge *rubno*	edge *rubno*	edge *rubno*	edge *rubno*	edge *rubno*	edge *rubno*	edge *rubno*	edge *rubno*
Altitude zone (m a.s.l) *Nadmorska visina (m n.v.)*	393	521	830	1091	1270	1300	1463	1535
Exposition *Ekspozicija*	SI	SZ	SI	SI	SZ	ISI	SI	SI
Inclination (°) *Inklinacija (°)*	12	6	19	10	7	12	11	24
Yield *Urod*	none *nikakav*	very good *vrlo dobar*	good *dobar*	good *dobar*	very good *vrlo dobar*	very good *vrlo dobar*	average *osrednji*	good *dobar*

Table 2 Data on viability, 1000 seed weight and laboratory germination of beech seed (*Fagus sylvatica* L.) from different altitudes in the area of Velebit in 2007
Tablica 2 Podaci o vitalitetu, težini 1000 sjemenki i laboratorijskoj klijavosti sjemena bukve (Fagus sylvatica L.) sa različitih nadmorskih visina na području Velebita u 2007. godini

Locality *Lokaliteti*	Altitude zone (m a.s.l) *Nadmorska visina (m n.v.)*	Viability (%) *Vitalno (%)*	Non stained *Neobojeno(%)*	Rotten (%) *Gnjilo (%)*	Damaged (%) *Oštećeno (%)*	Empty (%) *Šturo (%)*	1000 seed weight (g) *Težina 1000 sjemenki (g)*	Laboratory germination (%) *Laboratorijska klijavost (%)*
Pod Pogledalcem	521	90,0	4,0	1,5	1,5	3,0	150,6	36,5
Mala Snježnica	830	76,5	3,5	5,0	6,0	9,0	119,9	37,3
Velika Stražbenica	1091	80,5	1,0	0,0	9,5	9,0	99,5	34,8
Santinova Ložnica	1270	77,0	1,0	0,5	5,0	16,5	110,9	35,3
Snižnica	1300	78,0	5,0	0,0	3,5	13,5	119,2	39,0
Krecelj	1463	81,0	0,5	3,5	3,5	11,5	111,7	39,3
NP Sjeverni Velebit	1535	87,0	1,5	0,0	2,0	9,5	120,5	39,0

Data on the altitudes of the investigated localities and on some more important biological characteristics of beech (*Fagus sylvatica* L.) seed are given in Table 2.

The lowest viability (76.5%) was manifested by beechnut from the locality Mala Snježnica 830 m above the sea, and the highest (90.0%) by that from the locality Pod Pogledalcem 521 m above the sea. The average beechnut viability for all the seven investigated localities was high and amounted to 81.4%. The highest percentage of non-vital unstained seed was recorded in the locality Snižnica (5.0%). The highest quantity of decayed seed was recorded in the locality Mala Snježnica (5.0%), whereas seeds of this category were not identified in the localities Velika Stražbenica, Snižnica and NP North Velebit. An amount of 1.5% of decayed seed was registered on average. The highest percentage of insect damaged seeds was recorded in the locality Velika Stražbenica (9.5%) and the lowest in the locality Pod Pogledalcem (1.5%), with an average percentage of seeds of this category reaching 4.4%. The highest quantity of empty seeds were recorded in the locality Santinova Ložnica (16.5%), and the lowest again in the locality Pod Pogledalcem. There were on average 10.3% of empty seeds.

The weight of 1,000 seeds was the highest in the locality Pod Pogledalcem (150.6 g) and the lowest in the locality Velika Stražbenica (99.5 g). The average weight of 1,000 seeds was 118.9 g. The correlation between altitude and weight of 1,000 seeds was negative and significant (r= -0.6315).

Total laboratory seed germination was the highest in the locality Krecelj (39.3%) and the lowest in the locality Velika Stražbenica (34.8%), whereas average germination reached 37.3%. The correlation between altitude and weight of 1,000 seeds was positive and significant (r= 0.48350).

Categories of non-stained beech seed (*Fagus sylvatica* L.) obtained with the tetrazolium method are given in Table 3.

There were on average 1.0% seeds with a stained radicle and over one third of non-stained cotyledon surface. Only 0.07% of the seeds had completely non-stained radicles and cotyledons. An average of 0.57% seeds with non-stained radicles and completely stained cotyledons were also identified (most of them in the locality Pod Pogledalcem). Correlation analysis confirmed negative and significant

Table 3 Categories on non-stained beech seed (*Fagus sylvatica* L.) from different altitudes in the area of Velebit in 2007 obtained with the tetrazolium method

Tablica 3 Kategorije neobojenog sjemena bukve (Fagus sylvatica L.) sa različitih nadmorskih visina na području Velebita u 2007. godini dobivene tetrazol metodom

Category of non stained seed (%) *Kategorije neobojenog sjemena (%)*	Locality *Lokaliteti*						
	Pod Pogledalcem	Mala Snježnica	Velika Stražbenica	Santinova Ložnica	Snižnica	Krecelj	NP Sjeverni Velebit
1	0,0	0,0	0,0	0,5	0,0	0,0	0,5
2	0,5	1,0	0,0	0,5	0,0	0,0	0,5
3	0,5	1,5	0,5	0,0	4,0	0,5	0,0
4	0,0	0,0	0,5	0,0	0,5	0,0	0,5
5	3,0	0,5	0,0	0,0	0,5	0,0	0,0
6	0,0	0,5	0,0	0,0	0,0	0,0	0,0

Legend - *Legenda*:

1 - area of soft or necrotic tissue larger than 1/3 of the seed

1 - površina mekanog ili nekrotičnog tkiva veća od 1/3 sjemenke

2 - unstained radicle, more than one third of cotyledons unstained

2 - radikula neobojena, više od trećine kotiledona neobojeno

3 - radicle stained, more than one third of cotyledons unstained

3 - radikula obojena, više od trećine kotiledona neobojeno

4 - whole radicle unstained

4 - cijela radikula neobojena

5 - radicle unstained, cotyledons stained

5 - radikula neobojena, kotiledoni obojeni

6 - radicle and cotyledons completely unstained

6 - radikula i kotiledoni potpuno neobojeni correlation between altitude and non-vital non-stained beechnut seed (r=-0.5192). Positive and significant correlation (r=0.69452) between altitude and empty (non-vital beech seed was obtained (Figure 1).

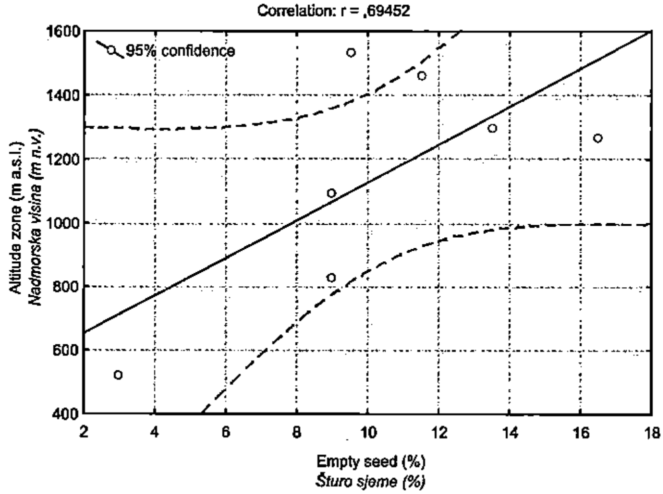

Figure 1 Correlation between altitude and empty (non-vital) beech seed.
Slika 1 Korelacija između nadmorske visine i šturog (nevitalnog) sjemena bukve

The results of descriptive statistics and weight of beech (*Fagus sylvatica* L.) seed from different altitudes in the area of Velebit in 2007 are given in Table 4.

Table 4 Descriptive statistics for weight of beechnut (*Fagus sylvatica* L.) from different altitudes in the area of Velebit in 2007
Tablica 4 Deskriptivna statistika za težinu sjemena bukve (Fagus sylvatica L.) sa različitih nadmorskih visina na području Velebita u 2007. godini

Locality-*Lokalitet*	Altitude zone (m a.s.l) *Nadmorska visina (m n.v.)*	N	Mean	Median	Minimum	Maximum	Variance	Std.Dev.
Pod Pogledalcem	521	30	0,31	0,32	0,10	0,45	0,01	0,08
Mala Snježnica	830		0,26	0,27	0,09	0,40	0,01	0,08
Velika Stražbenica	1091		0,21	0,22	0,06	0,40	0,01	0,09
Santinova Ložnica	1270		0,23	0,24	0,09	0,35	0,01	0,07
Snižnica	1300		0,23	0,24	0,05	0,36	0,01	0,09
Krecelj	1463		0,22	0,23	0,10	0,29	0,00	0,05
NP Sjeverni Velebit	1535		0,24	0,24	0,08	0,35	0,00	0,07

On average, beechnut from the locality Pod Pogledalcem was the heaviest (0.31 g), and that from the locality Velika Stražbenica was the lightest (0.21 g). The average weight of beechnut from all the seven localities amounted to 0.24 g. Variance analysis (Figure 2) yielded a statistically significant difference in beechnut weight in terms of the investigated localities (F=5.751, p=0.000015). Tukey's HSD test was used to establish statistically significant differences in beechnut weight between the local-

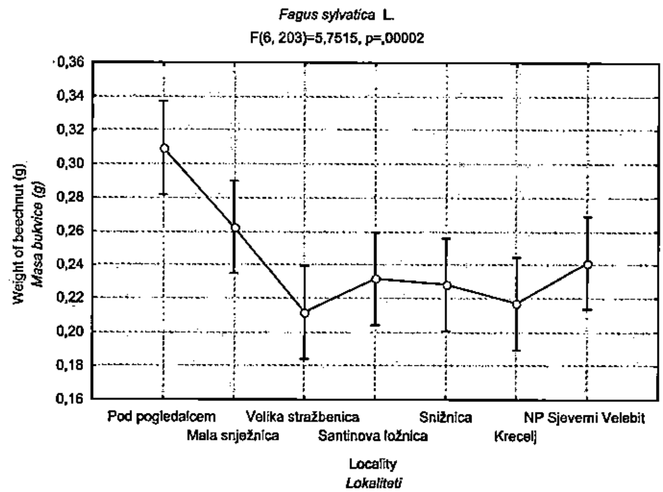

Figure 2 Weight of beechnut (*Fagus sylvatica* L.) from different altitudes in the area of Velebit in 2007
Slika 2 Težina sjemena bukve (Fagus sylvatica L.) sa različitih nadmorskih visina na području Velebita u 2007. godini

ity Pod Pogledalcem and all the other localities (p=0.000041; 0.001733; 0.000874; 0.000087; 0.010782), except Mala Snježnica (p=0.210751). Variance analysis did not confirm any statistically significant differences between beechnut weight and seed yield rate (F=2.921, p=0.056100).

The results of descriptive statistics for the length and width of beech (*Fagus sylvatica* L.) seed from different altitudes in the area of Velebit in 2007 are given in Table 5.

Table 5 Descriptive statistics for length and width of beechnut (*Fagus sylvatica* L.) from different altitudes in the area of Velebit in 2007
Tablica 5 Deskriptivna statistika za duljinu i širinu sjemena bukve (Fagus sylvatica L.) sa različitih nadmorskih visina na području Velebita u 2007. godini

Locality-*Lokalitet*	N	Lenght of beechnut (mm)-*Duljina bukvice (mm)*						Width of beechnut (mm)-*Širina bukvice (mm)*					
		Mean	Me-dian	Mini-mum	Maxi-mum	Va-riance	Std. Dev.	Mean	Me-dian	Mini-mum	Maxi-mum	Va-riance	Std. Dev.
Pod Pogledalcem	30	16,33	16,14	14,55	18,29	1,09	1,04	8,63	8,62	7,64	10,17	0,52	0,72
Mala Snježnica		15,33	15,52	10,70	16,89	1,22	1,11	8,97	9,07	6,92	10,14	0,84	0,92
Velika Stražbenica		14,50	14,58	12,05	17,40	1,61	1,27	7,47	7,57	5,24	10,20	0,96	0,98
Santinova Ložnica		16,21	16,02	13,81	18,43	1,48	1,22	8,20	8,15	7,32	9,49	0,24	0,49
Snižnica		15,54	15,85	12,00	17,74	2,34	1,53	7,87	7,94	6,38	9,06	0,53	0,73
Krecelj		14,72	14,94	11,84	16,09	0,60	0,78	7,91	8,00	6,03	8,66	0,33	0,58
NP Sjeverni Velebit		15,33	15,54	12,06	17,73	1,04	1,02	7,90	7,78	6,83	9,42	0,48	0,70

Beechnut from the locality Pod Pogledalcem was the longest (16.33 mm) and that from the locality Velika Stražbenica was the shortest (14.50 mm). The average beechnut length for the investigated localities was 15.42 mm. Beechnut from the locality Mala Snježnica was the widest (8.97 mm) and that from the locality Velika Stražbenica was the narrowest (7.47 mm). The average beechnut width for the investigated localities was 8.14 mm. Variance analysis provided a statistically significant difference in beechnut length ($F=10.50$; $p=0.000000$) and width ($F=14.04$; $p=0.000000$) in terms of the studied localities (Figure 3). Tukey's HSD test was used to establish statistically significant differences in beechnut length between the localities Pod Pogledalcem and Mala Snježnica ($p=0.014191$), Velika Stražbenica ($p=0.000026$), Krecelj ($p=0.000027$) and NP North Velebit ($p=0.013979$). Statistically significant differences were also obtained between the localities Mala Snježnica and Santinova Ložnica ($p=0.049107$), Velika Stražbenica and Santinova Ložnica ($p=0.000026$), Velika Stražbenica and Snižnica ($p=0.008836$), Santinova Ložnica and Krecelj ($p=0.000037$), and Santinova Ložnica and NP North Velebit ($p=0.048478$). Tukey's HSD test yielded statistically significant differences in beechnut width between the localities Pod Pogledalcem and Velika Stražbenica ($p=0.000026$), Snižnica ($p=0.001843$), Krecelj ($p=0.003608$) and NP North Velebit ($p=0.002830$). Statistically significant differences were also obtained between the localities Mala Snježnica and Velika Stražbenica ($p=0.000026$), Santinova Ložnica ($p=0.001278$), Snižnica ($p=0.000026$), Krecelj ($p=0.000026$) and NP North Velebit ($p=0.000026$). There was also a difference in beechnut width between the localities Velika Stražbenica and Santinova Ložnica ($p=0.002811$).

Variance analysis did not confirm any statistically significant difference between beechnut width and yield rate ($F=1.63$, $p=0.198194$), but a statistically significant difference was found between the variables beechnut length and yield rate ($F=20.95$, $p=0.000000$). The average beechnut width at medium yield was 7.91 mm, at good yield it was 8.11 mm, and at very good yield it was 8.23 mm. Tukey's HSD test found a statistically significant difference in beechnut length and medium and very good yield rates ($p=0.000022$), and between good and very good yield rate ($p=0.000022$). The average beechnut length at medium yield was 14.72 mm, at good yield it was 15.05 mm, and at very good yield it reached 16.03 mm (Figure 4).

Cumulative laboratory seed germination of beech (*Fagus sylvatica* L.) from different altitudes in the area of Velebit in 2007 is presented in Figure 3.

The highest germination percentage after 8 weeks of stratification was manifested by beechnuts from the locality Krecelj (9.00%), and the lowest by those from the locality Mala Snježnica (0.50%). The

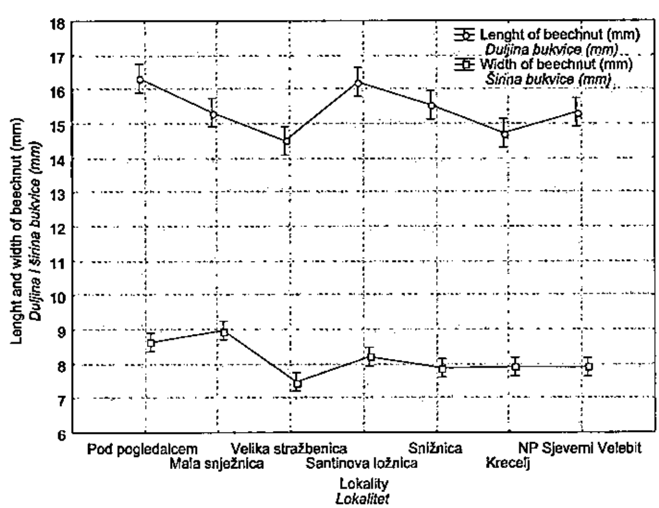

Figure 3 Length and width of beechnut (*Fagus sylvatica* L.) from different altitudes in the area of Velebit in 2007
Slika 3 Duljina i širina sjemena bukve (Fagus sylvatica L.) sa različitih nadmorskih visina na području Velebita u 2007. godini

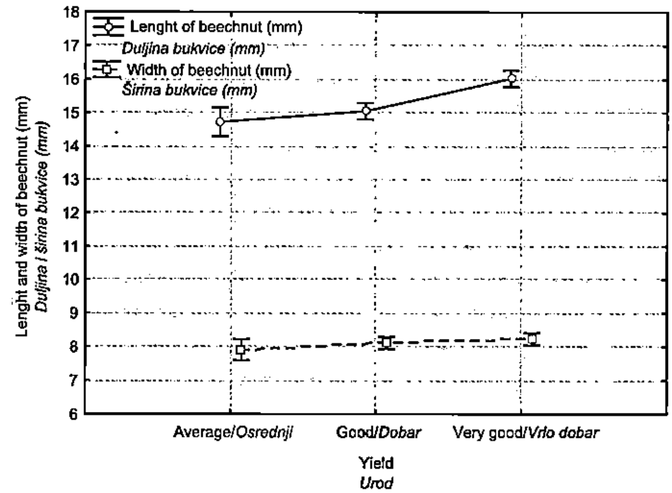

Figure 4 Length and width of beechnut (*Fagus sylvatica* L.) with regard to yield rate
Slika 4 Duljina i širina sjemena bukve (Fagus sylvatica L.) s obzirom na stupanj uroda

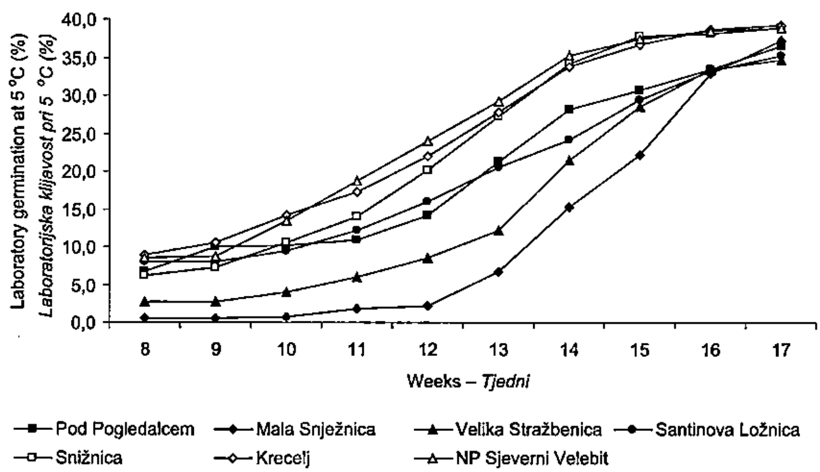

Figure 5 Cumulative presentation of laboratory seed germination of beech (*Fagus sylvatica* L.) from different altitudes in the area of Velebit in 2007
Slika 5 Kumulativni prikaz laboratorijske klijavosti sjemena bukve (Fagus sylvatica L.) sa različitih nadmorskih visina na području Velebita u 2007. godini

average beechnut germination at the end of 8-week stratification amounted to 5.96%. At the end of testing, the highest laboratory germination, equal to that in the eighth week, was exhibited by beechnut from the locality Krecelj (39.25%), and the lowest by beechnut from the locality Velika Stražbenica (34.75%) The average laboratory beechnut germination after 17 weeks of testing in sand at a constant temperature of 5°C was 37.29%. Correlation analysis showed a positive and significant correlation between beechnut viability and its germination on the eighth week in the stratifying medium (R= 0.39919). Correlation between beechnut germination on the eighth week in the stratifying medium and total laboratory germination is also positive and significant (R= 0.40364). Correlation between beechnut germination in the eighth week in the stratifying medium and the altitude of seed provenances is positive and significant (R= 0.53016), and so is correlation between total laboratory beechnut germination and the altitude of seed provenances (R= 0.47954). Accordingly, an increase in the altitude significantly increases total seed germination in the laboratory.

DISCUSSION
RASPRAVA

According to Rehder (1940), the genus *Fagus* L. comprises 10 species of medium tall trees which occur naturally in temperate zones of the northern hemisphere. Of all woody and economically important forest species in Croatian forests, common beech is the most widely spread and inhabits all the three regions (Pannonian, Dinaric and Mediterranean). It covers an area of 921,882 ha or 44.72% of the total forested area. There are no artificially raised beech forests or forest cultures in the Republic of Croatia, because natural regeneration is the only method of beech forest regeneration. Natural regeneration is the basic prerequisite for their biological diversity, productivity and stability (Matić 2003a).

Beech is a species that is currently in the optimum of its biological potential, as confirmed by the fact that it thrives on all lithological bedrock, including volcanic lava, eruptive and metamorphic silicate rocks, silicate sandstones, limestones and dolomites, etc. It colonizes all expositions, grows on a broad soil spectre ranging from distinctly acid to extremely basic and in the orographic sense occurs in all vegetation belts, from planar to subalpine one. According to Matić (2003b), beech is the only tree species in Europe that occurs naturally at altitudes from 100 m to as much as 2,000 m. Beech is found in all vegetation belts of continental Croatia in an altitudinal range from 100 to 1,500 m. In the pre-alpine belt of the Dinaric mountains, above beech-fir forests and before the belt of mugho pine, beech again builds pure stands which often form the upper boundary of forest vegetation, as is the case with the localities Bjelolasica (1.533 m a.s.l.), Risnjak (1,528 m a.s.l.), Viševica (1,428 m a.s.l.), and with numerous mountain tops of Velebit.

For this research, beech yield was registered and beech seeds were collected from 7 localities in the area of northern Velebit from altitudes ranging between 521 and 1,535 m a.s.l. According to Regent (1980), beech flowers from April to May and the fruit (beechnut) matures from September to October of the current year, to fall shortly after the first frosts. In terms of beech seed yield intervals, there are differences from author to author. Dirr and Heuser (1987) write that beech yield varies from year to year and that a good yield cannot be expected every year. Regent (1980) reports that beech bears fruit every 7-12 years, while Seletković and Tikvić (2003) state that intervals of ample seed yield occur approximately every 5-8 years. According to Young and Young (1992), common beech begins fructifying at 40-80 years of age, with intervals of 2-20 years. Oršanić et al. (2005) write that beech stands in Croatia put forth full mast every 5-10 years in warmer sites and every 8-12 years in colder ones. Övergaard (2010) states that until the end of the 17th century, the interval of full beech mast was about five years, but during the past 30 years it has been 2.5 years. Full beechnut yield is usually preceded by warm and dry July the previous year. Matthews (1955) obtained a positive correlation between beech yield rate and air temperature and the number of sunny days in July of the previous year. Among others, beech yield was investigated by Matyas (1965) and Schmidt (2006). Literature sources rarely mention seed quantities in good mast years; in other words, yield is often expressed only qualitatively. According to Henriksen (1988), it takes at least 500,000 vital seeds ha^{-1} (50 seeds m^{-2}), whereas Huss (1972) states that there should be a minimum of 20 seedlings per m^2 to ensure good quality natural regeneration. Övergaard (2010) writes that there is a big difference in beechnut quantity between good and bad mast years. During good years, beechnut yield is counted in millions or hundreds of thousands of seeds at least, whereas during bad years the quantity of seeds reaches only several thousand. Bilek et al. (2009) emphasise that a good beechnut yield in the current year negatively affects the yield of the following year. Late spring frost is the main limiting factor of seed fructification on the northern boundary of the natural distribution range of beech, while dry summers impede fructification on the southern boundary (Peters 1997). In the eastern part of the range, beech flowers more frequently from e.g. Western Europe, but this phenomenon does not affect yield frequency (Standovár and Kenderes 2003). Seed bearing and plant survival are also influenced by insects, fungi and birds. According to Suszka et al. (1996), full beech mast can be expected every 5-10 years and partial every 3-5 years. Yield periodicity depends on microclimatic conditions. In some years the yield is completely absent, even in solitary trees, while in other years only some individual trees may bear seeds; however, the seeds are generally empty. According to the same authors, beechnuts that fall off first in September are usually of very bad quality, empty or infected by insects. Šindelář (1993) also states that beechnuts begin to fall off in September and reach their maximum in the second part of October. Fructification is also affected by the phenology of individual trees (early or late flowering), the social position in a stand (height classes according to Kraft), and the position within a stand. Thus, yield quantities may vary significantly within one locality (Standovár i Kenderes 2003). Long-term investigations of beech seed yields in England revealed high variability between trees and mast years (Harmer 1994). Oddou-Muratorio et al. (2010) report on the limited capacity of beech seed dispersal to a distance of 10.4 m and pollen to a distance of 41.63 m. In their research into impacts of snow accumulations on the survival of *Fagus crenata* seed, Shimano and Masuzawa (1998) conclude that about 70% of the beechnuts are spared from mouse damage in the sites with a thicker snow cover during winter, and that about 70% of the survived seeds successfully germinate in the spring. In contrast, most beechnuts were mouse damaged in the sites with a thinner show cover, while the remaining, healthy beechnuts degenerated through desiccation during winter. This research can be correlated with our research, in which we recorded a smaller percentage of damaged seeds in the localities at higher altitudes, where there are thought to be fewer beech seed predators. In our research, the beech seed yield of 2007 ranged from none in the MU Senjska Draga to very good in the MU Bršljun, Lom and Senjsko Bilo, which coincides with claims by Harmer (1994) on high yield variability among the trees. In terms of the average yield in the study area, it can be concluded that it was between medium and good. It should be emphasised that yield rate did not drop with an increase in the altitude of the studied localities. In fact, it was completely absent at the lowest altitude.

Morphometric research by Yilmaz (2010) on 14 different provenances of oriental beech (*Fagus orientalis* Lipsky.) showed high variability within and between the populations. Beechnuts from some provenances were long and moderately heavy, whereas some others were thick, wide and heavy. Regardless of the provenances, the highest and the lowest correlations were found between the length and width (0.697), and weight and length (0.307). There is very little research into common beech seed of a similar kind. Gradečki (2003) reports the average seed length of 14.89 mm in the range of 11.31-16.20 mm, and the average width amounting to 9.53 mm in the range of 7.63 - 10.34 mm. Seed size showed good direct correlation with seed mass. In our research, beechnut length and width also differed significantly with regard to the studied localities. The average beechnut length for all the localities was 15.42 mm in the range of 14.50 - 16.33 mm, which is very similar to the results obtained by Gradečki (2003). The average width was 8.14 mm in the range of 7.47 - 8.97 mm. A statistically significant difference was obtained between beechnut length and yield rate. In other words, an increase in yield rates results in increased seed length.

Thomsen and Kjaer (2002) report on the significant difference in seed weight, germination percentage, dormancy and seed yield of beeches from different families. Seed weight was significantly correlated with the year of research, which may indicate the genetic hereditary property. Seed weight was also significantly correlated with seed germination from the yield of 1995, but not from that of 1993. According to Young and Young (1992), the average beechnut weight is 4.6 g, which is significantly more that the values obtained by our research, where beechnut weight ranged from 0.21 to 0.31 g, or 0.24 g on average.

Stjernquist (2010) reports that rain reduces endosperm mass and the total quantity of essential nutrients in the seed, but also stimulates the reduction of endosperm mass at all crown levels. Future research should include the chemical soil composition in the immediate vicinity of the trees from which the seeds are collected. According to Bonner and Leak (2008), one hl contains from 50 to 53 kg of fresh, or from 39 to 45 kg of air dried beech seed. The same source mentions that 1 kg has from 4,000 to 6,200 pieces of pure beech seed or 4,630 pieces on average. Gradečki (2003) reports that 2,000 g contains on average 4,098 seeds in the range of 6,062 – 10,000 pieces. It is reversely proportional to seed mass. According to the same author, the average seed weight in one hectolitre is 450 N in the range of 330 – 580 N. Suszka et al. (1996) write that 1 kg contains from 3,000 to 5,000 of beechnuts with a moisture content of 25%, or 3,500 to 5,800 pieces with a moisture content of 8%. According to this author, 1 hl contains about 50 kg of fresh or 39 to 45 kg of dry beechnuts. According to Šmelkova (2001), 1 kg of seeds contains an average of 4,300 beechnuts with average germination of 70%, purity of 90% and the proportion of full seeds of 90%. The WSL catalogue, Versuchsgarten (1991), lists 3,700 to 4,700 pieces of beechnut in one kg. According to Žgela (2002), there are on average 4,300 beechnuts in one kg, while Regent (1980) counted 4,600 pieces in one kg, which is slightly more than the quantity of 3,226-4,762, or 4,167 pieces on average obtained in our research. According to Šmelkova (2002), the weight of 1,000 seeds or the absolute beechnut weight is 234 g, and according to Suszka et al. (1996), the weight is between 150 and 300 g, or 250 g on average. In one of their investigations, Roth et al. (2005) found that 1,000 beech seeds weighed 237 g, whereas according to WSL h Versuchsgarten (1991), their weight varied between 210 and 270 g. Gradečki (2003) cites the average seed mass of 258 g in the range of 100.331 g. In our research, the average weight of 1,000 seeds was 118.9 g, which is much less that the data put forth by the above mentioned authors. The highest absolute weight of 1,000 seeds (150.6 g) was obtained in the locality Pod Pogledalcem at the lowest altitude, but this can be attributed to the method of seed collection in this area. A negative and significant ($r = -0.6315$) correlation was obtained between the altitude of a locality and the weight of 1,000 seed; in other words, an increase in the population altitude leads to a significant decrease in the weight of 1,000 seeds. This can, among others, be attributed to microclimatic features. The awareness of this pattern may be interesting from the aspect of nursery production of beech seedlings. In order to reach an accurate answer on the influence of altitude on beechnut weight, research should be repeated in several more localities. Research by Gračan et al. (2006) indicates the existence of differentiation, both between and within the provenances of common beech in Croatia.

Beech seed is characterized by deep embryo dormancy which blocks germination of a vital and mature embryo even when it is isolated from the seed and placed into optimal germination conditions (Bewley and Black 1994). Barthe et al. (2000) also report that at the moment of collection beechnut has well developed deep dormancy because of the embryo and the outer fruit structure. The fruit pericarp inhibits embryo germination because it prevents water uptake and gas exchange. Fluridone, a pyridine inhibitor of carotenoid synthesis, is important in ABA biosynthesis, which plays a key role in embryo dormancy of beech seed. According to El-Antably (1976) and Suszka et al. (1996), embryo dormancy can be overcome with cold-moist stratification at temperatures from 2-5°C, with or without a substrate. The time period required to overcome dormancy is long and lasts 5-8 weeks, and in some cases for as many as 12 weeks (Muller and Bonnet-Masimbert 1982). In our research, beechnut began germinating on week eight in the stratifier at a temperature of 5°C, which coincides with the data by Muller and Bonnet-Masimbert (1982). On the other hand, Gradečki (2003) reports that previous treatment of the seed in cold storage at a temperature of +3°C to +5°C for 60 days did not prove efficient in overcoming beech seed dormancy, because without additional moisture the seeds became too dehydrated. The key factor for successful beech seed stratification is the seed moisture content, which should not be above 30% (Muller and Bonnet-Masimbert 1983). Krawiarz and Szczotka (2008) stress that during stratification at 3°C, there is an abrupt increase in the activity of amino acid arginine (ADC) and enzyme ornithine decarboxilase (ODC) in the embryonic cavity (from week 7) and cotyledons (from week 8).

According to Kolářová et al. (2010) there is no statistically significant difference in germination capacity between non-treated dormant beechnuts, beechnuts treated with tap water and beechnuts treated with ethephon or GA_3. Ethephon and GA_3 treatment reduces germination capacity if applied to the seed previously subjected to stratification for four weeks. The effect of ethephon and GA_3 on germination rate or energy and on overcoming dormancy increases significantly if beechnuts are stratified for four weeks prior to the treatment. The effect of GA_3 on mean germination time of stratified beechnuts did not prove important in comparison with dormant non-treated beechnuts. A shorter mean germination time was achieved with seeds soaked in 1,000 mg·l^{-1} GA_3 prior to germination. Compared to the beechnut soaked in running water, the application of GA_3 shortens the period of cold stratification by three weeks. In our research, the largest amount of seeds germinated after 14 weeks in the stratifier on average, while average germination on week eight was 5.96%. On average, the smallest amount of seeds germinated in the ninth week in the stratifier (0.86%). According to ISTA (2006), beechnut germination is tested in sand and at a temperature of 3-5 °C, with a note that the duration of testing depends on dormancy and that in some exceptional cases it may last for 24 weeks. Since the seed is dormant, the ISTA rules (2006) suggest that, instead of testing germination, seed viability should be tested with the tetrazolium method. Foffová and Foff (2003) write that the tetrazolium test and the germination test of beech seed give variable results, which indicates subjectivity in the assessment of stained seed parts or regular, irregular and non-germinated seeds. In terms of drying and storage, Gosling (2007) classifies beech seed into the "*Intermediate*" group, while in terms of dormancy he places it in the group of deeply dormant species. To overcome dormancy, he proposes cold stratification of 16 (12-20) weeks at a temperature of 4°C. Young and Young (1992) report on germination of untreated beech seed of 14%. Seeds kept in cold stratification for 120 days manifested germination of 67%. The same authors write about 100%-germination of freshly collected and stored seeds, which were cold stratified for 5 months.

The key element of good seed germination is healthy seeds. The pericarp of a healthy seed is light brown in colour. Gradečki et al. (2006) report on the low average value of laboratory germination of beech seed, which was between 1 and 37%, or 12% on average. The average proportion of fresh, non-germinated seed ranged from 1-5%, or 5% on average, of diseased and rotten seed from 24-81% or 62% on average, and empty seed from 1-70% or 19% on average. Šmelkova (2001) reports on the average beechnut germination of 70%, whereas according to WSL Versuchsgarten (1991) it varies between 50 and 80%. The results of research by Rezaii et al. (2010) into the seed of *Fagus orientalis* Lipsky showed a favourable effect of stratification on germination. Significant variations were obtained between provenances and duration of seed stratification. In our research, average laboratory germination amounted to 37.3%, which coincides with the data by Regent (1980), who reports on average germination of 35%. Differences in seed germination in individual localities can be the result of varying degrees of seed dormancy. In the same locality, the degree of seed dormancy can vary from year to year or within the same year by provenances (Wang 1976, 1980). With this in mind, it would be advisable to repeat investigations in the same localities. The degree of dormancy is influenced by the nutrition status and water status of mother trees, as well as by climatic conditions during maturation. Accordingly, seeds should not be collected from several trees or one tree and the results should not be related with provenances. According to Villiers (1972), higher temperatures in the vegetation period may cause deeper dormancy than usual. In some tree species, seed dormancy may vary in relation to geographic distribution (Viliers 1972, Wang and Haddon 1978).

A positive and significant correlation was established between altitude and total laboratory seed germination (r= 0.48350), or laboratory germination on week eight (r= 0.53016), which suggests that laboratory beechnut germination increases significantly with an increase in the altitude of the populations. The trend is visible as early as the eighth week of testing. This can be explained, among others, by a lower average air temperature at higher altitudes in relation to the lower ones, and consequently, less developed seed dormancy at higher altitudes.

According to Ratajczak and Pukacka (2005) and Pukacka and Ratajczak (2007), substances that could play a key role in maintaining seed viability are phenols, α-tocopherol, sterols, ascorbic acid, glutathione and soluble proteins. Germination capacity is positively and strongly correlated with amounts of total phenolic compounds, ultraviolet (UV-) absorbing phenols and soluble proteins. According to Gradečki (2003), the average beechnut viability was 69 % (10-90 %), and the average proportion of empty seed was 19 % (1--50 %).

Procházková and Bezděčková (2008) provide data on the viability of three seed lots of beech from different altitudes. Beechnut viability of the first lot from an altitude between 601 and 700 m was 85%, of the second lot from an altitude between 551 and 600 m was 66-68%, and of the third lot from an altitude of 551-600 m was 83%. In this research, the highest viability (90%) was obtained at the lowest altitude of 521 m. Gradečki (2006) reports that beech seed viability tested with the tetrazolium method provided a higher result than that obtained with a germination test. Seed viability ranged from 10-90% or 72% on average, the proportion of empty seed was 0-50% or 15% on average, while the proportion of non-vital (diseased) seed amounted to 13% on average. Our research also confirmed the already established fact that much higher results are obtained with seed viability assessment than with germination tests. The average viability was 81.4%, which is somewhat higher than the data cited by Gradečki et al. (2006). An amount of 10.3% of empty and 4.4% of diseased seed was found on average, which is less than the results reported by Gradečki et al. (2006). According to Ratajczak and Pukacka (2005), the loss of viability in beechnuts strongly correlates with an increase in membrane permeability and the formation of lipid-hydroperoxide (LHPO) as an indicator of peroxidation of unsaturated fatty acids. During storage time, concentrations of individual phospholipids and fatty acids decrease proportionally with the loss of viability. Decreased vitamin E (α-tocopherol) and sterol concentrations were recorded, which have an important role in protecting the membrane from harmful environmental impacts. Beechnut germination capacity abruptly decreases at temperatures above 0°C and in high moisture conditions. This happens mainly because peroxidation of unsaturated fatty acids weakens the membranes.

Tylkowski (2002) writes that the majority of beech seed lots are characterized by different levels of infestation with pathogenic fungi of the genera *Fusarium, Rhizoctonia* and *Cylindrocarpon* spp. Research into beechnut mycoflora revealed the most frequent saprophytic fungi, as well as fungi causing diseases: *Alternaria alternata, Arthrobotrys superba, Botrytis cinerea, Fusarium* spp., *Penicillium* spp., *Trichotecium roseum, Mucor* sp., *Graphium* sp. and *Chaetomium* sp. (Novak Agbaba et al. 2003).

According to Wang (2003), the percentage of empty seeds from 91 trees in the same locality ranged from 4.8 to 40.9% and had the form of normal distribution. The average percentage of empty seed was 21.4%, which is much higher than the percentage obtained in our research. The percentage of repeatability of empty seeds was 43.4%. Self-pollination is an important cause of the occurrence of empty beech seeds. Our research found a positive and significant correlation between altitude and the percentage of empty seeds; with an increase in the altitude of a locality the quantity of empty seed increases significantly, which can, among others, be explained by a higher rate of self-pollination. In many forest tree species, controlled self-pollination results in a higher share of empty seed, unlike cross-pollination or wind pollination (e.g. Nielsen and Schaffalitzky De Muckadell 1954, Dieckert 1964, Sorensen 1971, Park and Fowler 1982, Kormutak and Lindgren 1996). Genetically speaking, empty seed in forest tree species can arise from incompatibility (Nielsen and Schaffalitzky De Muckadell 1954, Yazidani and Lindgren 1991) or embryo degeneration, which may be the consequence of inbreeding. Apart from genetic factors, empty seed in forest trees may also occur as the result of environmental factors, such as limited pollen quantities (Colangeli and Owens 1990) and insect damage (O'reilly and Farmer 1991). Cross-pollination in species that are wind-pollinated is positively correlated with stand size and flower density.

The average nursery germination of beech seed (Gradečki et al., 2006) is very low and reaches only 3%. The relationship between laboratory and nursery germination coincides relatively well. Future research should focus on investigating nursery germination of beechnut from different altitudes in order to gain a realistic picture of this important biological property of a seed. Insights gained by such research would be widely applicable in forest nursery production. A positive and significant correlation ($R= 0.39919$) was established in our research between beechnut viability and germination on week eight in the stratifier, and between beechnut germination on week eight and total laboratory germination ($R= 0.40364$). In other words, instead of the time-consuming procedure of testing beechnut germination in the laboratory, it is recommended to test beechnut viability. On the other hand, in the case of testing germination, a period of eight weeks can be considered a sufficiently reliable time to provide satisfactory results.

CONCLUSIONS
ZAKLJUČCI

Beechnut yield in the Velebit area in 2007 ranged from none in the MU Senjska Draga to very good in the MU Bršljun, Lom and Senjsko Bilo.

Beechnut from the locality Mala Snježnica 830 m a.s.l. demonstrated the poorest viability (76.5%) and that from the locality Pod Pogledalcem 521 m a.s.l. the highest (90.0%). The average beechnut viability for all the seven investigated localities was high and amounted to 81.4%. Correlation analysis established a negative and significant correlation between altitude and non-vital unstained beechnut seed (r=-0.5192). A correlation between altitude and empty (non-vital) beechnut seed was positive and significant (r= 0.69452).

The weight of 1,000 seeds was the highest in the locality Pod Pogledalcem (150.6 g) and the lowest in the locality Velika Stražbenica (99.5 g). The average weight of 1,000 seeds was 118.9 g. A negative and significant correlation (r= -0.6315) was established between altitude and weight of 1,000 seeds.

On average, beechnut in the locality Pod Pogledalcem was the heaviest (0.31 g), and that in the locality Velika Stražbenica was the lightest (0.21 g). The average beechnut weight from all the seven localities was 0.24 g. A statistically significant difference was confirmed in beechnut weight with regard to the studied localities.

Variance analysis did not establish any statistically significant difference between beechnut width and yield rate, whereas there was a difference between the variables beechnut length and yield rate. Tukey's HSD test revealed a statistically significant difference in beechnut length and yield rate, described as average and very good, and good and very good. The mean beechnut length at average yield was 14.72 mm, at good yield it was 15.05 mm, and at very good yield it was 16.03 mm.

Total laboratory germination of beechnut after 17 weeks of testing in sand at a constant temperature of 5°C was the highest in the locality Krecelj (39.3%) and the lowest in the locality Velika Stražbenica (34.8%), with average germination amounting to 37.3%. The correlation between altitude and laboratory seed germination was positive and significant (r= 0.48350). The correlation between beechnut germination on week eight in the stratifier and the altitude of seed provenances was positive and significant (R= 0.53016), and so was the correlation between total laboratory germination of beechnut and the altitude of seed provenances (R= 0.47954). This indicates that total seed germination in the laboratory increases with an increase in altitude.

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