Variability of acorn nutrient concentrations in pedunculate oak (*Quercus robur* L.) genotypes

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ABSTRACT: The objective of this study was to determine mineral element concentrations (N, P, K, Ca, Mg, Na, Fe, Zn, Mn, and Cu) in various parts of pedunculate oak acorns to estimate variability in the chemical composition of different genotypes. Acorns were collected during the 1998 harvest from seventeen *Quercus robur* L. genotypes. Trees originated from a vegetative seed plantation Banov Brod, belonging to the Forest Estate Sremska Mitrovica (Srem, Vojvodina, Serbia). The investigation of the nutritional status of acorns revealed significant differences between the studied genotypes. Considering all genotypes, the general sequence of nutrient amounts for the entire embryo was as follows: N > K > Ca > P > Mg > Na > Fe > Zn > Mn > Cu. Concentrations of Ca, Mg, Fe, and Mn were higher for the pericarp and seed coat than for the embryo. Pericarp and seed coat nutrient concentrations showed lower variability than in the embryo, with the exception of potassium concentration.

Keywords: chemical composition; embryo; mineral elements; seed; testa

Acorns are an important wildlife food resource and seed source for oak regeneration (Greenberg 2000). Successful forest regeneration from seeds requires a favourable combination of seed supply, seedbed and environmental conditions (Leadem et al. 1997). When revitalizing English oak forests, one must bear in mind that, among others, seed origin and quality are important factors affecting the development of future trees.

Mineral nutrient reserves in seeds play an important role during germination and seedling establishment. Macro- and micronutrients accumulated in seeds are essential for their regular metabolic activity; they are the main resource of mineral elements for young plants till their root system develops and begins to absorb soil nutrients (KASTORI 1984). During seed development, minerals from the mother plant move through the apoplast and accumulate in the embryo (WOLSWINKEL 1992). There is an absence of a general relationship between the effective

seed nutrient supply and the balance of minerals in the soil environment, and it seems that most seeds behave in a broadly similar way (KITAJIMA, FENNER 2000). Distribution of mineral nutrients in seed tissues is different (Moussavi-Nik et al. 1997). There is also a variability in nutrient uptake, transport and distribution in genotypes (Sarić 1981).

Seeds are a significant source of nutrients for human and wildlife nutrition, and plant physiologists are interested in materials stored in seeds as a source of nutrient material and energy for early development of seedlings (Crocker, Barton 1957). The cotyledon is very important for acorn germination and subsequent seedling establishment, growth and survival because it serves as the main nutrient resource for young seedlings (Ovington, Macrae 1960; Kormanik et al. 1998). The mineral composition of acorns in many oak species was investigated earlier and they contain 0.06–0.12% of P, 0.04–0.32% of Ca, and 0.07–0.14% of Mg (Bonner 1971, 1974).

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There are also numerous studies dealing with acorn food reserves (carbohydrates, lipids and proteins) in response to maturity level, metabolic activity during dormancy, stratification, germination and species (Bonner, Vozzo 1987; Connor et al. 1996). In contrast, information about Q. robur acorn chemical composition is poor, and no chemical analyses between varieties within species have been published (SAFFARZADEH et al. 1999). Such information is of a great value, knowing that within-species variability may improve plant fitness over a wide range of environmental conditions and resource availability (CASTRO-Díez et al. 1997). Plant fitness depends on the number of seeds produced and the proportion of those seeds that germinate, and both can be influenced by the environmental conditions (Allison 2002). Hence, the regulation of ion uptake and compartmentation is one of the adaptative mechanisms of many tolerant species to environmental stresses (BOHNERT et al. 1995).

The objective of this study was to determine nutrient concentrations (N, P, K, Ca, Mg, Na, Fe, Zn, Mn, and Cu) in various parts of English oak acorns to estimate variability in the chemical composition of different genotypes.

MATERIAL AND METHODS

Study site

Plant material used in this study originated from the seed plantation Banov Brod, belonging to the Forest Estate Sremska Mitrovica (Srem, Vojvodina, Serbia). The plantation is located on the left bank of the Sava river, Šančine location – Banov Brod forest, Bosut village (44°55′N, 19°23′E), at 81 m above sea level. The soil belongs to eutric Cambisol type. The analyses of soil basic chemical characteristics were performed at the Laboratory for Agroecology of the Institute of Field and Vegetable Crops (Report No. 08-96/837), and data are shown in Table 1. The seed orchard, formed of 85 English oak genotypes, was established by grafting. English oak scions were taken from plus trees chosen in the forests of the Eastern and Western Srem, and numbered (from 1 to 85). Two to five years old seedlings, produced from seeds, were the rootstocks. Seventeen genotypes were chosen for the analyses of acorn chemical composition: 4, 5, 6, 16, 18, 20, 21, 22, 25, 28, 29, 30, 33, 35, 38, 40, and 85.

Acorn collection

Acorns from seventeen 20-year-old Quercus robur L. trees were analyzed for the concentrations of mineral elements. Acorns were harvested from the ground after natural drop. Approximately two kilograms of healthy, undamaged (by insects or else) seeds were taken from a total yield for each genotype. Samples contained 330-700 acorns, depending upon their mass, which was variable in the particular genotypes (see Nikolić, Orlović 2002). The whole samples were used for preparing a representative sample for each genotype. After cleaning seeds were stored at 2-3°C in plastic bags. Before the chemical analyses, acorns were washed with demineralized water and cut in half, so that the pericarp and testa could be separated from cotyledons. The results show nutrient concentrations in the embryo (i.e. both cotyledons and embryo axis) and in the cover tissue (both pericarp and testa).

Nutrient contents

Nutrient concentrations were analyzed in the embryo as well as in pericarp and testa (i.e. seed coat). The different acorn tissues were dried at 100°C to constant weight, and milled before analysis. Total N concentration in dry matter was estimated by standard micro-Kjeldahl method (Nelson, Sommers 1973). After dry ashing at 450°C and treatment with HCl samples were analyzed for P, K, Ca, Mg, Na, Fe, Zn, Mn, and Cu concentrations. The yellow method for the determination of phosphorus was used (GERICKE, KURMIES 1952). Potassium and sodium concentrations were estimated using flame photometry (Marjanović, Krstić 1998), and magnesium, calcium, iron, zinc, manganese and copper were determined by atomic absorption spectrophotometry (VARIAN SPECTRAA-10). The concentrations of macronutrients and sodium were expressed in mg per g dry weight while those of microelements are given in $\mu g/g$.

Statistical analysis

The obtained results are means of three replications. The data were statistically processed using

Table 1. Soil chemical characteristics

CaCO ₃	Humus	Nitrogen	$AL-P_2O_5$	AL-K ₂ O	p	ьН
(%)	(%)	(%)	(mg/100 g)	(mg/100 g)	in KCl	in $\rm H_2O$
0.98	3.48	0.233	6.1	24.1	6.3	6.5

the analysis of variance (LSD-test). Duncan's multiple-range test (at $\alpha = 0.05$ significance level) was used for studying differences between the various genotypes. The mean values of the parameters were ranked and marked with letters. Values with the same letter did not differ significantly. Cluster analysis utilizing a hierarchical classification system was employed to examine if any of the studied genotypes could be grouped together according to the acorn chemical composition. The used computer program package STATISTICA (ver 5.0) was based on Euclidean distances, and computation of raw data led to joining tree clustering. The twenty variables in the clustering procedure were the mean values of element concentrations (mg/g) in various acorn tissues.

RESULTS

The embryo and the cover tissues differed in their chemical composition. Table 2 indicates the mean amounts of mineral elements in the acorn tissues for all studied genotypes. Compared to pericarp and testa, the embryo had a higher amount of nitrogen, phosphorus, potassium, zinc and copper, and lower concentration of calcium, magnesium, sodium, iron and manganese. For all studied genotypes, the following patterns were obtained for the embryo: N > K > Ca > P > Mg > Na > Fe > Zn > Mn > Cu. For the cover tissues these patterns were different: N > Ca > K > Mg > Na > P > Fe > Mn > Zn > Cu.

Nitrogen (N)

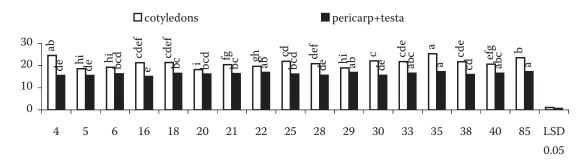
Among all assayed mineral nutrients the amounts of nitrogen were the highest. Nitrogen accumulation was higher in the embryo (Fig. 1). The concentrations of this element ranged between 25.36 (genotype 35) and 18.19 (genotype 20) mg/g in the embryo. These concentrations were lower in the cover tissue and varied from 17.25 (genotypes 35 and 85) to 15.14 (genotype 16) mg/g.

Table 2. Mean nutrient concentrations in various parts of acorn in seventeen *Q. robur* genotypes. Conc. – concentration (mg/g dry weight for N, P, K, Ca, and Mg; μ g/g for Zn, Fe, Mn, and Cu). CV% – coefficient of variation

		Embryo	Pericarp + testa
N	conc.	21.18	16.24
	CV%	3.00	2.40
P	conc.	0.90	0.15
	CV%	8.00	4.20
1/	conc.	7.84	3.19
K	CV%	5.80	8.30
	conc.	1.09	3.26
Ca	CV%	15.40	9.70
	conc.	0.74	0.96
Mg	CV%	11.50	5.60
NT-	conc.	0.13	0.17
Na	CV%	22.50	10.50
7	conc.	9.80	9.00
Zn	CV%	21.40	18.90
Г-	conc.	32.00	96.00
Fe	CV%	15.90	8.50
Mn	conc.	7.90	3.00
	CV%	6.80	3.30
C	conc.	6.20	6.00
Cu	CV%	6.30	4.70

Phosphorus (P)

Phosphorus concentration depended on the part of the seed (Fig. 2). Generally, these concentrations were nearly six times lower in the pericarp and testa compared to embryos. Phosphorus content was between 1.18 (genotype 35) and 0.75 (genotype 29) mg/g in the embryo tissue while the cover tissue contained from 0.23 (genotype 30) to 0.10 (genotypes 18, 29, and 40) mg/g dry matter.



Genotype

Fig. 1. Nitrogen concentration in various parts of Q. robur acorn (mg/g)

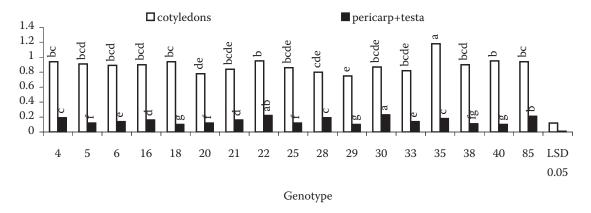


Fig. 2. Phosphorus concentration in various parts of Q. robur acorn (mg/g)

Potassium (K)

Fig. 3 summarizes the concentrations of potassium in different acorn tissues. The average potassium content for all genotypes amounted to $7.84\,\text{mg/g}$ in embryo, and $3.19\,\text{mg/g}$ in pericarp and testa. The respective minimum and maximum values were between 9.73 (genotype 35) and $6.58\,\text{mg/g}$ (genotype 28), and between 4.63 (genotype 21) and $0.88\,\text{mg/g}$ (genotype 20).

Calcium (Ca)

According to calcium content in different acorn tissues, the genotypes may be classified into several groups (Fig. 4). Among others, genotypes 6, 16, 18, and 22 showed significantly higher concentrations of calcium in the embryo. Its minimum and maximum values in this tissue were between 1.64 and 0.77 mg/g. The accumulation of this macronutrient was higher

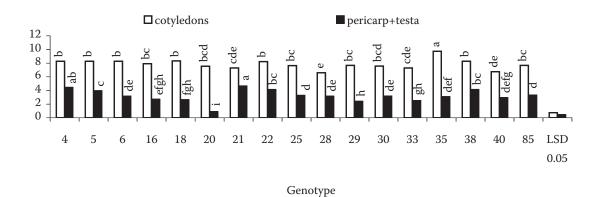


Fig. 3. Potassium concentration in various parts of Q. robur acorn (mg/g)

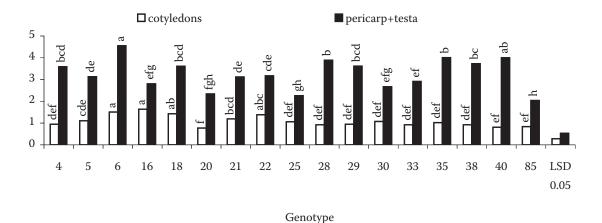


Fig. 4. Calcium concentration in various parts of Q. robur acorn (mg/g)

Table 3. Microelement content of *Q. robur* acorn: genotype variability of embryo concentrations^a

C t	Microelement concentrations (μg/g)				
Genotype -	Zn	Fe	Mn	Cu	
4	8 cd	28 defg	10 c	8 a	
5	10 c	40 bc	4 g	6 c	
6	10 c	30 defg	12 b	7 b	
16	10 c	22 g	6 e	6 c	
18	16 a	35 cd	7 de	7 b	
20	14 ab	26 defg	7 de	4 e	
21	10 c	24 fg	7 de	7 b	
22	11 bc	25 efg	14 a	8 a	
25	9 cd	52 a	7 de	6 c	
28	8 cd	33 cdef	4 g	4 e	
29	5 d	27 defg	12 b	6 c	
30	8 cd	34 cde	7 de	6 c	
33	9 cd	29 defg	5 f	6 c	
35	12 bc	45 ab	10 c	8 a	
38	8 cd	25 efg	8 d	5 d	
40	11 bc	32 cdef	7 de	5 d	
85	8 cd	33 cdef	6 e	7 b	

^a Each mean was calculated using three replications. Different letters in a single column indicate significant differences between genotypes (Duncan's test) at $\alpha = 0.05$

in the cover tissues, i.e. in pericarp and testa, for all studied genotypes.

Magnesium (Mg)

Considering all genotypes, magnesium concentrations were higher for the pericarp and testa compared to the embryo, with the exception of genotypes 6, 18, and 30 (Fig. 5). Magnesium content ranged from 0.56 (genotypes 21 and 28) to 1.08 (genotype

Table 4. Microelement content of Q. robur acorn: genotype variability of pericarp and seed coat concentrations^a

Comotumo	Microelement concentrations (μg/g)				
Genotype -	Zn	Fe	Mn	Cu	
4	16 b	44 ij	47 b	8 a	
5	7 ef	57 hi	17 k	4 e	
6	6 ef	109 d	32 f	6 c	
16	6 ef	99 de	23 i	6 c	
18	6 ef	86 ef	25 h	7 b	
20	13 c	112 cd	23 i	4 e	
21	9 e	124 bc	31 f	7 b	
22	6 ef	60 h	56 a	6 c	
25	9 de	157 a	27 g	6 c	
28	8 e	40 j	12 l	4 e	
29	31 a	164 a	46 b	7 b	
30	4 f	62 h	20 j	5 d	
33	4 f	77 fg	21 j	7 b	
35	7 ef	137 b	42 c	6 c	
38	12 cd	125 bc	38 d	6 c	
40	7 ef	71 gh	35 e	5 d	
85	7 ef	107 d	20 j	7 b	

^a Each mean was calculated using three replications. Different letters in a single column indicate significant differences between genotypes (Duncan's test) at $\alpha = 0.05$

18) mg/g in embryo, and from 0.60 (genotype 28) to 0.147 mg/g (genotype 40) in the cover tissues.

Sodium (Na)

Generally, relatively low concentrations (average value of 0.13 mg/g for embryo, and 0.17 mg/g for pericarp and testa) were recorded in acorn tissues of all investigated genotypes (Fig. 6). When compared

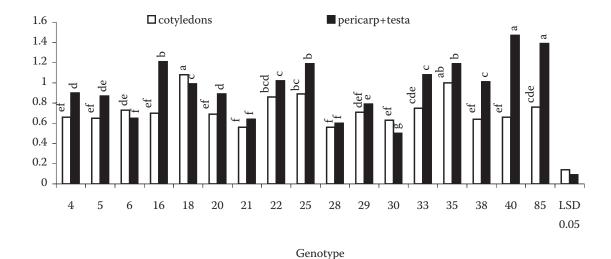


Fig. 5. Magnesium concentration in various parts of Q. robur acorn (mg/g)

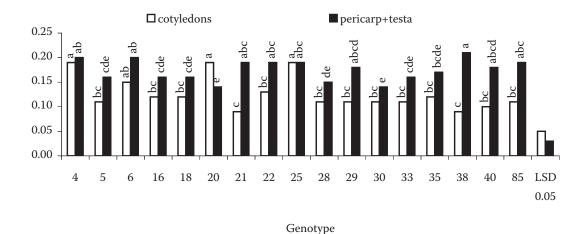


Fig. 6. Sodium concentration in various parts of Q. robur acorn (mg/g)

with the other elements, the genotypic variability of embryo Na concentration was more evident.

Microelements (Zn, Fe, Mn, Cu)

The determination of microelements revealed a higher variability for the embryo in comparison with concentrations in the pericarp and seed coat. On average, iron and manganese concentrations were lower in the embryo. The amounts of zinc and copper were slightly higher in the embryo, with respective concentrations around 9 and 6 μ g/g for the embryo and acorn cover tissues. The results summarized in Tables 3 and 4 show genotype variability of microelement contents in various tissues of *Q. robur* acorn.

In order to get better insight into differences between genotypes, we used cluster analysis.

Genotypes were grouped

A dendrogram in Fig. 7 illustrates the results of cluster analysis on the basis of average element concentrations in both the embryo and the cover tissues. The lines that parallel the axis, bearing genotype numbers, indicate the distance at which two clusters combine. According to the dendrogram (Fig. 7), genotypes were segregated into two main clusters. One of them consisted of genotype 4 and 35, indicating similarity in their chemical composition. The other consisted of all other genotypes, while genotype 20 was clearly separated from the others. The closest associations were observed for genotypes 25 and 30. Genotypes 28 and 40, 21 and 22, 6 and 29 were also closely associated.

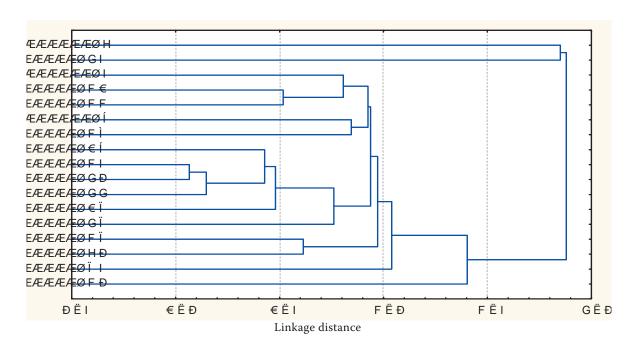


Fig. 7. Dendrogram of a horizontal hierarchical cluster analysis utilizing micro- and macroelement concentrations in various parts of acorn of seventeen *Q. robur* genotypes. G – genotype

DISCUSSION

Nutrient contents of plant seeds depend on mobilization from the soil, uptake by the roots, translocation and redistribution within the plant, import and deposition in the seeds, etc. (Grusak, DellaPenna 1999). Previous studies suggested that there existed differences between genotypes in the uptake, translocation, accumulation and use of mineral elements required for plant growth (CLARK 1983). There are numerous studies dealing with seed chemical characteristics of different species which approve the presence of all macroelements and microelements detected in plant tissues until now. The chemical composition of seeds of agriculturally important species has been studied in detail, whereas only poor data on the forest seed, particularly Q. robur acorns, is available.

The present results suggest that various acorn tissues contained different amounts of nutrients. On average, concentrations of Ca, Mg, Na, Fe, and Mn were higher for pericarp and seed coat than for embryo tissue. The relatively small remobilization of nutrients from the seed coat was shown in weed genotypes during imbibition, germination and early seedling growth, thus loading of nutrients predominantly into the seed coat represents a wasteful process for the plant (Moussavi-Nik et al. 1997). Considering that the element remobilization ability from acorn cover tissues during these processes was not previously observed (according to our knowledge), such studies are needed. Pericarp and seed coat nutrient concentrations showed lower variability compared with embryo, with the exception of potassium concentration. The chemical composition, among the structure of each of different parts of the seed, exerts an influence, either collectively or singly, on moisture absorption, which can be a limiting factor during germination (CROCKER, BARTON 1957).

In contrast to calcium and sodium amounts the nitrogen concentrations showed the lowest genotypic variability in various parts of acorn. Among all studied nutrients, the accumulation of nitrogen was the highest in *Q. robur* acorns. The supply of N to forest trees relies upon factors controlling organic matter decomposition and the release of mineral N (HARRIS, RIHA 1991), and environmental factors have a greater impact on element contents in vegetative than in reproductive plant organs (KASTORI 1984).

Genotype 35 may be separated from others by the highest accumulation of phosphorus in the embryo. It seems that the concentration of this nutrient has an important influence on acorn germination after storage. Previous studies revealed a positive cor-

relation between the vitality of acorns in storage, the content of phytic acid in acorns and the content of P in soil (ŽITNIK et al. 1999). The higher content of P in the leaves was followed by a higher acorn content of P and phytic acid and germination. The seed content of phosphorus, although constituting only a small proportion of total demand, may be essential for plant establishment and growth (TYLER, ZOHLEN 1998).

On average, potassium concentrations were higher for the embryo and less variable between studied genotypes than those recorded for cover tissues. The chemical composition of cotyledon tissue from macadamia nut, walnut, and hazel nut was studied previously (LOTT, BUTTROSE 1978). According to our results, it seems that the potassium content of *Q. robur* embryo was closer to hazel nut, while the other two species accumulated lower amounts of this nutrient.

The accumulation of calcium was higher in the cover tissues of acorns. On average, the triples of the embryo concentrations were recorded for pericarp and testa. Such distribution of calcium is quite reasonable, considering the pericarp anatomic structure. For all studied genotypes the estimated mean value for embryo tissue was 1.09 mg/g, while pericarp and testa contained 3.26 mg/g.

Mg concentration as well as concentrations of other nutrients studied in this paper showed variability between genotypes and different acorn tissues. When compared with acorn magnesium amounts of many other oak species (Bonner, Vozzo 1987), it could be seen that they are similar, with the exception of southern red oak (*Q. falcata* var. *falcata*).

Among analyzed macroelements, sodium accumulation was the lowest in *Q. robur* acorns. The highest accumulation of this nutrient in the embryo was recorded for genotypes 4, 20, and 25, while in the cover tissues for genotype 38. Belonging to the group of useful elements, sodium may have a favourable impact upon the growth and development of agricultural plants, whereas in forest plants such as oaks no data is available.

The chemical composition of *Q. branti* hulled acorns was investigated previously (SAFFARZADEH et al. 1999). Calcium, phosphorus, magnesium and potassium concentrations were lower, and sodium amounts were significantly higher than embryo amounts of these nutrients presented in this paper.

Considering the studied genotypes, zinc concentrations showed a high variability in all analyzed parts of acorn (Table 2). The values estimated for pericarp and seed coat (Table 4) varied between 4 (genotypes 30 and 33) and 31 μ g/g (genotype

29). Embryo concentrations (Table 3) ranged from 5 (genotype 29) to 16 μg/g (genotype 18). Considerably lower variability was estimated for copper concentrations, and the values ranged from 4 to 8 μg/g. The analysis showed that many genotypes were characterized by equal concentrations of this nutrient in all studied acorn parts (genotypes 4, 16, 18, 20, 21, 25, 28, 40, and 85). The accumulation of iron and manganese was three to nearly four times higher in the cover tissues than in the embryo. Considering different genotypes, iron concentrations showed higher variability in comparison with manganese. Genotype 29 could be separated from the others by the highest iron and zinc concentrations in acorn cover tissues. Compared to micronutrient concentrations in Q. branti acorns (SAFFARZADEH et al. 1999), the O. robur embryo contained higher concentrations of Mn, Cu, Zn, and Fe. The variability in mineral element distribution between the embryo and cover tissues could affect their availability for seedling growth. For example, iron in the embryo of the common bean seeds was directly available for newly emerging plants, while iron in the seed coat was not (Moraghan et al. 2002).

This study has shown that studied genotypes have a different ability to accumulate nutrients in the different seed parts. Variability was slightly but statistically significant. Genotype 35 may be separated from the others by the highest accumulation of nitrogen, phosphorus and potassium. The large reserves of nutrients in the embryo cells ensure that the embryo can grow during the initial stage independently of any nutrient supply from other seed tissues or from the external environment (Moussavi-Nik et al. 1997). Our results are supported by other studies suggesting that the mineral composition of genotypes from the same habitat and environmental conditions is most frequently specific, representing a genotypic character (SARIĆ, LOUGHMAN 1983). Considering that mother trees were grown under the same environmental conditions, our results illustrate the influence of certain genotypes on the accumulation of various nutrients within acorns. Such information is of great value in forestry and could be one of the guidelines enabling the choice of genotypes that meet desired criteria.

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Variabilita koncentrací živin v žaludech různých genotypů dubu letního (*Quercus robur* L.)

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ABSTRAKT: Cílem práce bylo stanovení koncentrací minerálních prvků (N, P, K, Ca, Mg, Na, Fe, Zn, Mn a Cu) v jednotlivých částech žaludů dubu letního a odhad variability chemického složení u různých genotypů. Žaludy jsme odebrali během sklizně v r. 1998 od sedmnácti genotypů druhu *Quercus robur* L. Stromy pocházely z vegetativně založeného semenného porostu v lokalitě Banov Brod, která patří do polesí Sremska Mitrovica (Srem, Vojvodina, Srbsko). Šetření stavu živin v žaludech ukázalo významné rozdíly mezi sledovanými genotypy. Když vezmeme v úvahu všechny genotypy, celkové pořadí množství živin v celém embryu bylo následující: N > K > Ca > P > Mg > Na > Fe >

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Zn > Mn > Cu. V perikarpu a osemení byly koncentrace Ca, Mg, Fe a Mn vyšší než v embryu. Variabilita koncentr živin v perikarpu a osemení byla s výjimkou koncentrace draslíku nižší než v embryu.				
Klíčová slova: chemické složení; embryo; minerální prvky; osivo; osemení				

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