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EFFECTS OF Mo, Zn, Sr AND Ba LOADS ON THESE ELEMENTS' UPTAKE AND OIL CONTENT AND FATTY ACID COMPOSITION OF RAPESEED

ABSTRACT: Studied in the present paper were the long-term effects of the application of high Mo, Zn, Sr and Ba rates (0, 90, 270, and 810 kg ha^{-1}) on rapeseed oil content and oil fatty acid composition. The trace elements were applied in the spring of 1991, while the rapeseed was sown on a calcareous chernozem soil in 2001. The trace elements differed significantly in their rates of accumulation in rapeseed plants. Relative to the control, the Mo content of the stem increased up to 1,000 times, that of the chaff over 100 times, and that of the seed around 60 times. The levels of the other trace elements increased considerably less relative to the control. The increases were typically twofold to threefold, depending on the plant part involved. The trace elements accumulated the most in the vegetative plant parts, except for Zn, a major quantity of which was found in the seed as well. The application of the high rates of Sr, Zn and, to an extent, Mo reduced the seed oil content of rapeseed. However, the differences were not statistically significant. The application of the trace elements had no significant effect on the fatty acid composition of the rapeseed oil, either. The increased levels of the trace elements found in the rapeseed plants indicate that 11 years after application significant amounts of the applied elements are still present in the soil in a form available to plants. However, the rates were not high enough to affect the synthesis of oil and its fatty acid composition.

KEY WORDS: Mo, Zn, Sr, Ba — loads, rapeseed, oil content, fatty acids

INTRODUCTION

Rapeseed (*Brassica napus* L. ssp. *oleifera*) is a highly productive oil crop. Brassica species constitute the world's third most important source of vegetable oil at present. The nutritive value, oxidative stability and melting point of vegetable oils depend on their levels of certain fatty acids (Beare-Rogers, 1988; Galliard, 1980). Oil content and fatty acid composition are quantitative traits of a plant. They are most often inherited intermediately and

they are controlled by minor genes. Plants' quantitative traits can be affected by numerous external factors. The environmental factor with the largest effect on fatty acid content is temperature during seed development (Beringer, 1971; Pleines and Friedt, 1988). Other factors found to have an effect on this trait include light (Trémoliéres et al., 1982), oxygen concentration in the atmosphere (Dompert, 1976), frost damage (Daun et al., 1985), and agronomic practices (May et al., 1994).

The objective of this study was to determine how high rates of Mo, Zn, Sr and Ba — trace elements having different physical-chemical properties and physiological and environmental importance — affect the oil content and fatty acid composition of rapeseed. Zn and Mo are biogenic, transition elements, and, like all heavy metals, they are toxic at higher concentrations, which means they are potential environmental pollutants (Kiekens, 1990; Jones et al., 1990). Sr and Ba are not biogenic elements, they belong in the group of alkaline earth metals and have no major environmental impact. In addition, these elements differ significantly when it comes to the intensity of their accumulation and distribution in plants (Marschner, 1995).

Knowing how excess concentrations of heavy metals affect the chemical composition of vegetable oils is particularly important not only from the academic standpoint but also from the point of view of actual agronomic practice and the production of biologically valuable food. Knowledge of the effects of environmental factors on the levels and composition of vegetable oils is also necessary for the design of informative genetic experiments as well as for the correct interpretation of the results of such experiments.

MATERIALS AND METHODS

Plant Material and Treatments

A small-plot field experiment was set up in the spring of 1991 on the loamy-textured calcareous chernozem soil formed on loess at the Nagyhőrcsők Experimental Station of the Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences, Budapest, to investigate the effect of high trace element rates. The plowed layer of the growing site contained approximately 5% $CaCO_3$ and 3% humus, was satisfactorily supplied with available Ca, Mg, Md and Cu, moderately supplied with N and K, and poorly supplied with P and Zn. The groundwater was at a depth of 15 m and the area had a negative water balance tending toward drought. Salts of the 13 trace elements examined were each applied at four levels in the spring of 1991 prior to maize sowing. In 2001, the winter oilseed rape variety Doublal was sown. The trace element levels were 0, 90, 270 and 810 kg ha^{-1}. Studied in the present paper were only the effects of Mo, Zn, Sr and Ba. These elements were applied as $(NH_4)_6MO_70_{24}$, $ZnSO_4$, $SrSO_4$, and $BaCl_2$, respectively. All the plots received basic fertilization with 100 kg ha^{-1} each of N, P_2O_5 and K_2O in the form of ammonium nitrate, superphosphate and potassium chloride, respectively.

Plant Analysis

Mo, Zn, Sr and Ba levels were determined separately in the seed, stem (stem plus leaves) and chaff. After breaking down the plant materials with cc HNO_3 + cc H_2O_2, the levels were determined using the ICP technique. The total seed oil content was determined by extraction using petroleum ether according to Soxhlet. In order to analyze the fatty acid composition, the oil was extracted by a hydraulic press, after which the preparation of fatty acid methyl esters of the oil was carried out using trimethyl sulfonium hydroxide according to Butte (1983). The qualitative and quantitative compositions of the mixture of fatty acid methyl esters were determined by gas chromatography with a flame-ionizing detector (HP 5890 with FID) and a capillary column (HP--INNOWax cat. No 19091N-133). The results were statistically processed by calculating the least significant difference.

RESULTS AND DISCUSSION

Mo, Zn, Sr and Ba Contents, Accumulation and Distribution

Plants differ in their capacity for the uptake, accumulation, translocation and use of different mineral elements. Among the trace elements studied, Mo in particular has an especially high accumulation rate. The results shown in Table 1 support this. The Mo content in the stem increased over 1,000 times relative to the check treatment, in the chaff over 100 times, and in the seed about 60 times. A significant characteristic of plant nutrition with Mo is a wide variation between the critical deficiency and toxicity levels. These levels may differ by a factor of up to 10^4 (Marschner, 1995). Molybdenum contents above 5—10 mg kg^{-1} dry wt are considered critical for humans and herbivorous animals. Values obtained in the present study significantly exceeded this threshold value. The levels of the other trace elements — Zn, Sr and Ba — increased to a considerably smaller extent as a result of their application, in most cases as little as two to three times, depending on the plant part involved. The zinc content of the vegetative plant parts was low, which may have been due to the low level of available zinc in the soil. Despite the fact that the mobility of Zn in plants is not large (Mengel and Kirkby, 1987), the seed Zn content was considerably higher than the Zn content of the vegetative plant parts. Strontium is not an essential element for plants, and it is not considered to be particularly toxic, either. Still, the uptake, distribution and accumulation of Sr in plants is extremely important in the soil-plant-human system. This problem has gained weight since the Chernobyl accident in 1986. In this connection, it is important to know the effects of single factors on Sr accumulation in plants. Agroecological factors have relatively little effect on the Sr content of rapeseed and wheat (Haneklaus, 1989). This is partly supported by the results of the present study too. Sr levels increased to about the same extent relative to the control in all rapeseed plant parts under study. The measured levels were quite below the toxicity threshold for plants (500 mg kg^{-1})

and were also lower than the toxic concentration for food, especially in the seed (150 mg kg^{-1}) (Pais, 1980). Similar Sr levels in rapeseed are reported by Haneklaus (1989). Similarly to Sr, the Ba content in our study increased to aproximately the same extent relative to the check treatment in all the rapeseed plant parts under investigation. Plant Ba levels vary across a wide range and this element is considered to be more toxic to plants than Sr (Scharrer, 1955).

Table 1. Effect of Mo, Zn, Sr and Ba loads on their levels in rapeseed

Plant part	Rate (kg · ha^{-1})*				LSD 5%
	0	90	270	810	
	Mo content (mg Mo · kg^{-1} DM)				
Stem	0.1	73	144	137	42
Chaff	0.5	78	164	266	48
Seed	0.5	6	13	29	3
	Zn content (mg Zn · kg^{-1} DM)				
Stem	3.5	8.0	9.6	13.0	1.2
Chaff	5.0	8.2	7.3	10.5	1.4
Seed	32.0	41.0	45.0	49.0	6.0
	Sr content (mg Sr · kg^{-1} DM)				
Stem	58	56	72	94	6
Chaff	88	99	124	211	10
Seed	16	22	19	32	4
	Ba content (mg Ba · kg^{-1} DM)				
Stem	6.0	7.0	11.8	20.1	2.7
Chaff	6.1	7.0	9.3	19.4	2.4
Seed	1.8	2.7	3.0	3.9	1.3

* The trace elements were incorporated into the soil 11 years before.

In some studies involving other crop species that had been carried out in previous years in the same trial as our experiment, the trace elements concerned were found to have similar effects on their levels in plants. In those studies, too, there was a particularly large increase of Mo levels resulting from this element's application in the trial (Kádár and Prokisch, 2000b; Kádár et al., 2000a; Kádár et al., 2000c, Kádár 2001).

The accumulation of the trace elements involved in our study varied (Figure 1). In relation to the check, Mo accumulated the most as a result of the treatment, followed by Ba, Zn, and, lastly, Sr. In the check treatment, it was Sr that accumulated the most, followed by Zn, Ba, and Mo. Rapeseed's great capacity for Sr accumulation was also confirmed by Haneklaus (1989), who reported that the Sr content of rapeseed leaves was six times larger than that of wheat leaves.

The large accumulation of the trace elements indicates that 10 years after application the soil still contained a significant amount of them in the form available to plants. Results of the 2000 soil analysis showed that the topsoil

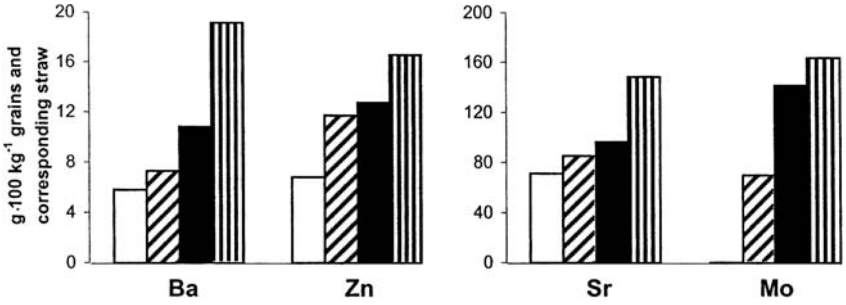

Figure 1. Accumulation of Mo, Zn, Sr and Ba in harvest unit of 100 kg grains and corresponding quantities of straw in rape. Analyses were done on plants grown on the soil loaded eleven years before with 0 (□, control), 90 (▨), 270 (■) and 810 (▥) kg of Mo, Zn, Sr and Ba per ha, respectively.

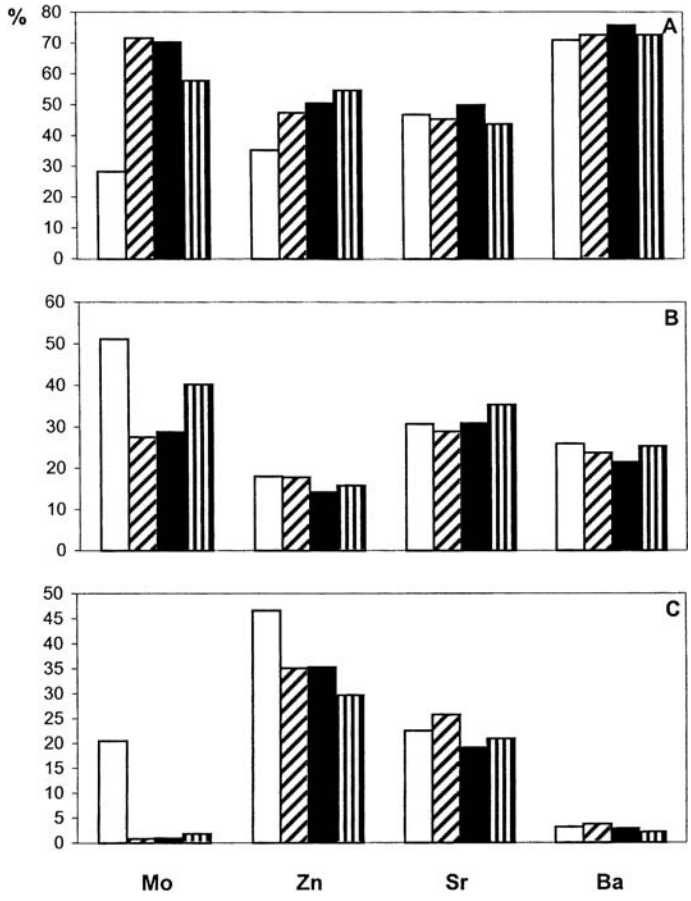

Figure 2. Distribution of Mo, Zn, Sr and Ba in stem (A), chaff (B) and seed (C) of rape (in % of total content). Analyses were done on plants grown on the soil loaded eleven years before with 0 (□, control), 90 (▨), 270 (■) and 810 (▥) kg of Mo, Zn, Sr and Ba per ha, respectively.

had 4—5% of Mo, 42—50% of Zn, 28—30% of Sr and 15—20% of Ba in a readily soluble form (extracted by NH_4-acetate + EDTA).

In rapeseed, storage lipids are synthesized in the seed tissue during sedd development. The accumulation of the trace elements and their overall distribution across the plant could therefore be of importance from the point of view of oil content and the oil's fatty acid composition (Figure 2).

The distribution of individual elements in plants is specific and dependant on numerous biotic and abiotic factors. The seed can be very rich in Mo (Rebafka, 1993). In the control treatment, around 20% of the total accumulated Mo were located in the seeds. The application of Mo led primarily to its accumulation in the stem. Of all four elements, it was zinc that accumulated the most in the seeds. Similar to what happened with Mo, the application of zinc increased the zinc level in the stem. Strontium accumulated the most in the stem, followed by chaff and, finally, the seeds. Similar distribution has been reported in wheat as well (Kastori et al., 1992; Lásztity, 1996). The application of Sr and Ba had no effect on their distribution, since their presence increased uniformly in the rapeseed parts concerned. Of the four elements, Ba accumulated the most in the stem, while only a small amount of it (only 3% of the total amount accumulated) was found in the seeds.

The uneven accumulation of the trace elements in the seed suggests that these elements potentially pose varying degrees of danger when it comes to their entry into the food chain. In this connection, a question also arises of the extent to which these trace elements actually end up in the products of rapeseed processing.

Oil content and its fatty acid composition

The oil content and its fatty acid composition both change in the course of plant growth and development and plant aging. Genetic, biotic and environmental factors play an important role in these changes. Environmental factors can affect plant lipid metabolism in several ways. They can cause changes of adaptive nature (nonhereditary variability) and stress-induced degenerative changes and affect biochemical processes that are not directly related to lipid metabolism (Nyitri, 1998). High concentrations of trace elements, especially heavy metals, may induce stress in the plant and affect plant metabolism and plant physiological processes (Kabata-Pendias and Pendias, 1984). It is therefore reasonable to expect that their excessive accumulation in the plant, especially the seeds, will cause changes in lipid metabolism and hence affect the seed oil content and its fatty acid composition.

Among the environmental factors, there has been relatively little study of the effects of mineral nutrition, particularly trace elements, on the levels and chemical composition of vegetable oils (Yermanos et al., 1964).

Most of the studies concern the effects of nitrogen, whose application most often reduces the oil content (Dybing, 1964; Yermanos et al., 1964). In Beringer (1966), the application of nitrogen reduced the oil content of oat grains to a negligible extent and practically had no effect on the oil's fatty acid composition. According to Kádár (2001), increasing nitro-

gen rates decreased the oil content of poppyseed, while increased phosphorus supply had no effect on this trait, although it reduced the oleic and linolenic acid contents and increased the linoleic acid level. Szirtes and Lukács (1980) reported that the foliar application of Mn, Cu and Zn had no major impact on the oil content of sunflower seeds. As for the effect of these elements on the oil's fatty acid composition, there was an increase in the linoleic acid content and a drop in the oleic acid one. In the absence of nutrient deficiencies, foliar applications of iron chelates did not affect the safflower and flax oil contents of seed and the iodine value of oil (Yermanos et al., 1964).

Table 2. Effect of Mo, Zn, Sr and Ba loads on oil content of rapeseed (%)

Element	Rate (kg · ha^{-1})*				LSD 5%
	Ø	90	270	810	
Mo	43.15	42.15	41.65	42.53	4.07
Zn	43.11	43.16	43.72	41.91	4.81
Sr	43.75	43.16	43.76	42.13	2.34
Ba	43.75	44.58	44.29	45.00	4.89

* The trace elements were incorporated into the soil 11 years before.

Table 3. Effect of Mo, Zn, Sr and Ba loads on the fatty acid composition of rapeseed oil (%)

Treatment* (kg ha^{-1})	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenic acid
	16:0	18:0	18:1	18:2	18:3	20:1
Mo Ø	3.89	2.04	65.17	17.25	10.02	1.50
Mo 90	3.81	1.99	65.99	16.76	10.12	1.44
Mo 270	3.87	2.01	66.20	17.06	9.85	1.42
Mo 810	3.86	2.01	66.05	16.92	9.80	1.46
LSD 5%	0.09	0.55	1.95	1.74	0.41	0.13
Zn Ø	3.85	2.06	66.89	16.80	9.65	1.46
Zn 90	3.93	2.03	66.25	16.79	6.46	1.43
Zn 270	3.80	2.07	67.08	16.57	9.36	1.45
Zn 810	3.89	2.07	66.87	16.56	9.17	1.45
LSD 5%	0.14	0.09	1.20	0.38	0.50	0.13
Sr Ø	3.86	2.10	66.92	17.02	9.84	1.44
Sr 90	3.96	2.14	67.35	16.37	9.40	1.42
Sr 270	4.07	2.07	66.78	16.35	9.27	1.40
Sr 810	3.93	2.06	66.15	17.04	9.31	1.42
LSD 5%	0.22	0.12	1.53	0.70	0.63	0.06
Ba Ø	3.86	2.06	66.32	17.02	9.82	1.45
Ba 90	3.90	2.06	65.29	17.35	9.53	1.42
Ba 270	4.01	2.07	66.83	16.45	9.35	1.40
Ba 810	4.04	2.06	66.65	16.42	9.41	1.41
LSD 5%	0.19	0.11	2.27	0.93	0.89	0.13

* The trace elements were incorporated into the soil 11 years before.

The application of high rates of Sr, Zn and, to an extent, Mo reduced the seed oil content of rapeseed (Table 2). However, the differences were not statistically significant. The application of these trace elements had no significant effect on the fatty acid composition of the rapeseed oil, either (Table 3). The accumulation of the trace elements in plants indicates that 10 years after application significant amounts of them are still present in the soil in the form available to plants. However, the rates were not high enough to affect plant metabolism and hence plant growth and development itself. This is supported by the fact that there were no significant differences between the yields in the trial treatments and those in the check treatment. The application of high Mo, Zn, Sr and Ba rates had no significant effect on the oil content of rapeseed and its fatty acid composition, in spite of the significant accumulation of these elements in the plant.

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УТИЦАЈ ПРИМЕНЕ ВИСОКИХ ДОЗА Mo, Zn, Sr и Ba НА ЊИХОВО УСВАЈАЊЕ, САДРЖАЈ УЉА И САСТАВ МАСНИХ КИСЕЛИНА У СЕМЕНУ УЉАНЕ РЕПИЦЕ

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Резиме

Уљана репица је високо продуктивна уљана биљка и по значају је трећа у свету. У раду је испитан утицај продуженог дејства примене високих доза Mo, Zn, Sr и Ba (0, 90, 270 и 810 kg/ha) на садржај уља и састав масних киселина у семену уљане репице. Микроелементи су примењени 1991. године. Оглед је изведен на земљишту типа чернозема, на огледном пољу Института за земљиште и агрохемију Мађарске академије наука. Примењени микроелементи су се у различитој мери накупљали у уљаној репици. Највеће је било накупљање Mo. Високе дозе Sr, Zn и донекле Mo смањиле су садржај уља у семену уљане репице. Настале разлике у односу на контролу у садржају уља и саставу масних киселина уља нису биле сигнификантне. Добијени резултати указују да 11 година после примене високих доза Mo, Zn, Sr и Ba на земљишту типа чернозема још увек значајна количина остане у приступачном облику за биљке. На то указује њихово велико накупљање у биљкама. Поред тога, није дошло до значајније промене у садржају уља и саставу масних киселина и семену уљане репице.

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EFFECT OF Cd ON CONTENT AND DISTRIBUTION OF SOME MACRO- AND MICRONUTRIENTS IN PEA PLANTS DIFFERING IN AGE

ABSTRACT: Contents and distribution of N, K, Mg, Cu, Mn and Zn in pea plants treated with Cd at different age was investigated. Plants were treated with 10^{-7} or 10^{-5} M Cd for 48h 25th or 63rd days after seed germination. Results showed that more Cd was accumulated in plants treated with Cd at latter stages of growth and development. Treatments with both concentration of Cd caused accumulation of Cd in roots. Contents and distribution of the investigated macro- and micronutrients depended on Cd concentration and plant age.

KEY WORDS: pea, cadmium, plant age, macronutrients, micronutrients

INTRODUCTION

Heavy metals (HM) present in nutrient solution can inhibit or enhance the uptake of mineral nutrients by plants (Sela et al., 1988). The effect of HM is based on the antagonism or synergism of mineral elements (Morghan, 1993). During uptake, structure and function of root cell membranes may be affected by HM present in nutrient solution. It is shown that the selectivity of cell membranes was reduced a few minutes after the exposure of plants to HM (Pandolfini et al., 1992). Also, contact with HM causes depolarization of cell membranes (Costa and Morel, 1994) and selectivity reduction of ion translators (De Vos et al., 1991).

HM could affect the uptake, transport (Hart et al., 1998) and distribution (Hernández et al., 1996a) of mineral nutrients in plants. The effect of HM depends on the element (Petrović et al., 1996), plant species (Obata and Umebayashi, 1997) and the length of treatment (Hernández et al., 1996b).

Cadmium (Cd) toxicity is also associated with the uptake and translocation of mineral nutrients. For example, leaf chlorosis observed in Cd presence is related to the deficiency of Fe (Root et al., 1975; Foy et al., 1978) or Zn (Turner, 1973). Cd present in nutrient solution can affect the uptakes of Zn, Fe, Cu and Mn acting as an antagonist (Root et al., 1975, Keck, 1978, Wong et al., 1984, Bjerre and Schierup, 1985) and/or synergist (Turner, 1973, Khan and Khan, 1983, Wong et al., 1984, Vasquez et al., 1989). However, opposite results of Cd effect on uptake of mineral nutrients have also been reported. For example, according to Wallace et al. (1980) and Sela et al. (1988), Cd present in nutrient solution increased Fe content, while Khan and Khan (1983) and Bjere and Schierup (1985) noted the decrease of Fe content in treated plants. Therefore, the aim of this study was to investigate whether the age of pea plants plays a role in the effect of Cd on the content and distribution of some macro- and micronutrients.

MATERIAL AND METHODS

The trials with the pea cultivar „Jezero" were conducted in a greenhouse under semi-controlled conditions. The seeds were germinated in vermiculite in a thermostat at 25°C. After germination, young plants were transferred to 2 l pots containing a nutrient solution of the following composition: (mM) 2.5 $Ca(NO_3)_2$; 2.5 KNO_3; 1.0 KH_2PO_4; 1.0 $MgSO_4 \times 7H_2O$; and (mM) 23.1 B; 4.6 Mn; 0.38 Zn; 0.16 Cu; 0.052 Mo and 8.59 Fe as Fe-Na-EDTA.

Two different Cd concentrations, 10^{-7} and 10^{-5} M, and their effect on plants differing in age were investigated. The first group of plants was treated on the 25th day after germination and the second group was treated on the 63rd day with 10^{-7} or 10^{-5} M Cd for 48 hrs. Cd was applied as $CdCl_2$.

During the 48-hr treatment, the nutrient solution of the control plants was replaced with deionized water. After treatment, plants were harvested and separated into roots and aboveground parts.

Dry weight (DW) of individual organs was determined after drying at 60°C to a constant mass. Content of N in plants was determined according to Kjeldahl method, while the contents of K, Mg, Cu, Mn, Zn and Cd were measured after dry ashing at 450°C, by AAS using Varian SpectrAA 10.

The results were statistically processed by the analysis of variance. Differences between the treatments were calculated using Duncan's multiple range test.

RESULTS AND DISCUSSION

Cadmium content

The increased Cd concentration caused greater Cd accumulation in roots and aboveground parts of pea plants. Regardless of its concentration in the so-

lution and plant age, Cd tended to accumulate in roots and this could be a pattern of protection of the aboveground parts. Cd treatment on the 63rd day caused an increased accumulation of Cd as compared with the treatment on the 25th day. Difference in Cd contents between the plants treated with Cd on the 25th and those treated on the 63rd day showed that the Cd content was age-dependent (Figure 1). These results are in accordance with the findings of Petrović et al. (1999), which showed an increase in Cd content in plants in response to the increases of Cd concentration in nutrient solutions and plant age.

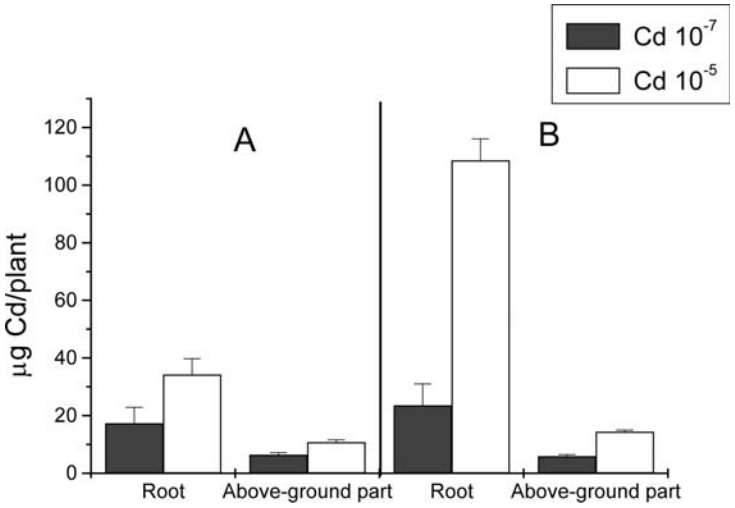

Figure 1. Cd content in roots and aboveground parts of pea plants differing in age after treatment with Cd. A — treatment on the 25th day after seed germination, B — treatment on the 63rd day after seed germination, ⊤ — Duncan's test 5%

Effect of Cd on distribution of N, K and Mg

Increased Cd concentrations in nutrient solution decreased the contents of N, K and Mg in the aboveground parts regardless of the day of plant treatment (Figure 2). Decreased levels of K in consequence to Cd treatment have also been observed in wheat (Trivedi and Erdei, 1992), cucumber (Burzynsky, 1998) and corn plants (Walker et al., 1977). K content decreased only in the aboveground parts. High levels of Cd in Cd-sensitive plants can damage root cell membranes causing decreases of water and K uptake or even a release of K from the root. Damage of cell membranes also means that proteins like H^+-ATP-ase are losing their function (Obata et al., 1996). There is a possibility that, following the same pattern, K^+ transport proteins also may lose their function.

The contents of the investigated macronutrients in roots depended of plant age. The treatment with Cd on the 25th day decreased K and Mg contents while the treatment on the 63rd day increased N, K and Mg contents in roots. As a consequence of Cd treatment, the content of all investigated macronutri-

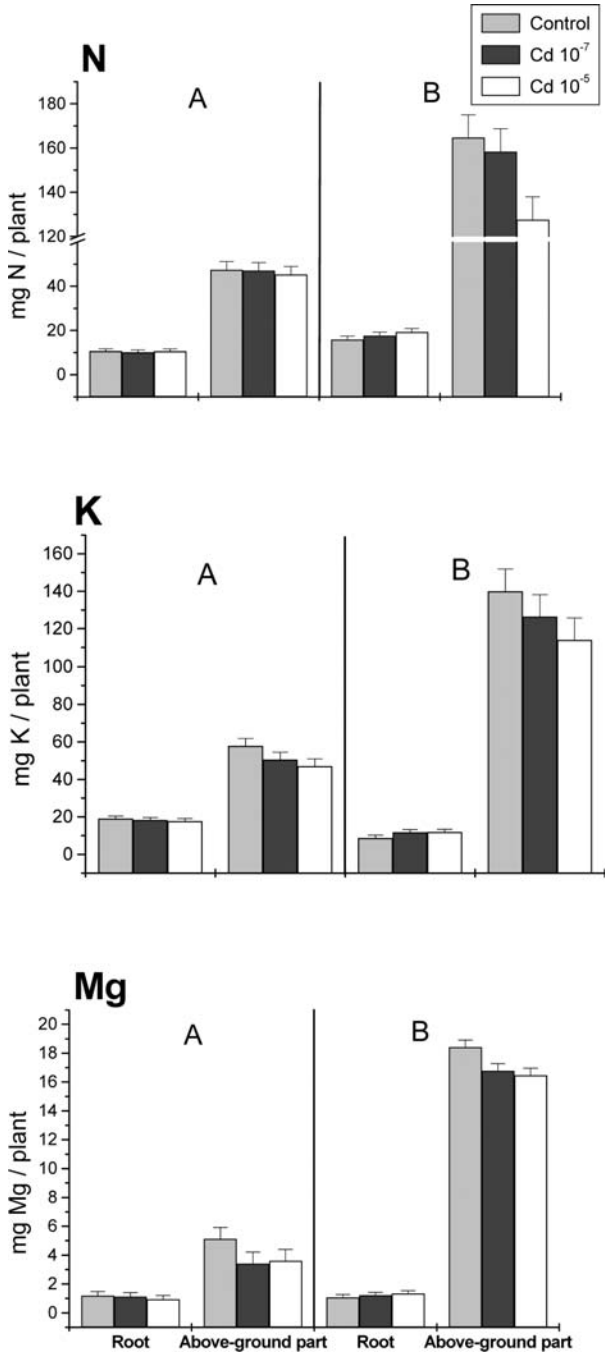

Figure 2. Effect of Cd on the N, K and Mg contents in roots and aboveground parts of pea plants differing in age. A — treatment on the 25th day after seed germination, B — treatment on the 63rd day after seed germination, ⊤ — Duncan's test 5%

ents decreased in the above-round parts of plants, regardless of plant age. The treatment on the 25th day did not change the content of N neither in roots nor in the aboveground parts (Figure 2).

Treatment with Cd affected the distribution of macronutrients between the aboveground parts and roots. The most significant effect on N distribution was caused by the higher Cd concentration and its application on the 63rd day. With the increase of Cd concentration in the nutrient solution, K and Mg tended to accumulate in roots regardless of plant age (Table 1). Also, the treatment of young corn plants with Cd has increased the Mg concentration in tissues (Nascimento et al., 1998).

Table 1. Effect of Cd on the aboveground/root content of N, K and Mg in pea plants differing in age

Cd (M)	N	K	Mg
Treatment on the 25th day after seed germination			
0	4.44	3.03	4.30
10^{-7}	4.66	2.86	3.86
10^{-5}	4.30	2.57	3.04
Treatment on the 63rd day after seed germination			
0	10.37	15.96	17.21
10^{-7}	9.00	9.71	13.49
10^{-5}	6.62	10.63	12.61

Effect of Cd on distribution of Cu, Mn and Zn

Cd treatment on the 25th day after germination lowered the contents of all investigated micronutrients in the aboveground parts and the Mn content in roots of the tested pea plants. The same treatment did not significantly alter the contents of Cu and Zn in roots. Cd treatment on the 63rd day after germination lowered the Mn content in roots while its content in the aboveground parts was not changed significantly. The Zn content decreased in roots and aboveground parts in consequence to Cd treatment on the 63rd day. The treatment on the 63rd day with the lower Cd concentration increased, while the higher Cd concentration decreased, the content of Cu in the aboveground parts. As in the aboveground parts, a more significant increase of Cu content in roots was observed in plants treated on 63rd day with the lower Cd concentration (Figure 3).

Distribution of the investigated micronutrients depended on Cd concentration and plant age. The treatment on the 25th day decreased the Cu transport and increased the Mn transport in the aboveground parts. The treatment on the 25th day with the lower Cd concentration increased the Zn transport while the higher Cd concentration did not affect Zn transport in the aboveground parts. The treatment on the 63rd day increased the Mn transport in the aboveground parts. Conversely, the Cu transport in the aboveground parts decreased with Cd treatment on the 63rd day, especially when the lower concentration was applied. The treatment on the 63rd day with the lower Cd concentration increased the Zn transport in the aboveground parts while the higher concentration stimulated the accumulation of Zn in roots (Table 2).

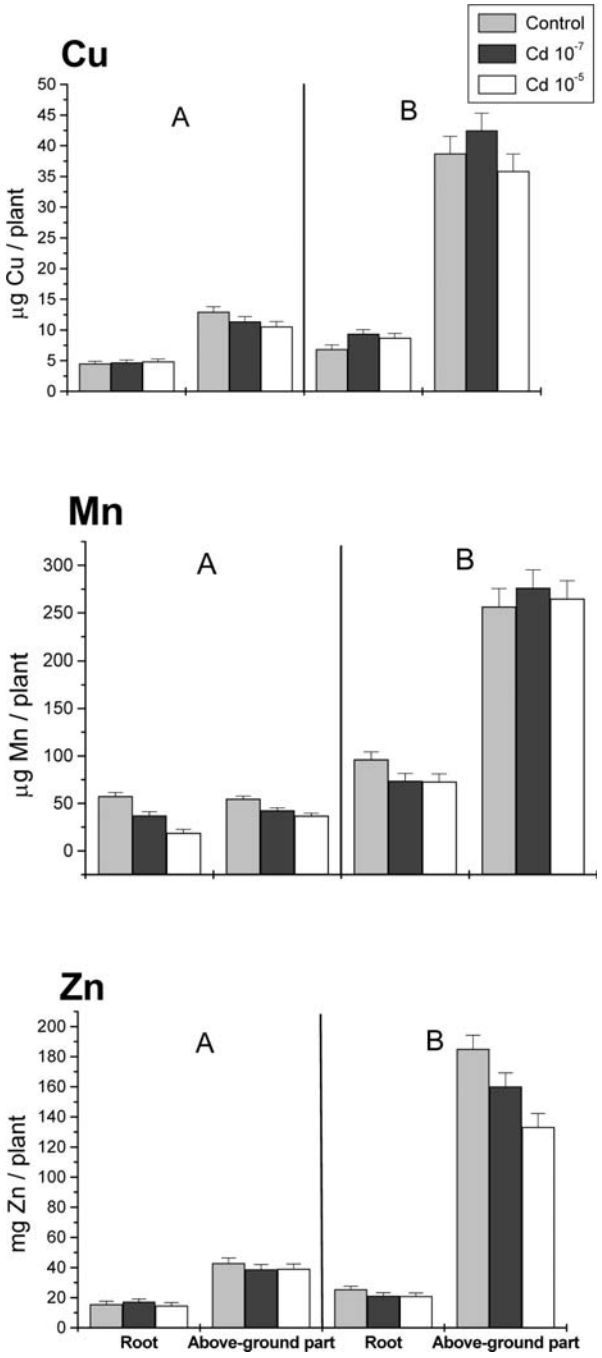

Figure 3. Effect of Cd on the Cu, Mn and Zn contents in roots and aboveground parts of pea plants differing in age. A — treatment on the 25th day after seed germination, B — treatment on the 63rd day after seed germination, ⊤ — Duncan's test 5%

Table 2. Effect of Cd on the aboveground/root content of Cu, Mn and Zn in pea plants differing in age

Cd (M)	Cu	Mn	Zn
Treatment on the 25th day after seed germination			
0	2.87	0.96	2.75
10^{-7}	2.41	1.14	2.25
10^{-5}	2.17	1.95	2.66
Treatment on the 63rd day after seed germination			
0	5.63	2.67	7.26
10^{-7}	3.83	3.75	7.57
10^{-5}	4.87	3.62	6.32

A decrease of Mn content in pea plants 48h after Cd treatment (Figure 2) was also reported in a study by Hernández et al. (1996a). They noted a decrease in Mn content with an increase of Cd concentration in nutrient solution in another pea genotype. Increase of Mn transport to the aboveground parts was also observed by Hernández et al. (1996b). The authors considered the increase in Mn transport after Cd treatment to be a defense mechanism that prevents Cd accumulation in the photosynthetically active parts of plant. The lower Mn accumulation in plants and its increased transport to the aboveground parts after a short exposure to Cd was also observed by other authors (Obata and Umebayashi, 1997).

CONCLUSION

The results obtained in our study showed that the uptake of Cd depended on its concentration in the nutrient solution and on plant age. The accumulated Cd was mainly retained in roots. Our results pointed to the dependence of the contents and distribution of the investigated macro- and micronutrients on the Cd concentration applied and on plant age. The effect of Cd on the distribution of nutrients in relation to plant age varied for all nutrients except Zn.

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УТИЦАЈ Cd НА САДРЖАЈ И ДИСТРИБУЦИЈУ МАКРО И МИКРОЕЛЕМЕНАТА КОД ГРАШКА РАЗЛИЧИТЕ СТАРОСТИ

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Резиме

У полуконтролисаним условима у стаклари, методом водених култура испитиван је третман Cd на масу суве материје и садржај Cd, N, K, Mg, Cu, Mn и Zn и њихова дистрибуција на грашку различите старости. Биљке су третиране Cd-ом у трајању од 48 сати у концентрацији од 10^{-7}M или 10^{-5}M 25. или 63. дана након клијања семена. Веће накупљање Cd уочено је у корену, третманом вишом испитаном концентрацијом и третманом у каснијим фазама. Садржај и дистрибуција испитиваних елемената зависила је од концентрације Cd као и од старости биљке у време третмана. Сем Zn, сви испитивани елементи су показали специфичну реакцију на присуство Cd у зависности времена његове примене.

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MINERAL COMPOSITION OF SELECTED *Salvia* SPECIES GROWING WILD IN THE VOJVODINA PROVINCE

ABSTRACT: Composition of mineral elements has been investigated in three *Salvia* species (*Lamiaceae*) growing wild in the Vojvodina Province (Serbia), as well as in a commercial phytopreparation (tea) of *Salvia officinalis* L. We determined concentrations of micro- and macroelements in dried plant material in order to evaluate their nutritive and medicinal value and to indicate an easily accessible natural source that could be used as a possible food supplement. The study showed that the plants were well supplied with essential elements. Concentrations of potentially harmful heavy metals (Pb, Cd, Ni, Cr) were below their respective toxic levels. From the nutritive and medicinal aspects, the investigated *Salvia* species may be considered as a natural source of essential microelements, especially Fe and Cu, with a low capacity for heavy metal accumulation.

KEY WORDS: *Salvia* spp., micro- and macroelements, heavy metals

INTRODUCTION

The genus *Salvia* is one of the largest members of the *Lamiaceae* family. It comprises more than 900 species, which are widespread all over the world. The Mediterranean, Central Asia, America and South Africa are the main centers of diversity of this genus. The genus is represented by 36 species in the European flora (H e d g e, 1972). In the flora of Serbia, 15 species have been described. They are distributed in the continental areas of southern, southeastern and northern Serbia, growing under complex and heterogeneous environmental conditions. In the northern parts of Serbia (the Vojvodina Province), which are a part of the Pannonian Plain, 9 species have been described, including *Salvia reflexa* Hornem., *S. nemorosa* L. and *S. glutinosa* L. They grow under semi-arid environmental conditions, mainly influenced by the Mediterranean and continental climates.

Some plants from the genus *Salvia* are well known for their medicinal, aromatic and antioxidant properties (T y l e r, 1993; M a l e n č i ć et al., 2000). The most popular species of the genus, sage (*Salvia officinalis* L.), is a well-known medicinal plant. Its dried leaves are used for preparing tea. The most significant medical applications of the common sage, prepared in a variety of pharmaceutical forms, are for inflammations and digestive system disorders. The sage acts as an antiphlogistic, stomachic, antiseptic, hypoglycaemic, antiasthmatic, carminative, cholagogic, emmenagogic, antihydrotic and astringent drug and it is used as spice (C h i e j, 1988; S t e f a n o v i ć - R a d i s a v l j e v i ć et al., 1998). However, the majority of wild-growing *Salvia* species have not been fully evaluated from the phytochemical point.

Investigation of wild-growing plants from the aspect of their potential nutritive and medicinal value and as indicator of environmental pollution also requires the determination of the mineral composition of plants. The study of the presence and concentration of toxic heavy metals (HM) in plant herba is of special interest, since the industrial pollution of agricultural land and forests is becoming a serious ecological issue in many parts of the world. It is well known that some wild-growing medicinal plants, such as *Hypericum perforatum* L., *Achillea milefollium* L., *Matricaria recutita* L., *Sambucus nigra* L. etc., could be characterized as HM accumulators. The determination of HM concentration in food products is important from the aspect of health safety, but also from the aspect of determination of their biological value. Beside organic compounds, the dissoluble mineral elements in plant teas and tinctures may have an additional therapeutic role (S á r k ö z i et al., 2003). The aim of this study was to determine the mineral composition of selected *Salvia* species, in order to evaluate their nutritive and medicinal value and to indicate an easily accessible natural source that could be used as a possible food supplement.

On the basis of their relative portions in plant dry material, elements which constitute plants are classified as: macroelements (2—60 mg g^{-1} DM), microelements (> 1 μg g^{-1}, < 1 μg g^{-1} DM) and ultramicroelements (< 1 μg g^{-1} DM) (K a s t o r i, 1998). From the toxicological point of view, the most important elements are heavy metals. HM represent a heterogeneous group of elements which differ in their chemical characteristics and biological functions. Some authors hold the opinion that more appropriate names for HM should be „trace elements" or „trace metals" (B a r c e l ó and P o s c h e n r i e d e r, 1990). Some HM are essential for living organisms (Zn, Fe, Mn, Co, Cu, Mo and Se), other may stimulate plant growth and development when present in certain concentrations to (Ni, V, W and Ti), while some elements are highly toxic substances in metabolism (Pb, Cd, Hg, Cr and As) (K a s t o r i, 1993). Beside pedogenesis, HM reach the soil also through industrial and agricultural production. Smelteries for metal extraction, steam power plants and mines are considered as the largest polluters of air, water and soils. Additional sources of HM contamination are mineral fertilizers, pesticides, metal-contaminated sewage and household refuse (V a n A s s c h e and C l i j s t e r s, 1990).

MATERIALS AND METHODS

Plant material has been collected during full flowering from different locations in the Vojvodina Province (Serbia), as shown in Figure 1.

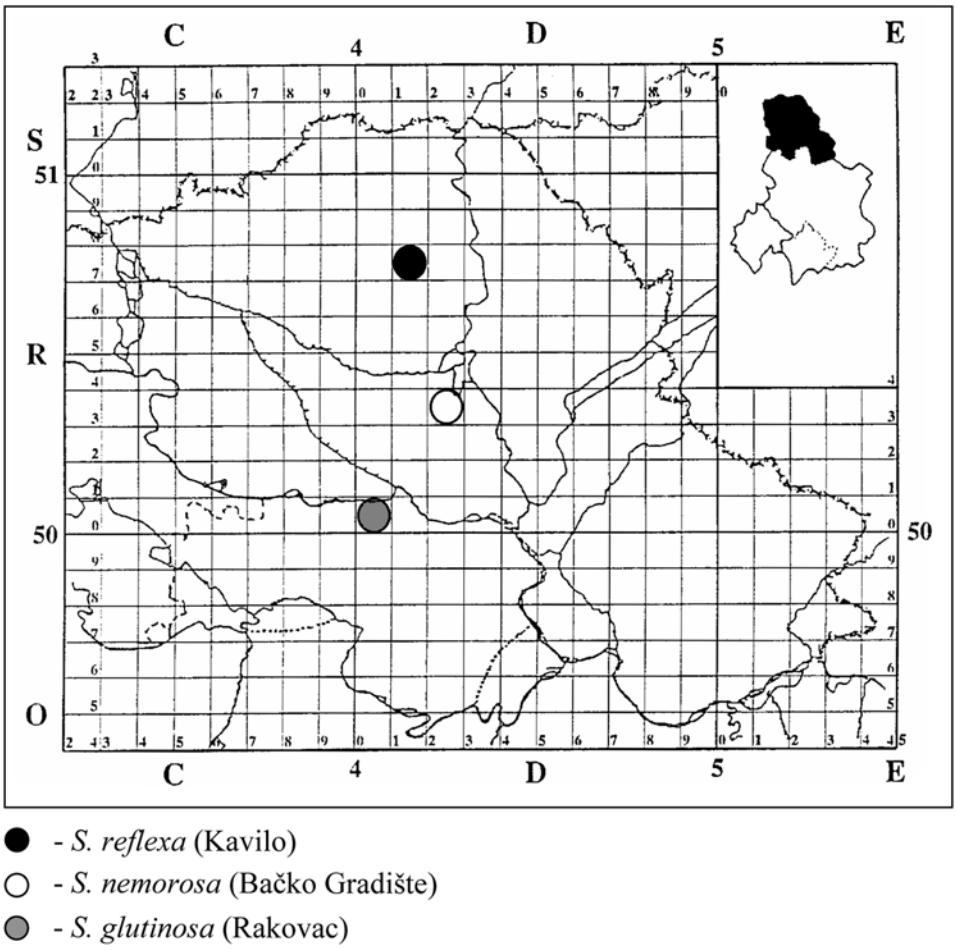

Figure 1 — Localities where plant material was collected

Voucher specimens have been taxonomically determined and deposited at the Department of Botany, Faculty of Natural Sciences and Mathematics, University of Novi Sad. Plant leaves were chosen for the experiment. In addition to this, plant material of *S. officinalis* was also analyzed. Sage leaves were obtained from a commercial phytopreparation (tea) of domestic origin supplied by a local herbal apothecary. Our intention was to analyze and compare the mineral composition of the wild-growing *Salvia* species with the composition of the common sage, which has been acknowledged as a medicinal plant both in folk medicine and in the official pharmacopoeia.

Dry matter mass was determined after oven drying at 60°C to constant mass. N content was determined by Kjeldahl method. The other elements were determined after dry ashing samples at 450°. K content was measured using flame photometry, P spectrophotometrically by V-Mo method, while the concentrations of Cd, Pb, Ni, Cr, Fe, Cu, Mn, Zn and Ca were determined by atomic absorption spectrophotometry (AAS, Varian SpectrAA-10). The obtained results were statistically evaluated and shown as mean ± S.E.M. Variation coefficient (Cv) was calculated as well.

RESULTS AND DISCUSSION

The mineral composition of the investigated wild-growing *Salvia* species is represented as the contents of micro- and macroelements in dry plant material. For the reason of comparison, the mineral composition of sage (*S. officinalis*) was also determined.

Microelements

The results for microelement contents are shown in Table 1. An average content of lead (Pb) in plant species is 5—10 ppm while the toxic level is above 30 ppm (Kastori and Petrović, 1993).

Table 1 — Microelement content of *Salvia reflexa*, *S. nemorosa*, *S. glutinosa* and *S. officinalis* (ppm in DM)

Element	*S. reflexa*	*S. nemorosa*	*S. glutinosa*	*S. officinalis*	Cv
Pb	3.99±0.52	4.75±0.24	9.00±0.69	3.05±0.29	43.85%
Ni	2.21±0.18	2.19±0.016	5.94±0.05	2.79±0.04	53.03%
Cd	2.87±0.45	2.62±1.15	2.51±0.32	0.34±0.07	48.78%
Cr	0.98±0.008	0.37±0.09	1.97±0.31	1.49±0.10	49.51%
Cu	34.18±0.93	36.70±1.38	59.75±1.85	25.10±0.75	32.80%
Zn	18.84±0.45	34.25±0.36	93.75±6.85	65.46±2.75	54.40%
Fe	340.54±35.7	167.62±11.4	508.26±60.3	453.77±710.9	35.45%
Mn	46.53±1.01	46.26±0.74	49.47±1.30	39.25±1.22	8.27%

Our results showed that Pb content was low and non-toxic, ranging from 3.99 to 9.00 ppm. An increased amount was detected only in *S. glutinosa*, but it did not exceed the maximum allowed concentration. This increase may be due to the vicinity of a road. It is known that motor vehicles are the leading factor in Pb contamination, and the intensity of plant contamination with Pb progressively decreases with the increase of plant distance from road. Compared with our results, high Pb contents were detected in some *Salvia* species from southeastern Serbia — a high Pb content in *S. pratensis* (12 ppm) and a toxic content in *S. officinalis* (69 ppm) (Blagojević et al., 1998).

Growing evidence has been gathered in recent years attesting that low concentrations of nickel (Ni) are beneficial for plant growth and development, respiration intensity and photosynthesis, as well as for the activity of antioxidant enzymes (Kastori and Petrović, 1993). An average content of Ni is 0.1—5.0 ppm, while the toxic level ranges from 10 to 100 ppm. The highest content of this HM was recorded in *S. glutinosa* which was in agreement with the results of other authors who reported the content of 29.3 ppm for *S. glutinosa* from the Vlasina region (Blagojević et al., 1998). Same as for Pb, our plant material was collected from a natural habitat, near a heavily trafficked road. It seems that the detected Ni content, although increased, did not have a toxic effect on this plant. This is in agreement with the results saying that non-essential elements for higher plants such as Ni may in certain cases stimulate plant growth and development.

Cadmium (Cd) is toxic for plants when present in high concentrations, due to its high affinity to -SH groups of enzymes and proteins. High Cd content in plants completely inhibits Fe metabolism causing chlorosis, which means that it affects photosynthesis. Also, it can inhibit respiration, electron transport and transpiration (Kastori and Petrović, 1993). The average and toxic Cd concentrations in plant leaves are 0.05—0.2 ppm and 3—30 ppm, respectively. Plants take up Cd from the soil and its higher contents have been detected near roads. It can also reach agricultural soils with phosphate fertilizers since phosphate ore contains 1—110 ppm of Cd (Ubavić et al., 1993). In the wild-growing *Salvia* species from the Vojvodina Province, Cd contents ranged from 2.51 to 2.87 ppm, which is bellow the toxic level. The lowest content of this HM (0.34 ppm) was found in the tea specimen of *S. officinalis*. Low contents of this element (< 0.1 ppm) in 16 samples of culturated (from Serbia) and wild-growing (from Montenegro) *S. officinalis* were also reported by Maksimović et al. (1998).

Chromium (Cr) content in the investigated plants ranged from 0.37 to 1.97 ppm. Once again, the highest content was found in the herba of *S. glutinosa*, but it was not above the maximum allowed concentration.

Copper (Cu) is an essential microelement for plants. An average content of Cu in dry plant material is 2—20 ppm (Fernandes and Henriques, 1991). Its function in plant metabolism is mainly catalytic. It affects many biochemical and physiological processes, such as respiration, metabolism of proteins, lipids and carbohydrates, chlorophyll and terpene biosynthesis, water balance, etc. In our specimen, Cu concentrations were increased, ranging from 25.10 to 59.75 ppm. This is in agreement with the results of other authors (Blagojević et al., 1998) who reported the Cu contents in different *Salvia* species from Serbia (*S. glutinosa*, *S. officinalis*, *S. pratensis*, *S. aethiopis*) from 22.66 to 84 ppm. When assimilated in excess by the plant, Cu can be phytotoxic. It inhibits the activity of a large number of enzymes, hence photosynthesis and pigment synthesis. Cu may block electron transport in photosynthesis which can lead to the production of oxygen-free radicals ($O_2^{\cdot -}$, $^{\cdot}OH$) and lipid peroxidation (Malenčić, 1996).

Zinc (Zn) activates or binds to more then 300 enzymes, including Cu, Zn-superoxide dismutase, carboanhydrase, phosphatases, some proteinases, etc. Hence, it influences many metabolic processes in the living cell. Zn concentra-

tion in plants may vary between 30—150 ppm, but usually it is 20—50 ppm. In the investigated *Salvia* species, it ranged from 18.84—93.75 ppm, the highest level being found in *S. glutinosa*. Similar results were obtained for Zn content in *S. glutinosa* from the Vlasina region in southeastern Serbia (86 ppm) (Blagojević et al., 1998). All of the examined specimens were well-supplied with Zn, except for the plants of *S. reflexa* in which a deficit in this essential microelement was detected.

Polyvalence and chelating capability are two main features of iron (Fe). They determine its role in plant metabolism: chlorophyll biosynthesis, photosynthesis, respiration, nitrogen fixation, nitrate and nitrite reduction, etc. (Kastori, 1998). Fe concentration in dry plant material typically reaches 1000 ppm or more. In the investigated plant material, Fe contents were from 167.42 to 508.26 ppm. These results seem to indicate that both soils and plants in the Vojvodina Province are well-supplied with this essential microelement. Contrary to our findings, a low Fe content (45.63 ppm) was reported for *S. officinalis* from the Sićevo gorge (southeastern Serbia) (Miladinović, 2000). In other *Salvia* species from Serbia and Montenegro, Fe content ranged from 26.66 up to 2000 ppm (Maksimović et al., 1998; Blagojević et al., 1998).

The most significant role of manganese (Mn) in life processes is enzyme activation. As a cofactor it activates more then 35 different enzymes, but it constitutes only two of them — Photosystem II Mn-protein and Mn-SOD. Mn is involved in water photooxidation, detoxification of oxygen radicals, CO_2 reduction, fatty acid oxidation, etc. (Kastori, 1998). In our plant species, Mn content was low, ranging from 39.25 to 49.47 ppm. Since the critical threshold for Mn deficiency in plants is < 10 ppm, it is obvious that investigated plants were sufficiently provided with Mn.

Macroelements

The results for the contents of macroelements are shown in Table 2. Nitrogen (N) is one of the most important macroelements in plant nutrition. It is an essential constituent of amino acids, proteins, nucleotides and nucleic acids, coenzymes, vitamins, pigments, alkaloids and other plant biomolecules (Popović et al., 1998).

Table 2 — Macroelement content of *Salvia reflexa*, *S. nemorosa*, *S. glutinosa* and *S. officinalis* (% in DM)

Element	*S. reflexa*	*S. nemorosa*	*S. glutinosa*	*S. officinalis*	Cv
N	2.82±0.28	2.26±0.33	1.60±0.29	0.68±0.17	43.13%
P	0.215±0.005	0.248±0.008	0.441±0.004	0.150±0.040	41.65%
K	3.75±1.19	1.58±0.12	2.97±0.25	1.92±1.46	33.62%
Ca	1.29±0.26	1.57±0.25	1.66±0.32	1.54±0.01	9.09%

N content in dry plant material is usually 1.5—4.0%. Among the examined *Salvia* species, the lowest content was recorded in *S. officinalis* (0.68%).

The highest content was found in wild-growing *S. reflexa* (2.82%), probably due to the fact that this plant species grows as a weed on agricultural soil, especially in maize fields.

Phosphorus (P) plays an important role in photosynthesis, respiration, glycolysis, pentose phosphate pathway, etc. It constitutes nucleic acids and coenzymes, such as ATP, UDP, CoA, PAL, $NADP^+$, FMN, FAD etc. P content in plants is usually 0.1—0.8% (dry weight) (K a s t o r i, 1998). In the investigated material, P content varied from 0.15 to 0.44%, which was an indication of a satisfactory level of P supply in plants. The lowest concentration was recorded in *S. officinalis*, the highest was in *S. glutinosa* herba.

Similarly to N and P, potassium (K) belongs to the group of essential macroelements which may be a limiting factor in plant production. Still, K is not a constitutional element, i.e., it neither constitutes organic compounds nor it influences enzymatic reactions in plant metabolism. Its primary role is based on its capacity to regulate water balance in plant cells. K content in dry plant material may reach 5—6% and more, but in the investigated *Salvia* species it ranged from 1.58 to 3.75%. The obtained results suggest that the plants were well-supplied with this essential element.

In the Vojvodina Province, which takes the southern part of the Pannonian Plain, calcium (Ca) content in the soil is not a limiting factor for plant growth and development. Its concentration in dry plant material of the investigated *Salvia* species was 1.29—1.66%.

CONCLUSION

The investigation of the mineral elements composition of the selected *Salvia* species showed that the plants were well supplied with essential micro- and macroelements. The concentrations of toxic HM (Pb, Cd, Ni, Cr) in plant material did not exceed their respective maximum allowed concentrations. The highest content of HM was detected in *S. glutinosa*, which confirmed the fact that plants growing along the roads should not be considered for human diet. From the aspect of medicinal value it seems that the investigated *Salvia* species from the Vojvodina Province may be considered as an easily accessible natural source of essential microelements, especially Fe and Cu. The results obtained for the mineral composition of *S. officinalis* justified the use of this plant as herbal remedy. Beside organic compounds with biological activity, *S. officinalis* is also rich in essential microelements and, contrary to some other medicinal plants, it has a low capacity for HM accumulation.

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ЕЛЕМЕНТАРНИ САСТАВ ОДАБРАНИХ САМОНИКЛИХ ВРСТА РОДА *Salvia* ИЗ ВОЈВОДИНЕ

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Резиме

У раду је истражен елементарни састав три врсте рода *Salvia* (*Lamiaceae*) које самоникло расту у јужним деловима Панонске низије, тј. на простору Војводине. Истовремено, одређен је елементарни састав фитопрепарата (чаја) жалфије (*Salvia officinalis* L.) у компаративне сврхе. Садржај микро- и макроелемената у сувом биљном материјалу одређен је применом спектроскопских метода, са циљем да се процени нутритивна вредност и потенцијална лековитост биљних дрога што би указало на лако доступне сировине природног порекла које би се могле користити као додатак у исхрани. Студија је показала да су биљке добро обезбеђене неопходним елементима на природним стаништима. Концентрације потенцијално штетних тешких метала (Pb, Cd, Ni, Cr) кретале су се испод максимално дозвољене концентрације. Са здравственог, и са аспекта исхране, истраживане врсте рода *Salvia* могу се сматрати природним извором неопходних микроелемената, нарочито Fe и Cu, које истовремено показују низак ниво акумулације токсичних тешких метала.

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MOLECULAR CHARACTERISATION OF GLUTENIN ALLELES AT THE *Glu-D1* LOCUS

ABSTRACT: It is well known that the composition of high-molecular-weight (HMW) glutenin subunits impacts the bread making quality. The HMW subunits 1Dx5-1Dy10 are typically associated with high dough strength and good bread making quality, contrary to 1Dx2-1Dy12 subunits. Bread wheat cultivars from Institute of Field and Vegetable Crops in Novi Sad have been screened for the alleles present at *Glu-D1* locus using traditional SDS PAG electrophoresis method and a new PCR based approach. The *Glu-D1* locus was screened for two main x-type alleles which code for HMW glutenins 2 and 5, and two main y-type alleles which code for HMW glutenin subunits 10 and 12. Among the analyzed cultivars, 55.6% expressed the presence of 1Dx5 and 1Dy10 alleles at the *Glu-D1* locus. These results confirmed that by using marker-assisted selection (MAS) it is possible to identify genotypes with alleles for good bread making quality, which could be successfully used in wheat breeding programs.

KEY WORDS: *Glu-D1* locus, marker-assisted selection, PCR, SDS PAG electrophoresis, wheat

INTRODUCTION

In hexaploid wheat, *Triticum aestivum*, the genes controlling the synthesis of high-molecular-weight glutenins are located on the long arms of the chromosomes 1A, 1B and 1D at the loci *Glu-A1*, *Glu-B1* and *Glu-D1*, respectively. Harberd et al. (1987) proved that there are two closely linked genes at each of *Glu-1* loci, one gene controlling the synthesis of x-type HMW glutenin subunits, another controlling the synthesis of y-type subunits. All glutenin genes are organized with a large central region containing repeat short motifs coding for the same amino acid flanked by unique N- and C- terminus domains (De Bustos et al., 2000).

The properties of wheat dough have been related to the allelic combination of the genes coding for the high-molecular-weight (HMW) subunits of

glutenin. Payne (1987) demonstrated that bread-making quality is associated with the presence of HMW subunits x5+y10 at *Glu-D1* locus providing good quality and x2+y12 providing poor quality.

Traditionally, HMW glutenins had been analyzed using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), the protein bands representing subunits have been numbered according to their mobility on the gel (Payne, 1987). More recently, D'Ovidio et al. (1994), Ahmad (2000), De Bustos et al. (2001) have demonstrated the usefulness of PCR-based analysis for distinguishing between cultivars with different HMW subunits.

The aim of this paper was molecular characterization of glutenin alleles at the *Glu-D1* locus of selected bread wheat cultivars using SDS PAG electrophoresis and new specific PCR based primers, in order to identify wheat genotypes carrying glutenin allelic combinations related with good bread making quality.

MATERIAL AND METHODS

Material. Eighteen bread wheat cultivars from Institute of Field and Vegetable Crops in Novi Sad were used in this study. Wheat cultivars Jugoslavija (x5+y10) and Baranjka (x2+y12) served as controls for HMW composition analysis.

Polyacrylamide gel electrophoresis. Extraction of proteins and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) was done on 10% gel, in Tris-glycine buffer (pH 8.3) according to the procedure described by Vapa and Savić (1988).

DNA isolation. Total genomic DNA was isolated from wheat grains according to Plaschke et al. (1995). The DNA concentrations were determined spectrophotometrically.

PCR analysis. PCR analysis was performed according to Ahmad (2000). The 50-µl amplification reaction mixture contained 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 300 mM dNTP (deoxyribonucleotide), 250 ng of each primer, 1U of Taq DNA polymerase and 50 ng genomic DNA. Primers used for identifying 2 vs. 5 x-type allele, designated as P1 and P2, and primer pair applied for identification of 10y vs. 12 y-type allele, designated as P3 and P4, were published in Ahmad (2000). Amplifications were performed in an Eppendorf Mastercycler programmed at 94°C for 5 minutes, followed by 45 cycles at 94°C for 1 min, 63°C for 1 min and 72°C for 1 min. After 45 cycles, the extension temperature was kept at 72°C for 10 min. PCR products were analyzed on 2% agarose gel containing 0.5 µg/ml ethidium bromide and visualized under UV light.

RESULTS AND DISCUSSION

During SDS PAG electrophoresis, proteins are separated into their subunits according to molecular weight. HMW glutenin subunits coded by the

Glu-1 loci are grouped in the upper part of the gel. In the examined cultivars, only subunits 5+10 and 2+12 controlled by the genes at the *Glu-D1* locus were determined (Figure 1). The subunits 5+10 were more frequent as a result of Novi Sad wheat breeding programs favoring the parents carrying these subunits (V a p a et al., 1995, 1997; V a p a and O b r e h t, 2000).

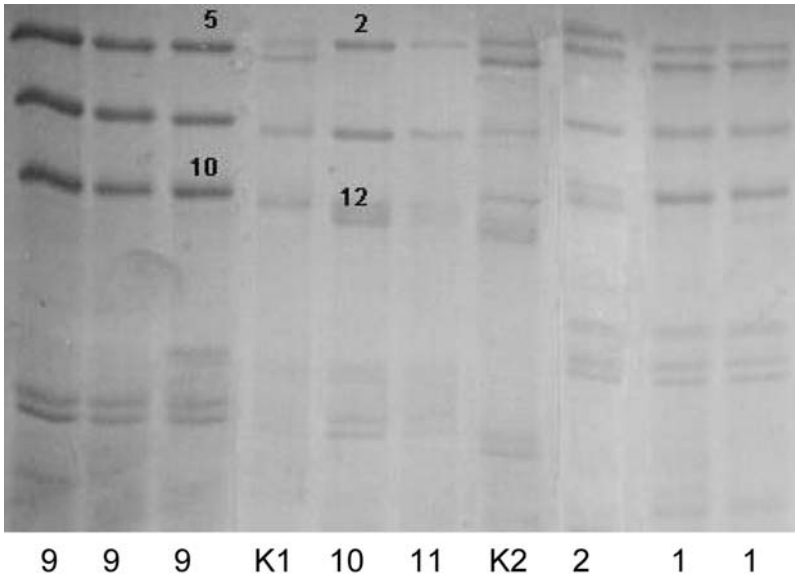

Figure 1. SDS PAGE of the analyzed wheat cultivars (K1 — Jugoslavija, K2 — Baranjka, 1 — Alfa, 2 — Anastasija, 9 — Dična, 10 — Draga and 11 — Dragana)

When primer combinations P1 and P2 were used, 450 bp amplification products were visible on the gel in ten out of the eighteen analyzed genotypes, indicating the presence of Dx5 alleles. In the other eight cultivars amplifica-

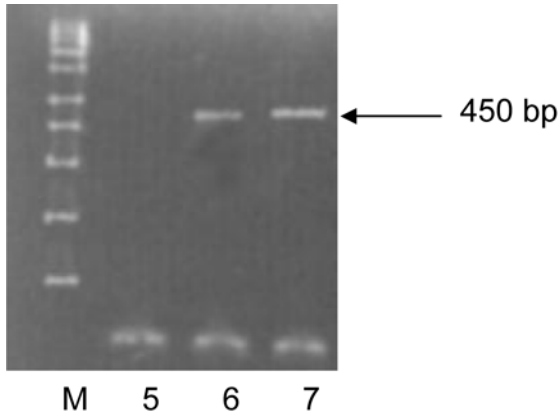

Figure 2. Separation of amplification products on agarose gel (2%) using the P1/P2 primer set (M — 100 bp marker 5, 6 and 7 are cultivars Bečejka, Cipovka and Dejana)

tion products were detected indicating that allele 2 at *Glu-D1* locus was not present (Figure 2).

With primer sets P3 and P4, specific 576 bp fragment characterized Dy10 allele and it was present in ten cultivars. The other eight cultivars expressed 612 bp amplification product showing the presence of Dy12 allele at the analyzed locus (Figure 3).

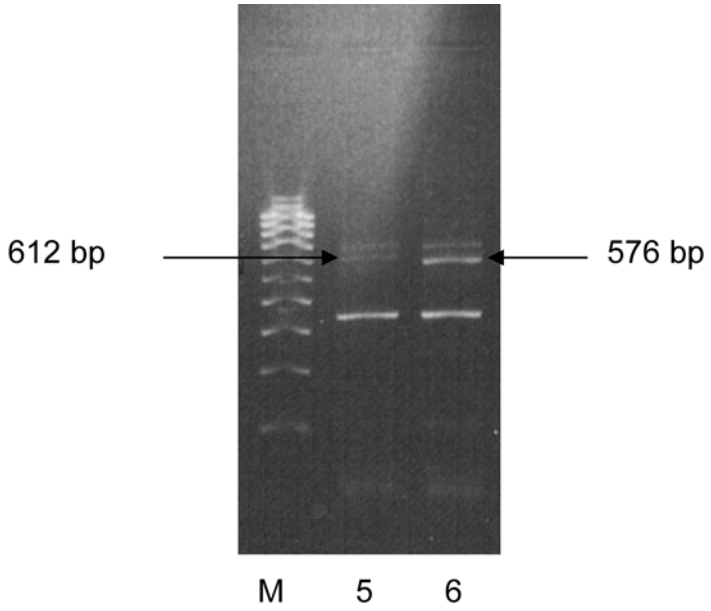

Figure 3. Separation of amplification products on agarose gel (2%) using the P3/P4 primer set (M — 100 bp marker and 5 and 6 are cultivars Bečejka and Cipovka)

Identification of HMW glutenin alleles at *Glu-D1* locus of the 18 selected bread wheat cultivars revealed that 10 cultivars possessed 1Dx5 and 1Dy10 alleles at the *Glu-D1* locus, while 8 cultivars possessed 1Dx2 and 1Dy12 alleles at the analyzed locus (Table 1).

Table 1. Glutenin alleles presence at *Glu-D1* locus in the selected bread wheat cultivars

No.	Cultivar	1Dx2 (none)	1Dx5 (450 bp)	1Dy10 (576 bp)	1Dy12 (612 bp)
1.	Alfa	—	+	+	—
2.	Anastasija	+	—	—	+
3.	Bajka	—	+	+	—
4.	Balkan	—	+	+	—
5.	Bečejka	+	—	—	+
6.	Cipovka	—	+	+	—
7.	Dejana	—	+	+	—
8.	Delta	—	+	+	—

9.	Dična	—	+	+	—
10.	Draga	+	—	—	+
11.	Dragana	+	—	—	+
12.	Evropa	+	—	—	+
13.	Evropa 90	+	—	—	+
14.	Fortuna	—	+	+	—
15.	Italija	+	—	—	+
16.	Ivanka	—	+	+	—
17.	Jarebica	—	+	+	—
18.	Jarka	+	—	—	+

Bread wheat cultivars typically possess Dx5+Dy10 or Dx2+Dy12 allelic combinations at the *Glu-D1* locus. In wheat cultivars of different origin, allelic combinations Dx5+Dy12 and Dx2+Dy10 were reported as well (P a y n e, 1987, M a r g i o t t a et al., 1993). Since HMW subunits x5+y10 at *Glu-D1* locus provide good quality and subunits x2+y12 provide poor quality, simultaneous marker-assisted selection for both alleles is crucial in wheat breeding. 1Dx2 and 1Dx5 alleles have a high degree of homology, similarly to 1Dy10 and 1Dy12 alleles (A n d e r s o n et al., 1989). Selected site-specific primers proved to be efficient in distinguishing 1Dy10 and 1Dy12 (A h m a d, 2000).

In this research each set of allele-specific primers amplified a single product, 450 bp in size, in the case of 1Dx5 allele, and specific 576 bp and 612 bp products, in the case of 1Dy10 and 1Dy12 alleles, respectively. Ten of the eighteen analyzed genotypes possessed Dx5+Dy10 allelic combination, representing potentially good bread making quality genotypes that can be used in wheat breeding programs. In order to completely qualify these genotypes as cultivars with good bread making quality characteristics, it is necessary to apply the same approach of marker-assisted selection and define the HMW glutenin allelic compositions at *Glu-A1* and *Glu-B1* loci.

The development of PCR-generated DNA markers in the concept of marker-assisted selection represents the approach that can help to avoid misleading interpretation of the results obtained by SDS-PAGE analysis and can help to identify genotypes for specific purposes in wheat breeding programs. Simplicity and speed make the new PCR-generated DNA markers a valid alternative to standard SDS PAGE method.

CONCLUSION

Eighteen bread wheat cultivars developed at Institute of Field and Vegetable Crops in Novi Sad were screened for the alleles present at Glu-D1 locus using SDS PAGE and PCR-based approach. The *Glu-D1* locus was analyzed for two main x-type alleles which code for HMW glutenins 2 and 5, and two main y-type alleles which code for 10 and 12 HMW glutenin subunits. Among the analyzed cultivars, 55.6% expressed the presence of 1Dx5 and 1Dy10 alleles at *Glu-D1* locus. They are potentially good bread making quality genotypes, which can be used as parents in wheat breeding programs.

Diagnostic PCR system with primers specific for nearly identical alleles represents a useful new tool in marker-assisted selection, enabling identification and selection of a single allele, instead of the polypeptide gene product.

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МОЛЕКУЛАРНА КАРАКТЕРИЗАЦИЈА ГЛУТЕНИНСКИХ АЛЕЛА *Glu-D1* ЛОКУСА

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Резиме

Познато је да композиција субјединица глутенина велике молекулске масе утиче на квалитет хлеба. Најзначајнија је чињеница да, насупрот субјединицама 1Dx2-1Dy12, субјединице 1Dx5-1Dy10 условљавају добру еластичност теста и доприносе квалитету хлеба. У раду су анализирани алели *Glu-D1* локуса сорти пшенице Института за ратарство и повртарство у Новом Саду. Примењена је стандардна натријум додецил сулфат полиакриламид гел електрофореза (SDS PAGE) и молекуларна анализа базирана на ланчаној реакцији полимеразе (PCR). У локусу *Glu-D1* пронађена су два основна типа х алела 2 и 5, као и два основна типа у алела 10 и 12. Утврђено је да 55,6% сорти поседује 1Dx5 и 1Dy10 алеле у *Glu-D1* локусу. Ови генотипови представљају потенцијално добре родитеље у оплемењивању пшенице на добар квалитет хлеба. Резултати селекције помоћу молекуларних маркера могу помоћи идентификацији генотипова за специфичну примену у програмима оплемењивања пшенице, при чему се предност даје PCR анализама које омогућују идентификацију и селекцију појединачних алела, а не протеинских продуката гена, добијених SDS PAGE методом.

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HMW GLUTENIN VARIATION AND RYE CHROMATINE PRESENCE IN WHEAT GENOME

ABSTRACT: For estimation of wheat end-product quality during wheat breeding programs, composition of high-molecular-weight glutenin subunits (HMW GS) and the presence of 1BL/1RS translocations serve as markers due to their profound effects on dough elasticity and viscous properties. Ninety-three wheat genotypes from Institute of Field and Vegetable Crops in Novi Sad have been analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in order to determine their HMW GS composition and 1BL/1RS translocation presence. Eleven alleles were found at the *Glu-1* loci. Subunits 1 and 2* and the null allele N were determined at the *Glu-A1* locus. Subunitis 7, 7+9, 7+8, 6+8, 20 and 21 were found at the *Glu-B1* locus, subunits 2+12 and 5+10 at the *Glu-D1* locus. The 1BL/1RS translocation was discovered in 28 cultivars, although three of them were heterogeneous.

KEY WORDS: electrophoresis, glutenins, rye chromatin, wheat

INTRODUCTION

Some potentially important traits of plant genotypes cannot be revealed by classical morphological and agronomical description. Molecular markers have been set up for the description of plant genetic diversity and genetic structure. Electrophoretic analyses of seed storage proteins — glutenins and gliadins have proven to be useful in wheat evaluation and characterization. It was shown that up to 60—65% of variability in bread making quality (BMQ) can be accounted for by differences in HMW glutenin composition (Payne et al., 1988, Rogers et al., 1989). The HMW glutenin subunits represent one of the prolamine fractions of seed proteins. They are encoded by homeologous *Glu-1* loci located on the long arms of group 1 chromosomes in cultivated bread and durum wheats as well as in wild species of the *Triticeae* tribe. Allelic variability at the *Glu-A1* locus in bread wheat cultivars is low when compared to the variability of the *Glu-B1* and *Glu-D1* loci. It is known that only three alleles in the *Glu-A1* locus are present in bread wheat cultivars,

whereas thirty and eleven alleles are present in the *Glu-B1* and *Glu-D1* loci, respectively.

The most widely spread translocations in wheat are those in which the short arm of rye chromosome 1 replaces the short arm of wheat group-1 chromosome. Wheat breeders worldwide have used rye as a source of genes for agronomic improvement of wheat. Unfortunately, some undesirable quality characteristics are often associated with the presence of the 1RS translocation in wheat. Hence, a long-term goal of breeders and geneticists has been to suppress the undesirable quality effects associated with 1RS whole-arm translocations. 1RS carries resistance genes to rusts (Lr26, Sr31, Yr9), powdery mildew (Pm8) and insects (Berzonsky et al., 1999; Graybosch, 2001). Also, 1RS may directly increase yield (Villareal et al., 1997). Conversely, depending of wheat genotype, 1RS chromatin can negatively impact wheat end--product, meaning reduction of flour yield or production of undesirable 'sticky dough' (McKendry et al., 1996).

The aim of this paper was to analyze genetic variability of HMW GS and to identify the presence of the 1BL/1RS translocation by the method of SDS PAG electrophoresis in 93 genotypes of hexaploid wheat originated from Institute of Field and Vegetable Crops in Novi Sad.

MATERIAL AND METHOD

Plant material. Single grains of 93 wheat genotypes developed at Institute of Field and Vegetable Crops in Novi Sad were used in this study. Twenty grains of each cultivar were analyzed. The cultivars Jugoslavija and Baranjka were used as controls for HMW composition analysis. The cultivars Chinese Spring and Proteinka were used as negative and positive controls for detection of the 1BL/1RS translocation, respectively.

Methods. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS--PAGE) on 10% gel, in Tris-glycine buffer (pH 8.3) was used for the analyses (Vapa and Savic, 1988). Total reduced seed proteins were separated in order to define HMW GS composition. The presence of the 1BL/1RS translocation in different wheat cultivars was detected by separation of unreduced total seed proteins.

RESULTS

Storage proteins of the 93 wheat genotypes were separated by SDS-PAG electrophoresis to determine their HMW glutenin subunits.

Eleven alleles were determined at the *Glu-1* loci: three alleles at the *Glu-A1* locus, six at the *Glu-B1* locus and two at the *Glu-D1* locus (Table 1). Subunits 1 and 2* and the null allele N, which did not form a visible band on the gel, were determined at the *Glu-A1* locus. Subunits 7, 7+9, 7+8, 6+8, 20 and 21 were found at the *Glu-B1* locus. Subunits 2+12 and 5+10 were determined at the *Glu-D1* locus (Figure 1).

Table 1. HMW GS composition and 1BL/1RS translocation presence in the analyzed wheat cultivars

HMW GS			Cultivar
Glu-A1	*Glu-B1*	*Glu-D1*	
2*	7+9	5+10	Nova Banatka, Partizanka, Podunavka, Vojvođanka, Bačvanka 1, Bačvanka 3, Banaćanka 1, Banaćanka 2, Banija, Dobro Polje, Podunavka 1, Balkan*, Posavka 2*, Kozara*, Podunavka 2, Posavka 1*, Zelengora*, Partizanka niska, Pomoravka*, Banatka niska, Staparka, Jednota, Poljana*, Subotičanka, Hlebna*, Jadranka, Kosovka, Lozničanka, NS 6001, Rodna, Studena, Tanjugovka, NS 3205*
N	7+9	2+12	Bečejka, Zrenjaninka, Nova Posavka, Evropa, Francuska, Italija, Sremka
1	7+9	2+12	Šiđanka, Ravnica, Žitnica,
N	7+9	5+10	Sremica, Tisa, Mačvanka 1*, Mačvanka 2*, Zvezda, Una, Košava, Kolubara, Apatinka*, Somborka, Pančevka, Viktorija, Ibarka*, Jedina*, Panonka, Tamiš*, Avala*, Belozrna*, Pomurka*, Rudničanka, Valjevka*, Vukovarka*
N	7+8	5+10	Lasta
1	7+9	5+10	Novosadska Brkulja*, Sutjeska*, Iskra*, Duga, Crvenkapa, NS 7000, NS 7014
2*	7+9	2+12	Nizija, Ličanka*
N	7	5+10	Nova rana*
N	6+8	2+12	NS 6389*
N	7	2+12	NS rana 1, NS rana 2, NS rana 3, NS rana 4, NS 3183, Zora
1	6+8	2+12	Novosadska Crvena,
1	7+8	5+10	NS6002, Jelica
1	7+8	2+12	Sava, Biserka, Drina, Senka*
2*	7+8	2+12	Vera*
N	21	2+12	NS 100
2*	20	5+10	Fruškogorka

* — indicating cultivars with the 1BL/1RS translocation

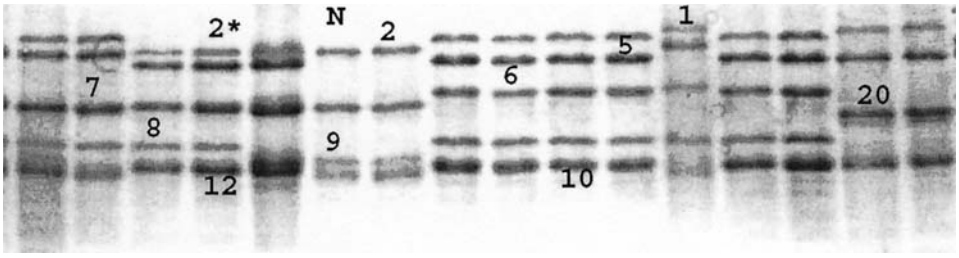

Figure 1. SDS-PAGE electrophoregram of HMW glutenin subunits

At the *Glu-A1* locus, subunits N and 2* were found at same frequency (40.86%), at the *Glu-B1* locus subunits 7+9 (79.57%) and at the *Glu-D1* locus subunits 5+10 (72.04%) were found at the highest frequency. The most frequent HMW glutenin composition was 2*, 7+9, 5+10 (33.33%).

Nine investigated genotypes were heterogeneous, showing one to two different electromorphs in addition to the predominant pattern. Six genotypes (Sutjeska, Zvezda, Iskra, Lasta, Nova posavka, Novosadska 6001) had two electrophoretic patterns, three of them (Zora, Novosadska 6002, Novosadska 6389) possessed three patterns.

Unreduced proteins from single grain of 93 bread wheat cultivars developed in Serbia were analyzed by the method of SDS PAG electrophoresis. When the 1BL/1RS translocation was present, secaline protein bands were visible in the upper part of the gel. Of the 93 wheat cultivars analyzed, 64 did not express secaline protein subunits, which meant that they did not posses the 1BL/1RS translocation. Twenty-eight cultivars possessed 1RS rye chromatine, detected as secaline bands on the electrophoregram (Table 1.). The cultivar Novosadska 100 possessed the 1B/1R substitution. Three cultivars, Tisa, Sutjeska and Novosadska 6389, showed intracultivar heterogeneity.

DISCUSSION

SDS-PAGE analysis of the 93 hexaploid wheat genotypes revealed the presence of 11 alleles at the *Glu-1* loci. Subunits 1 and 2*, as well as the null allele N, were determined at the *Glu-A1* locus. Subunitis 7, 7+9, 7+8, 6+8, 20 and 21 were found at the *Glu-B1* locus, subunits 2+12 and 5+10 at the *Glu-D1* locus. Subunit 21, which is rare, can be found only in wheat cultivars possessing the 1B/1R translocation or substitution (Vapa and Savic, 1988). This finding was confirmed in this research, by detecting subunit 21 only in the cultivar Novosadska 100, which had previously been characterized as a cultivar possessing the 1B/1R substitution (Petrovic et al., 1988).

Ample evidence has been provided (Dencic and Vapa, 1996; Redaelli et al., 1997) that the allelic composition of the *Glu-1* loci is one of the major determinants of bread-making quality in wheat. Payne (1986) determined *Glu-1* quality scores on the basis of the relationships of certain HMW glutenin subunits and the SDS sedimentation value. The maximum *Glu-1* quality score for glutenin subunits can be 10, the minimum can be 3. It was confirmed that glutenin subunits differing in the number and location of cysteine residues have variable disulphide bonding capabilities, thus contributing differently to the overall structure of gluten. For example, Anderson et al. (1988) found that additional cysteine residue in a good quality subunit 5 when compared with a poorer quality related subunit 2 supports a cysteine residues importance model in gluten macropolymer structure. Among the 93 analyzed wheat genotypes, 31 had a high value of *Glu-1*, score 9. The proportion of high quality genotypes proved a high potential of desirable technological properties. This germplasm can be used in future breeding programs for improved bread-making quality.

The presence of the 1BL/1RS translocation in wheat cultivars was successfully proved using different cytogenetic and biochemical methods (Petrovic et al., 1988; Javornik et al., 1991). SDS PAG electrophoresis confirmed the presence of the 1BL/1RS translocation in 27% of the analyzed cultivars from Serbia (Vapa et al., 1993). This research revealed the presence of the 1BL/1RS translocation in 30.11% of the cultivars developed in Institute of Field and Vegetable Crops in Novi Sad. Although alien chromatin may have negative impact on wheat bread-making performance (Hussain et al., 1997), the relatively high frequency of cultivars possessing the 1BL/1RS translocation was a consequence of the presence of beneficial genes controlling resistance that can be useful in wheat breeding programs (Graybosch, 2001).

Careful selection of parents for crossing with known 1RS lines, coupled with rigorous selection for end-use quality, can diminish negative impact of the 1BL/1RS translocation. In addition, the tools of genetic engineering can now be used to improve the quality of 1RS lines.

CONCLUSION

SDS-PAGE analysis of the 93 hexaploid wheat genotypes from Institute of Field and Vegetable Crops, Novi Sad, revealed the presence of 11 alleles at the *Glu-1* loci. Subunits 1 and 2* and the null allele N were determined at the *Glu-A1* locus. Subunits 7, 7+9, 7+8, 6+8, 20 and 21 were found at the *Glu-B1* locus, subunits 2+12 and 5+10 at the *Glu-D1* locus. The 1BL/1RS translocation was discovered in 28 cultivars, although three of them were heterogeneous.

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ВАРИЈАБИЛНОСТ ГЛУТЕНИНА ВЕЛИКЕ МОЛЕКУЛСКЕ МАСЕ И ПРИСУСТВО РАЖЕНОГ ХРОМАТИНА У ГЕНОМУ ПШЕНИЦЕ

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Резиме

У циљу утврђивања квалитета пшенице током процеса оплемењивања, одређује се композиција субјединица глутенина велике молекулске масе и присуство 1BL/1RS транслокације, који су маркери ефекта на еластичност и вискозност брашна. Одабрана 93 генотипа пшенице из Научног института за ратар-

ство и повртарство у Новом Саду анализирани су методом натријум додецил сулфат полиакриламид гел електрофорезе (SDS-PAGE) у циљу детерминације композиције субјединица глутенина велике молекулске масе и присуства 1BL/1RS транслокације. Пронађено је 11 алела у *Glu-1* локусима. У локусу *Glu-A1* откривене су субјединице 1 и 2*, као и нулти алел N. У *Glu-B1* локусу субјединице 7, 7+9, 7+8, 6+8, 20 и 21 су детектоване, и у *Glu-D1* локусу 2+12 и 5+10 субјединице. 1BL/1RS транслокација утврђена је код 28 сорти, док су три сорте биле хетерогене за анализирано својство.

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A COMPARATIVE ANALYSIS OF STOMATA AND LEAF TRICHOME CHARACTERISTICS IN *Quercus robur* L. GENOTYPES

ABSTRACT: The objective of this study was to determine genotype variability of leaf trichome and stoma characteristics. Leaves were sampled from seventeen pedunculate oak (*Quercus robur* L.) genotypes originating from clonal seed orchard Banov Brod (Srem, the Vojvodina Province). The pedunculate oak has hypostomatal leaves. Statistically significant differences were found for the dimensions and density of stomata. Genotype variability of stomatal dimensions was less pronounced in comparison with their density (CV = 8.88%). Stomata number ranged from 530 to 791 per mm^2 of leaf area; genotypes 18 and 25 could be distinguished from the others for the highest stomata number per leaf unit area, genotype 35 for the lowest number. In all genotypes, only solitary eglandular trichomes were observed on the adaxial leaf surface, while both solitary eglandular and uniseriate glandular hairs were present on the abaxial surface. Single glandular trichomes were observed in all genotypes, while some of them were characterized by the presence of two (genotypes 4, 5, 6, 16, 22, 25, 28, 29, 30, 35, 38, 40, and 85) or three (genotypes 16, 25, 35) hairs joined by their basal cells.

KEY WORDS: *Quercus robur*, genotype variability, leaf surface, stomata, trichomes

INTRODUCTION

The leaf epidermis has numerous functions (Senbush, 2003). Therefore, it has several differentiated cell types to serve various functions: basic epidermis cells, cells of the stoma complexes and cells of the trichomes. Leaf trichomes have been used for a long time in the recognition of species in numerous groups of angiosperms, either by the presence/absence or by the presence of different types of them (Penas et al., 1994). In addition to other characteristics, leaf trichomes are often used for classification and identification of *Quercus* species and their hybrids (Buck and Bidlack, 1998; Ishida et al., 2003). Llamas et al. (1995) and Penas et al. (1994) crea-

ted keys for identification of different oak species according to a detailed analysis of the foliar trichomes. Other authors studied the functions of leaf trichomes. Karabourniotis et al. (1998) found that nonglandular leaf hairs present a very effective barrier against abiotic (UV-B radiation) and probably biotic (pathogenic) stresses. Occurrence of a dense trichome layer may play a protective role against not only ultraviolet radiation damage but also high insolation (Karabourniotis et al., 1999). Results of Choi et al. (2001) indicated that trichomes might participate in cadmium detoxification.

Stomata number and their dimensions are attributes associated with genotype, with strong influence of ecological factors of the sites where the species predominates (Jošt, 1972). According to Bačić (1981), features of the guard cells of oaks can also be used for classification purposes within the genus *Quercus*. Interspecific differences in stomatal density, and, in some cases, in stomatal length, were reported by Ashton and Berlyn (1994) for three oak species belonging to section *Erythrobalanus*.

It is well known that the structure, density (i.e., number per leaf area unit), and pattern of distribution of stomata and trichomes on leaf surfaces differ greatly among plant species. To our knowledge, no such study has examined intraspecific variability of trichome and stoma characteristics in the pedunculate oak.

In this study, the structure, dimensions and density of stomata and the morphology of leaf trichomes were examined in the pedunculate oak (*Quercus robur* L.). To avoid environmental influences on these characteristics, we studied trees sharing the same ecological conditions. We expect these results to reveal the existence of genotype specificity for the studied leaf characteristics.

MATERIAL AND METHODS

Plant material

Leaf samples were taken in July 1998, from 20-year-old trees in the clonal seed orchard Banov Brod situated along the left bank of the Sava River (44°55' N, 19°23' E). The orchard had been established by grafting, and it includes 85 *Q. robur* genotypes. Seventeen genotypes were analyzed, with designation numbers 4, 5, 6, 16, 18, 20, 21, 22, 25, 28, 29, 30, 33, 35, 38, 40 and 85. To reduce the within-tree variability, one branch was taken from the middle of the crown of each genotype. Branches were transported to the laboratory in closed plastic bags. Three leaves were randomly chosen to represent each genotype. Only fully expanded, undamaged leaves without signs of scarring, disease, or herbivory were examined.

Stomata and trichome characteristics

The characteristics of stomata (characteristics of guard cells, number per square mm /stomatal density/ and dimensions /length and width/) and tricho-

mes (type, structure, distribution) were studied on prints of epidermal layer made by the „collodion method" (W o l f, 1954), using a light microscope. Prints were made on the middle portion of the sampled leaf laminae. Leaf blades were painted with colorless varnish on the adaxial and abaxial epidermis. After drying, varnish was removed by adhesive tape, which removed also a part of leaf epidermis. The specimens were mounted on glass slides for microscopic evaluation of stomata and trichomes. The number of stomata per mm^2 was counted in three randomly chosen fields of view of each print using an eyepiece micrometer. The size of stomata was calculated as average of fifteen measurements per print (five per field of view). Images were taken using Motic Image Analyzing System, Version 2.0.

Statistical analyses

The collected data were subjected to various statistical analyses including calculation of parameter means, LSD test and coefficients of variation. The comparison of genotypes was done by Duncan's test at a = 0.05 significance level.

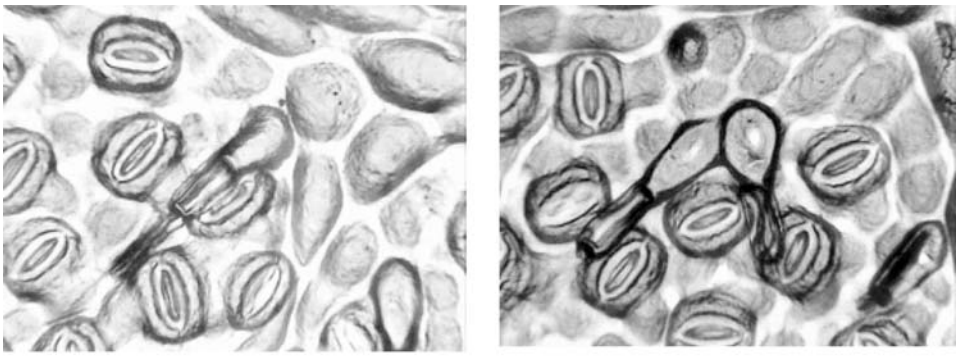
RESULTS

Trichome characteristics

In this paper leaf hairs were described and classified according to the complement of trichome types given by P e n a s et al. (1994) and L l a m a s et al. (1995). They accepted trichome nomenclature and morphology proposed previously by H a r d i n (1976).

In all pedunculate oak genotypes, the adaxial surface of mature leaves contained only solitary nonglandular type of trichomes. They were single,

Figure 1. Solitary type of trichomes on the adaxial leaf epidermis (x 400)



A B

Figure 2. Abaxial epidermal surface of a mature *Q. robur* leaf. A: glandular trichomes and stomata (x 1000); B: two trichomes joined at the base (x 400). Apical cells are often misshapen or missing.

erect or appressed, characterized by thin walls and variable length (Figure 1). These trichomes were situated only on the main vein. Two types of trichomes were observed on the abaxial surface: uniseriate (glandular) and solitary hairs. Unlike solitary hairs, uniseriate trichomes were distributed over the entire lamina surface, in the armpits that the secondary nerves form with the main vein. They were multicellular and uniseriate. Their cells were thin-walled, with exception of the oval-shaped basal cell characterized by thick walls. Going to the top of the hair, cells became smaller, the apical cells often being misshapen or missing. Trichomes were mostly single, but in some genotypes two or three were joined at the base (Figure 2, B).

Stomata characteristics

In the studied pedunculate oak genotypes, stomata were observed only on the abaxial leaf surfaces. The stoma complex was composed of two guard cells, characterized by thinner walls and extended lumina at their ends and markedly thick walls in the middle part, and a spindle-like form of the aperture (Figure 2).

Table 1. Stomatal density and their dimensions in *Quercus robur* genotypes

Genotype	Stomatal density	Stomatal dimensions (μm)	
	(no./mm^2)	Length	Width
4	606 cd	26.5 abc	19.4 abcd
5	606 cd	25.4 bcd	18.2 cde
6	593 cd	27.2 ab	19.5 abc
16	597 cd	25.5 bcd	18.6 cde
18	785 a	26.6 abc	18.4 cde
20	691 abc	26.0 abcd	18.9 cde

21	763 ab	25.6 bcd	19.1 bcde
22	687 abc	25.4 bcd	17.8 e
25	791 a	25.7 bcd	20.7 a
28	595 cd	26.7 abc	18.9 cde
29	682 abc	27.6 a	20.5 ab
30	619 cd	25.5 bcd	19.7 abc
33	613 cd	25.5 bcd	19.5 abc
35	530 d	26.4 abcd	18.6 cde
38	669 bc	25.5 bcd	19.4 abcd
40	612 cd	25.8 bcd	17.9 de
85	685 abc	24.7 d	18.8 cde
Average	654.4	25.98	19.05
CV%	8.88	3.49	4.11

[a] Letters after the mean values denote differences among the genotypes at letter do not differ significantly.

Statistically significant differences were observed for the dimensions and density of stomata (Table 1). The stomata number ranged from 530 to 791 per mm^2 of leaf area. Most of the studied genotypes had between 600 and 700 stomata per leaf area unit. The highest density of stomata on the abaxial leaf side occurred in genotypes 18 and 25, the lowest in genotype 35. This characteristic showed low genotype variability according to the coefficient of variation (8.88%). The variability of stomatal dimensions, i. e., length and width, was almost two times lower. Taking into account all genotypes, stomata had larger length than width. The longest stomata were found in genotype 29 (27.6 μm), the shortest in genotype 85 (224.7 μm). Stomatal width ranged from 17.8 μm (in genotype 22) to 20.7 μm (in genotype 25). The genotype with the highest density of stomata could be distinguished from others also by the widest stomata. In addition, stomatal density and length were correlated negatively (r^2 = —0.13), while positive correlation was found for density and width (r^2 = 0.24).

DISCUSSION

Forest trees, as well as other plant species, are exposed to fluctuating ecological conditions and other negative consequences which are in part caused by industrial development and other human activities. Their survival depends on the ability to avoid or reduce the effect of these factors. Studies on the impact of unfavorable factors of the environment, particularly of air pollution, on morpho-anatomical changes of leaves in different tree species (*Quercus*, *Tilia*, *Acer*) have shown that stomatal alterations are possible consequences (Janković, 1991). A dense trichome layer could be advantageous during leaf development under stress conditions (Karabourniotis et al., 1999). Its protective role against biotic and abiotic environmental factors is important during early leaf development stages, to be taken over later on by the epidermis

(Karabourniotis, Fasseas, 1996). This may be the reason for hair density decrease with age (Llamas et al., 1995).

Furthermore, leaf trichomes have often been used in taxonomy for species identification, but with variable success. While some authors reported that identification of some oak species could be based solely on trichome characteristics (Penas et al., 1994; Llamas et al., 1995; Lee et al., 1999), Bačić (1996) could not distinguish among *Q. brachyphylla* Kotschy, *Q. dalmatica* Radic, and *Q. pubescens* Willd. using the micromorphological characteristics of their trichomes, stomata, common epidermal cells and abaxial epicuticular wax.

Our results showed that trichomes were present on both leaf surfaces, although they were more abundant in terms of density and type on the abaxial leaf surface. Solitary nonglandular trichomes were observed on the adaxial epidermis, while uniseriate glandular and solitary nonglandular trichomes were present on the abaxial epidermis. Engel et al. (1993) reported that only the abaxial side of *Q. robur* leaves possesses numerous glandular trichomes „as long stretched tubes with an ovally shaped basis". Glandular trichomes of *Q. robur* leaves contain an essential oil, which dissolves the wax crystalloids after it has been released onto the leaf surface (Engel et al., 1993). According to Hardin (1976), uniseriate trichomes are quite common in the genus *Quercus*. Guttenberger (2000) found them on the abaxial epidermis in *Quercus x viridis*, while Penas et al. (1994) and Llamas et al. (1995) observed them in many other oak species and their hybrids. The length, diameter, and number and shape of cells of uniseriate trichomes are variable (Llamas et al., 1995).

Although the density of trichomes was not assessed in this study, we observed that the amount of glandular hairs varied among genotypes. For example, genotype 25 could be distinguished from the others for a very dense trichome layer. Furthermore, the genotypes differed also in the pattern of glandular trichomes arrangement. Uniseriate hairs were most abundant in all genotypes. In some cases, which differed in trichome density, two hairs would emerge from the same basal cells (genotypes 4, 5, 6, 16, 22, 25, 28, 29, 30, 35, 38, 40 and 85). Genotypes 16, 25, and 35 could be distinguished from the others for the presence of three joined glandular trichomes. The available literature on this subject makes no mention of similar observations. Bačić (1981) reported that the lower epidermis of *Q. robur* leaves has tufted hairs, which consisted of three or four cells joined only at the base, which were planted in the epidermis.

The stomata of oak leaves have certain structural features which make then mutually distinguishable. Considering the absence of accessory (subsidiary) cells, the stomata of oak leaves belong to the „асектний" type (Анели, 1975) or anomocytic type (Metcalfe and Chalk, 1957). According to the structure of guard cells (the markedly thick middle part of ventral walls, thin walls and wide lumina at terminal parts, the spindle-like form of the aperture), the stomata in *Q. robur* belong to „гантелегубовидний" type (Анели, 1975). Electron-microscopic scan of the stomata of oak leaves (Bačić, 1981) showed the presence of cuticular lamellae on the ventral guard cell walls. The-

se lamellae undoubtedly reduce the intensity of transpiration. Also, there is a protrusion arising from the outher walls of the guard cells, which overhangs the central part of the stoma and surrounds it almost completely, reducing air movement above the aperture.

There were significant differences in the number of stomata per unit leaf area. Considering all genotypes studied, the average number of stomata per leaf mm^2 was 654, while the average dimensions of stomata were 26.0 x 19.1 μm. Engel et al. (1993) reported that the stomata in mature *Q. robur* leaves were 25—30 μm long and 15—20 μm wide. Our values of stomatal length and width are closer to the results published by Bačić (1982) for the pedunculate oak (24.0 x 19 μm), but the number of stomata in the genotypes we studied was notably higher. It is well known that the number of stomata per unit leaf area varies among plant species as well as among individual *Quercus* species. Studying three oak species, Ashton and Berlyn (1994) obtained the highest stomatal density in *Q. coccinea*, followed by *Q. rubra* and *Q. velutina*. Spector (1956) suplied data on interspecific differences among several oak species. The variability of stomatal number and dimensions among *Q. robur*, *Q. cerris*, and *Q. fraineto* species was found by Bačić (1982) to depend on location, tree age and part of the leaf. Studying within- and between-tree variability of stomatal density and dimensions in *Q. petraea*, Brushi et al. (2003) found that the observed trends in the studied parameters reflected, among other environmental factors, the patterns of responsiveness to moisture within the tree crown. According to Ashton and Berlyn (1994), the studied oak species did not vary in the stomatal length of the shaded leaves and those exposed to sun, while the stomatal density was higher in the leaves exposed to full sun as compared with those growing in the shade.

Generally, atmospheric CO_2 concentration and temperature must be taken into consideration when studying stomatal density. Beerling and Chaloner (1993) reported that *Q. robur* leaves developed under the summer temperatures had a reduced stomatal density compared with their spring counterparts. Paoletti et al. (1998) studied modifications of leaf surface structures in *Q. ilex* L. due to natural CO_2 increase in the environment. Their results showed that CO_2 increase decreases stomatal frequency, the reduction curve almost equaling that obtained for the CO_2 change from the preindustrial age till the present day. Furthermore, after experiments with young *Q. petraea* seedlings grown at ambient and elevated carbon-dioxide concentrations, Kurschner et al. (1998) ascertained the same trend. According to Burgh et al. (1993), relation between atmospheric CO_2 increase and stomatal density decrease could be used as a bioindicator of paleoatmospheric CO_2 change during last 10 million years. So, when studying genotype variability of stomatal characteristics, one must have in mind that their number per unit leaf area as well as their dimensions are strongly influenced by ecological conditions (Bačić, 1982).

Some characteristics of leaf anatomic structure, including stomatal density, could influence biomass production in tree species. For example, while studying poplar clones, Orlović et al. (1994) found a positive correlation between stomatal frequency and leaf anatomical structure on one side and bio-

mass production on the other. Hence, parameters like these should be taken into consideration in the selection and breeding of tree species.

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КОМПАРАТИВНА ПРОУЧАВАЊА ОСОБИНА СТОМА И ЛИСНИХ ТРИХОМА КОД ГЕНОТИПОВА ХРАСТА ЛУЖЊАКА (*Quercus robur* L.)

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Резиме

У раду су проучаване карактеристике стома и лисних трихома у циљу утврђивања генотипске варијабилности. Узорци листова су узети са седамнаест генотипова храста лужњака (*Quercus robur* L.), који потичу из вегетативне семенске плантаже Банов Брод (Срем, Војводина). Храст лужњак има хипостоматалне листове. Утврђене су статистички значајне разлике за димензије и број стома. Генотипска варијабилност дужине и ширине стома била је мање изражена у поређењу са њиховим бројем (CV = 8.88%). Број стома по јединици површине листа се кретао од 530 до 791. Генотипови 18 и 25 су се издвајили по највећем, а генотип 35 по најмањем броју стома. Код свих генотипова, на адаксијалној површини листа утврђено је присуство нежлезданих трихома солитарног типа, док су на абаксијалној, поред нежлезданих, присутне и просте-једнонизне жлездане длаке. Појединачне жлездане длаке су најмногобројније и присутне су код свих генотипова. Неки од њих се карактеришу присуством две (генотипови 4, 5, 6, 16, 22, 25, 28, 29, 30, 35, 38, 40, 85) или три (генотипови 16, 25, 35) жлездане длаке, које су спојене својим базалним делом.

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A CONTRIBUTION TO THE STUDY OF STAND DEGRADATION PROCESS ON THE TERRITORY OF FRUŠKA GORA NATIONAL PARK

ABSTRACT: We analyzed the process of stand degradation of individual forest ecosystems in the region of the zonal community *Tilio-Carpino-Quercetum robori-cerris* Jov. 79 located in the western part of Fruška Gora National Park. Degradation occurred is consequence to regeneration felling and it progressed towards the extinction of oaks, first of all the valuable pedunculate oak and Vergilius's oak, and then also Turkey oak. The analyzed mature stands were degraded, coppice and mainly predominated by the Turkey oak in the tree stratum. In juvenile stands, often initially well regenerated with Turkey oak, the dominant species in the tree stratum are lime, hornbeam, flowering ash, field maple and other secondary species, predominantly of coppice origin. Restitution of the degraded stands is possible by applying an adequate regeneration system. Taking into account the primarily protective function of the analyzed forest ecosystems and the fact that these are degraded and coppice stands, the system of regeneration should be based on combined restoration.

KEY WORDS: Fruška Gora National Park, degraded stands, clear felling, regeneration felling, restitution, combined restoration

INTRODUCTION

The Fruška Gora Mountain is a complex ecological and vegetation system due to its geographical position in the southern part of the Pannonian Plain, the size of the massif and its well-developed relief, geological-petrographical, pedological as well as macro- and microclimate diversity, and rich paleobotanic and syndynamic histories. The regional spatial plan of the Fruška Gora Mountain till 2000 covers an area of 73,444 ha, with the national park zone of 25,094 ha and the protection zone of 48,350 ha. Based on an ecological-production study, the National Park has been divided into 65 forest ecosystems (ecological units). According to the similar development-production characteristics, they have been grouped in 43 forest types (Jović et al., 1983—1986). In the plan documents, forest management is prescribed on ecological bases. The principal management directives are prescribed based on the actual

state and based on the conditionally taken optimal state, under the targeted purposes.

According to the NP „Fruška Gora" management plan data (1987—1996), primarily protection forests occupy 77%, production-protection forests occupy 5% and the remaining area is set aside for special purposes. The forests of the Fruška Gora Mountain are mostly anthropogenic, formed by the conversion of high forests into coppice and degraded forms (Janković and Mišić, 1980). Coppice forests occupy 82% of the area, and the conserved coppice stands cover the highest percentage — 73% per area and 77% per volume. Mixed stands are dominant, however, the degradation processes favored the expansion of lime, predominantly white lime, which is presently the most frequent species. The dominating tendency of white lime is permanently present at the sites of numerous forest communities on the Fruška Gora Mountain. Vlatković and Grujić (1986) report that limes cover 33% of the area in communities of zonal oaks, and limes also invaded more than 40% of the sites of submontane beech communities. According to the data of current forest management plans, limes already occupy about 35% of the area and total volume of NP „Fruška Gora".

Due to inadequate and untimely regeneration felling, forest communities in NP „Fruška Gora" consist mostly of the species adapted to periodically repeated anthropogenic effects. However, despite the adaptability, it is also possible that some species are not represented in the community. One of the main reasons of the absence of the species regeneration is the method of regeneration felling. A characteristic example is clear felling in the period when the species did not bear fruit, so that regeneration could not occur in the felling areas. Also, in the initially well regenerated stands, stand degradation is caused by intraspecific competition most often between the less valuable but fast-growing coppice species and the slower-growing valuable oak species of seed origin. Identical anthropogenic effects and the mentioned processes of stand regeneration lead to still poorer, degraded stands, with the dominance of the most resistant species. This is also how the pure lime stands were formed (Vajda, 1956, Mišić, 1979; Bobinac and Radulović 1997).

The continuous functional utilization of forests in the region of the Fruška Gora Mountain depends on the maintenance of the natural composition of species in forest ecosystems, biological stability and longevity of stands. Biodiversity of numerous species in the NP „Fruška Gora" can be achieved by the maintenance and restitution of the natural composition of species in forest ecosystems. However, the trend of coppice lime dominance in numerous forest ecosystems does not contribute to that process. The invasion of white lime into individual xero-mesophilic forest ecosystems on the Fruška Gora Mountain was primarily characterized as the consequence of the intensive anthropogenic effect. However, the authors Mišić et al. (1997) and Dinić A. et al. (1999) do not characterize it as the degradation, but as the beginning of the progressive succession of vegetation in the direction of the more mesophilic forest types. Taking into account the harmonized decisions of the Regional Spatial Plan of the Fruška Gora Mt. till 2000 and the decisions of the ecological-typological plan of forest management, by which the realisation of numero-

us and complex forest functions depends on the conversion of low forests into high forests, and on the conservation and improvement of the natural stand composition, this study is focused on the study of actual processes of stand degradation and on the definition of the main causes on the sample of the selected study stands. The aim of the study is to objectify the attitudes with regard to this issue, which is not significant only for forestry.

STUDY AREA AND METHOD

We analyzed three stands in the region of Erdevik in the management unit „Gvozdenjak-Lice" (551), which belong to forest types in the area of the zonal community (*Tilio-Carpino-Quercetum robori-cerris* Jov. 79, Tomić, 1991, 1992) in the western part of NP „Fruška Gora". The singled out forest types are characterized by poly-dominant combination of several tree species and they are floristically rich (Jović et al., 1983—1986). The study stands are situated on a flattened, mildly sloping terrain and on the deluvium, up to the altitude of 200 m. According to the forest management plans (1987—1996 and 1997—2006), stand no. 1 is the forest of pedunculate oak, hornbeam and Turkey oak with limes in the higher-altitude valleys (*Tilio-Carpino-Quercetum robori-cerris collinum*) on deluvium (forest type 144, Compartment 1 *j*, area 2.58 *ha*). Stand no. 2 is the forest of pedunculate oak, hornbeam, Turkey oak and lime (*Carpino-Quercetum robori tilietosum*) on eutric cambisol to leached eutric cambisol (forest type 135, Compartment 9 b, area 7.80 *ha*). Stand no. 3 is the forest of Turkey oak and Vergilius's oak (*Quercetum cerris virgiliane xerophyllum*) on the slopes, on pararendzina on loess to shallow brown soil (forest type 381, Compartment 54 g, area 8.30 *ha*).

We analysed even-aged, coppice stands, conserved, aged 89—120 years, which were clear-felled in the final stage of harvesting. The presented elements of stand structure and the measures of management are based on the data from the Management Plan for the unit „Gvozdenjak-Lice" (1987—1996). The success of natural regeneration of oaks in the regenerated areas was assessed after 6—8 years on the sample strips spaced 50 *m*, by the method of ocular evaluation (Stojanović and Krstić, 2000). The abundance and spatial representation of oaks was assessed in the period when oak biological property of retaining the leaves longer than the other tree species was most pronounced. The presented photographs illustrate the characteristics of oak species natural regeneration, and also their extinction from the regeneration areas in the conditions of competition with other species.

RESULTS AND DISCUSSION

Due to inadequate and untimely regeneration felling in the past, which resulted in unequally favourable conditions for the regeneration of individual species, the study mature stands can be considered as the result of regressive succession. The most frequent species in the stands was Turkey oak, and the

more valuable oaks were represented as individual trees. Table 1 shows that the percentage of oaks in the total number of trees in the stands, above the taxation limit, was more than 50%, and the percentage of pedunculate oak, sessile oak and Vergilius's oak was 1.7—6.5%. As oaks are light demanders, the recruitment of their seedlings in an even-aged stand structure did not occur, so the presented number of oak trees is also the total number, starting from the saplings stage, which was represented in the stands. Other tree species with lower light requirements increased the abundance in the stands, thanks to more frequent seed yield and more intensive coppice regeneration, after periodic disturbances of the canopy (Figure 1).

Table 1. Stand Data according to Management Plan of „Gvozdenjak-Lice" 1987—1996

Species	Stand state per *ha*														
	Stand 1					Stand 2					Stand 3				
	N	*V*	*Iv*	*ds*	*hs*	*N*	*V*	*Iv*	*ds*	*hs*	*N*	*V*	*Iv*	*ds*	*hs*
Turkey oak	104	187.0	4.3	37.0	22.3	209	326.5	6.9	33.0	23.0	108	268.7	4.7	40.0	26.6
Pedunculate oak	6	13.0	0.3	42.0	23.4	—	—	—	—	—	—	—	—	—	—
Sessile oak	—	—	—	—	—	14	7.8	0.2	24.0	22.0	—	—	—	—	—
Vergilius's oak	—	—	—	—	—	12	6.3	0.2	24.0	20.0	3	4.5	0.0	40.0	28.0
Lime	62	42.0	1.1	30.0	18.5	88	40.7	1.2	24.0	19.0	60	9.1	0.7	15.0	13.7
Hornbeam	40	25.0	0.8	28.0	19.5	—	—	—	—	—	1	1.0	0.0	37.0	26.4
Flowering ash	—	—	—	—	—	58	6.6	0.2	13.0	14.0	3	0.3	0.0	13.0	15.0
Other	—	—	—	—	—	14	2.6	0.0	13.0	12.0	1	2.1	0.0	47.0	27.4
Total	212	267.0	6.5			395	390.5	8.8			176	287.7	5.5		
The priority stand functions, management aims and measures															
Priority function	soil protection 1st degree					soil protection 1st degree					soil protection 1st degree				
Origin	coppice stand					coppice stand					coppice stand				
Management group	stand for regular management					stand for transition management					stand for transition management				
Management System	Regeneration felling					Regeneration felling					Clear felling				
Felling type	*Final felling*					*Final felling*					—				
Age	89					89					120				
Year of felling	1993/1994					1994/1995					1995/1996				

Due to inadequate regeneration procedure, more valuable oaks are almost completely absent in the newly-formed stands today, and the dominant species are Turkey oak, lime, hornbeam, flowering ash, field maple, etc., predominantly of coppice origin. As reported in the Management Plan (1997—2006), the stands are classified in the management group for the transitional management. Based on the method of ocular assessment, the status of oaks in the newly-formed stands, is as follows:

Figure 1. Structure of a mature stand
(Erdevik, GJ-551, Compartment 54 g, Photo: M. Bobinac)

a. **regeneration area 1** — in the eighth year it was satisfactorily regenerated with Turkey oak, and not sufficiently with pedunculate oak. Turkey oaks were of seed origin and in the greater part of the area there were 5—10 *trees/m*^{2}, height 1.5—2.5 *m*, only individual trees were above 3 *m* high. In the initial stages of tree growth, tree height was lower than the potential height, due to cattle grazing (Figure 2);

b. **regeneration area 2** — in the seventh year it was partly satisfactorily regenerated with Turkey oak, and not sufficiently with other oak species. The regeneration was richly established, with the dominant lime coppice trees, height up to 8 *m*, diameter up to 13 *cm*, flowering ash, field maple, hornbeam and Turkey oak. Turkey oak of seed origin occurs in individual, smaller, densely formed groups, height 2.0—4.0 *m*. Individual Turkey oaks surrounded with other species are characterised by a high degree of slenderness (h:d 200) or retarded development (height 0.5—1.0 *m*). Other oaks are very rare and they have a subdominant position in the stand (Figure 3);

c. **regeneration area 3** — in the sixth year it was partly satisfactorily regenerated with Turkey oak. The dominant trees in the regeneration area are coppice trees of lime, height up to 6 *m*, flowering ash, field maple, hornbeam and Turkey oak, etc. Turkey oak of seed origin occurs in individual groups, height 2,0—3,0 *m*. Individual Turkey oak trees are above 3 *m* high, with a high degree of slenderness (h:d 200). A great number of trees are retarded, height 0.5—1.0 *m*. Other oaks are not represented in the regeneration area (Figure 4).

Figure 2. Abundantly formed Turkey oak regeneration in the whole area (Erdevik, GJ-551, Compartment 1*j*, December 2001, photo N. Stanković)

Figure 3. Sessile oak and Turkey oak retarded growth compared to lime coppice (Erdevik, GJ-551, Compartment 9*b*, December 2001. Photo: N. Stanković)

Figure 4. Groups of Turkey oak in the fifth year, surrounded with coppice lime, flowering ash, etc. (Erdevik, GJ-551, Compartment 54 *g*, photo: M. Bobinac)

The study stands belong to vital forest ecosystems that prevent site degradation, because of their fast natural regeneration after clear felling, but they are subject to the processes of regressive succession. The main reason for Turkey oak dominance in the study stands, regenerated in the late 19th century, is the interaction between the specific purpose of the forest complex and management measures in that period in and the Turkey oak regenerative potential. Juvenile Turkey oak grows in height somewhat faster than pedunculate oak and sessile oak (Jovanović, B. 2000), so it is considered as an aggressive species in the communities with these species. The competitive force of Turkey oak in the juvenile period, compared to other oaks, can be explained also by the better adaptation to different site conditions (Bobinac, 1997, Bobinac, 2001a, Bobinac, 2002a, Bobinac and Vilotić, 1998) and by fast growth in favourable conditions (Bobinac, 2000, Bobinac et al., 2001).

During the last decades, due to frequent sanitation felling, as the consequence of forest dying (Jović et al., 1995, Marinković and Stojanović, 1995, Marković et al., 1995), the structure of the stands was disturbed and the species which are more shade-tolerant in the juvenile period occupied the underwood layer. Clear felling in the study stands was synchronised with the years of abundant seed yield of Turkey oak trees and with the degree of Turkey oak natural regeneration, defined in the conditions of the stand canopy. Due to difficult conditions of forest management operations in stands 2 and 3, although individual parts were initially well regenerated with Turkey

oak, the development of many Turkey oaks gradually slowed down and the dominant position was taken by the coppice species. Turkey oak is the dominant species today in stand 1, thanks to a more favourable structure of the underwood layer, better preparation measures and the synchronisation of clear felling with the more abundant acorn yield of a greater number of trees. Turkey oak is also superior in the competition with other species. Tending measures which were directed at enhancing the least valuable oak — Turkey oak in the regeneration areas 2 and 3 were not profitable procedures. As they could not be performed timely and frequently enough, the stands were left to the spontaneous development till the beginning of the first thinning, i.e. the first income. After the first thinning, we can expect the dominance of lime, and the other species are going to be reduced to single tree or group representation (Bobinac, 1996, Bobinac and Radulović, 1997).

The prevailing percentage of coppice regenerated species (lime, hornbeam, flowering ash, field maple, etc.) in the regeneration areas results from their higher number in the mature stands (Figure 1) and the absence of preparatory measures in the regeneration phase. In such conditions, naturally or artificially regenerated pedunculate oak and other oaks barely survive, because they are heliophytes. Pedunculate oak is also highly susceptible to the effect of various factors (Bobinac and Karadžić, 1994, Bobinac, 1999). Therefore, at the site potentials enabling the production of highly valuable oak wood, the dominating management system is similar to that which was implemented on the territory of the Fruška Gora Mountain in the previous rotations, although the present period has better technical-technological means for establishing the conditions for the progressive succession in forest ecosystems.

There are frequent examples, as in the study stands, that degradation process is the consequence of difficult conditions of forest management. However, based on the analysis of forest management guidelines in the Regional Spatial Plan till 2000 for the territory of the National Park Fruška Gora and based on the analysis of the prescribed silvicultural procedure in forest management plans, the reasons of the degradation process in the generally difficult management conditions, are also the imprecise management guidelines and imprecisely prescribed measures of forest management in the stands intended for regeneration. Due to the imprecise (and thus unbinding), not harmonised in time and often inadequate measures of forest management in the valid legal documents (management plans), and due to the identical or corrected decisions in the implementation documents (annual working plans), the trend of stand degradation has continued for several decades. Thus, for example, Forest Management Plans (Table 1) in the stands 1 and 2 prescribe regeneration method in the form of regeneration felling with a short regeneration period, and the type of felling in the plan period is final felling. Final felling in the temporal series of regeneration fellings means the final cutting (Jovanović, 1988), i.e., it means a measure for the final removal of the remaining crop trees, aiming at the complete liberation and undisturbed development of the targeted established seedlings of the present species or species designated in the typological plan. When this is not the case, in the adopted system of forest management, final felling should not be a priori prescribed in legal documents (nor should it

be implemented even if prescribed in legal documents) if regeneration is not made possible in the stand or if the development of seedlings is not possible at the time of final felling. Also, as the main function of the analysed stand on ecological principles is the first degree of protection against water erosion, or the first degree of water conservation, and as the stand is the tourist-recreation centre, this requires the prescription of measures which will optimally contribute to the above purposes in practice.

According to plan documents, forest management in the National Park Fruška Gora is based on general ecological principles and special requirements that result from its status as a protected natural resource, and also in agreement with the demands of forestry profession. Among the demands of forestry profession, which change with numerous effects, innovation processes and increasing needs, silvicultural measures harmonised with site conditions and stand state, by which the above demands are expressed in forest ecosystems, are traditionally clear, both terminologically and essentially, and they are also harmonised with general ecological principles. Taking into account Turkey oak dominance in study stands and the biological potential of Turkey oak seed yield (B o b i n a c, 2002 b), the continuity of this species can be maintained in the newly established stands by the prescribed system of forest management (regeneration felling) and felling type (final cutting) in forest management plans, under the change of silvicultural form, as shown in stand 1. Regeneration felling must be synchronised with the primary and the most significant factor of natural regeneration — abundant yield of a great number of trees on the prepared areas. However, this procedure only partially improves the previous state of the stands and the production level, and it does not improve the natural composition and the biological stability, as the base of the functional utilisation of forest resources in future. According to the typological (ecological) base and the general ecological principles, the system of forest management by regeneration felling in such stands requires the prescription of the combined regeneration.

In the phase of stand regeneration, forest management plans in NP „Fruška Gora", as the real demands of forestry profession for stand harvesting and stand improvement, must be based on the precisely prescribed regeneration system. The imprecisely prescribed system and the inadequate restoration lead to the continuation of degradation processes in the newly established stands. Such stands will not contribute to the future rational utilisation of the site production potential or to numerous other functions of forests in the National Park Fruška Gora.

Lime is an important and valuable tree species and a factor of progressive succession in closed stands, but also it is a potential factor of regressive succession in the phase of oak stand regeneration and a factor of regressive succession when it dominates if it is vegetatively reproduced on regeneration areas. In individual oak ecosystems on Fruška Gora, the apparently contradictory role of lime can best be controlled by the adoption of management system prescribing the cultivation of highly valuable oaks according to the typological base.

CONCLUSIONS

Stand degradation of individual forest types in the region of the zonal community (*Tilio-Carpino-Quercetum robori-cerri*s J o v. 79) in western part of NP „Fruška Gora" is the consequence of clear felling. It develops in the direction of oak extinction, primarily more valuable pedunculate oak, Vergilius's oak, and then also Turkey oak.

The stands established in the previous rotation are characterised by the dominance of Turkey oak, predominantly of coppice origin. The dominant species in the newly established „naturally regenerated" stands in the initial phase are often lime, hornbeam, flowering ash and field maple, predominantly of coppice origin, with the marked tendency of lime dominance.

The coenological structure of stands after clear felling is decided by the increased percentage of the more sciophytic species in the mature stands and their biological potential, predominantly, vegetative regeneration.

The natural composition of species of seed origin can ensure the production of highly valuable oaks, biological stability and longevity of stands, as opposed to more or less degraded forms. The restitution of the degraded stands with more or less disturbed natural composition of species is possible in the phase of regeneration by prescribing the adequate regeneration system and by the implementation of modern technological solutions in the restoration procedure.

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ПРИЛОГ ПРОУЧАВАЊУ ПРОЦЕСА ДЕГРАДАЦИЈЕ САСТОЈИНА НА ПОДРУЧЈУ НП „ФРУШКА ГОРА"

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Резиме

Анализиран је процес деградације састојина које припадају појединим шумским екосистемима у региону зоналне заједнице у западном делу НП „Фрушка гора" (*Tilio-Carpino-Quercetum robori-cerris* Jov. 79). Деградација је последица примењиваних сеча при обнављању састојина, а одвија се у правцу нестајања храстова, прво вреднијих лужњака, крупнолисног медунца, а затим и цера. Састојине настале у претходној опходњи су са доминацијом цера у првом спрату, претежно изданачког порекла, а у новоформираним састојинама, често и почет-

но добро подмлађеним са цером, при даљем спонтаном развоју у првом спрату доминирају липа, граб, црни јасен и друге врсте, претежно изданачког порекла. На ценолошку изграђеност младих састојина опредељујуће утиче повећано учешће врста дрвећа које више подносе засену у зрелим састојинама и њихов биолошки потенцијал, превасходно, вегетативне регенерације. Заустављање регресивне сукцесије у састојинама и њихово реституирање могуће је у фази обнављања прописивањем одговарајућег система обнављања и доследном применом савремених технолошких решења у обновном поступку. Имајући у виду приоритетну заштитну функцију састојина и чињеницу да доминирају изданачке-очуване, а ценолошки осиромашене састојине, систем обнављања путем оплодне сече треба заснивати на комбинованој обнови. У појединим храстовим екосистемима на Фрушкој гори наизглед контрадикторна улога липе, важне врсте дрвећа и као фактора прогресивне сукцесије у склопљеним састојинама, али тиме, и као потенцијалног фактора регресивне сукцесије у фази њиховог обнављања и као фактора регресивне сукцесије када доминира вегетативно регенерисана на подмладним површинама, најбоље се може превазилазити усвајањем и прописивањем система газдовања при којем ће се гајити високо вредно дрво различитих храстова према типолошким карактеристикама.

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THE EFFECT OF USE OF NITROGEN-FIXATORS IN FORAGE PEA PRODUCTION

ABSTRACT: Microorganisms are the most numerous group of living organisms in the pedosphere. They encompass bacteria, viruses, fungi, algae, protozoa and lichens. Their numbers amount to several million per one gram of absolutely dry soil while their biomass amounts to 5—20 tons per hectare.

The aims of this investigation were to examine the effect of application of root nodule bacteria (single strain, mixture of strains, microbiological fertilizer „Nitragin") on the total number of microorganisms, the numbers of fungi, actinomycetes, azotobacters, free nitrogen-fixing bacteria and ammonifiers and the activity of dehydrogenase, as well as how the application of bacteria affects some parameters of nitrogen fixation (dry mater mass, percentage and content of nitrogen).

In the variant with „Nitragin", the total number of microorganisms and the numbers of fungi, azotobacters and free N-fixing bacteria increased. The largest number of actinomycetes was found in the variant with the mixture of strains. The largest number of ammonifiers was found in the variant with the single strain. The dehydrogenase activity, dry mater mass, percentage and content of nitrogen were increased in the variants with the single strain and the mixture of strains.

KEY WORDS: microorganisms, soil, nitrogen fixation

INTRODUCTION

Microorganisms are the most numerous living organisms in the pedosphere, which exert influence on soil fertility. Soil microorganisms include bacteria, fungi, algae, protozoa, viruses and lichens. The number of microorganisms goes up to several million per one gram in absolutely dry soil, and their biomass amounts to five to twenty tons per hectare. The number and activity of certain groups of microorganisms are specific for each soil type (Govedarica and Jarak, 1995). Nitrogen is a major element that influences the production of organic substances and yield performance of agricultural crops. The atmosphere is an important and inexhaustible source of nitrogen. According to *FAO* records (1993), about 175×10^5 tons of nitrogen a year return to the ground in the process known as nitrogen fixation. This process can be performed

without the presence of microorganisms or it may be helped by microorganisms — nitrogen-fixing bacteria. Depending on the relation between the microorganism and the plant, biological nitrogen fixation is classified as symbiotic, free and associative. Soil pH, moisture, temperature, mineral and organic fertilizers and agricultural chemicals are factors that exert influence on the process of nitrogen fixation (G o v e d a r i c a and J a r a k 1995).

The goal of this research was to examine and determine how the use of root nodule bacteria affects the number and activity of microorganisms, as well as how it affects some parameters of nitrogen fixation (plant mass, percent and content of nitrogen in the plant).

MATERIAL AND METHODS

The experiment was carried out in the location of Kljajićevo. The type of soil was chernozem.

Seeds of the forage pea variety *Junior* were inoculated with „Nitragin" (a commercial microbiological preparation made at the Institute of Field and Vegetable Crops, Novi Sad), *Rhizobium leguminosarum bv. viciae* strain 20 GH and a mixture of strains (20GH, D1 and 897) obtained from the microbiology laboratory of the Faculty of Agriculture, Novi Sad. The control variant was not inoculated.

Soil samples for microbiological analyses were taken during pea flowering from a soil layer of 0—30 cm. The samples were analyzed for the total number of microorganisms, the number of fungi, actinomycetes, free N-fixing bacteria and ammonifiers and dehydrogenase activity. The total number of microorganisms was determined by the method of P o s h o n and T a r d i u x (1963). The number of fungi was determined on Czapek-Dox medium, the number of actinomycetes on the synthetic Waksman-Carey medium. The number of free N-fixing bacteria was determined on Feodorov's medium with microelements. The number of azotobacters was determined on Feodorov's medium by the fertile drop method (A n d e r s o n, 1965).

The number of microorganisms was calculated in 1 gram of absolutely dry soil. The activity of dehydrogenase was determined using the spectrofotometric method of T h a l m a n n (1968). The effectiveness of nitrogen fixation was determined using the dry mass of plant (g), percent and content of nitrogen in the plant.

RESULTS AND DISCUSSION

Microorganisms and products of their metabolism are the living component of soil and they are one of the parameters of soil fertility.

Live cells of microorganisms are introduced into the soil by bacterization of legumes. This changes the relations inside the microbiological population in the rhizosphere (J a r a k et al., 1994). Beside nitrogen fixing, nodular bacteria also produce organic matter of polysaccharide and protein type, which increa-

ses the content of nutrients needed by the resident soil microorganisms (E g e - r a a t, 1975).

The bacterization of seeds of the pea varieties Karina and Pionir with effective strains of *Rhizobium leguminosarum bv. viciae* (S t r n i š a, 1999) indicated the positive influence of bacterization on the total number of bacteria, number of actinomycetes and the activity of dehydrogenase in rhizospheric soil at the beginning of growing season.

In this study, the numbers of all examined groups of microorganisms were extremely high. According to numerous authors, chernozem is the soil type that provides favorable conditions for the growth of microorganisms (G o v e d a r i c a et al., 1993, M i l o š e v i ć, 1990).

In the examined location, the total number of bacteria and the numbers of fungi, azotobacters and free nitrogen-fixing bacteria were significantly increased in the variant with „Nitragin" application (Tables 1 and 2).

Table 1. Total number of microorganisms (TN), numbers of actinomycetes and fungi and the activity of dehydrogenase in pea rhizosphere

Variant	TN 10^7 g^{-1} of soil	Actinomycetes 10^5 g^{-1} of soil	Fungi 10^4 g^{-1} of soil	Dehydrogenase μg TPF per g of soil
Control	791.62	9.18	4.92	193.67
„Nitragin"	954.81	4.43	12.44	308.00
Strain 20GH	606.15	5.06	2.79	315.33
D1+897+20GH	730.53	12.54	6.78	345.00
LSD 5%	11.34	0.65	1.34	7.69
LSD1%	17.18	0.99	2.04	11.65

Table 2. Numbers of azotobacter, free N-fixing bacteria and ammonifiers in pea rhizosphere

Variant	Azotobacter 10^2 g^{-1} of soil	Free N-fixing bacteria 10^5 g^{-1} of soil	Ammonifiers 10^6 g^{-1} of soil
Control	51.49	423.43	1050.70
„Nitragin"	55.02	738.85	884.72
Strain 20GH	35.36	417.37	1379.90
D1+897+20GH	31.80	357.88	1138.40
LSD 5%	2.25	37.86	9.52
LSD 1%	3.41	57.35	14.28

The inoculations performed with strain 20 GH and the mixture of strains showed that the latter was more effective. The application of nodular bacteria, both directly by inoculation and as microbiological fertilizer, tended to stimulate the activity of dehydrogenase.

Rhizobium leguminosarum bv. viciae strains develop nodules on pea roots which bind elemental nitrogen — 20 kg—200 kg N ha^{-1} are bound annually (W a n i et al., 1995). Significant amounts of organic matter are left in the soil after harvest. After mineralization, forms of nitrogen are made which are

accessible to the next crop. Effective symbiotic relationship leads to an increased protein content in the seed (Jarak et al., 1997), which is of great importance for agricultural production.

In this study, the values of plant mass, the content and percent of nitrogen were inconsistent (Table 3).

Table 3. Effect of bacterization on plant mass, % and content of nitrogen in forage pea plants

Variant	Plant dry mass (g)	% of N	N content (mg per plant)
Control	4.06	3.94	162.47
„Nitragin"	3.33	4.27	138.80
20GH strain	4.20	4.76	183.10
D1+897+20GH	4.66	4.65	216.46
LSD 5%	1.42	1.52	58.94
LSD1%	2.15	2.30	89.29

Increase in plant mass and the content and percent of nitrogen occurred in the variants with strain 20 GH and the mixture of strains while „Nitragin" did not produce such effects (Table 3).

An important factor that affects the process of nitrogen fixation is mutual compatibility between the host and nodular bacteria (Lie, 1974; Ohlendorf, 1986). Compatibility depends on lectin, a root exudate that makes the structure of the surface layer of root hairs, and on polysaccharides that are synthesized by microorganisms. The importance of compatibility between plant variety and bacterial strain was confirmed for other legumes, too (Jarak et al., 1992, Milić et al., 1999). In the soil that contains a high amount of nitrogen (the chernozem soil), the efficiency of nitrogen fixation was reduced, as implied by the results of Bolman and Wassey (1997).

CONCLUSION

The numbers of all examined group of microorganisms were high, which is characteristic of the chernozem soil.

In the variant with „Nitragin", the total number of microorganisms ad the numbers of fungi, azotobacters and free N-fixing bacteria significantly increased.

The number of actinomycetes was the largest in the variant with the mixture of strains; the number of ammonifiers was largest in the variant with the single strain.

The mixture of strains and strain 20GH had a positive influence on dehydrogenase activity in the soil and plant mass and the content and percent of nitrogen in the plant.

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ЕФЕКАТ ПРИМЕНЕ АЗОТОФИКСАТОРА НА МИКРОБИОЛОШКУ АКТИВНОСТ У РИЗОСФЕРИ СТОЧНОГ ГРАШКА

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Резиме

Оглед је постављен на локалитету Кљајићево. Тип земљишта је био чернозем.

Семе сточног грашка сорте *Јуниор* инокулисано је са *Нитрагином* (микробиолошки препарат Научног института за ратарство и повртарство Нови Сад), са сојем *Rhizobium leguminosarum bv. viciae*, 20 GH и смешом сојева 20 GH, D1 и 897 у истим односима (из микробиолошке лабораторије Пољопривредног факултета у Новом Саду). Контролна варијанта није инокулисана. Свака варијанта је постављена у три понављања на укупној површини од једног хектара.

Узорци земљишта за микробиолошке анализе су узимани у фази цветања грашка из ризосферне зоне до дубине од 30 cm. У испитиваним узорцима земљишта одређиван је укупан број микроорганизама, гљива, актиномицета, азотобактера, олигонитрофила, амонификатора и активност дехидрогеназе. Ефективност азотофиксације одређивана је преко суве масе биљке (г), % и садржаја азота у биљци (мг).

Бројност свих испитиваних група микроорганизама била је висока и карактеристична за чернозем. На варијанти где је примењен Нитрагин добијено је статистички значајно повећање укупног броја микроорганизама, гљива, азотобактера и олигонитрофила. Бројност актиномицета била је најзначајнија на варијанти инокулисаној са појединачним сојем. Активност дехидрогеназе била је највећа на варијантама где је примењен појединачан сој или смеша сојева. На повећање масе биљке, садржај и проценат азота позитивно је деловала смеша сојева и сој 20GH.

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MINERALOGICAL COMPOSITION OF SOLOD

ABSTRACT: The objective of the present study was to investigate in detail the mineralogical soil composition in order to obtain a clearer picture of the extent to which it and the related pedogenetic process taking place in locations marked as solods on the R = 1:50,000 soil map of the Vojvodina Province (Nejgebauer et al., 1971) correspond to the solodization process described in the literature.

In the profiles of the soils we studied in the regions of western Bačka and southern Srem, illite was the dominant clay mineral, but some specific phenomena were also observed that from the pedogenetic point of view are typical of a soil type previously described in the literature (solod), namely the greater contribution of quartz in the surface horizons of the total soil sample (which is indicative of clay mineral decomposition and destruction), the predominance of illite and kaolinite in the surface horizons of the clay fraction, and the fact that in the Bt,g horizon the kaolinite content decreased considerably and illuviation occurred of chlorite and vermiculite, two highly dispersive minerals from the smectite group.

KEY WORDS: mineralogical soil composition, mineralogical composition of clay fraction, solod

INTRODUCTION

Minerals account for around 60% of the volume of most soils. The decomposition of primary minerals releases substances necessary for plant nutrition. These substances are retained in secondary minerals, which are indicators of the rate of decomposition, while the presence or absence of a particular mineral is indicative of processes that lead to the development of a given soil type.

Problems of halomorphic soils study, amelioration and utilization are as relevant today as ever both internationally and domestically. An overview of our country's previous research in the field shows that solod has received less attention than the other types of halomorphic soils (solonchak and solonetz).

According to Bazilevich (1967), the solodization process involves the decomposition of the mineral portion of the soil, relocation of the decomposition products and their retention in the illuvial horizon, and the formation of secondary clay minerals.

Rode (1969) reports that intensive changes of secondary clay minerals continue within the eluvial horizon during the solodization process, while the primary minerals (feldspars) stay almost intact.

Aleksandrović et al. (1973) studied the mineralogical composition of solod near the village of Čelarevo and found that montmorillonite (smectite) had leached to a greater depth than in solonchak and solonetz. Carbonates, too, leached away completely down to 65 cm depth. Among clay minerals, illite dominated throughout the profile, followed by chlorite and vermiculite. There was no clear evidence of kaolinite, while quartz was found in traces.

According to Adam (1983), the mineralogical composition of the clay fraction of Slavonia-Baranja solod is characterized by the presence of montmorillonite (smectite), chlorite, vermiculite, illite, kaolinite and quartz. Based on the mineral distribution down the profile, the author concluded that a complete leaching of montmorillonite from the A/E horizons had occurred in the solod concerned.

Explaining the solodization process, Glazovska (1981) argues that clay minerals saturated with sodium under occasional surface overwetting conditions will gradually disperse and leach away. Also, in part due to their large specific surface area, the clay minerals will undergo hydrolytic decomposition under the influence of water saturated with H_2CO_3.

According to Kovda and Rozanova (1988), the mineral composition of the clay fraction of solods formed on loess is dominated by hydromica and mixed-layer silicates of the smectite — hydromica type, and kaolinites and illites are also present. The amount of smectite, an expanding (swelling) mineral, is smaller in the eluvial part of the profile compared with the illuvial one.

The objective of the present study was to investigate in detail the mineralogical soil composition in order to obtain a clearer picture of the extent to which the pedogenetic process taking place in locations marked as solods on the R = 1:50,000 soil map of the Vojvodina Province (Nejgebauer et al., 1971) correspond to the solodization process described in the literature.

MATERIALS AND METHODS

A total of 21 pedological profiles were opened in representative locations marked as solods or solods and solonetz on the R 1:50,000 soil map of Vojvodina and sampled for soil laboratory tests. Twelve of these profiles were located in the agroclimatic region of western Bačka and nine in southern Srem.

Western Bačka locations:
Profile 1 — Deronje, Branjevina site
Profile 2 — Deronje, Cerić site
Profile 3 — Mali Bač, Pužar site

Profile 4 — Mali Bač, Pužar site
Profile 5 — Bođani, Bođanski Hrastik site
Profile 6 — Bođani, Komorska Šuma site
Profile 7 — Mladenovo, Sv. Petar and Pavel site
Profile 8 — Bačka Palanka, Novi Majur site
Profile 9 — Čelarevo, Čibska Šuma site
Profile 10 — Veternik, Pašnjak site
Profile 11 — Veternik, Pašnjak site
Profile 21 — Bezdan, Sekeš site

Southern Srem locations:
Profile 12 — Ogar, Lošinci site
Profile 13 — Ogar, Matijevica site
Profile 14 — Ogar, Široka Bara site
Profile 15 — Obrež, Ševinjak site
Profile 16 — Ašanja, Pustara site
Profile 17 — Petrovčić, Draška Bara site
Profile 18 — Petrovčić, Dobanovački Zabran site
Profile 19 — Vitojevci, Jančev Do
Profile 20 — Grabovci, Grabovačko Ostrvo—Đurđevo Polje site

The soil unit characterization was done according to the principles of the current classification of Yugoslav soils (Škorić et al., 1985), in which solod is not mentioned as a soil type.

Based on detailed field and laboratory analyses, it was determined that all of the profiles exhibited clear signs of hydromorphism caused by surface and ground water action and had prounounced textural differentiation between the Aoh, Eg, Aoh/Eg horizon and the Btg horizon as well as that in some of the profiles the presence of adsorbed Na exceeded 15% of T, all of which resulted in the following soil unit categorization:

ORDER: HYDROMORPHIC
CLASS: GLEYIC
TYPE: PSEUDOGLEY SOIL (formerly solod)

This type includes two subtypes:

— one with a prounounced Eg horizon of the Aoh-Eg-Btg-BCG-CG composition, which encompasses profiles 3, 6, 7, 12 and 13 (solod 1); and

— one with no prounounced Eg horizon of the Aoh/Eg-Btg-BCG-CG composition, which encompasses profiles 5, 9, 14, 19 and 20 (solod 2).

ORDER: HALOMORPHIC
CLASS: SOLONETZ
TYPE: SOLONETZ

This type includes two subtypes:

— a pseudogley one with the Aoh-Eg-Btg, na-BCG-CG composition (solonetz 1), variety: typical, form: deep, which includes profiles 1, 2, 4, 10 and 11; and

— a typical one with the Aoh/Eg-Btg, na-BCG-CG composition (solonetz 2),

variety: non-saline (profiles 8, 15, 16, 17 and 21)
saline, chloride-sulphate (profile 18)
form: shallow (profile 21)
medium deep (profile 17)
deep (profiles 8, 15, 16 and 18)

The mineralogical composition of the total soil sample was determined using selected representative samples of profiles 1, 4, 6, 7, 8, 9, 11, 15, 17 and 20, while the mineralogical composition of the clay fraction was determined using all the horizons of profiles 7 and 17. During the sample selection, we made sure that all soil units from the soils of both western Bačka and southern Srem were represented.

Analyses of the mineralogical soil composition were performed in the laboratory of the Faculty of Agriculture in Zemun. The mineralogical composition of the total soil sample was determined by the X-ray-diffraction (XDR) analysis of non-oriented powdered samples after crushing in a McCronne cylindrical mill at 10 μm and lyophilization in a vacuum. Determination of clay minerals (< 2 μm) was carried out on oriented preparations: air-dry, saturated with ethylene glycol and potassium and progressively heated (300°C and 500°C) according to Brindly and Brown (1980). The X-ray diagrams were obtained using SIMENS D-5000, 2 kW diffractometer, in Co Kα radiation.

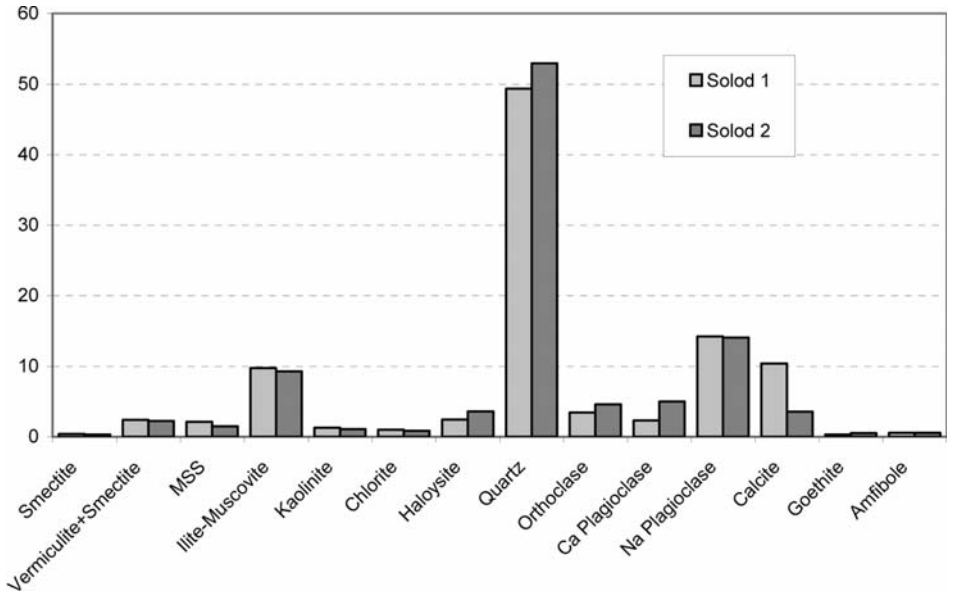
To identify and quantify soil minerals and clay fractions, we used the DRX Win 1.4c (1996) computer package, while the deconvolution subpackage was used for the separation of complex peaks. Semiquantitative analysis of the levels of clay minerals was performed using the adiabatic method, while clay mineral identification was carried out by the clay method of Thorez J. (1989).

RESULTS AND DISCUSSION

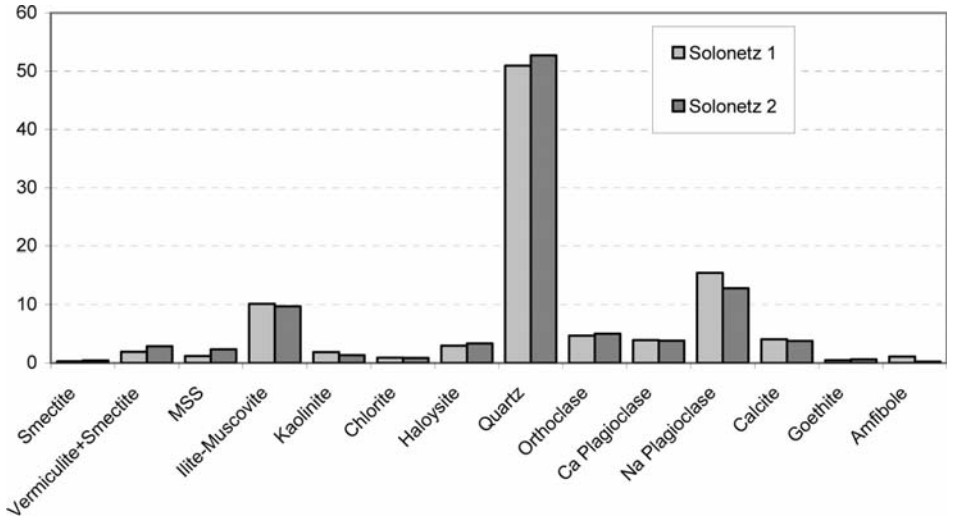
Mineralogical composition of the soil sample

Looking at the average mineralogical composition of the soil across the profile depth, great similarity can be noticed in all of the four soil types involved in the study. Such composition is typical of soils formed on loess, which was the parent substrate of all the profiles studied.

Reflecting the parent substrate, the soil composition was dominated by quartz, feldspar, and mica, accompanied by smaller quantities of clay minerals, calcites, chlorites, goethites and amphiboles (Graphs 1 and 2).



Graph 1. Mineralogical composition means (%) of total soil sample of pseudogley (solod 1 and 2) for the entire profile



Graph 2 Mineralogical composition means (%) of total soil sample of solonetz (solonetz 1 and 2) for the entire profile

The quartz content of pseudogley soils (solod 1 and 2) ranged from 34.44 to 61.37%, while that of solonetz varied between 42.57 and 58.94%. In all of the profiles under study, highest quartz contents were found in the surface horizons (Aoh and Aoh/Eg), with gradual decreases of quartz levels with depth (Table 1).

Tab. 1 Quartz/plagioclase ratio in total soil sample

Profile	Location	Horizon	Depth, cm	Quartz	Plagioclase	Ratio
PSEUDOGLEY — GLEY ZONE				%	%	
6	Bođani	Aoh	3—14	56.42	21.14	3.66
		Eg	14—26	52.40	18.93	2.77
		Bfe	26—66	49.76	18.97	2.62
7	Mladenovo	Aoh	0—8	52.29	18.02	2.90
		Eg	8—20	53.63	18.66	2.87
		Btg	20—71	53.70	18.81	2.85
9	Čelarevo	Aoh/Eg	0—40	53.68	19.39	2.77
		Btg	40—88	47.94	21.22	2.26
20	Grabovci	O	0—10	61.37	14.06	4.36
		Aoh/Eg	10—25	53.56	20.12	2.66
		Btfe	25—85	48.11	20.52	2.34
SOLONETZ SOIL						
1	Deronje	Aoh	0—5	57.77	16.72	3.46
		Eg	5—19	51.06	22.80	2.24
		Btg, na	19—60	50.03	22.33	2.24
4	Mali Bač	Aoh	0—9	56.93	16.06	3.54
		Eg	9—19	54.53	17.93	3.04
		Btg, na	19—58	45.26	18.81	2.40
11	Futog	Aoh	0—10	51.32	16.96	3.02
		Eg	10—24	49.18	21.53	2.28
		Btg, na	24—67	42.57	21.54	1.98
8	Mladenovo	Aoh/Eg	0—18	53.99	21.36	3.40
		Btg, na	18—71	50.16	17.66	2.84
15	Obrež	Aoh/E	0—24	58.94	17.05	3.46
		Bt, na	24—40	54.35	18.76	2.89
		Btfe, na	40—95	48.17	17.13	2.66
17	Petrovčić	Aoh/Eg	0—12	57.09	16.62	3.44
		Btg, na	12—36	56.42	14.22	3.96
		Bt, na	36—70	45.38	12.95	3.50

The most common among the feldspars from the study were sodium plagioclases. The total plagioclase content (Ca plagioclases + Na plagioclases) of pseudogley soils ranged between 14.06 and 21.14% and that of solonetz between 7<.19 and 12.71%.

Other clay minerals commonly found in pseudogley soils include kandites (halloysite and kaolinite), vermiculites and smectites as well as small amounts of mixed layer silicates (MLS).

According to B a z i l e v i c h (1967), the solodization process involves the decomposition of the soil's mineral component, relocation of the decomposition products and their retention in the illuvial horizon, and the formation of secondary clay minerals. This theory is supported by:

— a clear difference between the E and B horizon in the mineralogical composition of the colloidal fraction. Typical for the E horizon are hydromica, and there is a noticeable presence of kaolinite and quartz, while montmorillonite is characteristic of the B horizon;

— a higher quartz to plagioclase ratio of the E horizon compared with the B and CG horizons;

— the existence of characteristic ortstein formations in the E horizon;

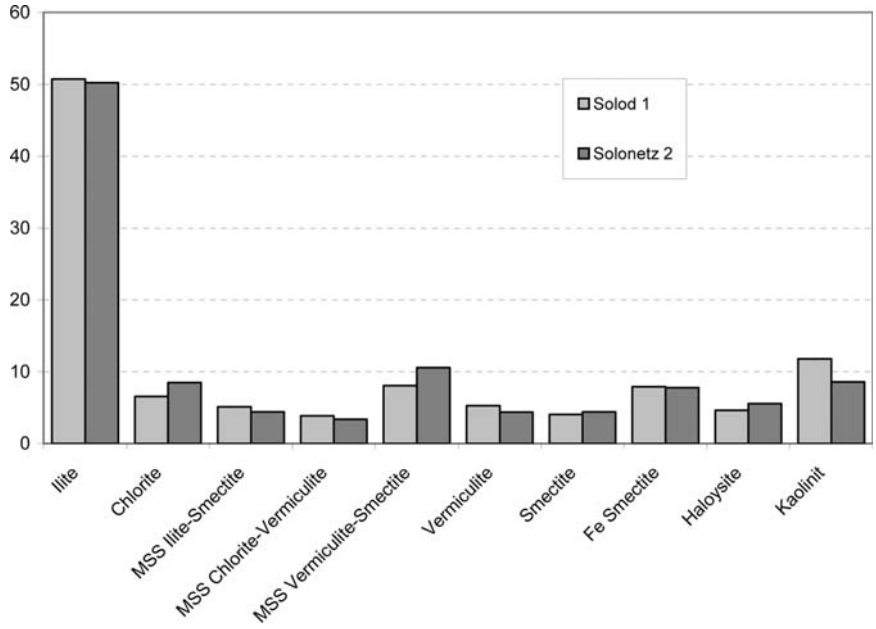
— the variation of migrating capability of various chemical elements around the profile;

— the high concentrations in both the soil and the colloidal fraction of mobile forms of SiO_2 and R_2O_3, which originate from the degradation of the soil's mineral component.

After calculating the quartz/plagioclase ratio (Table 1), we can see that it was higher in all of the surface horizons than in the Bt,g and Bt,g ones in the pseudogley soils as well as in solonetz, which, according to Bazilevich, is one of the indicators of solodization, i.e., of the degradation of the mineral component of a soil's surface horizons.

Mineralogical composition of the clay fraction

Clay minerals greatly influence many soil properties and many processes taking place in the soil. Hence, they are an important factor in determining soil fertility.



Graph. 3. Mineralogical composition means (%) of clay fraction for the entire profile of pseudogleys (solod 1, profile 7) and typical solonetz (solonetz 2, profile 17)

The mineral content of the clay fraction was analyzed in all the horizons of profile 7 (Mladenovo, Z. Bačka) as a representative of pseudogley (solod) soils and profile 17 (Petrovčić, J. Srem) as a representative of solonetz soils.

A look at the mean values of the clay fraction mineral content in both of the profiles (Graph 3) reveals great similarity between them. In both profiles, minerals from the illite group were the most common component (50%), followed by different varieties of MLS (17—18%), kandites (halloysite and kaolinite, 15—16%), smectites and Fe smectites (around 12%) and minor amounts of chlorites and vermiculites.

Looking at the individual values within the profiles, we can notice different distributions and redistributions of particular mineral groups across the horizons, although there was a similarity between the two profiles in this regard as well (Table 2).

Minerals from the illite and kandite groups (kaolinite and halloysite) were found mostly in the surface horizons. Their levels dropped gradually with depth and were at their lowest in the transitional horizon (BC) or the parent substrate/re-deposited loess (C horizon).

The mixed-layer silicates (MLS) had a more complex composition than the simple clay minerals and were formed by pedogenetic processes where they underwent interstratification in different ratios. Among the MLSs studied, the most common were vermiculite-smectite ones. Their levels increased with depth and were the highest in the parent substrate.

According to K o s t i ć (2000), the decomposition of mica may bypass the vermiculite phase during pedogenesis and lead to the formation of smectites. In soils formed under temperate climate conditions, smectites are highly important minerals because of their small dimensions, high specific surface area and great adsorptive capacity. Smectites dehydrate faster, that is, they lose water easily, whereas vermiculites rehydrate faster, i.e., they readily reabsorb the lost water into their structure. During drying, smectites shrink and the soil cracks, but wetting makes them swell again. Such behavior (shrinking/swelling) is most pronounced in smonitza and vertic soils rich in smectites (K o s t i ć, 2000).

The levels of smectite, chlorite and vermiculite minerals, which were lowest in the surface horizons, gradually increased with depth. Significant increases in the concentrations of these minerals were observed in the Btg horizon of the pseudogley soil (solod) relative to the rest of the horizons, especially in the case of Fe smectites, whose presence may be indicative of isomorphic series of newly formed smectites.

The increased presence of smectites and vermiculites in the Btg horizon of solod and the Btg,na horizon of solonetz is closely linked with the highly unfavorable water-air properties of these soils.

Numerous smectite varieties can be found in nature as a result of ionic changes in the tetrahedral and octahedral positions of minerals. The smectite minerals are always found in small-grain particles and dioctahedral varieties are the predominant type found in the soil. Their chemical composition varies among three members of the isomorphic series: montmorillonite, beidellite and nontronite (feri smectite). In some environments characterized by high acidity

Tab. 2. Mineralogical composition of clay fraction (%)

Profile N°	Location	Horizon	Depth cm	Illite	Chlorite	MLS Illite--Smectite	MLS Chlorite--Vermiculite	MLS Vermiculite--Smectite	Vermiculite	Smectites	Fe Smectites	Halloysite	Kaolinite
7	Mladenovo	Aoh	0—8	56.60	3.77	4.85	4.31	5.39	2.83	2.02	6.33	4.04	16.17
	Solod 1	Eg	8—20	55.70	4.88	4.48	3.98	4.39	4.18	1.99	5.97	4.99	15.40
		Btg	20—71	50.10	8.50	3.41	3.64	8.53	7.25	6.07	9.71	3.41	9.10
		C	71—110	48.20	7.03	7.23	4.02	9.64	6.33	4.02	8.04	4.52	9.04
		CG	110—138	43.00	8.61	5.53	3.28	12.3	5.74	6.15	9.43	6.15	9.22
			Mean value	50.72	6.56	5.10	3.85	8.05	5.27	4.05	7.90	4.62	11.79
17	Petrovčić	Aoh/Eg	0—12	55.70	7.43	3.58	4.24	5.31	1.86	1.99	6.23	7.96	11.94
	Solonetz 2	Btg. na	12—36	53.50	7.49	4.21	3.57	6.56	3.99	2.67	6.24	8.02	10.02
		Bt. na	36—70	51.08	9.06	3.64	3.24	9.71	6.80	4.85	8.09	4.85	6.07
		BC	67—80	47.40	8.69	6.96	3.64	12.86	4.61	5.93	9.57	3.95	5.93
		C	80—110	43.40	9.66	3.55	2.10	18.4	4.52	6.58	8.68	2.96	8.87
			Mean value	50.22	8.47	4.39	3.36	10.57	4.36	4.40	7.76	5.55	8.57
	Both profiles		min.	43.00	3.77	3.41	2.10	4.39	1.86	1.99	5.97	2.96	5.93
			max.	56.60	9.66	7.23	4.31	18.40	7.25	6.58	9.71	8.02	16.17
			Mean value	50.47	7.51	4.74	3.60	9.31	4.81	4.23	7.83	5.09	10.18

and aggresive humus (the mor type), gaps occur in the tetrahedral sheet, leading to the formation of special transformational smectites. The cation saturation of such smectites with potassium immediately restores the structure of the parent (initial) illite. However, in the presence of higher concentrations of K ion in the solution, a partial fixation of these ions takes place in the interlayer accompanied by either structural contraction or collapse.

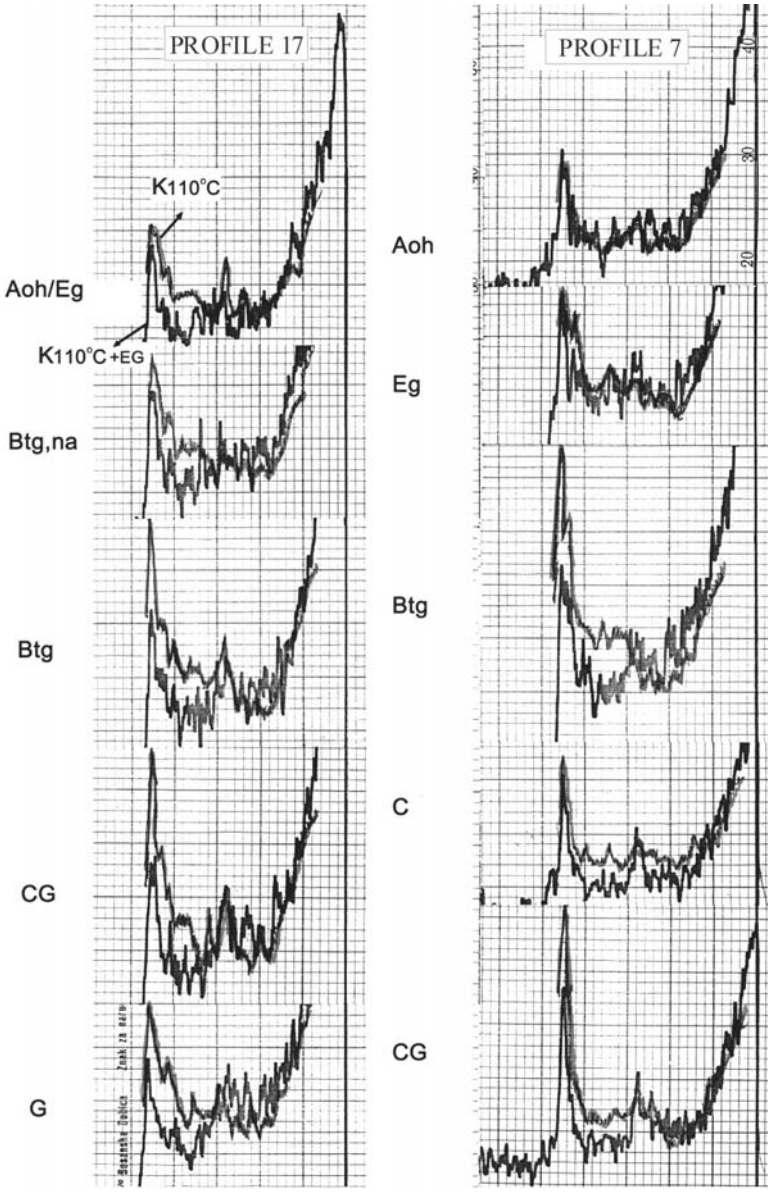


Figure 1. X-ray diagrams of clay fraction in horizons of profiles 7 and 17

In some soil types, continuous isomorphic series can be observed between beidellites, via fero-beidellites to nontronites. In that case, we can no longer speak of transformational smectites; what we have instead are minerals created *in situ* in the soil, i.e., smectites newly formed from water solutions. Proof of their presence is provided by treating the minerals with KCl (introduction of K ions, the K-test). In such a case, the newly formed smectites do not shrink irreversibly (their structure does not collapse) but they retain the capability for expansion of the interlayer space, i.e., the ability to swell (K o s t i ć, 2000).

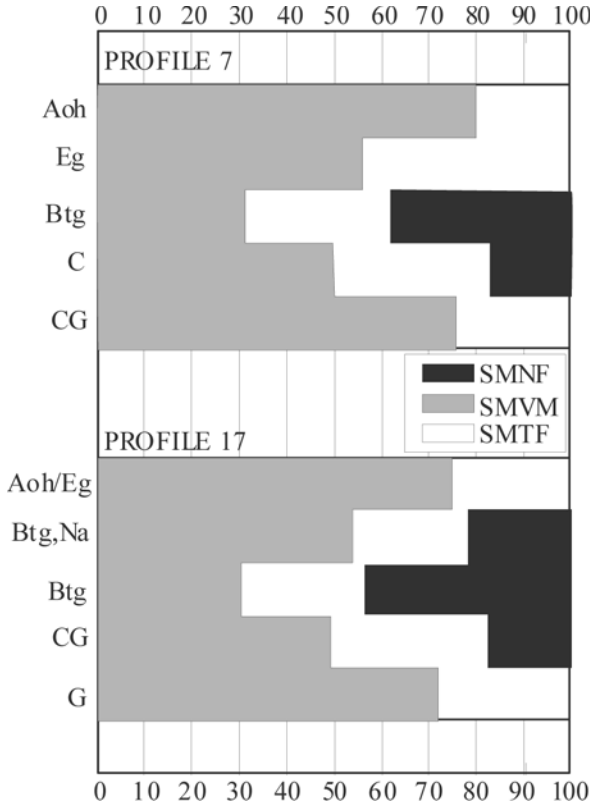


Figure 2. Distribution of smectite phases in clay fraction in horizons of profiles 7 and 17

Figures 1 and 2 show the results of the study of the distribution of the smectite phases in pseudogley soil (profile 7) and solonetz (profile 17). Figure 2 clearly shows that the Aoh and Eg horizons of the pseudogley soil contained transformational smectites and the smectite/vermiculite-type MLSs (SM-VM), while the Btg and BCG horizons also included newly formed smectites (SMNF) formed *in situ* by argillosynthesis from water solutions. In solonetz, the newly formed smectites appeared in the Btg,na, Btg and BG horizons.

CONCLUSIONS

Based on our study of mineralogical soil composition, it can be concluded that illite was the dominant clay mineral in the pseudogley profile. However, the following specific phenomena were also observed that from the pedogenetic point of view are typical of solod, a soil type previously described in the literature:

— The greater contribution of quartz in the surface horizons of the total soil sample, which may be indicative of clay mineral decomposition and destruction;

— Illite and kaolinite dominated in the surface horizons of the clay fraction, while in the Bt,g horizon the kaolinite content decreased considerably and illuviation occurred of chlorite and vermiculite, two highly dispersive minerals from the smectite group. The increased presence of smectites and vermiculites in the Btg horizon of the pseudogley soil was closely connected with its clayey mechanical composition and highly unfavorable air-water properties;

— The increased concentrations of the Fe-smectite mineral and the results of the K-test (distribution of smectite phases) in the Btg horizon of the pseudogley soil relative to the other horizons suggested that there appeared isomorphic series of the newly formed smectites;

— The great similarity in mineralogical composition between the pseudogley soil and solonetz indicated a genetic linkage between these two soil types.

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МИНЕРАЛОШКИ САСТАВ СОЛОЂА

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Резиме

Присуство или одсуство појединих минерала у земљишту указује на процесе који су довели до стварања одређеног типа земљишта. Суштина процеса и начина образовања солоћа (осолоћавање), ни данас нису довољно јасни. Према литературним подацима процес осолоћавања одвија се распадањем минералног дела земљишта, премештањем продуката распадања и њиховим задржавањем у илувијалном хоризонту и образовањем секундарних глинених минерала.

Циљ овог рада је да се на основу детаљног проучавања минералошког састава земљишта, на локалитетима која су на педолошкој карти Војводине P = 1:50 000 (Нејгебауер и сар., 1971), означени као солоћи, јасније сагледа у којој мери минералошки састав, а с тим у вези и присутни педогенетски процеси, одговарају процесу осолоћавања описаном у литератури.

У профилима испитиваних земљишта на подручју Западне Бачке и Јужног Срема, од глинених минерала доминантан је илит, али су испољене и специфичне појаве које су са педогенетичког гледишта карактеристичне за раније у литератури описивани солоћ, тј. већа заступљеност кварца у укупном узорку земљишта, у површинским хоризонтима која указује на распадање и деструкцију минерала глине, као и доминација илита и каолинита у фракцији глине у површинским хоризонтима, док се у Btg хоризонту знатно смањује садржај каолинита и врши илувијација врло дисперзних минерала из групе смектита, хлорита и вермикулита.

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EFFECT OF HUMIDITY ON THE LIFE CYCLE AND REPRODUCTION OF *DERMACENTOR RETICULATUS* (IXODIDAE) IN LABORATORY MICE

ABSTRACT: The reproductive cycle of *Dermacentor reticulatus* (Ixodidae) is described in this paper. Experimental infestation was carried out on immobilized (fixed) RAP mice and those that could move freely. *Dermacentor reticulatus* (*D. pictus*) larvae, nymphs and adults were obtained from eggs placed in glass test tubes in two variants: dry medium (T° = 28—30°C, R.H. = 49—51%) and wet medium (T° = 28—31°C, R.H. = 94—95%).

In the mice that were experimentally infested with the eggs incubated in wet medium, there were more fixed larvae that came off after sucking (8—786 larvae) compared with 0—7 larvae that were observed in the mice that could move freely. At the same time, more larvae were attached to the immobilized mice (2—786) compared with the number of larvae observed in the mice that could move freely (0—11). Infestation was not registered in the experimental animals that could move freely when the larvae were incubated in the dry medium. Experimental infestation with *Dermacentor reticulatus* failed in the RAP mice. The infested mice tolerated as much as 786 *Dermacentor reticulatus* larvae, but they died when 12—30 nymphs were fed on them.

KEY WORDS: *Dermacentor reticulatus*, reproductive cycle, experimental infestation, RAP mice, humidity

INTRODUCTION

Dermacentor reticulatus is a three-host tick affecting sheep, horses and, occasionally, cattle and dogs. Larvae and nymphs feed on rodents and insectivores and adults feed on large mammals (Cosoroabă, 1994; Gilota, 1974).

Dermacentor reticulatus is widespread in Europe. In Romania, this species is spread almost in the whole country (Cernăianu, 1957; Dominguez Penafiel, 1999).

Economic losses caused by *Dermacentor reticulatus* are multiple. The initial loss is due to blood sucking, during the biological cycle, at the expense of host animals. Expenses for tick treatment and control should be added to this. *Dermacentor reticulatus* can transmit a series of diseases to humans and animals (Cernăianu, 1957; Cosoroabă, 1994; Rodhain and Perez, 1985).

Studies concerning the biology and ecology of other Ixodidae species are numerous (Aeschlimann, 1967; Cilton et al., 2000; Cosbie and Boyce, 1998). However, studies of *Dermacentor reticulatus* are not many, especially in Romania. Zahler and Gothe (1995) examined the effect of temperature and humidity on *Dermacentor reticulatus* eggs, larvae, nymphs and adults. On the basis of these experiments, they concluded that temperature and humidity affected the hatching, larvae and nymphs molting and longevity, adult longevity as well as the oviposition of *Dermacentor reticulatus* females.

The primary objective of this study was to monitor the biological cycle of ticks (*Dermacentor reticulatus*) in laboratory mice. Another objective was to establish the effect of humidity, under relatively stable temperature, on the egg incubation time and the numbers of *Dermacentor reticulatus* larvae, nymphs and adults that attached themselves and fed on the mice.

MATERIAL AND METHODS

Engorged females of *Dermacentor reticulatus* were collected from parasitized horses from S.C. Izvin S.A. stud. Individual females were stored in glass test tubes in two variants:

a) Metianu test tubes with a 6 cm water column, 2 cotton-wool layers in contact with water, a 0.5 cm lint layer and an air column above; these were considered as wet medium due to high humidity.

b) Ordinary glass test tubes. These were considered as dry medium due to low humidity.

Both types of test tubes were closed with a plug wrapped with lint.

The two mediums, dry and wet, were used to observe the incubation and molting of satiated larvae and nymphs (Tables 3 and 5).

In all cases, temperature and humidity were measured two times a day, in the morning and evening, with the help of an electronic thermohygrometer.

RAP mice were used for experimental infestation with *Dermacentor reticulatus*. Infested forms were obtained from dry medium (T° = 28—30°C, R.H. = 49—51%) and wet medium (T° = 28—31°C, R.H. = 94—95%).

The mice to be infested were either left free-moving in cages (non-immobilized) or were immobilized with small leather straps.

The number of larvae used for infesting the mice ranged between 300 and 1000, the number of nymphs ranged between 50 and 56, and the number of adults ranged between 7 and 9. The numbers of fixed and satiated larvae, nymphs and adults taken out from the mice were monitored (Tables 2, 4 and 6).

RESULTS

Table 1 shows the results concerning *Dermacentor reticulatus* egg incubation. In laboratory conditions, under almost identical temperature regimens, the incubation took a longer time when the medium was wet (94—95%).

Table 1. Obtaining *Dermacentor reticulatus* larvae „in vitro"

Incubating eggs	T	R.H.	Incubating time
Dry medium	29—30°C	50%	13—17 days
Wet medium	30—31°C	94—95%	16—20 days

Legend: T — temperature; R. H. — relative humidity

More larvae raised in the wet medium remained attached to mice as compared with those raised in the dry medium (Table 2). Not a single larva from the dry medium, at the same dose per mouse (300 larvae), remained attached to the non-immobilized mice. On the other hand, when the mice were immobilized, 2—7 larvae remained attached and sucked blood.

Table 2. Infestation of RAP mice with *Dermacentor reticulatus* larvae

Source of the larvae	Mice infestation	Larvae per mouse	Mouse number	Attached larvae	Feeding time
Dry medium	Nonimmobilized	300	1	0	0
			2	0	
			3	0	
			4	0	
	Immobilized	300	5	7	
			6	7	
			7	2	
Wet medium	Non-immobilized	300	8	8	4—5 days
		300	9	11	
	Immobilized	300	10	143	5—6 days
		1000	11	786	3—6 days

In the case of the larvae from the wet medium, at the same dose per mouse (300 larvae), 8 to 11 of them remained attached to the non-immobilized mice. Attaching was easier to accomplish with the immobilized mice. At the same dose per mouse (300 larvae), 143 larvae remained attached. When infestation was carried out with 1000 larvae, 786 of them remained attached and fed on the mice.

Dermacentor reticulatus larvae incubated at 28—31°C in dry and wet mediums alike, the molting occurring after 8—10 days. The number of the obtained nymphs is shown in Table 3.

Table 3. Obtaining *Dermacentor reticulatus* nymphs „in vitro"

Incubating larvae	Number of incubating larvae	T	R.H.	Molting time	Nymphs obtaind	
					Nr.	%
Wet medium	40	29—30°C	95—96%	9—10 days	22 + 8	75
	40	28—30°C	95—96%	9—10 days	23 + 6	72,5
Dry medium	40	29—30°C	49—51%	8—9 days	28	70
Wet medium	250	30—31°C	94—95%	8 days	103	41,2
Dry medium	400	29—30°C	49%	8—9 days	101	25,25

Dermacentor reticulatus nymphs, irrespective of the type of medium in which they were raised, could not remain attached to the non-immobilized mice (Table 4). Regarding the rate of attachment on the immobilized mice, the nymphs from the wet and dry mediums were attached in almost equal numbers, 11—28 and 12—30, respectively.

Table 4. Infestation of RAP mice with *Dermacentor reticulatus* nymphs

Source of the nymphs	Mice infestation	Nymphs per mouse	Mouse number	Attached nymphs	Feeding time	Observations
Wet medium	Non--immobi-lized	56	1	0	—	—
		56	2	0	—	—
Dry medium		50	3	0	—	—
		50	4	0	—	—
Wet medium	Immobi-lized	50	5	11	7—8 days	—
		56	6	28	5 days	died on the 5th day p.i.
Dry medium		50	7	12	5—8 days	died on the 8th day p.i.
		56	8	30	6 days	died on the 6th day p.i.

Three out of each four infested mice died.

The nymphs incubated in the wet and dry mediums molted at 28—30°C and adults were obtained (Table 5). The period of time necessary for molting varied between 14 and 16 days.

Table 5. Obtaining *Dermacentor reticulatus* „in vitro"

Incubating nymphs	Number of incubated nymphs	T	R.H.	Molting time	Adult ticks obtaind	
					Nr	%
Dry medium	14	28—30°C	49—50%	14—15 days	14 (10* + 4**)	100
	21				20 (14* + 6**)	95,23
Wet medium	14	28—29°C	95—96%	15—16 days	14 (9* + 5**)	100
	21				21 (13* + 8**)	100

Legend: * — female, ** — male.

The percentages of the obtained adults ranged between 95.25 and 100. The number of females doubled that of males in all cases.

The results of mice infestation with *Dermacentor reticulatus* are presented in Table 6. Not a single adult tick remained attached to the immobilized and non-immobilized mice alike, no mater if raised in the wet or the dry medium.

Table 6. Infestation of RAP mice with *Dermacentor reticulatus* adults

Source of the adults	Mice infestation	Adults per mouse	Mouse number	Attached adults
Wet medium	Non-immobilized	7	1	0
		9	2	0
Dry medium		7	3	0
		9	4	0
Wet medium	Immobilized	7	5	0
		9	6	0
Dry medium		7	7	0
		9	8	0

DISCUSSION

Under laboratory conditions, at 28—31°C and variable relative air humidity, the reproduction cycle of *Dermacentor reticulatus* was successful. Although the incubation of larvae was successful both in the wet medium (R.H. = 94—95%) and the dry medium (R.H. = 50%), the period of incubation was shorter in the later case. However, the larvae obtained from the wet medium proved to be more active and they were more easily attached to mice. Comparing the influence of humidity on the different development phases, it seemed that increased relative air humidity was more necessary for egg incubation than for the incubation of larvae and nymphs. This fact was demonstrated by the higher capacity of the *Dermacentor reticulatus* larvae raised from eggs kept in the wet medium to stay attached and feed on the host.

If infestation by larvae succeeded with both non-immobilized and mobilized mice, the infestation by nymphs succeeded only with immobilized mice. Moreover, the infestation by *Dermacentor reticulatus* adults did not succeed at all. These results are partially contradictory to those of Metianu (cited by C e r n ă i a n, 1957) who claimed that the larvae and nymphs were easily attached while adults were more difficult to attach to the host. Perhaps he used another Ixodidae species or his experiments were performed under different conditions.

The mice allowed a large number of larvae to feed on their bodies (up to 786) but the number of nymphs was smaller (11—30). Furthermore, the mice infested by 12—30 nymphs invariably died. No adults were found attached to mouse skin. These results again partially contradict Metianu's report (cited by C e r n ă i a n, 1957) stating that a mouse allows 200—500 larvae or 100—250

nymphs or 5—10 adult ticks to feed on it. According to Cosoroabă (2000), however, only the larvae and nymphs feed on rodents while adults attach and feed on large animals.

CONCLUSION

Under the laboratory conditions provided in this study, longer time was needed to obtain *Dermacentor reticulatus* larvae from eggs when the relative air humidity was increased.

The rate of attachment of *Dermacentor reticulatus* larvae to mice was increased when the larvae were raised under higher relative humidity.

The larger the density of *Dermacentor reticulatus* larvae in the incubation medium, the smaller the percentage of nymphs obtained.

Infestation with *Dermacentor reticulatus* nymphs was successful only with the immobilized RAP mice.

The RAP mice tolerated the infestation by *Dermacentor reticulatus* larvae rather well, but it was not the case with nymphs.

The success of raising *Dermacentor reticulatus* adults „in vitro" was not influenced by relative air humidity.

The infestation of RAP mice with *Dermacentor reticulatus* adults failed altogether.

A general conclusion was drawn that under the laboratory conditions provided in this study RAP mice are not suitable hosts for *Dermacentor reticulatus* nymphs and adults.

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УТИЦАЈ ВЛАЖНОСТИ НА РЕПРОДУКТИВНИ ЦИКЛУС *DERMACENTOR RETICULATUS* (IXODIDAE) КОД ЛАБОРАТОРИЈСКИХ МИШЕВА

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Резиме

У раду је описан утицај влажности на репродуктивни циклус *Dermacentor retikulatus (ixodidae)*. Експериментална инфестација је спроведена на R. A. P. мишевима имобилисаним (фиксирани) и који су се могли слободно кретати по кавезу. Ларва *Dermacentor reticulatus* (*D. pictus*), чауре и одрасле су добијене од јаја која се постављају у стаклене епрувете у две различите средине: у суви медијум (T° = 28—30°C R. H. = 49—51%) и у влажни медијум (T° = 28—31°C; R. H. = 94—96%).

Код мишева који су експериментално заражени јајима инкубираним у влажном медијуму евидентирано је више ларви које су биле фиксиране, које су се након сисања одлепиле (8—786 ларви), у поређењу са 0—7 ларви које су евидентиране када су јаја инкубирана у сувим условима. Истовремено више ларви регистровано је на имобилисаним мишевима (2—786 ларви) у поређењу са бројем ларви које су евидентиране код мишева који су имали слободу кретања (0—11 ларви). Инфестација није забележена код слободно држаних експерименталних животиња када су ларве инкубиране у сувом медијуму. Инфестација ларвама успела је само код имобилизованих мишева, готово у једнаком односу, без обзира на медијум, суви или влажни. Експериментална инфестација *Dermacentor reticulatus* са одраслим ларвама није регистрована код R. A. P. мишева. Заражени мишеви су преживљавали инфестацију и до 786 ларви *Dermacentor reticulatus*, међутим мишеви заражени са 12—30 нимфи су угинули.