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Natalija E. Ellanskaya

National Botanical Garden „M. M. Grishko" NAN Ukrainian
Timirjazevska St. 1
Kyiv, 252014, Ukraine

STRUCTURE ANALYSIS OF SOIL MICROMYCETES IN SECTIONS OF THE UKRAINIAN NATURAL STEPPE RESERVE

ABSTRACT: The soil micromycetes found in Khomytivski Step and Kamyani Mohyly Ukrainian Natural Steppe Reserve were studied. A total of 53 species of soil micromycetes belonging to 19 genera were isolated. Species distribution included *Zygomycetes* (18.9), *Pyrenomycetes* (3.8) and *Hyphomycetes* (77.4). The widely spread genera were *Penicillium, Aspergillus, Trichoderma, Fusarium*. Species richness was much higher on the Khomytivski Step than the Kamyani Mohyly.

KEY WORDS: micromycetes, rhizosphera, reserve, species distribution.

INTRODUCTION

The steppes occupy large territories and have played a relevant role in the development of social-historical cultures with peculiar household activities and national traditions. Therefore, Ukraine is substantially the steppe state.

The large anthropogenic loads emphasize the problem of guarding the steppe. Due to the diligence of several scientists such as U. Kleopov, M. Klokov, M. Kotov, A. Lavrenko (Tkachenko, 1998), series of steppe reserves, representative of different typological variants of virgin plants were organized on the territory of Ukraine. The Ukrainian Natural Steppe Reserve (UNSR) is one of them.

The analysis of soil mycobiota is the integral part of researches of the functioning of steppe ecosystems and gives the useful information on biological variety of fungi conditioned in this case by the nature-climatic factors. The characteristic of a micromycetes complex supplements obtained before the early of information on number and taxonomic structure of bacteriums of soils of sections UNSR (Andreyuk, 1992).

The estimation of density of the population micromycetes rhizosphera of steppe plants by a method of crop on mediums is the most conventional ap-

proach in modern micologicale researches permitting to receive the full enough information on structure of a complex micromycetes on strict the quantitative basis with using index of the frequency of occurrence of species and similarity measurements (Mirchink, 1988, Dudka et al., 1982).

MATERIAL AND METHODS

The problem of the present research consist in comparing of complexes micromycetes in rhizosphera of vegetative communities of two sections USNR — Khomutivski Step and Kamyani Mohyly. Khomutivski Step is the section most remote to the south, of reserve and concerns to the xerophilous version of herb-fescue-feathergrass steppe. To typological tags Kamyani Mohyly as a whole are close to those in Khomutivski Step. In structure of steppe phytocenosises here dominate same turf cereals, as Khomutivski Step, to which one in many places are admixed rhizome cereals. However this section is characterized by high specificity, which one is conditioned by an output of crystal strains on a surface. The climatic parameters Khomutivski Step and Kamyani Mohyly are close enough, both are in dry to enough warm zone. For introduced reserves are characteristic chernozem soil.

Soil samples were gathered under different plants associations before termination a vernal vegetation in 1996—1997 years. Soil micromycetes studies were conducted as previously described (Waksman and Fred, 1922).

In genera, 10 g soil was placed in 90 ml sterile water and shaken for 5 min. A series of dilutions 1—10, 1—100, and 1—1000 was made and placed on plates containing malt extract agar with 50—100 mg/l of streptomycin or tetracycline was added to medium at 40°C. Plates were incubated at 25—27°C, and isolated colonies were transferred to agar plates for 3—21 days growth for further examination, characterization and identification.

Both qualitative and quantitave observations were made for species identification on the selected agar media. In addition to species identification, data were examined on the frequency of occurrence of some species.

The Dice (or Czekanovski) similarity measure of species content of fungi of two ecotopes was estimated according to the formula Dice S=2a/(2a+b+c), were *a* represents the number of common species, *b* represents the number of species in the soil of the one sample, and *c* represents the number of species in the soil of the another sample (SPSS, 1990).

Taxonomic literature was used for identification of the fungal isolates to species (Booth, l971; Ellis, 1971; Bilai, 1977; Domsch et al., 1980; Raper et al., 1949; Raper and Fennel, 1965).

RESULTS AND DISCUSSION

The species content of the soil micromycetes collected on two sections of UNSR is shown in Table 1.

Tab. 1. — Frequency of occurence of micromycetes in rhizosphera of two reserves of UNSR

Taxa	Frequency of occurence (%)	
	Khomutivski Step	Kamyani Mohyly
1	2	3
Zygomycotina, Zygomycetes, Mucorales		
Absidia spinosa Lender	0	23,1
Actinomucor elegans (Eidom) c.k. Benjamin	0	30,8
Cunningamella echinulata Lendner	26,7	30,8
Mortierella alpina Peyronel	40,0	30,8
M. policephata Coem	33,3	0
Mucor hiemalis Wehmer	40,0	0
M. mucedo Mich.: St.	13,3	0
M. pusillus Lindt.	20,0	0
Rhizopus orysae Went. et Prins. Geerlings	46,7	0
R. stolonifer (Ehrenb.: Lk) Lind.	33,3	30,8
Ascomycotina, Pyrenomycetes, Sphaeriales		
Chaetomium globosum Kunze: Fr	33,3	23,1
Melanospora zobelii (Corda) Fuckel	20,0	30,8
Deuteromycotina, Hyphomycetes, Hyphomycetales		
Acremonium rutilum W. Gams	26,7	15,4
Ac. strictum W. Gams	33,3	38,5
Alternaria alternata (Fr.) Keissler, Ellis	33,3	0
Aspergillus alutaceus Berk et Curt.	33,3	23,1
As. amstelodami (Mang.) Thom et Church	0	38,5
As. fumigatus Fres.	33,3	0
As. niger Teighem	33,3	0
As. terreus Thom	40,0	38,5
As. versicolor (Vuill) Tiraboschi	33,3	38,5
Gliocladium roseum (Link.) Bain	60,0	61,5
Gl. varians Pidopl	33,3	0
Paecilomyces lilacinus (Thom). Sam	20,0	0
Penicillium brevi-compactum Dierchx	33,3	30,8
P. canescens Sopp.	40,0	30,8
P. chrysogenum Thom	46,7	0
P. citrinum Thom	26,6	23,1
P. decumbens Thom	20,0	23,1
P. fellutanum Biourge	26,6	23,1
P. frequentans Westl.	40,0	23,1
P. funiculosum Thom	20,0	23,1
P. implicatum Biourge	33,3	30,8
P. jantinellum Biourge	26,6	15,4
P. lanosum Westl.	38,3	38,4
P. nigricans (Bain.) Thom	46,7	15,4

P. olsoni (Bain.) et Sart.	0	15,4
P. raciborskii Zal.	33,3	38,5
P. rugulosum Thom	20,0	23,1
P. spinulosum Thom	20,0	15,4
P. variabile Sopp	13,3	15,4
P. varians Smith	33,3	15,4
P. verrucosum Dierckx var *cyclopium* (Westl.) Sam.	40,0	38,5
P. viridicatum Westl.	33,3	38,5
Trichoderma album Press	33,3	0
Tr. koningii Oudem	23,3	38,5
Tr. viride Pers.	60,0	53,8
Botrytis cinerea Pers.: Fr.	0	23,1
Cladosporium herbarum (Pers.) Lk: Gray	26,7	30,8
C. cladosporioides (Link.) Bain	20,0	0
Doratomyces stemonitis (Pers.) Monton et Sm.	40,0	38,5
Tubercularіales		
Fusarium gibbosum App. et Wr	26,7	15,4
F. javanicum Koord.	20,0	30,8
F. moniliforme Sheld var. lactis (Pir. et Rg.)	20,0	38,5
F. oxysporum (Schlecht.) Snyd. et Hans	63,3	61,5
F. solani (Mart.) App. et Wr/	40,0	30,8
F. sporotrichiella Bilai	33,3	0

A total of 53 species of soil micromycetes belonging to 19 genera were isolated. From a systematic point of view, the identified species were divided as follows: *Zygomycetes* (10 species) belonging to six genera; *Pyrenomycetes* (2 species) belonging to two genera; *Hyphomycetes* (46 species) belonging to ten genera.

From *Hyphomycetes* the most prominent genus was *Penicillium* with 21 species with the following most common species *P. canescens, P. chrysogenum, P. frequentans, P. raciborskii*.

The special concern introduces presence of large number of species pigment-free and light *Penicillium*. Peculiar feature is also presence *Penicillium*, having teleomorpha stage. Notably, the representatives of the genus *Penicillium* were widely distributed, due to their high sporulation activity, and they are common in southern regions and cultivated soils they easily transfer both high and low temperatures, the spores save them a vitability in toxigenous soils under extremal conditions and growthes at rather low moisture.

The second most common genus was *Aspergillus* with six species from which *A. alutaceus, A. terreus* and *A. versicolor* were the most common. The diffusion *Aspergillus* is connected to definite environmental factors, where edafical conditions are play a key role (Zvyagincev, 1984).

The genus *Fusarium* was the next prominent with six species, from which *F. oxysporum, F. solani* and *F. gibbosum* appeared with similar frequencies. The common presence of *Fusarium* species reflect their preference for soil with high organic content and the ability of the organisms to produce

a large number of chlamydospores resistant to adverse environmental parameters (Bilai, 1977).

The colones of micromycetes with intensive growth are presented by species from genera *Trichoderma, Gliocladium, Chaetomium*: *Tr. viride, Tr. koningii, Gliocladium roseum* and *Chaetomium globosum*. Micobiota of Khomutivski Step differed on a specific structure a little — dominated *Tr. album* and *Gl. varians*. The great many of species from a genus *Trichoderma* frequently is connected to its opposing and competitive capacities (Domsch et al., 1980).

In Khomutivski Step and Kamyani Mohyly representative of the dark coloured hyphomycetes, were found. These species exit in unfavourable environments, where a dry continental climate with hot summer and in the winter without snow (Zhdanova, 1982),

Differences were noted in species distribution when comparing species content from the Khomutivski Step and Kamyani Mohyly. These distribution differences were influenced by soil, climatic conditions, character of higher plants, humidity, pH, temperature and other factors which influence soil micromycetes species content. Species richnes was much higher on Khomutivski Step than Kamyani Mohyly. However, some species from the order Mucorales displayed similar distribution on the both reserves. From Khomutivski Step to Kamyani Mohyly, respectively, the frequency of occurrence of *Mucor hiemalis* was 40,0% and 38,5%, and *Rhizopus stolonifer* was 33,35 and 30,8%. It should be noted that Mucorales species were more prevalent on the Khomutivski Step due to the presence of greater number of higher plant species and the high content of soil humus compared (Mirchink, 1988).

The soil micromycetes identified were divided into three groups according to the frequency of occurrence. Group 1 included species with the frequency of occurrence exceeding 50%. Group 2 included species with the frequency of occurrence at 25—50%. The majority of the species from reserves belonged to this group. Group 3 included species with the frequency of occurrence of < 25%. The role of rare micromycetes species not commonly found in soil habitats should not be underestimated since these species enrich the species diversity of any ecological niche by their quantity. Such species in our investigations were: *Paecilomyces lilacinus, Botrytis cinerea, Doratomyces stemonitis* and many others.

Index of resemblance of reserves was 75%. It can be explained by the similar soil-climatic and botanical conditions of these two districts.

In summary, species content, frequency of occurrence, common species, and index of resemblance, were examined for the two ecotopes. The analysis of the present material allows to draw a conclusion, that ecosystems, which littely differs by the type of soil and vegetation are characterized by minor divergences in structure of micromycetes complexes.

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СТРУКТУРНА АНАЛИЗА МИКРОБИОНТА У ДВА ЛОКАЛИТЕТА СТЕПЕ УКРАЈИНСКОГ ПРИРОДНОГ РЕЗЕРВАТА

Наталија Еланскаја
Национална ботаничка башта „М. М. Гришко" Украјинске академије наука,
Тимирјазевска 1, Кијев, 252014, Украјина

Резиме

У раду су проучаване микомицете на два локалитета степе: Хомутовске степе и Камене могиле (Камени гробови). Издвојено је 53 врсте микомицета које су сврстане у 19 родова. Процентуално су највише биле заступљене *Hyphomycetes* (77.4%), затим *Zygomycetes* (18.9%), а најмање *Pyrenomycetes* са свега 3.8%. Широко распрострањене врсте биле су: *Penicillium, Aspergillus, Trichoderma* и *Fusarium*.

Упоређивањем локалитета по броју врста запажа се да је локалитет Хомутовске степе много богатији у односу на Камене могиле, што је вероватно условљено земљишно-климатским чиниоцима и ботаничким особинама, односно већим бројем виших биљака и високим садржајем хумуса у земљишту.

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Zorica B. Svirčev, Igor N. Balaž

Institute of Biology, Trg Dositeja Obradovića 2
21000 Novi Sad, Yugoslavia

CO-CULTIVATION OF CYANOBACTERIA AND WHEAT PLANTS ON AGARIZED MEDIUM

ABSTRACT: A comparison of the interactions of eight cyanobacterial strains (*Anabaena* and *Nostoc*), isolated from different soils of Vojvodina with four wheat cultivars, carried out in the controlled agarized cultures have been made. The beneficial effect of solid surfaces in co-cultivation arises from the retention of bioactive products of cyanobacteria and rhizosphere at a position close to the producers. The contribution of solid media lies in possibility of visual observation of those interactions which are based on strains motility and root growth direction.

Various types of interactions have been noticed arranged to the strains and cultivars involved. The investigation showed that C3, C5 and LC2 strains are characterized by intensive clustering around root surface of all four examined wheat cvs. „Lasta" wheat cultivar roots excrete certain substance or substances that have attractive effect on the cyanobacterial filaments, able to direct their movement. In the case 2S7b of strain, cyanobacterial filaments were accumulated at the surface of the medium, keeping distance from the roots. The investigation also displayed that 2S3b and LC2 strains were able to direct growth of wheat roots as characterized by cyanobacterial production of extracellular substances provoking the cyanobacteria-dependent roots growth direction response.

Although in certain cases no colonization of the roots occurred, it did not imply absence of interactions at all. In few case of co-cultivation, no root colonization was observed while cyanobacteria were found to penetrate some root cells.

KEY WORDS: agarized medium, biologicaly active compounds, co-cultivation, cyanobacteria, wheat

INTRODUCTION

Many cyanobacteria may occur in the rhizosphere in varying degrees of intimacy with the root system. It has been become clear that cyanobacteria release many kinds of bioactive substances to their surrounding either actively or passively, such as: algicides, antibiotics, toxins and plant growth regulators. At the same time, it could be expected that they are influenced by the wide range of compounds excreted by the root system.

In spite of the fact that these interactions have been known for a long time (Pascher, 1929), great interest in novel associations between the higher

plants and diverse N_2-fixing microorganisms has developed on the scientific scene in recent years, arising from the prospects and possibilities of their potentially beneficial effects (Stewart, 1982; Rowell and Kerby, 1991) following application (Rodgers et al., 1979; Goyal, 1987). There are instances of marked interactions between cyanobacteria and eukaryotes, and among these the range of symbiotic associations involving the higher plants is of particular interest (Whitton, 1982). The extracellular release of nitrogen compounds and also the release of certain biostimulative substances are obvious ways in which cyanobacteria may benefit the higher plants (Metting and Pyne, 1986). Other studies have shown how plants may aid the growth and increase the N_2-fixation of cyanobacteria during co-cultivation (Rai, 1990).

A positive effect of an ammonia-excreting mutant of *Anabaena variabilis* on the growth of wheat, one of the most important crops in temperate regions, has been demonstrated (Spiller and Gunasekaran, 1990). Both the nitrogenase activity of this mutant strain and plant growth were enhanced by direct association of the cyanobacterium with plant roots (Spiller et al., 1993). We have also described the beneficial effects of N_2-fixing cyanobacteria on the growth and nitrogen content of wheat seedlings grown hydroponically with their roots colonized by soil isolates of cyanobacteria (Obreht et al., 1993).

In this paper we reported the movement of filamentous cyanobacteria towards the wheat roots resulting in cluster formation and root growth direction influenced by the cyanobacteria. This is an approach to examining the rhizosphere and cyanobacteria in relation to their interactive effect on plants.

MATERIAL AND METHOD

Organisms and growth conditions:

Nostoc and *Anabaena* strains 2S3b, 2S6b, 2S7b, 2S9b and S1, LC2, C3, C5, respectively, were isolated from different soils from the Vojvodina Province (Gantar et al., 1991a) and were maintained on BG-11 medium (Rippka et al., 1979).

The four wheat (*Triticum vulgare* L.) cultivars, „Zitnica", „Rana niska", „Lasta" and „Jugoslavija", were a gift from the Institute of Field and Vegetable Crops, Faculty of Agriculture, University of Novi Sad.

The cultivation of wheat seedlings and cyanobacterial isolates was carried out at 24°C with continuous illumination at a photon fluency rate of 95 $Em^{-2}s^{-1}$ for three weeks. The pH of the media was measured daily by microelectrodes.

Co-cultivation on agarized medium:

For the purpose of this study a new method of co-cultivation was designed, to enable visual observation of expected wheat roots-cyanobacteria interactions. In order to co-cultivate wheat seedlings and cyanobacteria in solid volumes, the inoculum of a fully-grown culture of the examined strain was suspended in melted, moderately cooled agarized BG-11 medium placed in 150

ml glass pots. After solidification, three-day-old wheat seedlings were transferred onto cyanobacteria-trapped agar volume.

In addition, to determine potential cyanobacterium-dependent roots growth direction, a gutter was made on the surface of solid medium placed in Petry dishes and inoculated with cyanobacteria. At the same time, wheat seedlings were placed on the opposite side of the medium.

The pH of the media was measured daily by microelectrodes. All types of examined interactions were determined by visual observation.

RESULTS AND DISCUSSION

Depending on strains and cvs. involved, several types of cyanobacterial motility over the solid volume were observed (Table 1). All strains grown under conditions as described above showed dense filaments aggregation in the superficial zone of the medium. It could be attributed to the highest light intensity and to the intensive diffusion of gasses in that area.

A complete colonization of the roots of cv. „Lasta" by six of the eight examined strains could be observed (Table 1). Intensive colonization of the whole root occurred after the aggregation of cyanobacterial cells at the bottom of the experimental vessels, in spite of lower light intensity inside the solid medium. The filaments of strain S1 were able to colonize the roots only in the superficial zone of the solid medium, but mostly were indifferent in relation to the presence of cyanobacteria. In the case of strain 2S7b, cyanobacterial filaments were accumulated at the surface of the medium, keeping distance from the roots.

Cyanobacterial strains C3, C5 and LC2 showed intensive clustering around the root surface of all wheat cvs. On the basis of the motility of other strains (2S3b and 2S9b), a positive chemo-topotaxis (C a s t e n h o l z, 1973) was established during the co-cultivation with cvs „Rana niska" and „Lasta". It could be assumed that the roots of the examined wheat cvs. excrete certain substance or substances which attract motile trichomes, causing their orientation and movement towards the roots.

A special type of association between cyanobacteria and wheat roots was observed at the end of the cultivation period. It was manifested as the development of an active growing mass of cyanobacterial cells around the root surface at the bottom of experimental vessels, where most of the wheat roots were settled (Table 1). In spite of the lower light intensity and diffusion of gasses inside the solid medium, intensive growth of cyanobacterial cells was observed. It was related to the stimulative effect of root exudates.

Within 24 hours after inoculation of wheat seedlings, a thin space with no cyanobacterial cells was recorded around the roots deep in the agarized medium. Such behavior of strains C3 and C5 seemed to determined by the pH value of the medium (Table 1). The pH of a thin zone nearest to the roots was 5.3 while the other parts of media characterized by dense populations of cyanobacteria showed pH ranges from 7.8 to 8.0. After three days of co-cultivation, cyanobacterial colonies were observed all over the medium, related to

Tab. 1. — Different types of interactions during the co-cultivation of cyanobacteria and four wheat cvs. root at the deep agarized medium (glass pots) and Petry plates

Strain	The ring around the root at the surface of agar				Colonization along the whole lenght of root				Cyanobacterial filaments at the bottom of the bottle				The movement of roots toward the Cyanobacteria (Petry plates)			
	L	J	Z	RN	L	J	Z	RN	L	J	Z	RN	L	J	Z	RN
S1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2S3b	—	—	—	—	+	—	—	+	+	—	—	—	+	—	—	+
2S9b	—	—	—	—	+	—	+	+	+	—	—	—	—	—	—	—
2S6b	—	—	—	—	+	—	—	—	+	—	—	—	—	—	—	—
C3	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—
C5	+	+	+	+	+	+	—	+	+	+	+	+	—	—	—	—
LC2	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	+
2S7b	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Legend:
L — „Lasta" wheat cv.
J — „Jugoslavija" wheat cv.
Z — „Zitnica" wheat cv.
RN — „Rana niska" wheat cv.

pH changes. The reduction of the pH values can be attributed to the excretion of H^+ by the root system or, according to Raven (1990) and Raven et al. (1991), to the process of ammonia ion consumption from the medium. The data obtained during the co-cultivation of strains C3 and C5 without combined nitrogen provide evidence that when N_2-fixation occurred, the pH value was influenced by the extracellular release of ammonia and its uptake by the plant. After several days, the pH values across the medium were stabilized and intensive colonization of wheat root took place.

The above results, when taken collectively, seem to provide evidence of the production of cyanobacteria-growth-stimulating substances by roots. The investigation also displayed that strains 2S3b and LC2 were able to direct the growth of wheat roots, due to the cyanobacterial production of extracellular substances that provoked the cyanobacterium-dependent root growth direction response (Table 1 — Petry plates).

Our previous results showed that the cyanobacterium-wheat root interaction resulted not only in the colonization of plant root by cyanobacteria, but also in the nitrogenase activity of the cyanobacterial strains associated with wheat roots (Svirčev et al., 1995) and significant changes in the length, weight and nitrogen content of wheat shoots and roots (Gantar et al., 1991b; Obreht (Svirčev) et al., 1993; Gantar et al., 1995; Svirčev et al., 1995; Svirčev et al., 1997).

During the co-cultivation in agarized medium, cyanobacterial cells, filaments or packages were found inside the rhyzodermal cells and intercellular space, mostly in combination with the wheat cv. „Lasta". Although in certain cases no colonization of the roots occurred, it did not imply a total absence of interactions. For instance, during „Lasta" and strain S1 co-cultivation, no ro-

ot-colonization was observed while cyanobacteria were found to have penetrated some root cells. A possible way of penetration was described according to Gantar et al. (1993). As indicated by our earlier studies, the penetration of roots by cyanobacteria occurred during the co-cultivation of cyanobacteria and several agronomically important plants in liquid and sand cultures (Gantar et al., 1991c; Svirčev et al., 1997).

The results obtained revealed a variety of interactions between cyanobacteria and roots of wheat plants, which depended on the motility of filamentous cyanobacteria and cyanobacterium-dependent root growth direction. The investigation also indicated that cyanobacterial strains were able to direct the growth of wheat roots, resulting from the cyanobacterial production of extracellular substances, which provoked the cyanobacterium-dependent root growth direction response.

It was evident that most of the examined strains were able to colonize the whole root of the cv. „Lasta". The strains C3, C5 and LC2 colonized the roots of all examined cvs, indicating that the type of interaction depends directly on the strain and cv involved.

The type of movement and cyanobacterium-wheat root interaction did not imply the presence of an intimate contact between cyanobacteria and root cells. A clear evidence of cyanobacterial penetration inside the root cells was observed not only in colonized roots but also in rhizodermal cells where clustering of cyanobacteria was not observed.

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ЗДРУЖЕНО ГАЈЕЊЕ ЦИЈАНОБАКТЕРИЈА И БИЉАКА ПШЕНИЦЕ НА АГАРИЗОВАНОЈ ПОДЛОЗИ

Зорица Б. Свирчев, Игор Н. Балаж
Институт за Биологију, Трг Доситеја Обрадовића 2,
21000 Нови Сад, Југославија

Резиме

На основу испитивања међусобног утицаја осам филаментозних цијанобактерија и корена четири сорте пшенице током узгајања на агаризованој подлози, утврђени су различити типови интеракција. Цијанобактеријски сојеви C_3 и C_5 су показали интензивно накупљање у зони ризосфере код све четири испитиване сорте пшенице. Према понашању већине испитиваних сојева, може се претпоставити да сорта „Ласта" има у коренским излучевинама супстанцу или супстанце које привлаче покретне цијанобактеријске филаменте. Потпуна колонизација кореновог система биљака сорте пшенице „Ласта" остварена је у присуству шест од осам испитиваних сојева. Индиферентно понашање у односу на присуство корена испитиваних биљака пшенице уочено је код соја S_1, чији су филаменти били равномерно распоређени у агаризованој подлози. За разлику од овог и свих осталих испитиваних сојева, у случајевима здруженог гајења биљака пшенице и соја $2S_{7b}$ уочен је врло специфичан ефекат који се испољио у кретању цијанобактеријских филамената што даље од корена све четири сорте пшенице. Што се тиче продукције цијанобактеријских ванћелијских продуката, једино су сојеви LC_2 и $2S_{3b}$ показали способност усмеравања раста кореновог система према растућим колонијама ових цијанобактерија. Уочен је утицај C_3 и C_5 сојева на смањење ацидитета у зони кореновог система и око саме биљке током почетног дела култивације. И поред тога што у неким случајевима није дошло до колонизације корена пшенице, не ради се о потпуном одсуству интеракције јер је продирање цијанобактерија у корен пшенице примећено и у неколонизованом корену.

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Vera M. Milić[1], *Nastasija B. Mrkovački*[1], *Dragiša S. Milošev*[2]

[1] Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Yugoslavia
[2] Faculty of Agriculture and Institute of Field and Vegetable Crops, Trg Dositeja Obradovića 8, 21000 Novi Sad, Yugoslavia

ACCUMULATION OF GROWTH SUBSTANCES IN THE APICAL LEAVES INDUCED BY SOYBEAN INOCULATION

ABSTRACT: The present paper represents an attempt to establish whether or not nodular bacteria (in this case, two strains with a different capacity for nitrogen fixation) are responsible for the level of growth substances in soybean leaves. Analyses of plant hormones were performed on a HPLC with a changeable wavelength detector. Six to eight peaks with different retention times (RT) were obtained from the hydrolyzed samples of the leaf tips of soybean plants inoculated with *B. japonicum* strains 1 and 2b and uninoculated, control plants. According to the RT standard, the peaks were equivalent to GA_3 with an RT of 3.63, abscisic acid (ABA) with RT 17.91, protocatechuic acid with RT 7.35, coumarone with RT 17.09, and L-tryptophane with RT 15.57. The area of the peaks varied according to the strain. The results of the study have shown that the two *B. japonicum* strains do affect the levels of growth substances in apical soybean leaves. No peaks corresponding to protocatechuic acid with RT 7.62 were found in the leaves of the uninoculated plants. The area of the peak corresponding to L-tryptophane was the largest in the control plants and the plants inoculated with strain 1, whereas in the plants inoculated with strain 2b it was only half as large. This indicates that the effective strain 2b caused a much more rapid conversion of L-tryptophane than the less effective strain 1, in which L-tryptophane content was the same as in the uninoculated plants.

KEY WORDS: *Bradyrhizobium japonicum*, growth substances, soybean, strain.

INTRODUCTION

Many plant developmental processes are mediated by plant hormones (growth substances), small molecules that are widespread in the plant and that can rapidly diffuse across membranes (Hirsch, 7). Some plant hormones, such as ethylene, influence microtubule orientation (Lang et al., 11), while others (gibberellins) elicit changes in GA^{2+} levels (Bush et al., 4). Plant hormones, or growth regulators, of indole type are involved in plant growth and

represent precursors that are enzymatically converted into indole-3 acetic acid (IAA). IAA is considered to be the main substance responsible for plant growth.

According to Badenoch-Jones et al. (1), IAA is formed in young leaves and apical buds and transported into nodules. However, there is evidence that IAA is transported from nodules and root into other plant parts (Bouma, 3; Badenoch-Jones et al., 2). Only a part of IAA is present in the form of free IAA in plant tissue. A large part of it is either bound or conjugated to other compounds (Cohen and Bandurski, 5).

Phenol compounds that originate from the plant side of the plant-bacterium leguminous association have an inhibitory effect on nodular bacteria (Kandasamy and Prasada, 9). Aromatic phenols, from simple phenols to benzene acids (Reynolds, 18), or in the form of glucoside, inhibit plant growth, germination, and mitochondrial metabolism (Desmos et al., 6) as well as root growth (Roy et al., 19).

The results of our previously study confirm that nodule dry matter mass and nitrogen content in soybean plants are correlated with growth substances produced by the tested strains of *B. japonicum* (Milić et al., 13, 14).

When we used the HPLC method for determination of growth substances (indole, phenole and gibberellin types) in pure culture of *B. japonicum* 1 and *B. japonicum* 2b, the effective strain 2b synthesized about eight times more IAA than the poorly effective strain (Milić et al., 15).

The present study representes an attempt to determine whether nodular bacteria (in this case two strains with a different capacity for nitrogen fixation with soybean plants and different IAA production in pure culture) are responsible for the level of growth substances in soybean leaves.

MATERIALS AND METHODS

I Bacterial cultures

Two wild-type strains of *Bradyrhizobium japonicum* — strain 1 and strain 2b — from the collection of the Department of Microbiology of the Institute of Field and Vegetable Crops in Novi Sad were cultivated in a liquid Demolon medium (Vincent, 20) up to the logarithmic phase of growth (10^9 cells per 1 ml).

II Plant materials

Soybean seeds (variety NS-16) developed at the Institute of Field and Vegetable Crops in Novi Sad were first sterilized in ethyl alcohol, then in 0.1% acid solution $HgCl_2$, after which they were rinsed with distilled water and germinated in water agar for five days. Soybean seedling were transferred into 12x2.5 cm tubes that contained 1/4 stength sterilized Jensen solution (Vincent, 20). The trial included three treatments with 10 replications each (each

tube contained one plant). Treatments were as follows: Treatment 1 (control) — uninoculated seedlings; Treatment 2 — seedlings inoculated with 0.5 ml of liquid culture containing strain 1; and Treatment 3 — seedlings inoculated with 0.5 ml of liquid culture containing strain 2b. The plants were grown in a greenhouse under semi-sterile conditions for 35 days (until flowering).

The apical leaves from each treatment were harvested separately and 1 g of fresh leaves extracted according to Hunter (8). We determined the type of the bound derivatives in the apical leaves of inoculated and uninoculated plants after alkali hydrolysis, i.e., the total amount of IAA derivatives and the type of phenolic and gibberellic compounds. Quality analyses of quality plant hormones (auxines, phenols, gibberellins) were performed on the HPLC. A liquid chromatographic apparatus Hewlett Packard 1048B and a detector of changeable wavelength were used as described in Milić et al. (15) and Milić and Mrkovački (16).

RESULTS AND DISCUSSION

Levels of growth substances in the apical leaves of soybean

As shown in the chromatographs (Figures 1—3), 6—8 peaks with different retention times were obtained from the apical leaves of soybean plants inoculated with *Bradyrhizobium japonicum* strains 1 and 2b and uninoculated soybean plants.

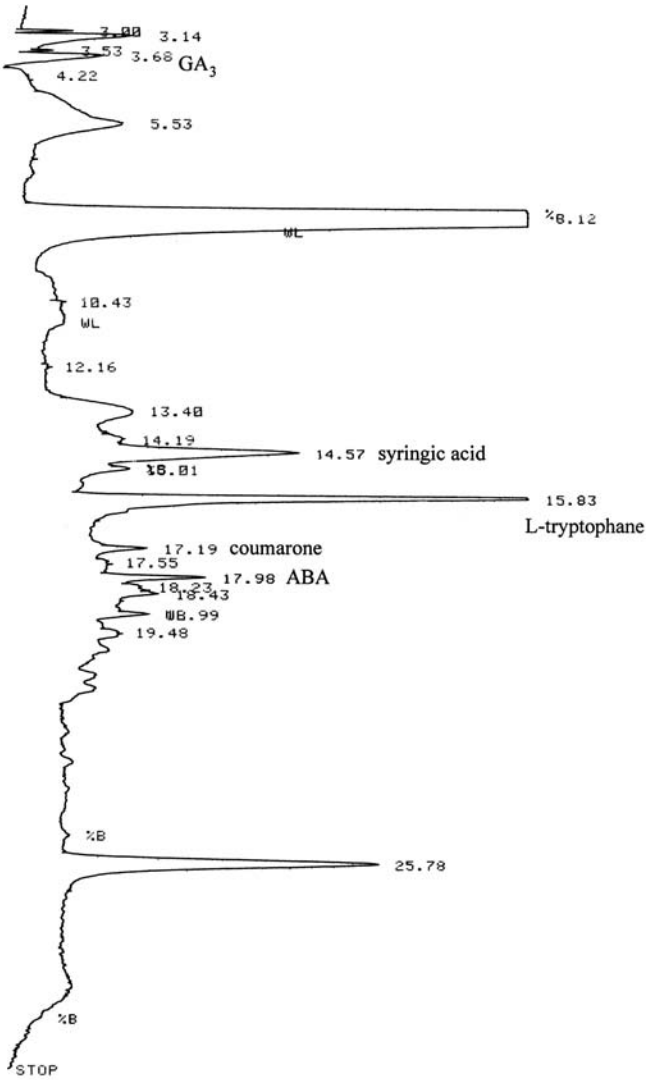
The results of this study provide evidence that in the hydrolyzed samples of uninoculated plants (Figure 1), peaks of compounds identical to syringic acid (RT 14.57), coumarone (RT 17.19), L-tryptophane (RT 15.83), abscisic acid (ABA) (RT 17.98), and gibberellic acid (GA_3) (RT 3.68) were found. The peaks of the other compounds could not be identified (RT 8.12, RT 13.40, RT 25.78).

In the leaves of soybeans inoculated with *B. japonicum* strain 1, we found peaks that correspond to syringic acid (RT 14.21), coumarone (RT 17.10), L-tryptophane (RT 15.66), abscisic acid (ABA) (RT 17.91), and gibberellic acid (GA_3) (RT 3.68) (Figure 2) as well as protocatechuic acid (RT 7.62), which was not present in the uninoculated soybean plants.

The leaves of plants inoculated with the highly effective strain 2b contained all the compounds that were found in the soybeans inoculated with *B. japonicum* strain 1 (protocatechuic acid RT 7.35, coumarone RT 17.09, L-tryptophane RT 15.57, abscisic acid (ABA) (RT 17.91) (Figure 3).

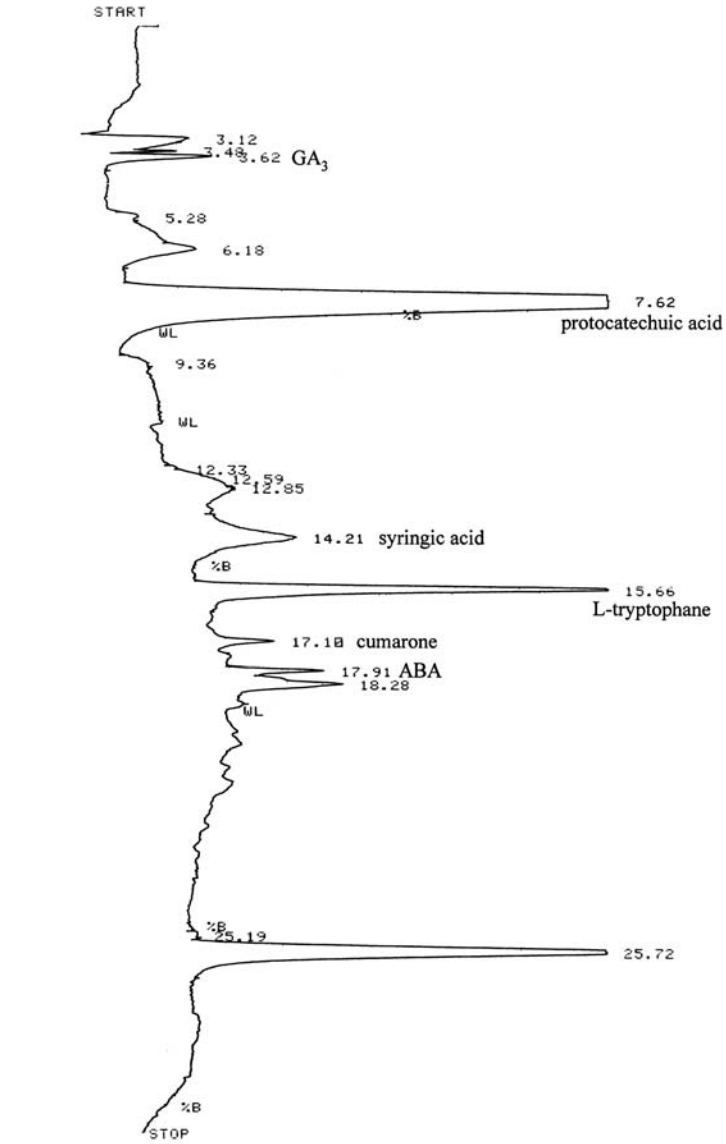
The other peaks found in the uninoculated and inoculated soybeans could not be identified.

The area of the peak corresponding to L-tryptophane was largest in the control plants and plants inoculated with strain 1, whereas in the plants inoculated with strain 2b it was only half as large. This indicates that the effective strain 2b caused a much more rapid conversion of L-tryptophane than the less effective strain 1, in which L-tryptophane content was the same as in the uninoculated plants.




```
hP 1080 3
   BTL:  15
AREA %
   RT          AREA       AREA %
 3.14          5392        2.513
 3.53           502        0.234
 3.68          4225        1.969
 5.53         12040        5.610
 8.12        126500       58.947
13.40          5099        2.376
14.19           657        0.306
14.57         12440        5.797
15.01          1800        0.839
15.83         21750       10.135
17.19           983        0.458
17.98          2146        1.000
18.99           954        0.445
25.78         20110        9.371
```

Fig. 1. — Chromatogram of growth substances found in hydrolyzed samples of the apical leaves of uninoculated plants



** 1000 B

BTL: 30

AREA % RT	AREA	AREA %
3.12	1392	0.835
3.62	3240	1.943
6.18	2359	1.415
7.62	85200	51.105
14.21	10160	6.094
15.66	21900	13.136
17.10	1686	1.011
17.91	2559	1.535
18.28	6081	3.648
25.72	32140	19.278

Fig. 2. — Chromatogram of growth substances found in hydrolyzed samples of the apical leaves of plants inoculated with *B. japonicum* strain 1

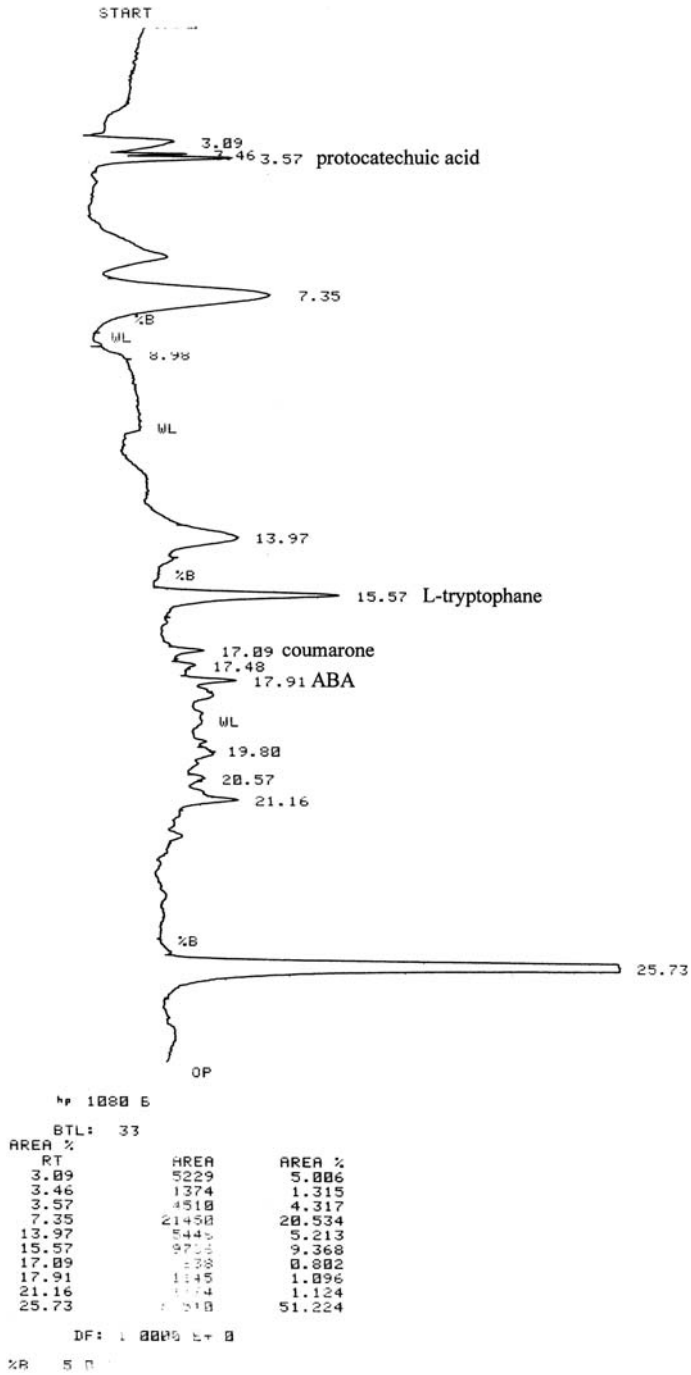


Fig. 3. — Chromatogram of growth substances found in hydrolyzed samples of the apical leaves of plants inoculated with *B. japonicum* strain 2b

Many indolyl derivatives, both „free" and „bound" (released after alkaline hydrolisis), possess an apparent growth-promoting activity and it is generally belived that, these compounds, such as indol-3-acetonitril (IAN), indole aldehyde (IAAld), triptaphol and gramine, represent precursors wich are enzymatically converted to IAA, the principal substance involved in the growth response (Pegg, 17).

The peak of syringic acid was not identified in the apical leaves of soybeans inoculated with strain 2b. The peak of protocatechuic acid was not found in the uninoculated plants, while in plants inoculated with strain 2b it was four times smaller than in the case of strain 1. The largest peak of coumarone was recorded in the apical leaves of plants inoculated with strain 1, while in soybeans inoculated with strain 2b it had a similar area as in the uninoculated plants, suggesting the presence of a special mechanism that inhibits coumarone synthesis in the more effective strain of *B. japonicum*.

Syringic acid, protocatechuic acid, and coumarone belong to the group of phenols that have an inhibitory effect on the plant, so it can be assumed that these substances influence the development of a symbiotic association of a particular level of effectiveness. Acording to the previously results, the two strains (2b, 1) of *B. japonicum* effected the symbiotic association with soybean differently. This can be explained by the effect of growth regulators on the plant. Strain *B. japonicum* 2b which produced several types and a large number of growth regulators in culture and accumulated much less phenolic compounds in the apical leaves of soybean than strain *B. japonicum* 1 (Milić, 12). Phenolic compounds originating from the plant have an inhibitory effect on nodular bacteria (Kanda-samy and Prasada, 9) and can therefore be assumed to influence nodulation and nitrogen fixation.

The amount of the compound with RT 25.78, which could not be identified, was largest in the leaves of plants inoculated with strain 2b, followed by strain 1, and, finally, the uninoculated, control plants. We suppose that it is an indole aldehyde (IAAld). IAAld is derived from tryptophan via indole pyruvic acid and is a native constituent of plants. It can, therefore, be assumed that inoculation promotes the synthesis of this compund, which, in turn, prompts the question of what its exact role in the plant may be.

The results showed that, according to the RT standards, the values RT 3.63 and RT 17.19 correspond to gibberellic acid (GA_3) and abscisic acid (ABA), respectively. The level of compounds with gibberellic activity is much higher in soybean nodules than in the roots without nodules (Williams and De Mallorca, 21; Milić, 13).

It is known that the level of ABA within the plant is related to the type of organ tissue and upon time. When plants are cultured under non-stressed conditions, cyclic phenomen or at least visible indications of their presence may be minimal (Koukkari and Warde, 11).

The results in this paper shows that the areas of separated peaks according to the ABA in the appical leaves of soybean plants were similar so we can say that here was no stress effect.

CONCLUSION

The results of the study show that *B. japonicum* strains 1 and 2b have an effect on the levels of growth substances in the studied parts of soybean leaves.

As shown in the chromatographs (Figures 1—3), 6—8 peaks with different retention times were obtained from the apical leaves of soybean plants inoculated with *Bradyrhizobium japonicum* strains 1 and 2b and uninoculated soybean plants from the control treatment.

According to the RT standard the obtained peaks correspond to to GA_3, abscisic acid (ABA), protocatechuic acid, coumarone and L-tryptophane.

The other peaks found in the uninoculated and inoculated soyebeans could not be identified.

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АКУМУЛАЦИЈА МАТЕРИЈА РАСТА У ВРШНИМ ЛИСТОВИМА ИНОКУЛИСАНЕ СОЈЕ

Вера М. Милић[1], Настасија Б. Мрковачки[1], Драгиша С. Милошев[2]
[1] Институт за ратарство и повртарство,
Максима Горког 30, 21000 Нови Сад
[2] Пољопривредни факултет, Институт за ратарство и повртарство,
Трг Доситеја Обрадовића 8, 21000 Нови Сад

Резиме

У вршним листовима инокулисаних и контролних неинокулисаних биљака одређивани су везани деривати после алкалне хидролизе, односно укупна количина деривата IAA као и фенолна једињења. Ова једињења посредују у расту биљака, а такође представљају прекурсоре који се ензиматски конвертују у IAA.

Анализа присутних биљних хормона урађена је на течном хроматограму под високим притиском (HPLC) са детектором промењиве таласне дужине. У испитиваним хидролизованим узорцима вршних листова биљке соје инокулисане сојевима *B. japonicum* 1 и 2b и код контролних неинокулисаних биљака, издиференцирано је 6—8 пикова различитих према времену ретенције (RT). Издиференцирани су пикови који према RT стандарду одговарају GA_3 са RT 3.63, абсцисинској киселини (ABA) са RT 17.91, протокатехинској киселини са RT 7.35, кумарину са RT 17.09, L-триптофану са RT 15.57. Површина добијених пикова разликовала се у зависности од употребљеног соја *B. japonicum* за инокулацију поника биљке соје.

У неинокулисаним листовима биљке није издиференциран пик који према стандарду одговара протокатехинској киселини са RT 7.62. Пик који одговара L-триптофану има највећу површину код узорака неинокулисаних биљака као и код инокулисаних сојем *B. japonicum* 1, док је код биљака инокулисаних сојем *B. japonicum* 2b био упола мањи. Ово указује да је конверзија L-триптофана под утицајем ефективног соја (*B. japonicum* 2b) много бржа него у слабо ефективног соја (*B. japonicum* 1) код којег је садржај L-триптофана био као код неинокулисаних биљака.

Сви остали издиференцирани пикови нису идентификовани. Инокулација стимулише синтезу једињења са RT 25.78. У свим испитиваним узорцима издиференциран је овај пик, али је његова површина највећа у узорку листа инокулисане биљке сојем *B. japonicum* 2b (високоефективан соj), затим у листовима инокулисаних са сојем *B. japonicum* 1, а најмања у неинокулисаним контролним биљкама.

Добијени резултати показују да квржичне бактерије — сојеви *B. japonicum* (1, 2b) који се међусобно разликују у синтези материја растења утичу на садржај ових материја у испитиваним деловима листова, односно својом продукцијом утичу на биохемијске и физиолошке процесе у биљци.

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Natalija P. Didyk, Vladimir P. Grakhov, Valerij L. Brechko

National Botanical Garden „M. M. Gryshko"
Nat. Acad. of Sci. of Ukraine Timiryazevskaja Str. 1
252014, Kiev, Ukraine

AUTOALLELOPATHIC STRESS IN NATURAL AND MODEL POPULATIONS OF QUACKGRASS (*Elytrigia repens* L.)

ABSTRACT: Development of autoinhibition in quackgrass populations and the role of allelopathic mechanism in the process were studied in four habitats of low urban pressure as well as in four-year plot experiment, modeling initial density of 0, 4, 16, 64, 256 quackgrass plants on 1 m^2.

In the natural quackgrass populations the development of indications of autoinhibition (decrease in shoot and rhizome densities, morphometric and physiological parameters of shoot growth, photosynthesis, vegetative and generative reproduction) correlated with intensity of recent intraspecific interactions (which were judged by quackgrass litter thickness) as well as with rhizosphere soil allelopathic activity.

In the model quackgrass populations the development of indications of autoinhibition and accumulation of increasing amounts of allelochemicals observed in natural populations were reproduced. The initial density of model quackgrass populations influenced the rate of achievement of maximum of the populations density and shoot growth parameters. In subsequent seasons self-thinning and decrease in shoot growth, photosynthesis, water uptake, vegetative and generative reproduction, changes in the metabolism of polyphenolics occurred. At that time rhizosphere soil demonstrated the highest allelopathic activity in laboratory tests.

Further investigations of the particular mechanisms of autoallelopathic inhibition in quackgrass populations are considered to be promising in terms of the development of new ecological methods for its control.

KEY WORDS: *Elytrigia repens* L., populations, plot experiment, autoinhibition, allelopathic activity.

INTRODUCTION

Quackgrass (*Elytrigia repens* L., Nevski) is a widespread cosmopolitan dominant in grassy communities and a noxious weed, recognized as economically important in many regions of Eurasia and North America (Fisjunov, 1984). In natural communities quackgrass usually forms dense monodominant

patches with small representation of other species. In favorable conditions quackgrass patches actively expand horizontally replacing neighboring vegetation. Then in the older patches self-thinning occurs and on the vacant sites other plant species penetrate. Quackgrass clumps, growing on the old patches show inhibition of shoot height, spike-formation, occlusion of xylem vessels (Bogdan, 1981; Grodzinsky, 1991).

Among suggested causes of the old quackgrass patches degradation, accumulation of phytotoxic concentrations of allelochemicals seems to be most credible (Bogdan, 1981; Grodzinsky, 1991). Numerous laboratory experiments showed, that exudates of living and especially of decaying quackgrass plants are highly allelopathic (Bogdan, 1981; Weston et al, 1986; Gorobets et al., 1990; Grodzinsky, 1991). Incubations of decaying quackgrass shoots and rhizomes in various types of soils results in the accumulation of phytotoxic concentration of organic, especially phenolic acids (Lynch et al., 1980) and ethylene (Harvey et al., 1978). Phytotoxic concentrations (up to 1000 ppm) of phenolic acids (p-coumaric, vanilic, p-hydroxybenzoic and others) were reported to be present in the rhizosphere soil associated with quackgrass (Whitehead et al., 1982; Gorobets et al., 1990; Grodzinsky, 1991).

No further investigation of autoallelopathic inhibition in quackgrass was conducted. Nevertheless understanding of this process would allow more deep insights into the quackgrass coenotic strategy and the development of new ecological methods for its control in agrocoenoses.

Our work is devoted to the regularities of development of autoinhibition in quackgrass natural and model populations and the role of allelopathic mechanism in the process.

MATERIALS AND METHODS

Natural quackgrass populations were located in the four habitats of low urban pressure within the limits of Kiev city and suburbs. Two of the investigated habitats represented dry meadows, with medium-humic loamy soils, other two — bottomland meadows, with low-humic sandy soils. The populations consisted of dense monodominant patches (cover of quackgrass was 70—90%), other species were present in insignificant amounts. The thickness of litter from decaying quackgrass shoots of the present and previous years varied from less then 1 cm in the invasional parts of the populations to 12—16 cm on the oldest parts. Assuming the amount of the litter to be proportional to the intensity of recent intraspecific interactions (which include allelopathy), we studied the dynamics of the parameters of quackgrass density and vitality as well as rhizosphere soil allelopathic activity along the gradient of litter thickness. The measurements of morphometric and physiological parameters of quackgrass vitality and collection of rhizosphere soil samples were conducted on sample plots (0.2x0.25 m^2), established in sites with different litter thickens, in June-July, 1997.

The development of autoinhibition in model quackgrass populations was studied in a four-year plot experiment established on the territory of the National Botanical Garden (Kiev city, Ukraine), on medium-humic loamy soil, in June, 1996. In the experiment initial densities of 0, 4, 16, 64 and 256 quackgrass plants per 1 m^2 were modeled. Before commencement of the experiment tillage and clearing were conducted. Plots (1x1 m^2) were dug around with trenches 20 cm in depth to eliminate quackgrass penetration from one plot to another, as the quackgrass rhizomes do not penetrate to a higher depths (Fisjunov, 1984). Quackgrass plants (clumps, with the rhizome fragment of 2—3 cm) from neighboring clones served as material for planting. Other plant species were constantly weeded out when they appeared. We used four replications for each treatment.

The morphometric measurements in the plot experiment were conducted in June and August of 1997—1999 (the beginning and the end of quackgrass flowering). The contents of photosynthetic pigments (chlorophylls a and b, carotenoids) and anthocyanins in quackgrass leaves were determined in July, 1998—1999. The rate of quackgrass shoot water uptake and activity of o-diphenoloxidase in leaves were determined in July, 1999. Soil samples for extraction and testing of allelopathic activity were collected in July, 1998—99.

The following morphometric characteristics were measured: parameters of growth and photosynthetic production (shoot height, number of shoots per clump, mean leaf length), vegetative and generative reproduction (percentages of generative and juvenile shoots in clumps). In the natural populations length of rhizome internodes and number of rhizome branchings were also registered.

The contents of photosynthetic pigments and anthocyanins in quackgrass leaves were determined by spectrophotometric methods (Solov'yova, 1988; Tretiakov et al., 1990). Activity of o-diphenoloxidase in leaves was determined by the spectrophotometric method based on the rate of pyrocatechol oxidation (Tretiakov et al., 1990). The rates of water uptake were determined on the basis of the amount of water, absorbed during 3 days by freshly cut quackgrass shoots located in testtubes with 0.1% $Ca(NO_3)_2$ (Grakhov, 1991).

The soil samples, air-dried (40°C) and sifted through 2 mm sieve, were extracted with methanol, and after that with 1% trilon B (soil-extragent volume ratio was 1:2) (Grakhov, 1991). Allelopathic activity of methanol and trilon extracts (substrate-extract volume ratio was 1:2) were evaluated in the bioassays on quackgrass seed germination, seedlings growth and rhizome buds sprouting (Grodzinsky, 1991).

The analysis of pair relations was conducted by the method of correlation relations (section of correlation analysis, non-parametric statistics), and one--way ANOVA. The strength of relations between variables was calculated as correlation relation (η^2) (Zajtsev, 1984).

RESULTS AND DISCUSSION

In the studied natural populations the parameters of quackgrass density, shoot growth, photosynthesis, vegetative and generative reproduction showed

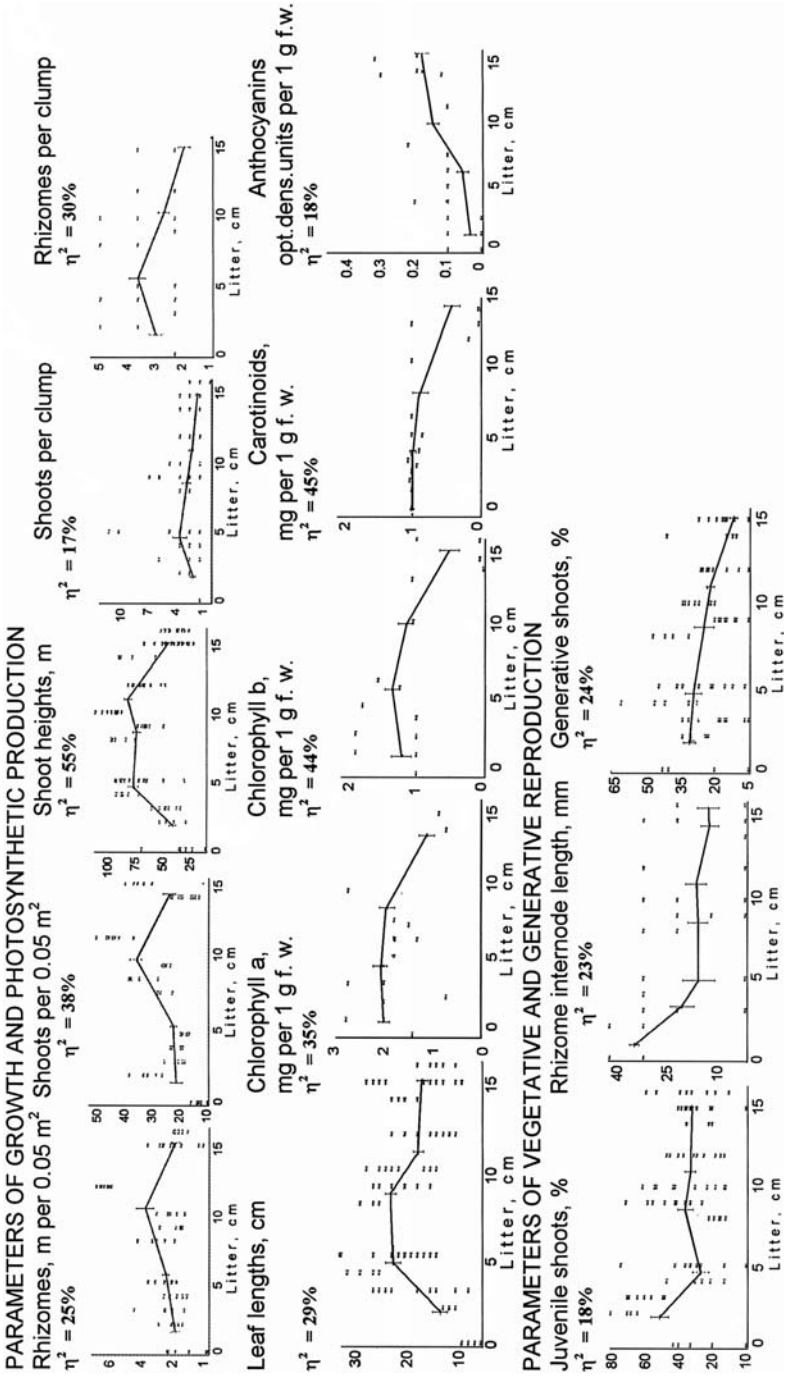

Fig. 1 — Dependence of quackgrass density and parameters on litter thickness in the natural populations (summarized data on four populations)

markable dependence on the intensity of recent intaspecific interactions which was judged by litter thickness (Figure 1). The optimum of the parameters of quackgrass density and shoot growth was observed on the sites with medium values of litter thickness (5—10 cm). The optimum of the parameters of quackgrass vegetative and generative reproduction were observed on the sites with low values of litter thickness (0—5 cm). On the sites with high values of litter thickness (10—15 cm) the parameters of shoot growth, photosynthesis, vegetative and generative reproduction in quackgrass plants were at pessimum, and the contents of anthocyanins in leaves were considerably increased.

The differentiation of natural populations of quackgrass and some other rhizomatus grasses into groups with prevalence of one of the functions of accumulation of biomass, or reproduction, or reserve (plants with inhibited growth and reproduction) was shown by other authors (Lubarsky et al., 1984). However, no mechanism of such differention was considered. On the other hand there are many examples of autoregulation of plant populations structure and functioning by allelochemicals (Friedman et al, 1985; Grakhov, 1991; Grodzinsky, 1991; Moroz, 1996). High allelopathic potential of quackgrass living and especially decaying parts was confirmed by many laboratory tests (Bogdan, 1981; Lynch et al., 1980; Weston et al., 1986). Phytotoxic concentrations of allelochemicals were shown to be present in rhizosphere soil associated with quackgrass (Whitehead et al, 1982; Gorobets et al, 1990; Grodzinsky, 1991). In connection with this it was interesting to determine allelopathic potential of the rhizosphere soil of the studied populations and whether it correlates with indications of autoinhibition in quackgrass plants.

The results of bioassaying of allelopathic activity of methanol and trilon extracts of soil of the studied populations analyzed by ANOVA (factor — litter thickness) are shown on Figure 2. Methanol extracts demonstrated higher allelopathic activity in all bioassays than trilon extracts. Allelopathic activity of both methanol and trilon extracts showed marked correlation with litter thickness and the development of indications of stress and inhibition in the studied populations. The allelochemicals extracted by methanol and trilon from the rhizosphere soil of the degrading sites of the populations markedly reduced quackgrass seedlings growth, delayed seed germination and rhizome buds sprouting in laboratory experiments. The results obtained confirmed the role of autoallelopathic mechanism in the degradation of the studied quackgrass populations.

The tendencies observed in the natural quackgrass populations were reproduced in the four-year plot experiment (Figure 3). The initial density of model quackgrass populations influenced the rate of achievement of maximum of the populations density (1000—1100 shoots per 1 m^2) and shoot growth parameters. Self-thinning and decrease in shoot growth, photosynthesis, water uptake, vegetative and generative reproduction, changes in the metabolism of polyphenolics occurred in subsequent. In the model populations with initial density of 0 plants per 1 m^2 quackgrass appeared in May of the third season, from the seeds air-drifted from neighboring clones. In this population the parameters of shoot density and growth had not achieved such high levels as in the

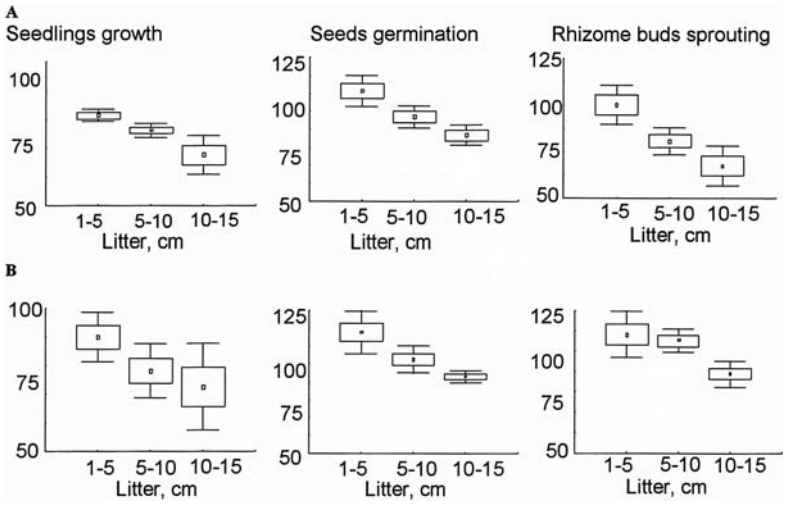

Fig. 2 — Dependence of allelopathic activity of methanol (A) and trilon (B) extracts of rhizosphere soil of the natural quackgrass populations on litter thickness (summarized data of four populations)

other treatments. Nevertheless some indications of stress (decrease in shoot growth, photosynthesis, generative and vegetative reproduction) were observed here in the fourth season. Thus unfavorable changes occurred in the thin long-existing quackgrass populations as well.

This conclusion was confirmed by the results of bioassaying of methanol and trilon extracts of rhizosphere soil of the model quackgrass populations (Figure 4). During the development of the model quackgrass populations increasing quantities of allelochemicals were accumulated. The allelochemicals extracted with methanol and trilon from rhizosphere soil of the populations with high initial density (64, 256) in the III—IV season and in the other populations in IV season markedly inhibited seedlings growth, delayed seeds germination and rhizome buds sprouting in the laboratory bioassays.

Thus, the development of autoinhibition as well as accumulation of increasing amounts of allelochemicals observed in the natural populations were reproduced by modeling quackgrass populations of different initial densities. The indications of stress and inhibition (decrease in shoot density, shoot growth, vegetative and generative reproduction, photosynthetic pigments contents and o-diphenoloxidase activity in leaves) in the model quackgrass populations showed positive correlation with the stage of their development, which in turn closely correlated with the build-up of allelopathic activity in rhizosphere soil. Autoinhibition in the model populations was observed in the following seasons after the achievement of maximum of shoot density and growth parameters or after more lasting vegetation of thin quackgrass populations. At the same time the soil accumulated phytotoxic concentrations of allelochemicals, which markedly reduced quackgrass growth and development in the laboratory bioassays.

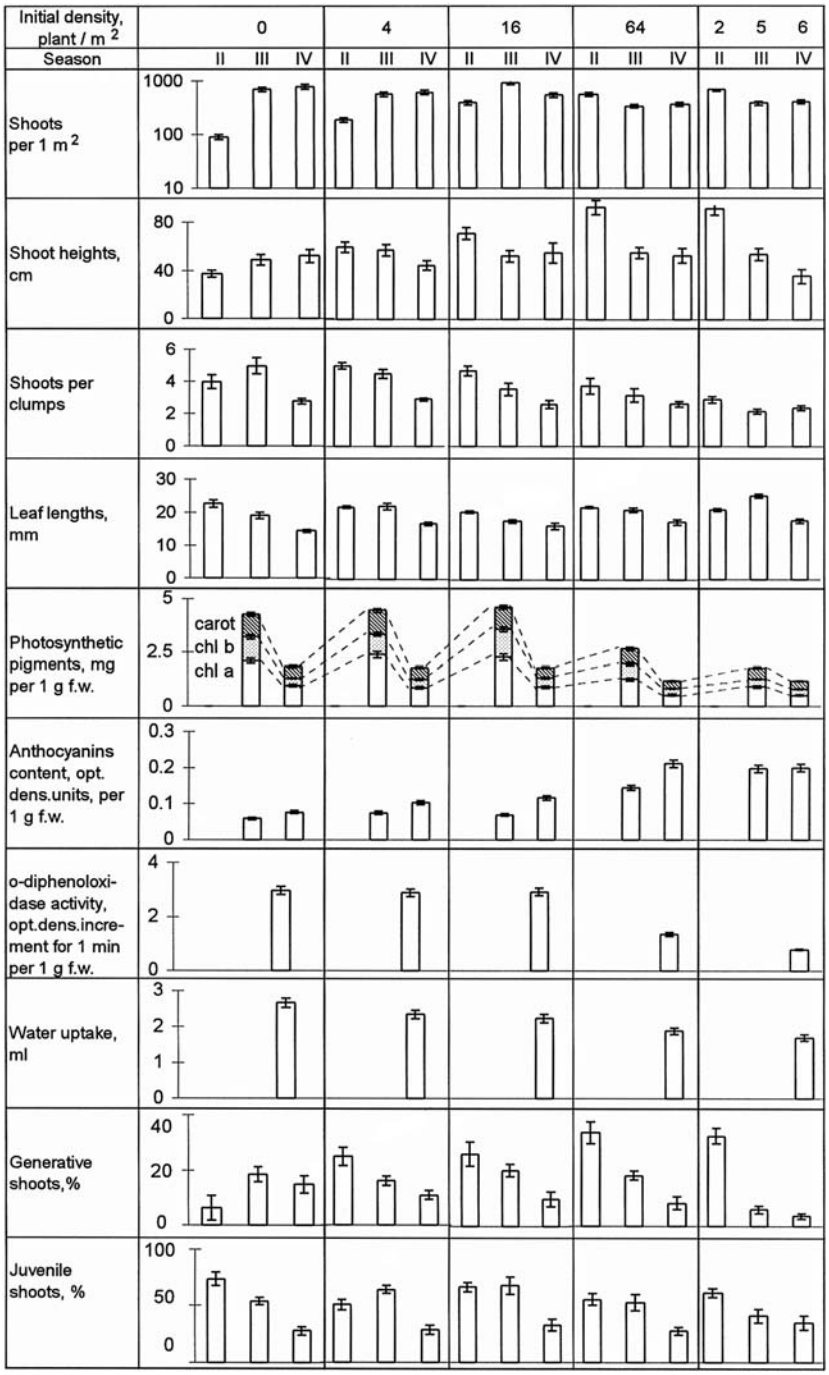

Fig. 3 — Year-to-year dynamics of quackgrass density and vitality parameters in the plot experiment

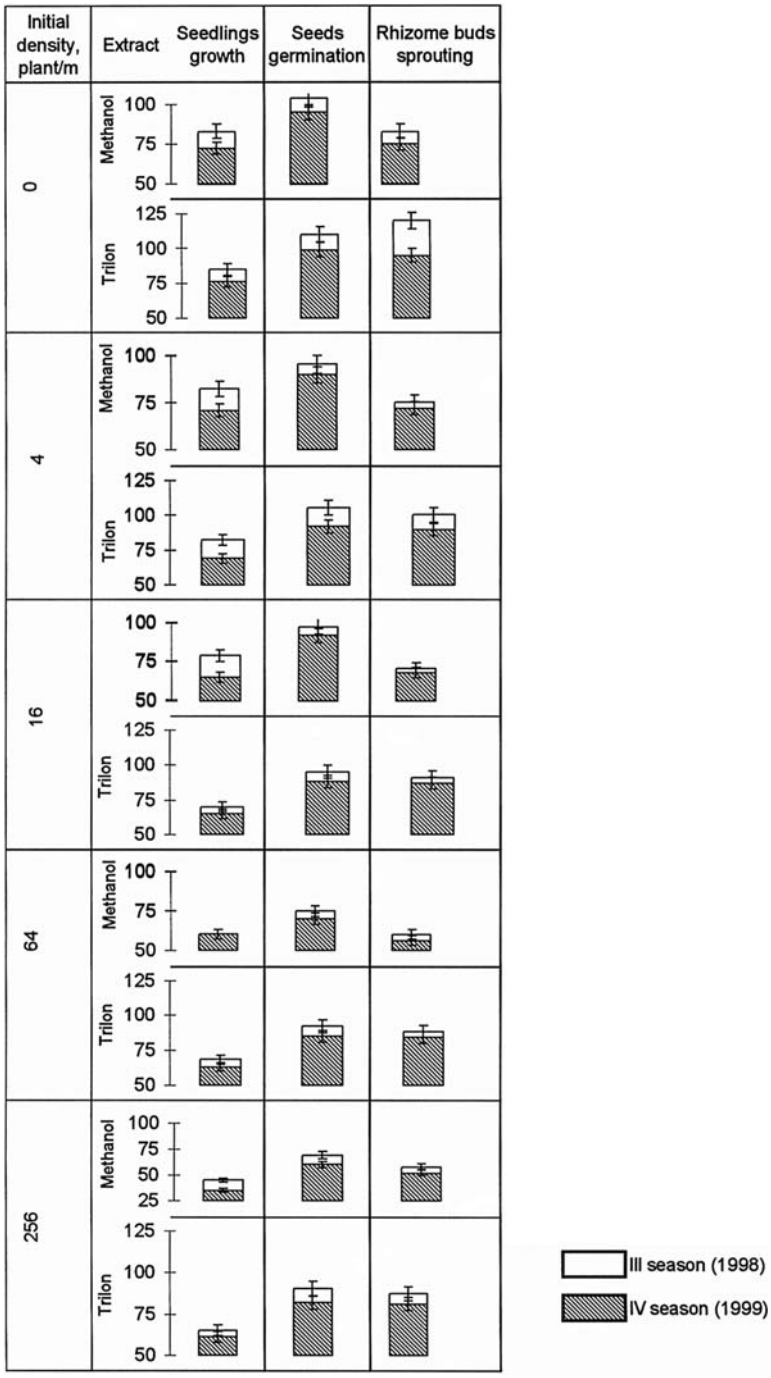

Fig. 4 — Year-to-year dynamics of allelopathic activity of methanol and trilon extracts of rhizosphere soil of the plot experiment

The results obtained present evidence of the role of autoallelopathic mechanisms in the regulation of plant population development and functioning. The revealed tendencies are in agreement with the traditional concept of autoallelopathic soil sickness by plant species with high allelopathic potential (Friedman et al., 1985; Grakhov, 1991; Grodzinsky, 1991; Moroz, 1996).

Further investigations of the particular mechanisms of autoallelopathic inhibition in quackgrass populations appear to be promising in terms of the development of new ecological methods for its control.

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АУТОАЛЕЛОПАТСКИ СТРЕС ПИРЕВИНЕ (*Elytrigia repens* L.) У ПРИРОДНИМ И ЕКСПЕРИМЕНТАЛНИМ УСЛОВИМА

Наталија П. Дидик, Владимир П. Грахов, Валериј Л. Бречко
Национална ботаничка башта „М. М. Гришко" Националне академије наука Украјине, Тимирјазевскаја 1, 252014 Кијев, Украјина

Резиме

Аутоинхибиција пиревине испитивана је на природним стаништима и у пољском експерименту при густини биљака од 0, 4, 16, 64 и 256 по m^2. На природним стаништима пиревине запажена је аутоинхибиција у расту корена, броју ризома, морфофизиолошким карактеристикама раста бусена, фотосинтетичкој продукцији, као и вегетативној и генеративној репродукцији. У експерименталним условима земљишни екстракт из ризосфере пиревине индуцира као и у природним условима, горе наведени стрес и супресију популације. Интензитет стреса је у функцији броја биљака по јединици површине.

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Sonja Z. Ilin[1], *Rudolf R. Kastori*[2], *Đorđe R. Malenčić*[3]

[1] Institute of Field and Vegetable Crops, Maksima Gorkog 30 21000 Novi Sad, Yugoslavia
[2] Faculty of Agriculture and Institute of Field and Vegetable Crops Trg Dositeja Obradovića 8, 21000 Novi Sad, Yugoslavia
[3] Faculty of Agriculture, Trg Dositeja Obradovića 8 21000 Novi Sad, Yugoslavia

THE EFFECT OF NICKEL ON SUPEROXIDE DISMUTASE ACTIVITY, LIPID PEROXIDATION INTENSITY AND OXYGEN RADICAL QUANTITY IN YOUNG PLANTS OF WHEAT AND MAIZE

ABSTRACT: Effect of different Ni concentrations (10^{-7}, 10^{-5} and 10^{-3} mol dm^{-3}) on the activity of an antioxidant enzyme, superoxide dismutase, lipid peroxidation intensity and oxygen radical quantity in young plants of wheat and maize has been studied using the method of water cultures. The obtained results have shown that the lower Ni concentrations increased the enzyme's activity, while the higher concentrations decreased its activity in both wheat and maize. Consequently, the oxygen radicals quantity was significantly lower at lower Ni concentrations. At high Ni concentration, in wheat leaves, the quantity increased. In the leaves of maize the oxygen radicals quantity increased with increase in Ni concentrations. Lipid peroxidation process occurred in both examined species and the content of its final product, malonyldialdehyde, increased with increase in Ni concentrations in the solution.

KEY WORDS: wheat, maize, nickel, superoxide dismutase, lipid peroxidation, oxygen radicals.

INTRODUCTION

Heavy metals (HM) are characterized by different chemical, physical and biological features. Knowing the ecology and mechanisms of uptake, accumulation, distribution and effects of HM on life proccesses of plants is of great importance, both from the physiological and the ecological aspect (Alloway, 1990).

Increased concentrations of HM, among which nickel (Ni) is classified, tend to cause phytotoxicity. One of the primary effects of HM on plants is changing the structure and function of cell membranes and organelles (Ken-

nedy and Gonsalves, 1987). At higher concentrations, HM could cause increased reduction of molecular oxygen (O_2) which is relatively inactive, and therefore produces toxic oxygen species such as: superoxide radical ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\cdot OH$). These highly reactive species of O_2 react with unsaturated fatty acids to cause peroxidation of membrane lipids (LP) in the plasmalemma or cell organelles causing the leakage of cellular contents, rapid desiccation and cell death (Scandalios, 1993).

All aerobic organisms are exposed to oxidative stress. In order to survive in the presence of high concentrations of HM, plants developed antioxidant defence system to counteract oxidative damage from reactive oxygen species (ROS) (Larson, 1988; Gašić, 1992). These mechanisms comprise enzymatic and non-enzymatic reactions: 1) reactions of antioxidants with ROS, keeping their concentrations at low level, and 2) reactions of transformation of ROS into stable molecules or molecules with low reactivity (Smirnoff, 1993; Štrbac, 1995). Catalases (CATs) and superoxide dismutases (SODs) belong to the first group of antioxidants and are known as the most efficient antioxidant enzymes. Due to action of these enzymes, potentially harmful $O_2^{\cdot -}$ and H_2O_2 are reduced to H_2O without cell damage. At the same time, the formation of the most potent oxidant known, the hydroxyl radical ($\cdot$ OH), is avoided:

$$1)\ O_2 + e^- \rightarrow O_2^{\cdot -}$$

$$2)\ H_2O_2 + O_2^{\cdot -} \rightarrow \cdot OH + OH^- + O_2$$

$$3)\ O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

$$4)\ H_2O_2 + H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$$

$\cdot$ OH indiscriminately and rapidly attacks virtually all macromolecules, leading to serious damage in cellular components, DNA lesions and mutations (Scandalios, 1993).

One of the primary effects of phytotoxic amounts of HM in higher plants is also the inhibition of enzyme activity. Two mechanisms of enzyme inhibition predominate: a) binding of the metal to functional groups of the enzyme, such as sulphydryl group, involved in the catalytic action or structural integrity of enzymes, and b) deficiency of an essential metal in metalloproteins or metal-protein complexes, eventually combined with substitution of the toxic metal for deficient element (Van Assche and Clijsters, 1990).

Investigations on plant material showed that the increase in antioxidant enzyme activity increases stress tolerance level (Štajner et al., 1995). Bearing that in mind, and since a number of experiments have shown that Ni in lower concentrations is beneficial to plant growth (Ilin and Kastori, 1995; Ilin, 1997), the aim of this study was to investigate the effect of different Ni concentrations on the activity of the antioxidant enzyme SOD, which is an important component of the antioxidant defence system. In correlation with this,

the effect of different Ni concentrations on $O_2^{\cdot -}$ quantity and LP intensity was investigated as well.

MATERIALS AND METHODS

Plant material for the experiments were the Yugoslav wheat cultivar Evropa 90 (*Triticum aestivum* L.), and the maize hybrid NSSC 640 (*Zea mays* L.). Seeds were germinated for 48 h in a thermostat at 25—27°C. Seedlings were grown in nutrient solution Reid and York (1958), pH 5.5 (controlled every other day), under conditions of greenhouse. There were eight plants of wheat and six plants of maize per pot. After 25 days, plants were treated with three concentrations of Ni (10^{-7}, 10^{-5} and 10^{-3} mol dm^{-3}), for six days. Ni was supplied in the form of $NiSiO_4 \cdot 6H_2O$. Control treatment was the nutrient solution without Ni added. In Figures 1—3, number 1 represents control, 2—10^{-7}, 3—10^{-5} and 4—10^{-3} mol dm^{-3} Ni. The experiment was set in seven replications. Activity of the antioxidant enzyme SOD, LP intensity and $O_2^{\cdot -}$ quantity were determined in the obtained plant material.

SOD activity was determined by the method of Misra and Fridovich (1972), based on the autocatalytic transformation of epinephrine-adrenochrome at pH 10.2. The enzyme was extracted from 1 g of fresh plant material (leaves).

The quantity of $O_2^{\cdot -}$ was studied by the inhibition of adrenaline autooxidation in the presence of plant extracts obtained from wheat and maize leaves (Misra and Fridovich, 1972).

LP intensity was measured as malonyldialdehyde (MDA) production at 532 nm. MDA is one of the major final products of lipid peroxidation. MDA was extracted from plant leaves with a mixture of thiobarbituric and trichloroacetic acids, as described by Placer et al. (1968).

The results of the investigation were statistically processed, using the analysis of variance, regression and correlation analysis.

RESULTS AND DISCUSSION

SOD activity

The total SOD activity in leaves of wheat and maize treated with different Ni concentrations is shown in Figure 1.

Significant increase in SOD activity in wheat leaves was established for the lowest Ni concentration as compared with the control. SOD activity increased with further increases in Ni concentration, but not significantly. Similar tendency was also established in leaves of maize.

Increase in SOD activity under conditions of low Ni concentration could be explained by the assumption that Ni activates some enzymes but it does not participate in catalytic enzyme reactions (Kastori et al., 1996a).

The induction of some enzymes is considered to play a significant role in the stress metabolism induced by metal phytotoxicity (Van Assche and

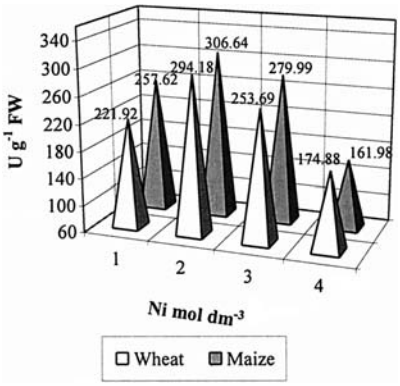

Fig. 1 — Effect of Ni on superoxide dismutase activity

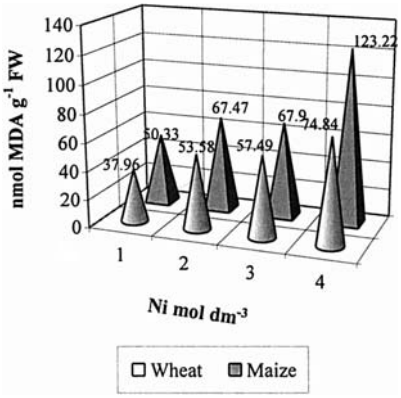

Fig. 2 — Effect of Ni on lipid peroxidation intensity

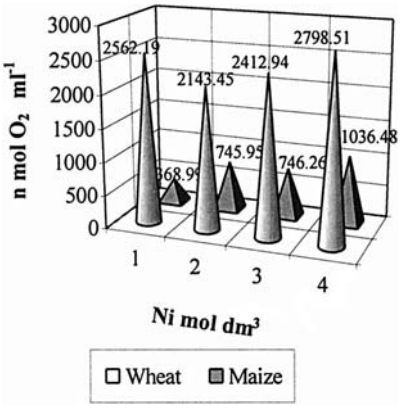

Fig. 3 — Effect of Ni on superoxide radicals quantity

Clijsters, 1990). According to our findings, it seems that metalloenzymes from the SOD group increase to some extent (at low Ni concentration) the resistance of wheat and maize plants to Ni toxicity.

Opposite to our results, the investigation of Pandolfini et al. (1996) showed that Ni did not affect SOD activity in examined wheat cultivars.

Lipid peroxidation intensity and superoxide radicals quantity

LP intensity (given in nmol MDA g^{-1} fresh weight) in leaves of wheat and maize subjected to different Ni concentrations is shown in Figure 2.

Results of this investigation showed that MDA concentration, as a result of peroxidation of membrane lipids, increased in both examined species with the increase of Ni concentration in the solution. Similar LP intensity levels in wheat and maize were probably caused by analogue SOD activity at high Ni concentrations. The increase in LP intensity under conditions of high HM presence was previously observed by other authors (Gašić et al., 1992; Kastori et al., 1996b).

The quantity of $O_2^{\cdot -}$ in leaves of wheat and maize plants exposed to different Ni concentrations is shown in Figure 3.

The lowest Ni concentration showed stimulative effect on SOD activity in wheat. Thus, the quantity of $O_2^{\cdot -}$ was significantly lower in this treatment compared with the control. At higher Ni concentrations the quantity of $O_2^{\cdot -}$ was also lower compared with the control but not significantly. As a result of lower SOD activity at the highest Ni concentration applied, the quantity of $O_2^{\cdot -}$ significantly increased as compared with the control. This means that at this Ni concentration the antioxidant system could not cope with the oxidative stress and prevent LP, caused by increase in quantity of toxic oxygen species such as $O_2^{\cdot -}$.

In leaves of maize, the quantity of $O_2^{\cdot -}$ significantly increased with all Ni concentrations used, as compared with the control.

Decrease in SOD activity caused by high Ni concentrations led to an increase in free radicals quantity. They cause damage in vitally important structures such as cell membranes (degradation of fatty acids) and DNA. Beside that, $O_2^{\cdot -}$ and its highly reactive derivatives such as hydroxyl radical ($^{\cdot}OH$) and singlet oxygen ($^1O_2$) damage proteins and chlorophylls (Navarri-Izzo et al., 1993; Štajner et al., 1997).

It can be concluded from the results of the study on the effect of Ni on SOD activity, LP intensity and $O_2^{\cdot -}$ quantity in leaves of wheat and maize, that the lower Ni concentrations increase SOD activity and decrease the quantity of $O_2^{\cdot -}$. Higher Ni concentrations decrease SOD activity and increase $O_2^{\cdot -}$ quantity. LP intensity increased in both examined species with the increase in Ni concentration in the solution.

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УТИЦАЈ НИКЛА НА АКТИВНОСТ СУПЕРОКСИД-ДИСМУТАЗЕ, ИНТЕНЗИТЕТ ЛИПИДНЕ ПЕРОКСИДАЦИЈЕ И КОЛИЧИНУ КИСЕОНИЧНОГ РАДИКАЛА У МЛАДИМ БИЉКАМА ПШЕНИЦЕ И КУКУРУЗА

Соња З. Илин[1], Рудолф Р. Кастори[2], Ђорђе Р. Маленчић[3]
[1] Научни институт за ратарство и повртарство,
Максима Горког 30, 21000 Нови Сад, Југославија
[2] Институт за ратарство и повртарство и Пољопривредни факултет,
Трг Доситеја Обрадовића 8, 21000 Нови Сад, Југославија
[3] Пољопривредни факултет, Трг Доситеја Обрадовића 8,
21000 Нови Сад, Југославија

Резиме

Методом водених култура испитано је дејство различитих концентрација никла (10^{-7}, 10^{-5} и 10^{-3} mol dm^{-3}) на активност антиоксидантног ензима супероксид-дисмутазе (SOD), интензитет липидне пероксидације (LP) и количину кисеоничног радикала ($O_{2}^{\cdot -}$) у листовима младих биљака пшенице и кукуруза. Резултати истраживања указују да су ниже концентрације никла повећале активност SOD, док су више концентрације никла смањиле активност овог ензима како код пшенице тако и код кукуруза. Као последица тога, количина $O_{2}^{\cdot -}$ је при нижим концентрацијама никла била значајно смањена, док је при високим концентрацијама никла дошло до повећања количине $O_{2}^{\cdot -}$ у листовима пшенице. У листовима кукуруза количина кисеоничног радикала је расла са повећањем концентрације никла. Интензитет липидне пероксидације се повећавао код обе испитиване врсте, са повећањем концентрације никла у раствору.

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Aleksa S. Knežević[1], *Pal P. Boža*[2], *Branislava Z. Butorac*[3], *Mirjana S. Vučković*[2]

[1] Institute of Field and Vegetable Crops, Faculty of Agriculture
Trg D. Obradovića 8, 21000 Novi Sad, Yugoslavia
[2] Institute of Biology, Faculty of Science
Trg D. Obradovića 2, 21000 Novi Sad, Yugoslavia
[3] Institute for Protection of Nature of Serbia
Radnička 20, 21000 Novi Sad, Yugoslavia

LEPIDIO CRASSIFOLIO — FESTUCETUM PSEUDOVINAE ASSOC. NOVA OF THE HALOBIOME IN YUGOSLAVIA

ABSTRACT: In the northwestern part of the Yugoslavia region, on the solonshak soil, between the Čonoplja and Svetozar Miletić settlements, the stands of the association which we described as *Lepidio crassifolio — Festucetum pseudovinae* assoc. nova were recorded. Within this association, the stands of *Lepidio crassifolio — Festucetum pseudovinae phragmitetosum communis* subassoc. nova and *Lepidio crassifolio — Festucetum pseudovinae camphorosmetosum annuae* subassoc. nova subassociations were differentiated.

KEY WORDS: association, characteristic taxon, stand, synecology, syntaxonomic status.

INTRODUCTION

The Čonoplja and Svetozar Miletić settlements were established in the loess terrace of the northwestern part of the Yugoslavia region. In the surroundings of these settlements, the halobiome with the investigated stands is of solonshak type (Živković et al., 1972). According to data of the meteorology station in the Sombor City, the average annual temperature of the investigated area has been of 10.6°C, and during the vegetation period (April—October) of 17.5°C. The average annual precipitation has been of 576 mm, and during the vegetation period of 319 mm. The semiarid period has extended from the beginning of July to the first third of October, and the arid one from the middle of August to the middle of September (Janjatović et al., 1991).

Phytocenological analyses of the investigated stands were done according to Braun—Blanquet method (1951). Plants were determined after Hegi (1965), Josifović (1970—1977), Soó (1964—1985) and Tutin (1964—1980). The system of floral elements after Gajić (1980), ecological indices

after Landolt (Landolt, 1977; Knežević 1994) and biological spectrum after Raunkiaer (Knežević, 1994) were used.

RESULTS AND DISCUSSION

Syntaxonomic status of the recorded association is as follows:

Class: *Festuco — Puccinellietea* Soó 1968
Order: *Artemisio — Festucetalia pseudovinae* Soó 1968
Alliance: *Festucion pseudovinae* Soó 1933
Suballiance: *Halo — Festucenion pseudovinae* Vučković 1985
Association: *Lepidio crassifolio — Festucetum pseudovinae* assoc. nova
Subassociation: *Lepidio crassifolio — Festucetum pseudovinae phragmitetosum communis* subassoc. nova
Subassociation: *Lepidio crassifolio — Festucetum pseudovinae camphorosmetosum annuae* subassoc. nova

Association *Lepidio crassifolio — Festucetum pseudovinae* is of semidesert character. In its stands abundance is 80—90%, and flora is composed of 22 taxa.

Species that are characteristic for the association *Fectuca pseudovinae* and *Lepidium crassifolium* W. et K. /*L cartilagineum* (May.) Thell./ have edificatorial and subedificatorial role.

These two species, together with *Podospermum canum, Odontites rubra* and *Camphorosma annua*, make a characteristic group.

Besides above mentioned species, another eight taxa, appearing in the floristic composition of *Festuco — Puccinellietea* class, were recorded.

Qualitative and quantitative participation of the taxa of *Festuco — Brometea* class and „Other taxa" were low (Tab. 1.).

Phytocenological Tab. 1 — Association *Lepidio crassifolio — Festucetum pseudovinae* assoc. nova

Life form	Floristic element	Subassociation	*phragmitetosum communis*			*camphorosmetosum annuae*				Presence
Size of sample area in m²			25	25	25	25	25	25	25	25	
Coverage %			80	80	90	90	90	90	90	90	
		Character taxa of ass.									
H	Eua	*Festuca pseudovina* Hack.	3.3	2.2	3.4	3.4	4.4	4.3	4.4	5.4	V
H	ST	*Lepidium crassifolium* W. et K.	2.2	2.2	2.1	2.3	3.3	3.3	2.2	3.3	V
		Differential taxa									
H	K	*Phragmites communis* Trin. var. *stolonifera* (G. F. W. Mey.) Hegi	2.1	1.1	1.1	—	—	—	—	—	II
T	Pt-P	*Camphorosma annua* Pall.	—	—	—	2.2	+.1	3.3	+.1	1.1	IV

		Festuco-puccinellietea **Character taxa**									
H	ISM	*Podospermum canum* C. A. Mey.	2.1	1.1	1.1	—	—	—	—	—	II
T	SSE	*Odontites rubra* Gilib.	—	+.1	2.2	2.1	1.1	+.1	2.1	1.1	V
H	Eua	*Plantago maritima* L.	1.1	2.2	+.1	+.1	—	—	—	—	III
H	SP	*Puccinellia limosa* (Schur.) Hol.	—	+.1	—	+.1	—	+.1	—	+.1	III
T	SPt-SM	*Cerastium dubium* (Bost.) Schw.	—	—	1.1	2.1	1.1	+.1	—	—	III
H	P	*Aster tripolium* L. var. *pannonicum* Jacq.	—	+.1	+.1	+.1	—	—	—	—	II
T	SM	*Hordeum maritimum* With. subsp. *gussoneanum* Parl.	—	—	—	—	+.1	+.1	—	—	II
T	Eua	*Atriplex litoralis* L.	—	—	+.1	—	—	—	—	—	I
H	SSE	*Lotus tenuis* Kit.	—	—	—	+	—	—	—	—	I
T	Spt	*Plantago tenuiflora* W. et K. f. *depauperata* Domin.	—	—	—	—	—	—	—	2.2	I
		Festuco-brometea **Character taxa**									
T	SM	*Bromus mollis* L.	—	—	—	—	1.1	+.1	+.1	—	II
H	Eua	*Achillea millefolium* L. subsp. *collina* (Becker) Weiss	—	—	—	+.1	—	—	—	—	I
T	SM	*Crepis setosa* Holl.	—	—	—	+.1	—	—	—	—	I
T	SSE	*Carduus acanthoides* L.	—	—	—	+	—	—	—	—	I
		Other taxa									
T	SEua	*Atriplex tatarica* L.	+.1	—	—	—	—	—	—	—	I
T	SSE	*Bromus commutatus* Schrad.	—	—	—	—	+	—	—	—	I
T	SPt-SM	*Lactuca saligna* L.	—	—	—	—	+	—	—	—	I
T	SEua	*Daucus carota* L.	—	—	—	—	—	—	+.1	—	I

The stands of *Lepidio crassifolio — Festucetum pseudovinae* assoc. nova association are not good for haymaking and cattle do not like to graze them.

At the beginning of the vegetation period, the soil on which these stands develop, was considerable wet. With the end of the spring rainfalls, the underground water level gradually decreased, thus during the semiarid period we recorded its average value at about 120 cm. During that period, due to capillary rising of the saline underground water, in the lower terrains around an artificial canal soil was rather wet, but with an increased salt contents in the rhisosphere layers. At the same time, due to rinse, in the elevated terrains salt was concentrated in the deeper layers.

Under such conditions, the association divided into two following subassociations: *Lepidio crassifolio — Festucetum pseudovinae phragmitetosum communis* subassoc. nova, with *Phragmites communis* Trin. var. *stolonifera* (G. F. W. Mayer) Hegi as a differential taxon, and *Lepidio crassifolio —*

Festucetum pseudovinae camphorosmetosum annuae subassoc. nova, with *Camphorosma annua* Pall. as a differential taxon.

Floristic diversity of *Lepidio crassifolio — Festucetum pseudovinae phragmitetosum communis* subassoc. nova subassociation was lower, and proportional participation of the taxa characterized by ecological index S_+ was higher (90.91%), comparing with *Lepidio crassifolio — Festucetum pseudovinae camphorosmetosum annuae* subassoc. nova subassociation characterized by the presence of the taxa of *Festuco — Brometea* class and higher number of the „Other taxa", while, in its stands, proportional participation of the taxa having S_+ ecological index was lower (63.16%).

Stratification was recorded only during the spring within the stands of *Lepidio crassifolio — Festucetum pseudovinae phragmitetosum communis* subassoc. nova subassociation with straightened shoots of *Phragmites communis* var. *stolonifera* making the upper stratum. During the semiarid and arid periods, when the reed was bent, there was no stratification within the stands of the investigated association.

Due to low floristic diversity, seasonal dynamism of the association was poorly expressed. The early summer aspect, with the gloomy violet clusters of *Festuca pseudovina*, mosaic distributed clusters of *Lepidium crassifolium* and less frequent and smaller yellow clusters of *Podospermum canum*, was the most characteristic and the most apparent.

The area type spectrum was characterized by 68.18% participation of the narrow spread taxa, 4 of them being Submiddeuropean, 3 Submediterranean, 1 East submediterranean, 2 Subpontic-Sumediterranean, 1 Subpontic, 1 Pontic-Pannonian, 1 Subpannonian, 1 Pannonian and 1 Subturan, and by 31.82 % participation of the wide spread taxa, among them 4 being Eurasian, 2 Subeurasian and 1 Cosmopolitan. The diversity and dominant role of the narrow spread taxa speak about the influence of the different climates interweaving and physical and chemical features of the soil on the association development. Presence of the taxa, which grow only within the Pannonian Plain, confirms endemic character of the association, while subdominant role of *Lepidium crassifolium* indicates semidesert character.

Biological spectrum of the association is characterized by 59.10% therophytes participation and 40.90% hemicryptophytes participation. The hemicryptophytic-therophytic character is a result of a rather dry and saline soil.

On the basis of the ecological indices average values it can be concluded that the association is characterized by domination of the open habitat plants (L — 3.91; T — 3.93) which overgrow mostly arid (F — 2.38) and moderate alkaline (R — 3.46) soil of the heavy mechanical structure (D — 3.99) with low nutrient and humus contents (N — 2.36; H — 2.18).

Comparison of the stands of *Lepidio crassifolio — Festucetum pseudovinae* assoc. nova association with floristically the most similar stands of *Plantagineto — Festucetum pseudovinae* Parabućski 1980 association from the western part of Vojvodina, described by Parabućski (1980), and the stands of *Artemisio — Festucetum pseudovinae* (Magyar 1928) Soó 1945 association recorded by Kabić (1985) in the western part of Vojvodina, points to the following facts:

The edificator species of the compared associations is a wide spread Eurasian taxon — *Festuca pseudovina* (Eurasian floral element of the continental character — Soó 1973).

A narrow spread East Submediterranean taxon — *Podospermum canum* (Pontic — Mediterranenan floral element — Soó 1970) is a member of the characteristic group of all associations.

The subedificator species of the compared associations, narrow spread taxa: *Lepidium crassifolium* (Pannonian endem — Soó 1968), *Plantago schwarzenbergiana* (Pannonian floral element — Gajić 1980; Transilvanian-Pannonian endem — Soó 1968) and *Artemisia maritima* subsp. *salina* (Continental floral element — Soó 1970) exclude each other.

The floristic composition of *Lepidio crassifolio — Festucetum pseudovinae* assoc. nova association is characterized by the presence of 22 taxa, 68.18% of them being characterized by S_+ ecological index. Qualitative and quantitative participation of the taxa of *Festuco — Brometea* class and „Other taxa" are low (Tab. 1).

The floristic composition of *Plantagineto-Festucetum pseudovinae* Parabućski 1980 association is characterized by the presence of 42 taxa, 59.09% of them being characterized by S_+ ecological index. Qualitative and quantitative participation of *Festuco — Brometea* class taxa, and particularly „Other taxa" in certain stands, are higher (Parabućski, 1980).

Fourteen taxa, 65.21 % of them being characterized by S_+ ecological index, make floristic composition of *Artemisio — Festucetum pseudovinae* (Magyar 1928) Soó 1945 association. Qualitative participation of *Festuco — Brometea* class taxa and „Other taxa" is low, and their quantitative participation is minor (Kabić, 1985).

The comparative review indicates that, from the synecological and syntaxonomic point of view, *Lepidio crassifolio — Festucetum pseudovinae* assoc. nova association is an intermediate association between *Plantagineto — Festucetum pseudovinae* Parabućski 1980 and *Artemisio — Festucetum pseudovinae* (Magyar 1928) Soó 1945 associations.

CONCLUSION

In the northwestern part of the Yugoslavia region, on the solonshak soil, between the Čonoplja and Svetozar Miletić settlements, the stands of *Lepidio crassifolio — Festucetum pseudovinae* assoc. nova association were recorded. Within these stands *Lepidio crassifolio — Festucetum pseudovinae phragmitetosum communis* subassoc. nova subassociation, with *Phragmites communis* Trin. var. *stolonifera* (G. F. W. Mayer) Hegi as a differential taxon, and *Lepidio crassifolio — Festucetum pseudovinae camphorosmetosum annuae* subassoc. nova subassociation, with *Camphorosma annua* Pall as a differential taxon, were differentiated.

The stands of *Lepidio crassifolio — Festucetum pseudovinae phragmitetosum communis* subassoc. nova subassociation were characterized by lower floristic diversity. They developed in lower terrain where, due to capillary ris-

ing of the saline underground water to the rhizosphere layers, during the semiarid period the soil was rather wet, but with an increased salt content.

The stands of *Lepidio crassifolio — Festucetum pseudovinae camphorosmetosum annuae* subassoc. nova subassociation were characterized by somewhat higher floristic diversity. They developed in the elevated terrain where, during the semiarid period, the salt was rinsed into the deeper layers.

The low floristic diversity, absence of apparent stratification and seasonal dynamism, high participation of the therophytic plants and spread only within the Pannonian Plain speak about extreme conditions and endemic character of the association.

From the synecological and syntaxonomic point of view, *Lepidio crassifolio — Festucetum pseudovinae* assoc. nova association is an intermediate association between *Plantagineto — Festucetum pseudovinae* Parabućski 1980 and *Artemisio — Festucetum pseudovinae* (Magyar 1928) Soó 1945 associations of *Halo — Festucenion pseudovinae* Vučković 1985 suballiance of *Festucion pseudovinae* Soó 1933 alliance of *Artemisio — Festucetalia pseudovinae* Soó 1968 order and of *Festuco — Puccinellietea* Soó 1968 class.

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LEPIDIO CRASSIFOLIO — FESTUCETUM PSEUDOVINAE ASSOC. NOVA НА ХАЛОБИОМУ ЈУГОСЛАВИЈЕ

Алекса С. Кнежевић[1], Пал П. Божа[2], Бранислава З. Буторац[3], Мирјана С. Вучковић[2]
[1] Институт за ратарство и повртарство, Пољопривредни факултет Нови Сад, Трг Д. Обрадовића 8, 21000 Нови Сад, Југославија
[2] Институт за биологију, Природно-математички факултет Нови Сад, Трг Д. Обрадовића 2, 21000 Нови Сад, Југославија
[3] Завод за заштиту природе Србије, Одељење у Новом Саду, Радничка 20, 21000 Нови Сад, Југославија

Резиме

У пределу северозападне Југославије, на солончаку између насеља Чонопља и Светозар Милетић, констатоване су састојине заједнице *Lepidio crassifolio — Festucetum pseudovinae* asoc nova.

У оквиру њих издиференциране су субасоцијације *Lepidio crassifolio — Festucetum pseudovinae phragmitetosum communis* subasoc nova чији је диференцијални таксон *Phragmites communis* var. *stolonifera* и *Lepidio crassifolio — Festucetum pseudovinae camphorosmetosum annuae* subasoc nova чији је диференцијални таксон *Camphorosma annua*.

Састојине субасоцијације *Lepidio crassifolio — Festucetum pseudovinae phragmitetosum communis* су флористички сиромашније. Обрастају нижа станишта која су током полусушног периода, услед капиларног доспевања слане подземне воде до ризосферних слојева земљишта, нешто влажнија и заслањенија.

Састојине субасоцијације *Lepidio crassifolio — Festucetum pseudovinae camphorosmetosum annuae* су флористички нешто богатије. Развијене су на вишим стаништима заједнице из којих су током полусушног периода соли испране у дубље слојеве земљишта.

Сиромашан флористички састав, слабо изражена спратовност и сезонска динамика, обилно учешће терофита и ареал ограничен на подручје Панонске низије указују на екстремност услова развоја и ендемичан карактер заједнице.

У синтаксономском и синеколошком погледу заједнице *Lepidio crassifolio — Festucetum pseudovinae* asoc. nova је интермедијерна заједницама *Plantagineto — Festucetum pseudovinae* Parabućski 1980 и *Artemisio — Festucetum pseudovinae* (Magyar 1928) Soó 1945 подсвезе *Halo — Festucenion pseudovinae* Vučković 1985, свезе *Festucion pseudovinae* Soó 1933, реда *Artemisio — Festucetalia pseudovinae* Soó 1968 и класе *Festuco Puccinelietea* Soó 1968.

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Ružica S. Igić, Pal P. Boža, Ljiljana S. Merkulov, Neda M. Mimica-Dukić[1], *Dragana M. Vukov, Goran T. Anačkov, Danijela D. Pavlovičova*

Institute of Biology, Trg D. Obradovića 2
21000 Novi Sad, Yugoslavia
[1] Institute of Chemistry, Trg D. Obradovića 4
21000 Novi Sad, Yugoslavia

DISTRIBUTION OF SPECIES OF THE GENUS *Salvia* L. 1754 (Sect. *Pletiosphace* Bentham.) IN THE VOJVODINA PROVINCE, YUGOSLAVIA

ABSTRACT: The Vojvodina Province is located in the southeastern part of the Pannonian Plain. It covers an area of 215,099 km^2 of which 76.6% are cultivated. That is why it is called the breadbasket of Yugoslavia. Owing to this fact, investigations of the native flora of the Province have special significance. The paper presents the results of a distribution survey of species of the genus *Salvia* in the Vojvodina Province. Of 36 *Salvia* species in the European flora, 9 occur in the Vojvodina Province: *Salvia aethiopis* L., *S. glutinosa* L., *S. pratensis* L., *S. nemorosa* L., *S. amplexicaulis* Lam., *S. nutans* L., *S. austriaca* Jacq., *S. verticillata* L. and *S. reflexa* Hornem. There are also records of a large number of infraspecific taxa and of one fixed hybrid. UTM maps show the distribution of the recorded taxa. *S. nutans* and *S. amplexicaulis* have disappeared from the Vojvodina Province. Importance of protecting these species is in their potential use as medicinal plants.

KEY WORDS: native flora, chorological distribution, infraspecific taxa, potential medicinal plants, protection.

INTRODUCTION

The genus *Salvia* belongs to subfamily *Lamioideae*, family *Lamiaceae*, order *Lamiales*, subclass *Lamiidae*, class *Magnoliopsida*, and section *Magnoliophyta* (Tahtadjan, 1987). Comprising about 500 species, it is the largest genus in the family *Lamiaceae*. Out of thirty six species occurring in Europe (Hedge, 1972), fourteen grow in Serbia (Diklić, 1974). Nine *Salvia* species occur in Vojvodina. The paper presents the distribution of five *Salvia* species of Sect. *Plethiosphace* Bentham, *Salvia pratensis* L., *S. nemorosa* L., *S. amplexicaulis* Lam., *S. nutans* L. and *S. austriaca* Jacq., and of their infraspecies taxa.

MATERIAL AND METHODS

Plant systematics has been based on *Systema magnoliofitorum* (T a h t a - d j a n , 1987). The description of each taxon is supplemented with its floral elements (S o ó , 1968). Chronological distribution of each *Salvia* species is also presented.

RESULTS AND DISCUSSION

In the Vojvodina Province, the genus *Salvia* is represented by nine species, *Salvia aethiopis* L., *S. glutinosa* L., *S. pratensis* L., *S. nemorosa* L., *S. amplexicaulis* L a m . , *S. nutans* L., *S. austriaca* J a c q . , *S. verticillata* L. and *S. reflexa* H o r n e m . A hybrid *Salvia nemorosa x pratensis* (*S. silvestris* L.) has also been found. These taxa are classified into five sections: Sect. *Aethiopis*, Sect. *Drymosphace*, Sect. *Plethiosphace*, Sect. *Hemisphace* and Sect. *Calophace*.

A chorological distribution of the species and infraspecific taxa of Sect. *Plethiosphace* in the Vojvodina Province is presented. Location data were based on the available literature, herbarium specimens of the Institute of Biology, Novi Sad, and our own field reconnaissance. The chorological distribution of the species is given in Figure 1.

Five species of Sect. *Plethiosphace*, *S. pratensis* L., *S. nemorosa* L., *S. amplexicaulis* L a m . , *S. nutans* L., and *S. austriaca* have been reported for the Vojvodina Province. The above hybrid also belongs to this section.

S. pratensis is a sub-Central European floral element. Its distribution in Vojvodina has been reported for the regions of Bačka, Banat, and Srem (Figure 1). In Bačka, in addition to *S. pratensis* subsp. *pratensis* var. *pratensis* f. *pratensis*, S. *pratensis* subsp. *pratensis* var. (S c h m .) R c h b ., S. *pratensis* l. *bicolor* B o ž a et O b r a d o v i ć , *S. pratensis* l. *rosea* L a t u r and *S. pratensis* l. *variegata* (W . et K . in W i l l d .) Maly have also been recorded.

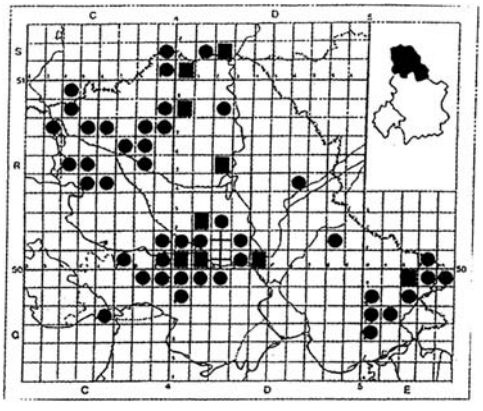

Fig. 1 — Distribution of *S. pratensis* L., in Vojvodina

Salvia pratensis* L. subsp. *pratensis* var. *pratensis* f. *pratensis

Bačka: Subotica CS 90; Hajdukova pustara, Daščan, Jasenovac, Hrastovača, Daščan, Kelebija, Čavolj CS 91; Palić, Ludaška pusta DS 00; Horgoš DS 11; Kamaraš DS 21; Bački Monoštor, Bezdan, Štrbac CR 37; Svilojevo CR 45; Gakovo CR 48; Rastina CR 49; Srpski Miletić CR 54; Doroslovo, Sonta CR 55; Sombor CR 57; Riđica CR 59; Odžaci CR 64; Kljajićevo CR 67; Sivac CR 76; Kula CR 85; Lipar CR 86; Gornja Rogatica, Orešković

CR 87; Futog CR 91; Vrbas CR 94; Bajša, Bačka Topola CR 97; Žednik, Mali Beograd CR 98; Veternik DR 01; Čantavir DR 08; Kać — Budisava DR 11; Temerin DR 12; Kovilj DR 20; Budisava, Šajkaš DR 21; Žabalj, Gospođinci DR 22; Stari Bečej DR 25; Senta DR 28; Lok DR 30; Mošorin, Mošorin—Vilovo DR 31; Titel—Tisa, Titel DR 40

Banat: Banatski dvor DR 64; Jarkovac DR 81; Puževo brdo, Gudurica ER 30; Deliblato EQ 06; Rošijana EQ 07; Brandibul EQ 08; Sušara EQ 17; Guzajne EQ 28; Magareći vrh EQ 29; Mesić, Dubrava, Sočica, Jablanka EQ 39; Karaula EQ 49

Srem: Neštin, Vizić CR 70; Banoštor, Čerević, Testera, Čerević—Katanske livade, Ravne, Andravlje, Brankovac CR 90; Rakovac, Osovlje DR 00; Stražilovački breg, Stražilovački breg—Lipe, Bukovac, Selište—Direk, Stražilovo, Banstol, Venac, Širine, Paragovo, Karlovci DR 10; Čortanovci, Kalakač DR 20; Koševac DR 30; Ristovača CQ 67; Čalma CQ 89; Mutalj, Sanča, Grgurevci, Glavica CQ 99; Pavlovci DQ 08; Kukavica, Vrdnik, Vrdnički hrub, Rovača, Rivica DQ 09; Kajnovac, Ševinjak, Irig, Maksimovac, Prnjavor, Velika Remeta DQ 19; Šelovrenac, Kalakač—Beška, Čortanovci—Beška DQ 29

Species variability:

***Salvia pratensis* subsp. *pratensis* var. *rostrata* (Schm.) Rchb.**

Bačka: along the road to Jasenovac CS 91.

***S. pratensis* L. l. *bicolor* Bož a et Obradović**

Bačka: Lipar CR 86; Orešković CR 87.

***Salvia pratensis* L. l. *rosea* Latur**

Bačka: Hrastovača CS 91; Lipar CR 86; Gornja Rogatica CR 87.

***Salvia pratensis* L. l. *variegata* (W. et K. in Willd) Maly**

Bačka: Telečka CR 77; Crvenka CR 85; Lipar, Lipar—Bajša CR 86.

S. nemorosa belongs to the sub-Pontic floral element. It is a widely distributed species and the most frequent *Salvia* species in the Vojvodina Province (Figure 2). In Bačka, the type species is accompanied by infraspecific taxa *S. nemorosa* f. *aprica* (Schur.) Soó, and *S. nemorosa* l. *purpurea* Priszter, while *S. nemorosa* l. *albiflora* Schur., *S. nemorosa* l. *badaconyensis* Soó and *S. nemorosa* f *submollis* Borb. also occur in the regions of Bačka and Banat. Hybridization is frequent between *S. pratensis* and *S. nemorosa* in Bačka and Srem, which resulted in the development of a hybrid species (Figure 3).

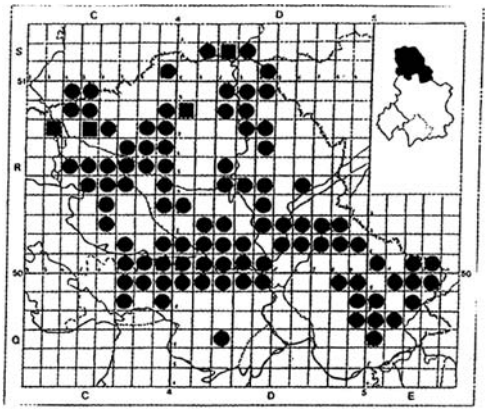

Fig. 2 — Distribution of *S. nemorosa* L. in Vojvodina

***Salvia nemorosa* L.**

Bačka: Subotica CS 90; Horgoš DS 11; Kamaraš DS 21; Ristovača, Bezdan, Monoštor CR 37; Apatin—Kurjačica, Svilojevo CR 45; Gakovo CR 48; Rastina CR 49; Karavukovo, Srpski Miletić CR 54; Sonta, Doroslovo CR 55; Sombor CR 57; Stanišić CR 58; Riđica CR 59; Bač CR 62; Deronje CR 63; Odžaci, Bački Gračac CR 64; Bački Brestovac CR 65; Kljajićevo, Čonoplja CR 67; Bačka Palanka CR 71; Ruski Krstur, Lalić CR 74; Kruščić CR 75; Sivac CR 76; Kula, Crvenka—Panonija CR 85; Srednji Salaš, Lipar CR 86; Rogatica, Orešković CR 87; Veternik—Futog CR 91; Stepanovićevo CR 93; Vrbas CR 94; Feketić, Lovćenac CR 95; Mali Iđoš CR 96; Bajša, Karađorđevo CR 97; Zobnatica, Žednik, Mali Beograd CR 98; Novi Sad—Veternik, Rimski šančevi, Rumenka DR 01; Sirig DR 03; Čantavir DR 08; Kać—Budisava DR 11; Temerin DR 12; Kovilj DR 20; Budisava, Šajkaš DR 21; Žabalj, okolina Đurđeva, Gospođinci DR 22; Bačko Gradište DR 24; Bečej DR 25; Senta DR 28; Senćanski Trešnjevac DR 29; Lok—Vilovo DR 30; Mošorin—Vilovo; Mošorin—Tisa DR 31; Lok—Titel, Titel—Tisa DR 40.

Banat: Đala DS 31; Kumane DR 34; Padej DR 37; Čoka, Jazovo DR 38; Filić—Novi Kneževac DR 39; Mali Siget—Veliki Siget, Banatsko Aranđelovo DS 40; Aradac—Tisa DR 42; Elemir—Okanj DR 43; Melenci—Ostrovo DR 44; Novo Miloševo DR 46; Sajan DR 47; Crna bara DR 49; Belo Blato DR 51; Zrenjanin, Mužlja DR 52; Orlovat DR 61; Lazarevo DR 62; Čestereg DR 64; Tomaševac DR 71; Banatski Despotovac—Deračka bara DR 72; Boka, Jarkovac DR 81; Sečanj DR 82; Ilandža DR 90; Konak DR 91; Lokve ER 00; Vatin ER 20; Malo središte ER 30; Deliblato, Deliblatska bara EQ 06; Flamunda, Palošće, Tilva—Sušara EQ 07; Brandibul, pašnjak Mala čoka EQ 08; Konstantinova bara EQ 16; Grebenac EQ 17; Vlajkovac, Pavliš EQ 19; Kuštilj EQ 28; Vršac, Kapelin breg, EQ 29; Široko bilo EQ 39; Tilva—čoka, pašnjak oko Kravana DQ 97; Devojački Bunar—Tilva DQ 89—DQ 98; Devojački Bunar DQ 89; Kravan DQ 97; Tilva DQ 98; Alibunar DQ 99.

Srem: Neštin, Plandište CR 70; Susek CR 80; Andrevlje, Čerević, Testera CR 90; Kamenica, Zmajevac, Beočin, Rakovac DR 00; Karlovci, Banstol, Remeta—Stražilovo DR 10; Petrovaradin, Krstatica, Mali šveb DR 11; Kalakač, Čortanovci DR 20; Koševac DR 30; Slankamen—Koševac DR 30—DQ 49; Koševac—Kalakač DR 20—DR 30; Kuzmin CQ 78; Bačinci—Erdevik CQ 79; Čalma CQ 89; Vranjoš, Plakor, Čikaš, Stejanovački Gat CQ 98; Glavica, Bešenovo, Mutalj kod Šuljma, Mala Remeta CQ 99; Kukavica, Pavlovci, Vrdnički hrub, Vrdnik DQ 09; Krušedol, Ševinjaka, Iriga, Kajnovac DQ 19; Karlovčić DQ 26; Šelovrenac, Beška DQ 29; Stari Slankamen DQ 49.

Species variability:

***Salvia nemorosa* L. f. *aprica* (Schur) Soó**

Bačka: Sirig DR 03; Bačko Gradište DR 24.

***Salvia nemorosa* L. f. *submollis* Borb.**

Srem: Stari Slankamen—Krčedin DR 30.

***Salvia nemorosa* L. l. *albiflora* Schur.**

Bačka: Kanjiža—Horgoš DS 11—DS 20; Mali Iđoš CR 96; Rimski šančevi, Novi Sad DR 01; Sirig DR 03; Titelski breg DR 40.

Banat: Tomaševac—Botoš DR 71.

***Salvia nemorosa* L. l. *badacsonyensis* Soó**

Bačka: Srednji salaš CR 86; Lovćenac CR 95; Novi Sad DR 01; Titel DR 40.

Banat: Tomaševac DR 71.

***Salvia nemorosa* L. l. *purpurea* Priszter**

Bačka: Titel, Titelski breg DR 40.

***Salvia nemorosa x pratensis* (*S. silvestris* L.)**

Bačka: Srednji Salaš CR 86; Žabaljska pustara DR 22.

Srem: Čerević CR 90; Karlovci DR 10; Petrovaradin DR 11; Čortanovci DR 20; Beška DQ 29; Slankamen DQ 49; Vrdnik DQ 90.

Today, the species ***S. amplexicaulis*** and ***S. nutans*** of Sect. *Plethiosphace* may be considered as extinct in the Vojvodina Province.

According to the *Bulgarian Flora* (Markova, 1989), a sub-Mediterranean species ***S. amplexicaulis*** occurs in the Balkan Peninsula while it is rare in Rumania and Hungary. Wagner (1914) and Javorka (1925) quoted this species for the Deliblato Sands in the region of Banat. Since there have been no subsequent reports, the species may be presumed extinct from the flora of the Vojvodina Province (Figure 5).

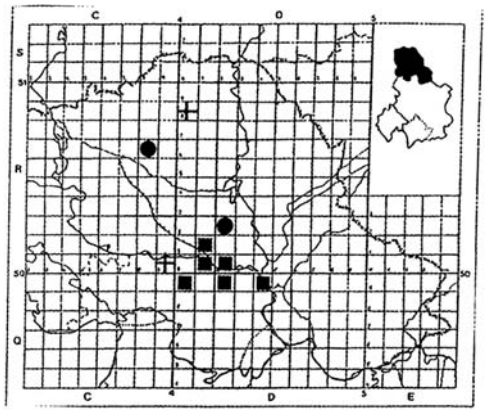

Fig. 3 — Distribution of *S. pratensis x S. nemorosa* in Vojvodina

***Salvia amplexicaulis* Lam.**

Banat: Deliblatska peščara EQ 06.

S. nutans is a Pontic species. In the Vojvodina Province, its occurrence is confined to several locations in Bačka and two in Srem (Figure 5). The last record of this species, made in 1966, was not confirmed by later field reconnaissances; therefore, the species is presumed extinct from its natural habitats.

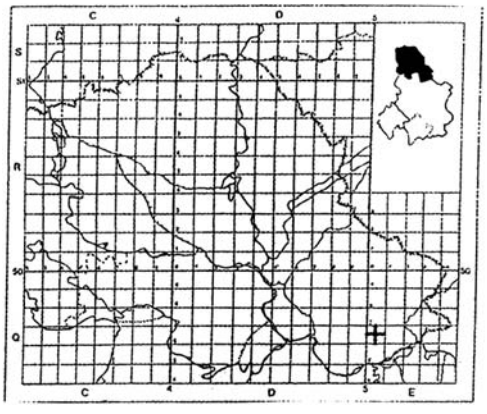

Fig. 4 — Distribution of *S. amplexicaulis* in Vojvodina

***Salvia nutans* L.**

Bačka: Bogojevo CR 54; Sombor CR 57; Sivac CR 76; Futog CR

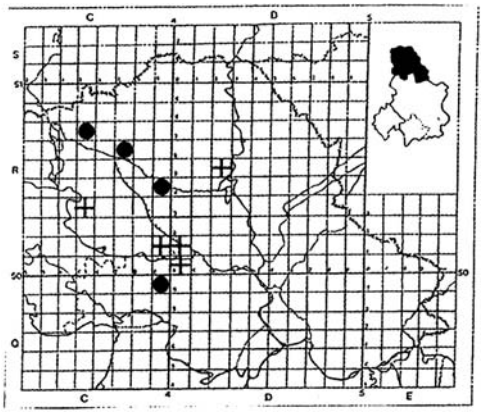

Fig. 5 — Distribution of *S. nutans* in Vojvodina

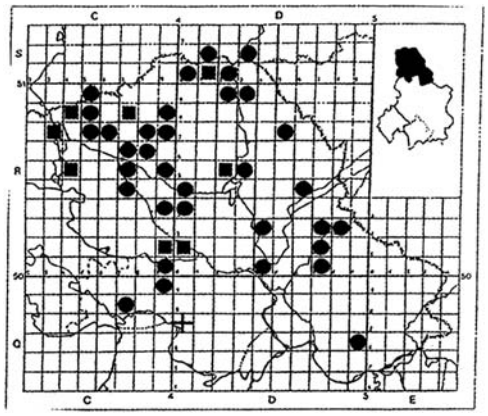

Fig. 6 — Distribution of *S. austriaca* in Vojvodina

91; Vrbas CR 94; Rimski šančevi, Novi Sad, Novi Sad—Futog DR 01; Kisač DR 02; Bečej DR 25.

Srem: Glavica CQ 99.

S. austriaca belongs to the Pontic-Pannonian floral element. In the Vojvodina Province, it is widespread in Bačka, Banat, and Srem. According to the distribution data, it is most frequent in Bačka and somewhat less frequent in Banat and Srem, possibly due to the steppe character of the species. An argument in favor of its character are beautifully conserved steppe fragments in the region of Bačka (Figure 4).

Salvia austriaca **Jacq.**

Bačka: Palić, Ludaško jezero DS 00; Bački Vinogradi DS 10; Horgoš DS 11; Horgoš—Kanjiža DS 20; Štrbac, Bezdan CR 37; Apatin CR 45; Gakovo CR 48; Sombor CR 57; Stanišić CR 58; Riđica, Stanišić—Riđica CR 59; Kljajićevo, Čonoplja CR 67; Ruski Krstur CR 74; Kruščić CR 75; Sivac CR 76; Pačir CR 78; Srednji Salaš, Lipar CR 86; Orešković, Rogatica CR 87; Futog CR 91; Stepanovićevo CR 93; Lovćenac, Feketić CR 95; Bačka Topola, Bajša CR 97; Zobnatica, Žednik CR 98; Novi Sad DR 01; Sirig DR 03; Srbobran DR 14; Bečej DR 25; Kanjiža—Senta DR 29.

Banat: Đala—Rabe DS 31; Novi Bečej DR 35; Čoka—Novi Kneževac DR 39; Zrenjanin—Aradac DR 42; Kikinda DR 57; Čestereg DR 64; Velike Livade DR 65; Tomaševac—Uzdin DR 70—DR 71; Banatski Despotovac—Sutjeska DR 72; Sečanj DR 82.

Srem: Čerević, Čerević—Katanske livade, Katanske livade CR 90; Glavica—Majur CR 90—CQ.

Two valuable species, *S. amplexicaulis* and *S. nutans* were irrevocably lost. *S. austriaca* is a vulnerable species which deserves our attention. *S. pratensis* and *S. nemorosa*, although still frequent in the Vojvodina Province, necessitate a future action towards their protection and conservation.

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РАСПРОСТРАЊЕЊЕ ВРСТА РОДА *Salvia* L. 1754 (Sect. *Pletiosphace* Bentham.) У ВОЈВОДИНИ (ЈУГОСЛАВИЈА)

Ружица С. Игић, Пал П. Божа, Љиљана С. Меркулов,
Неда М. Мимица-Дукић[1], Драгана М. Вуков, Горан Т. Аначков,
Данијела Д. Павловичова
Институт за биологију, Трг Д. Обрадовића 2, 21000 Нови Сад, Југославија
[1] Институт за хемију, Трг Д. Обрадовића 4, 21000 Нови Сад, Југославија

Резиме

Војводина се налази у југоисточном делу Панонске низије. Захвата површину од 21509 km² од чега је чак 76.6% обрађено, те представља житницу Југославије. Због тога флористи посебан значај придају истраживањима аутохтоне флоре која се сачувала само на малим фрагментима природних станишта (степе, слатине, шуме, пескови).

У раду је дат приказ распрострањења врста рода *Salvia* на подручју Војводине. Од 36 врста које расту у флори Европе, у Војводини је констатовано 9 врста: *Salvia nemorosa* L., *S. pratensis* L., *S. reflexa* Hornem, *S. amplexicaulis* Lam., *S. nutans* L., *S. verticillata* L., *S. austriaca* Jacq., *S. aethiopis* L., *S. glutinosa* L. Такође је забележен и већи број инфраспецијских таксона и један устаљени хибрид.

Њихово распрострањење приказано је на UTM картама. Неке од констатованих врста (*S.austriaca* и *S. aethiopis*) данас су ретки становници у нашој флори те их је неопходно заштитити. *S. nutans* и *S. amplexicaulis* нестале су из флоре Војводине. Значај заштите ових врста огледа се у томе да се оне могу користити и као потенцијалне лековите биљке. Диверзитет рода *Salvia* на нашим просторима обогаћују неке усељене врсте као што је *S. reflexa* Hornem., која је пореклом из Северне Америке, а расте само на два локалитета.

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Igor M. Stojanov, Dušan B. Orlić

Scientific Institute for Veterinary Medicine, Novi Sad, Yugoslavia
Rumenački put 6, 21000 Novi Sad, Yugoslavia

TYPES OF *CAMPYLOBACTER* SPECIES IN LAYING HENS WITH AND WITHOUT DIARRHOEA

ABSTRACT: The existence of the bacteria from the kind of *Campylobacter* in the organisms of animals and people, as well as their presence on different places in nature, is a reason why this bacteria is a subject of many investigations. Finding the *Campylobacter* kind in poultry and recognizing the importance of their presence is very important, from the view of medical preventing of the poultry, as well as from the view of human health, having in mind that this has to do with the zoonosis.

The subject of our study was bacterial examination of the digestive track of laying hens. In our work two groups of laying hens were examined. In the first group was the one in which, after the clinical examination presence of diarrhoea was stated, and in the second group were three examples where such diagnostical symptoms were not found. Importance of the laying hens in the production cycles of the poultry made us decide to investigate this kind of animals as a potential possibility of transferring the *Campylobacter* on people. The aim of the work was to try to isolate and identify *Campylobacter* in the digestive tract of the laying hens, and to see if there are some differences in their presence and in their kinds in the two above mentioned groups of laying hens.

For bacteriological investigation we used cloacal swabs from laying hens in two already mentioned groups. For isolation *Campylobacter* we used Columbia agar + campylosel, using Gener box microaer to provide microaerophile conditions. Identification of the isolated bacteries was done with the help of API strips and by using software for reading.

Altogether 60 cloacal smears were examined (36 from the first group and 24 from the second). Presence of the *Campylobacter* kind in the first group was 88.88% or 32 positive hens, and in the second group were 33.33% or 8 positive hens. In the first group of the laying hens in 32 positive hens *Campylobacter jejuni* subsp. *jejuni* was isolated in 32 cases (100%) and in one case *Campylobacter coli* as a mixed isolate with *Campylobacter jejuni* subsp. *jejuni.* In the second group of the analyzed laying hens out of 8 known cases all 8 were isolated by *Campylobacter coli*.

The results point to big presence of *Campylobacter* types in the sick laying hens and to the presence of different kinds of diarrhoea what certainly has to be investigated.

KEY WORDS: laying hens, diarrhoea, *Campylobacter* sp.

INTRODUCTION

The presence of the bacteria from the species of *Campylobacter* in the organisms of animals and people, as well as their presence in different places in

nature, is a reason why this bacteria is a subject of many investigations. Former investigation has shown that the presence of *Campylobacter* in the organisms of the hosts may cause a disturbance in health condition. At the same time in quite a big number of findings, it is either noticed that there are nonclinical symptoms, although *Campylobacter* is isolated from certain materials, or the relation between bacteriological, present isolated species of *Campylobacter* and the clinical symptoms cannot be proved. The findings of *Campylobacter* kinds in poultry material and recognizing the importance of their presence is significant from the standpoint of protecting health condition of animal, as well as from the standpoint of protecting health condition of people, because it causes zoonosis.

The subject of our investigation was bacteriological flora of the digestive tract in the laying hens. The importance of the laying hens in the production cycles made us decide to choose these animals and to research the potential possibilities of transmitting *Campylobacter* on people.

The aim of the work was to isolate and determine *Campylobacter* in digestive tract of laying hens. We wanted to see if there were differences caused by their presence and if there existed different kinds of *Campylobacters* in two already mentioned groups of laying hens.

MATERIAL AND METHODS

In the work two groups of laying hens were analyzed. In the first group was the one in which the presence of diarrhoea was stated after clinical research and the second where were the ones without such symptoms. For the bacteriological investigation we used cloacal swabs of the laying hens from both above-mentioned groups. Swabs were directly streaked on ready nutrient culture medium. For that purpose was used Columbia agar with 5% defibrinated ovine blood where the antibiotic medium *Campylosel* was added. In this waz prepared plates we incubated in anaerobic jars (McIntosh) where gas sacks of Gener box microaer was added. In such a way necessary microaerofil conditions were provided (Smibert, 1984; Pener, 1991). Identification of the isolated bacterias was performed by using API Strips and software for their analyzing.

THE RESULTS OF THE INVESTIGATION AND DISCUSSION

A total of 60 cloacal swabs were examined (36 from the first group and 24 from the second group).

In the Table 1 we find the results obtained by the bacteriological examination. They present the findings of the isolated *Campylobacter* in both groups of laying hens.

Tab. 1. — The results of the isolated *Campylobacter*

Group		Total of the examined	Positive finding	% of the positive findings
1	Laying hens with diarrhoea	36	32	88.88%
2	Laying hens without diarrhoea	24	8	33.33%

In Table 1 we clearly see that the positive findings of *Campylobacter* in the laying hens with diarrhoea was 88.88% or 32 chicks out of 36 examined, and the percentage of the positive samples of the hens without diarrhoea was 33.33% or 8 out of 24 observed.

In the works of the authors who investigated these problems was written about such results in the poultry material. Findings of *Campylobacter* in different materials, among which is also poultry, write (Smibert, 1978; Smibert, 1981; Skirrow and Benjamin 1980). They describe the importance of the presence of these bacterias in animals and people. In his work (Jacobs-Reitsma, 1995) presented the results of findings of *Campylobacter jejuni* in the breeding flock and the importance of their presence. They specially point out the presence of *Campylobacter* in this category of poultry because there is a possibility of vertical transmission of these bacterias on the chicks. About the presence of *Campylobacter jejuni* and *Campylobacter coli* was written in the work of (Glunder, 1995) who isolated these bacterias from poultry material.

In Table 2 are given the results of the findings of some kinds of *Campylobacter* in both observed groups of the laying hens.

Tab. 2. — The results of the isolated *Campylobacter*

Group		Total of the examined chicks	Positive findings	*Campylobacter jejuni* s. *jejuni*	%	*Campylobacter coli*	%
1	Laying hens with diarrhoea	36	32	32	100%	1	3.12%
2	Laying hens without diarrhoea	24	8	—	—	8	100%

In the Table 2 one can see that the presence of *Campylobacter jejuni* subsp. *jejuni* in the first group was 100% from the total of 32 positive material, but in one case it was a mixed infection with *Campylobacter coli*, what represented 3.12% of the total number of the findings. In the same way, one can see that when talking about the second group of the observed samples of *Campylobacter jejuni* subs. *jejuni* was not isolated in any sample. Findings of *Campylobacter coli*, as it has already been mentioned in the first group, was a part of the mixed infection with the *Campylobacter jejuni* subsp. *jejuni*, and in the second group *Campylobacter coli* was isolated in 100% of the total number of positive findings.

The findings of *Campylobacter* kind in the poultry, especially on their trunks, is stated in the work of (Ivanović, Snežana, 1990) where were reported some facts about *Campylobacter jejuni* subsp. *jejuni* and *Campylobacter coli* on the corps of the slaughtered poultry. Presence of these bacterias is very important from the standpoint of the potential infection of people having in mind that we speak about zoonoses. Finding *Campylobacter* species on the farms of poultry is also mentioned in the work of (Stern et al. 1995).

In the available literature we have not found any information that discussion about the importance of some species of *Campylobacter* and their influence on the appearance of diarrhoea in the poultry. We have not found information that would give us more precise answer whether the presence of *Campylobacter jejuni* subsp. *jejuni* causes some clinical symptoms, which we found and had taken them as our starting point of the research. We also have not found that on the samples without clinical symptoms of diarrhoea is found only *Campylobacter coli*.

CONCLUSION

— In the research we used the methodology, which was used by other authors as well, so the received results, comparing them to the experience of the previous investigation, can represent valid findings.

— After 36 checked samples of the laying hens with diarrhoea, 32 were positive on the presence of *Campylobacter* kind what makes 88.88% out of the total of the research material.

— After 24 checked samples of the laying hens without diarrhoea, 8 were positive on the presence of *Campylobacter* kind what makes 33.33% of the total number of the researched material.

— In the laying hens with diarrhoea *Campylobacter jejuni* subsp. *jejuni* was isolated in all the positive samples (100% of the total of 32 positive samples), while *Campylobacter coli* was isolated in one case (what makes 3.12% from 32 positive findings) as a mixed bacterial flora together with the above mentioned *Campylobacter*.

— In the laying hens without diarrhoea *Campylobacter coli* was isolated in 100% cases, while *Campylobacter jejuni* was not found.

— The findings of different *Campylobacter* of the two observed groups shows that there is a possibility of pathogen appearing of these bacterias and this is a good reason for further investigation. This is even more the case because we speak about zoonoses that can in direct or indirect way cause the infection of people.

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ВРСТЕ РОДА КАМПИЛОБАКТЕР КОД КОКА НОСИЉА СА ДИЈАРЕЈОМ И БЕЗ ДИЈАРЕЈЕ

Игор М. Стојанов, Душан Б. Орлић
Научни институт за ветеринарство Нови Сад
Руменачки пут 6, 21000 Нови Сад, Југославија

Резиме

Присуство бактерија из рода кампилобактер у организмима животиња и људи, као и њихово присуство на различитим местима у природи, представља разлог због којег је ова бактерија предмет интересовања истраживача. Налаз кампилобактер врста у живинским материјалима и сагледавање значаја њиховог присуства важни су како са становишта здравствене заштите живине тако и са становишта угрожавања здравља људи с обзиром да се ради о зоонози.

Предмет нашег истраживања је анализирање бактеријске флоре дигестивног тракта кока-носиља. У раду су испитане две групе кока-носиља. У првој групи се налазе јединке код којих је после клиничког прегледа утврђено присуство дијареје, а у другој групи су јединке код којих нису дијагностиковани такви симптоми. Значај кока носиља у производном циклусу живине определио нас је на овај избор животиња као и потенцијална могућност преношења кампилобактера на људе. Циљ рада је да се покушају изолација и идентификација кампилобактера из дигестивног тракта кока-носиља, као и да се сагледа да ли постоје разлике како у присуству тако и у врстама кампилобактера код две наведене групе кока носиља.

За бактеријско испитивање користили смо клоакалне брисеве кока носиља узетих из две споменуте групе. За изолацију кампилобактера користили смо Колумбија агар + campilosel уз обезбеђење микроаерофилних услова Gener box mic-

roaer. Идентификацију изолованих бактерија обавили смо уз помоћ API стрипова и софтвера за њихово очитавање.

Укупно је прегледано 60 клоакалних брисева (36 из прве групе и 24 из друге групе). Присуство кампилобактер врста у првој групи је било 88,88% или 32 позитивне јединке, а у другој групи је било 33,33% или 8 позитивних јединки. Код прве групе кока носиља од 32 позитивне јединке изолована је *Campylobacter jejuni* subsp. *jejuni* у 32 случаја (100,00%) и у једном случају *Campylobacter coli* као мешани изолоат са *Campylobacter jejuni* subsp. *jejuni*. Код друге групе анализираних кока-носиља од 8 позитивних случајева у свих 8 је изолована *Campylobacter coli*.

Резултати указују на значајно присуство кампилобактер врста код оболелих кока носиља и на различитост заступљених врста, што свакако заслужује нова истраживања.

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Branka M. Vidić, Branko M. Bjelajac, Stanko F. Boboš, Dušan B. Orlić*

Veterinary Institute „Novi Sad"
Rumenački put 6. 21000, Novi Sad
* Veterinary Centre for Animal Breeding
Knjaza Miloša 21, Banja Luka

PREVALENCE OF *HAEMOPHILUS SOMNUS INFECTION* IN CATTLE

ABSTRACT: *Haemophilus somnus* is known to be an etiological agent in several disease sindromes of cattle. A great number of countries in the world have confirmed existance of this disease. It is realistic to expect it's presence in our country as well.

The investigation included 93 samples of breeder bulls, 167 sera of the cows with reproductive disturbance (abortion, stillborn calves, repeated estrus, endometritis and others). Besides this, blood sera from 865 cows, 242 heifers, 294 young bulls and 117 calves were examined. For proving antibodies in blood sera method of agglutination and m-CF test have been applied. By the method of tube agglutination positive results were registred in 2.5% of the bull sera and by the m-CF in 4.3% of the samples. The highest positive percentage was reached by the method of microagglutination (9.68%), when was also the highest antibody titer, up to 1:128. The method of microagglutination was used for serological investigation of cattle sera from larger area. Agglutinin for *H. somnus* was discovered in all the categories of cattle. It is important to state that the highest positive percentage was found in the steers (18.03%), while the antibody titer was in the range from 1:4 to 1:512. Positive results were proved in 84 cow's sera sample (9.71%), was considerably lower (5.78%) in heifer and was the lowest in the calves, only in 2 sera samples (1.71%).

KEY WORDS: *H. somnus*, cattle, epidemiology.

INTRODUCTION

Haemophilus somnus is known to be an etiological agent in several disease sindromes of cattle including thromboembolic meningoencephalitis (Griner et al. 1956; Pritchard et al. 1979), respiratory disease (Canto et al. 1983; Bryson et al. 1990), abortion and possibly infertility (Humphrey et al. 1982a; Stuart 1990). In adition to being an important pathogen, this organism can be routinely isolated from mucosal surfaces of apparently normal animal. Most bulls carry the organism asymptomatically in the prepuce, many cows are vaginal carriers, and nasal carriers also occur (Humphrey et al.

1982b; Garoiu et al. 1982; Stefaniak 1993). Setting *Haemophilus* diagnosis is very complicated, and depends on combining the information from the clinical and laboratory investigations. Isolation of *H. somnus* is possible only under specific conditions, selective media and in atmosphere in 5% or 10% CO_2. Besides this, bacteria isolation itself is not a sure sign of pathogen influence, having in mind different virulence of the isolated strains. Certainly, in detecting of this disease serological methods would be of great help. First of all because of their variety in epizootiological investigations. Opinions about the values of the agglutination method are not the same, first of all when the degree and the length of immunological response is at stake. Besides this, serological methods are not standardized both concerning antigen and height of antibody titer that are evaluated as positive.

A great number of countries in the world have confirmed existing of this disease. It is realistic to expect its presence in our country as well. Up to now there have not been done investigations on the presence of infection with *H. somnus* in cattle, nor in the breeder bulls for artificial insemination.

MATERIAL AND METHOD

Material: serological investigation included 93 sera from breeder bulls, 167 sera samples of the cows with reproductive disturbances (abortion, stillborn calves, repeated estrus, endometriosis and others). Anamnestic information was received from the veterinary service. Besides this, blood sera from 865 cows, 242 heifers, 294 young bulls and 117 calves were examined. In the sera investigation samples were freely chosen from the field.

Methods: For proving agglutinin in blood sera of bulls and cows with reproductive disturbances only two agglutination methods have been applied so far: tube agglutination test (TAT), microagglutination (MA) and m-CF test. For every procedure an appropriate antigen was applied. The antigens were prepared out of the referential strain *H. somnus* 628 CAPM-Brno, The Czechs Republic.

a) tube agglutination test, modified method according to Shigidi and Hoerlein (1970). *Antigen*: we used 48 hour old culture of *H. somnus* containing blood agar (10% bovine blood) incubated at 37°C in 10% CO_2. Grown colonies were rinsed with buffered physiological solution pH 8,00 and than filtrated through fire. The suspension was inactivated for 1 hour at 60°C in a water bath. Bacterial suspension was afterwards centrifuged at 4500 r/m for 30 minutes, and the residue was suspended with 1% phenol-bufferphisiological dilution. This „shock" antigen may be used for 6 months if stored at +4°C. For investigation it was used diluted with 0,5% phenol-PBS that had optical density 0,39 at 550 nm.

Method: reaction is performed in tubes (13x130) that were prepared with double diluted inactivated sera with 0,5% phenol-PBS where 0,5 ml antigen was added and then incubated for 48 hours at 37°C. The end titer was marked as 50% agglutination (clear supernatant and sediment at the bottom of the tu-

be). Positive result was considered a finding of agglutinin in diluted sera from 1:80 upwards.

b) microagglutination: the procedure was performed in a microtitar plate with the bottom shaped as letter „V".

Antigen: For applying antigen we used 24 hours old culture of *H. somnus* developed on the brain-heart agar containing 10% calf fetal sera and 0,5% yeast extract, at 37°C with 10% CO_2. Colonies were rinsed with 0,6% formaline-phisiological solution and filtrated over layers of sterile gauze. We used antigen diluted with PBS that has optical density of 0,75 on 550 nm, died in methyl blue (0,1% dilution). Antigen was conserved for 6 months at +4°C adding thimerosal in the final concentration 1:10000.

Method: two time soluted sera was prepared that had previously been inactivated for 30 minutes at 56°C in PBS pH 8,0 (0,025 ml) adding 0,025 ml of antigen. Microplates were shaken and incubated for 4 hours at 37°C, and then stored over night at +4°C.

In sera investigation several kinds of control were present: positive and negative sera and control of antigen. Findings of agglutinin in sera dissolved in proportion 1:64 and higher were considered positive.

c) Complement-Fixation test (CF): applied micro method in microtitar plates with the bottom shaped as letter „U".

Antigen: as an antigen a 48 hours old culture of *H. somnus* was multiplied on the brain-heart agar containing 10% calf fetal sera and 0,5% yeast extract at the temperature of 37°C with 10% CO_2. Colonies were rinsed with physiological dilution, and bacteria suspension was centrifuged at 6000 r/m for 20 minutes. Clear supernatant in solution 1:20 was used in a reaction, and pH values were adjusted on 7,3.

Method: Investigated sera were inactivated for 30 minutes at the temperature of 58°C, and then prepared with Veronal-bufferphisiological solution in proportion 1:2 to 1:256. In the dissolved sera (0,025 ml) the same complements and antigens were added in appropriate titer. The plate was shaken and incubated for 20 hours at +4°C. Equal parts of hemolizin in titer and 2% ram erythrocyte (0,05 ml) were mixed and than incubated for 30 minutes at 39°C. Afterwards the results were read. In the work the following control was present: positive and negative sera, control of sera and antigens. Findings of antibodies in sera solution 1:8 and higher was considered positive.

RESULTS

The results of serological investigations are presented in Tables 1, 2, 3, 4 and on the Graphics 1, 2. Control investigation applied in the agglutination procedure preceded these investigations together with appropriate antigens and reactions of the bounded complements (mRVK), that had been performed on rabbit sera (Table 1). It can be seen from the table that the antigens for *H. somnus* were proved by this method, although in the procedure of agglutination higher level of antigen was present, comparing to the antibodies that bound complement.

Tab. 1 — Titer agglutinin and CF-antibodies in rabbit bloodsera immunized with strain 6280 *H. somnus*

method	antibody titer		
	rabbit 1	rabbit 2	rabbit 3
Tube agglutination	1 : 320	1 : 160	1 : 320
Microagglutination	1 : 512	1 : 512	1 : 1024
micro CF	1 : 32	1 : 16	1 : 64

The results of the investigated bull blood sera are shown in Table 2. By the method of agglutination positive results were registred in 2.15% of the investigated sera, and by the method of mRVK in 4.3% of the samples. The highest positive percentage was by the method of microagglutination, 9.68%, when was also the highest antibody titer, up to 1:128.

Tab. 2 — Findings of agglutinin and CF-antibodies in bull blood sera

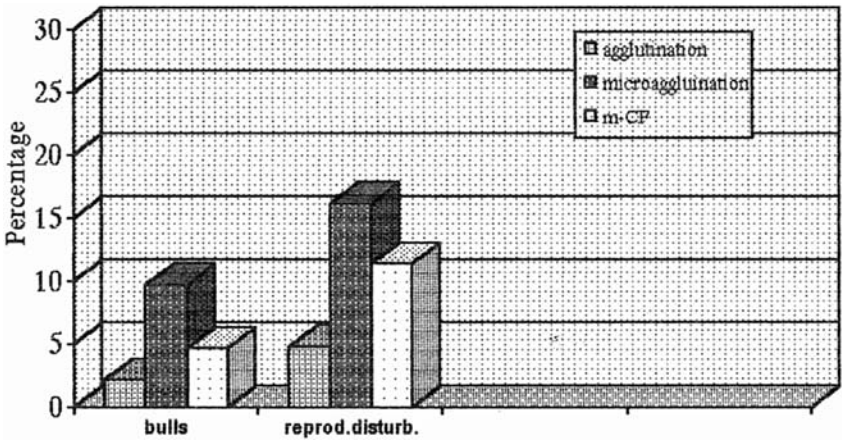
method	tube agglutination						total	positive
titer	≤10	20	40	80	160	320		
No. of anim.	74	14	3	2	0	0	93	2
%	79.57	15.05	3.23	2.15	0	0		2.15
method	microagglutination							
titer	≤4	8	16	32	64	128		
No. of anim.	25	17	23	19	6	3	93	9
%	26.88	18.28	24.73	20.43	6.45	3.23		9.68
method	micro CF							
titer	≤4	8	16	32	64	128		
No. of anim.	89	4	0	0	0	0	93	4
%	95.70	4.30	0	0	0	0		4.63

In Table 3 are shown the results of investigating blood sera from cows with reproductive disturbances. It is obvious that the highest percentage of the positive findings has been obtained by the method of microagglutination 16.18%, but considerably lower percentage of positive findings was obtained by the method of spore agglutination (4.79%). Analyzing these results of the bull blood sera and blood sera from cows with reproductive disturbances (Table 2, 3 and Graph. 1) it has been noted that by the method of microagglutination the greatest number of positive findings and the highest values of titer antibodies were established.

In accordance to this statement, the method of microagglutination was used for serological investigation of cattle sera from larger area. This investigation includes 865 cow sera, 242 heifer sera, 294 steer sera and 117 calf samples. Agglutinin for *H. somnus* was discovered in all the categories of cattle. It is important to state that the highest positive percentage was found in the steers (18.03%), while the antibody titer was in the range from 1:4 to 1:512. Positive results were proven in 84 cow's sera sample (9.71%), was considerably lower in heifer (5.78%) and was the lowest in the calves — only in 2 sera samples (1.71%).

Tab. 3 — Findings of agglutinin and CF-antibodies in blood sera from cows with reproductive disturbances

method	tube agglutination							total	positive
titer	10	20	40	80	160	320	640		
No. of anim.	83	51	25	7	1	0	0	167	8
%	49.70	30.54	14.97	4.19	0.60	0	0		4.79
method	microagglutination								
titer	4	8	16	32	64	128	256		
No. of anim.	32	30	41	37	20	3	4	167	27
%	19.16	17.96	24.55	22.15	11.98	1.80	2.39		16.18
method	micro CF								
titer	4	8	16	32	64	128	256		
No. of anim.	148	14	4	1	0	0	0	167	19
%	88.62	8.38	2.39	0.60	0	0	0		11.38

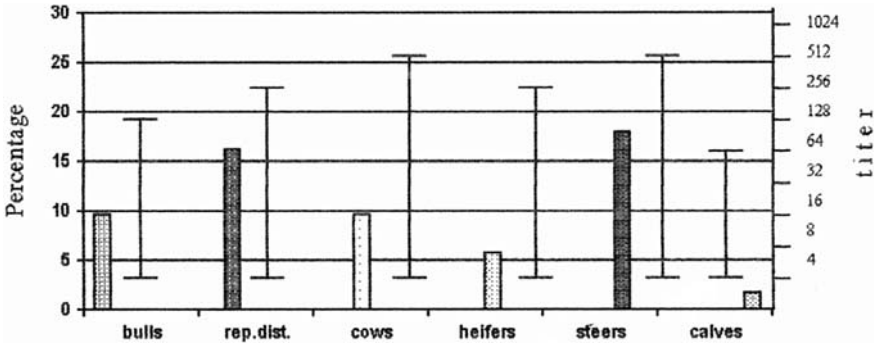


Graph. 1 — The results of investigated sera from bulls and cows with reproductive disturbances when three methods were applied

Tab. 4 — Findings of agglutination against *H. somnus* in cattle sera from different categories

category	titer 1:								Σ	+
	4	8	16	32	64	128	256	512		
cows	214	166	217	184	49	21	13	1	865	84
%	24.74	19.19	25.09	21.27	5.66	2.43	1.50	0.11		9.71
heifers	48	89	52	39	13	0	1	0	242	14
%	19.83	36.78	21.49	16.11	5.37	0	0.41	0		5.78
steers	55	74	60	52	31	17	4	1	294	53
%	18.71	25.17	20.41	17.69	10.54	5.78	1.36	0.34		18.03
calves	34	46	21	14	2	0	0	0	117	2
%	29.06	39.32	17.95	11.97	1.71	0	0	0		1.71

If we compare the results of serological investigation obtained by applying the method of microagglutination in beef, we can (having in mind the established groups of animals) conclude the following: it is obvious that the steers in a feed lot and the cows with reproductive disturbances have the highest percentage of positive findings. In cows and bulls percentage of positive findings is similar or the same, but at the same moment it is almost two times lower comparing to two other categories of animals (Graph. 2).



Graph. 2 — Findings of investigations of blood sera from diferent cattle categories by the method of microagglutination

DISCUSSION

For proving specific antibodies in animals, when it is the case with natural and artificial infection with *H. somnus*, several different serological methods can be applied: complement-fixation test (Canto et al. 1983; Yarnall, Corbeil 1989), indirect hemagglutination (Miller 1975), precipitation, latex test and different agglutination actions (Hoerlein et al. 1973; Stephens et al. 1981; Koblet et al. 1989). Since *H. somnus* shows cross-reaction with *P. multocida, P. haemolytica* and *Haemophilus agni*, but in low titre, this has to be taken into consideration when reading the results (Miller 1975).

Antigens of *H. somnus* are not known to be shared with other bacteria pathogenic for cattle. The agglutination test appears to be quite specific and the titres may be taken to reflect an antibody response due to exposure to the organism. It is considered that significantly high titre may be found in individuals of numerous groups of apparently healthy cattle, adding repport to the view that *H. somnus* infection of cattle is widespread and often not clinically apparent. Findings of antibodies for *H. somnus* in clinically healthy animals suggest that high level of antibodies in clinically ill samples is a consequence of secondary immunological response. The sporadic occurrence of clinical disease attributable to this organism must, therefore, depend on circumstances that are concomitant to the infection. Thromboembolic meningoencephalitis is an acute disease in which the clinical illness has a short course and is quickly

terminated by death due to the massive destruction of brain tissue. Antibody titre may reach values up to 1:400.

The occurrence of high serum antibody titre in cattle with septicaemia may simply be a consequence of fulminant infection stimulating a maximum immune response. It may be a clue to the pathogenesis of TEME. The occurrence of bacteriemia in the face of high serum antibody titre indicates that the host's phagocyte systems are no longer functioning effectively. Antibody may react with the antigen in the blood to form antigen-antibody complexes that are not phagocytes, resulting in continually increasing amounts of circulating antigen and immune complexes. Therefore, according to Stephens et al. (1981) changes in blood vessels are a consequence of hypersensitive reactions of type III.

The results of investigating the level of antibodies in sera of artificially infected animals differ, and this is conditioned by the choice of applied serological method and by the applied manner of inoculating the agenses (Bryson et al. 1990; Koblet et al. 1989; Stuart et al. 1990). In experimentally provoked infection Stephens et al. (1981), when inoculation was subcutaney, detected high level of agglutinin and low level of complement-fixation test. However, in the experimentally caused infection, when done intravaginally, applied serological tests proved not to be enough sensitive and specific (Stuart et al. 1990). Poor immunological respond was found also in asimptomatic carriers, as a result of inadequate antigen stimulation of the immune system in the host. This is in correlation with the place of antigen on the surface of mycosis of the genital tract (Koblet et al. 1989). Yarnall et al. (1989) showed that for serological diagnosing of haemophilosis IgG_2 antibodies are important to be proved, because in this way one can separate infected from not infected animal, aimptomatic reservoirs and the sick animals. Beside the mentioned method, indirect immunofluorescent method has also been used (Pritchard et al. 1979). ELISA test is used for proving IgG, IgM and IgA antibodies, when preputial discharge and blood sera are investigated, and also for proving IgG and IgA in semen plasma (Canto et al. 1983; Stefaniak 1993).

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РАШИРЕНОСТ ИНФЕКЦИЈЕ СА *HAEMOPHILUS SOMNUS* У ГОВЕДА

Бранка М. Видић, Бранко М. Бјелајац*, Станко Ф. Бобош, Душан Б. Орлић
Научни институт за ветеринарство „Нови Сад”,
Руменачки пут 6, 21000 Нови Сад
* Ветеринарско-сточарски центар, Књаза Милоша 21, Бања Лука

Резиме

Haemophilus somnus је познат као етиолошки узрочник неколико синдрома у говеда, тако да се могу разликовати три основна облика болести: септикемич-

ни, респираторни и генитални облик. Хемофилоза је заразна болест говеда, која захвата све категорије животиња и представља веома значајан здравствени и економски проблем. Велик број земаља у свету потврдио је постојање ове болести. Постављање дијагнозе хемофилозе је сложено, а заснива се на комбиновању података добијених клиничким и лабораторијским испитивањима. Изолација *H. somnus* могућа је само у посебним условима уз коришћење одговарајућих селективних подлога, тако да су серолошке методе од користи пре свега због могућности ширих епизоотиолошких испитивања. Досад у нашој земљи није испитивано присуство ове заразне болести, али је реално очекивати да је хемофилоза говеда присутна и у нас.

Серолошким испитивањем обухваћено је 93 узорка серума бикова и 167 серума крава са репродуктивним сметњама. Поред тога испитано је и 865 серума крава, 242 серума јуница, 294 јунади у тову и 117 серума телади, одабраних по слободном избору, са ширег подручја Покрајине. За доказивање антитела у серумима користили смо два поступка аглутинације (макро и микро) и mRVK. Титар аглутинина у серумима бикова кретао се од 1:8 до 1:128, а највећи проценат позитивних налаза добијен је методом микроаглутинације, 9,68%. Код крава са репродуктивним сметњама утврђено је 16,18% серопозитивних методом МА, а применом mRVK 11,38%. Значајно је истаћи да је највећи проценат позитивних налаза утврђен код јунади у тову (18,03%) са титром и до 1:512. Позитивни резултати установљени су код 84 узорка серума крава, или 9,71%, знатно нижи код јуница 5,78%, а код телади само у 2 испитана узорка серума или 1,71%.

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Ester J. Popović, Olivera N. Bjelić-Čabrilo, Ružica M. Mićić

Institute of Biology, Faculty of Natural Sciences
University of Novi Sad, Trg Dositeja Obradovića 2
21000 Novi Sad, Yugoslavia

HOST-INDUCED MORPHOLOGICAL VARIABILITY OF *PLEUROGENOIDES MEDIANS* (OLSON, 1876) TRAVASSOS, 1921 (TREMATODA: *LECITHODENDRIIDAE*)

ABSTRACT: Morphological variability of the frog trematoda *Pleurogenoides medians* in terms of host-induced variation was studied. Morphometric analysis included 90 adult parasites (*P. medians*) isolated from intestines of *Rana ridibunda; R. kl. esculenta* and *R. lessonae* from Obedska bara. The dimensions of body, oral and ventral suckers, ovary, testes and eggs were compared.

The host species has a large influence on the structure of adult trematodes. Largest differences were found in *P. medians* dimensions between marsh and pool frogs, lowest between the edible and pool frog specimens.

KEY WORDS: Trematoda, morphological variability, *Rana*, Obedska bara.

INTRODUCTION

Pleurogenoides medians is a well-known species, for a long time being frequently noted in various parts of Europe. According to Vojtkova (1974), it is a common intestinal parasite of Amphibia and certain lizards. Its distribution area probably covers that of its main host-frogs from the genus *Rana (R. lessonae, R. ridibunda, R. kl. esculenta).*

Importance of variability of morphological characters of trematodes has been frequently noted, but not studied enough. In Poland, Grabda-Kazubska (1967, 1981, 1984) analyzed the variability of the species *Opisthioglyphe ranae, Haplometra cylindracea* and *Prosotocus mirabilis*. In Yugoslavia, Popović et al. (1995) conducted a detailed morphometric analysis of the trematode *Pleurogenoides medians* isolated from the pool frog. The hosts were collected from three different types of aquatic ecosystems: pools, marshes and stagnant waters. Authors reported a strong influence of habitat conditions. In

the present study, attention is paid to morphological variability as dependent on host-induced variations.

MATERIAL AND METHODS

In the period 1987—1990, the sample of 134 individuals of *Rana lessonae* (57), *R. kl. esculenta* (56) and *R. ridibunda* (21) was collected from Obedska bara. Dissection and parasitological analyses of hosts, isolation and depositing of trematodes were done by standard methods.

Presence of 1.179 specimens of *Pleurogenoides medians* was recorded in the small intestine of the hosts. The morphometric analysis included 90 adults of *P. medians*, 30 from each frog species. The measurements involved: total length and maximum width of fluke body, length and width of oral and ventral suckers, ovary, left and right testes and egg dimension. Data were statistically analyzed (Petz, 1981): mean value (X), mean error (ME) and variation coefficient (VC). Also, minimum-maximum values were taken. Results were tested by the t-test at the level of 1% (P>0,01).

Features of Obedska bara: Obedska bara is a former meander of the Sava river. Together with adjoining areas, it makes a unique complex of water, marsh and land ecosystems with pond-marsh vegetation *Scirpo-Phragmitetum* W. Koch (1926), floating *Nymphaeo-Stratiotetum aloidi* and submerged community *Ceratophyllo-Myriophylletum verticiliati* (acc. to Janković, 1974), and forest community *Fraxino angustifoliae — Quercetum roboris* B. Jov. et Tom. (1978/79).

RESULTS AND DISCUSSION

The results presented in this paper are based on the taxonomic characters of the parasites. As mentioned earlier, only mature trematodes were examined while young individuals undergoing rapid growth were excluded. Main body dimensions of *P. medians* from all examined hosts are given in Table 1.

A significant diversity was found in the body shape. Lancet-shaped specimens were encounted most often and were characteristic for *Rana ridibunda* and *R. kl. esculenta* (Figures 1, 2). Trematodes isolated from *R. lessonae* are characterized by a rather short, slightly oval body (egg-shaped body) (Figure 3). The body shape proved to be dependent on various external and internal factors. First of all, the host species has a large influence on the development of trematodes. Popović et al. (1995) reported a strong influence of habitat conditions, presence of certain species of intermediate hosts, as well as the diet regime of the definitive host. The whole trematode body surface was covered with spines. The highest values of the measured characters, except for egg size, were obtained with a sample of marsh frog, the smallest with a fluke sample of pool frog. Trematodes find better development conditions in marsh frog, attaining in them more rapid growth, and more massive body and interior organs. Deviation of characters of parasites, related to pool frog, was obtained

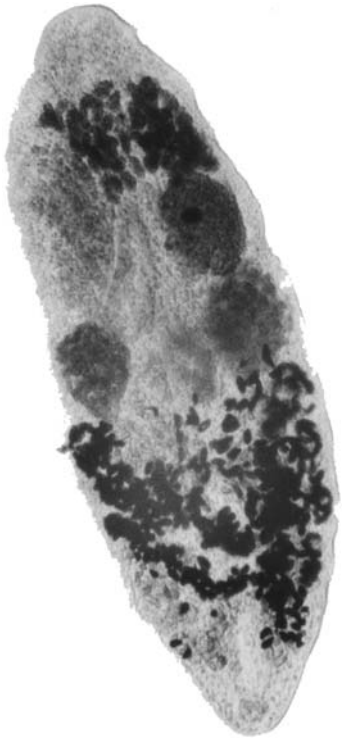

Fig. 1 — *Pleurtogenoides medians* from *R. ridibunda* (6.3 X 10 orig.)

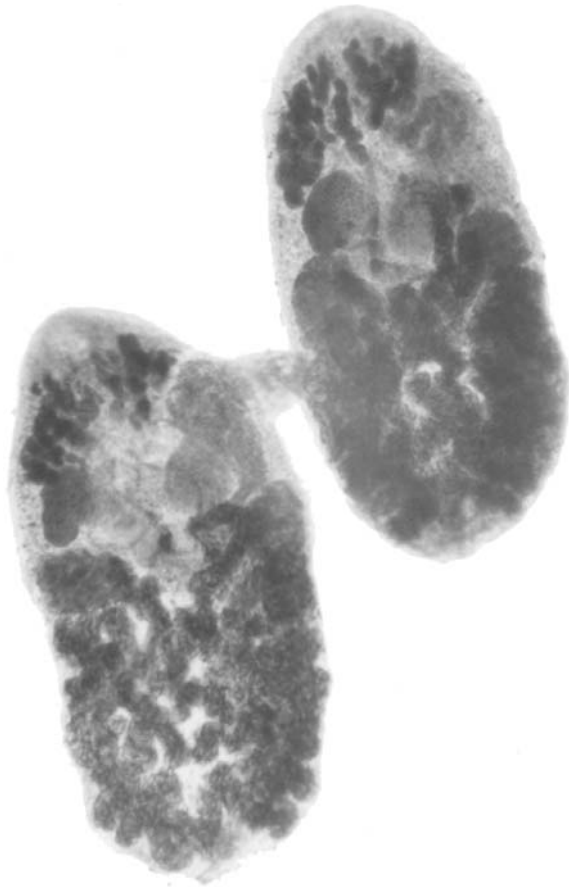

Fig. 2 — *Pleurogenoides medians* from *R. kl. esculenta* (6.3 X 10 orig.)

Fig. 3 — *Pleurogenoides medians* from *R. lessonae* (6.3 X 10 orig.)

only in the size of eggs, and related to edible frog, in the dimension of testes and eggs.

Comparing the results of measured characters of *P. medians* isolated from edible and marsh frogs, highly significant values were established for the width of ventral sucker, ovary and eggs and the length of body and testes (Table 1).

Tab. 1. — Results of the morphometric analysis of *P. medians* (t-test: significant and highly significant values)

		BODY DIMENSION		ORAL SUCKER	
		R. kl. esculenta	*R. lessonae*	*R. kl. esculenta*	*R. lessonae*
R. Lessonae	Length	5.4203**		3.4575**	
	Width			5.7482**	
R. ridibunda	Length	3.7961**	10.9682**		13.4946**
	Width		3.3975**		4.5211**
		ABDOMINAL SUCKER		OVARY	
		R. kl. esculenta	*R. lessonae*	*R. kl. esculenta*	*R. lessonae*
R. lessonae	Length	3.2413**			
	Width	2.4740*			
R. ridibunda	Length	2.2152*	8.5657**	2.0558*	2.8027**
	Width	2.8137**	7.4006**	2.6932**	
		LEFT TESTES		RIGHT TESTES	
		R. kl. esculenta	*R. lessonae*	*R. kl. esculenta*	*R. lessonae*
R. lessonae	Length	2.4173*		2.0862*	
	Width	2.2390*			
R. ridibunda	Length	5.2404**	3.4585**	3.2804**	
	Width	2.3683*		2.1961*	
		EGGS			
		R. kl. esculenta	*R. lessonae*		
R. lessonae	Length	4.8000**			
	Width				
R. ridibunda	Length	2.0930*			
	Width	4.4333**	5.2500**		

Related to the measured characters of marsh and pool frogs, we obtained highly significant values for dimension of body, oral, ventral suckers, length of left testis, ovary and the width of eggs (Table 1).

Smallest differences were found between the edible and pool frog sample. Highly significant values for the dimension of oral sucker, body length, ventral sucker and eggs, were recorded (Table 1).

Highest values were obtained in the variation coefficient for the dimension of ventral suckers (pool, edible frogs), oral suckers (marsh frog) and ovary (marsh frog), lowest for the width of eggs (Table 1). According to Grabda-Kazubska (1967), the dimension, shape and shell thickness of

eggs are characteristic of particular trematode specimens and are independent of their age and host species.

A detailed morphometric analysis of *P. medians* samples, collected from three different host species, showed highly significant differences. Specific conditions existed among the species of green frogs. They were associated with the physiology of the host, which has a large share in the formation of morphological characteristics of the parasite. The structure and the length of host intestine could influence the development of trematodes. Also, interpopulation relations among *P. medians*, as well as the influence of other species of intestinal parasite of hosts, may play an important role.

CONCLUSIONS

A detailed morphometric analysis was conducted on 90 specimens of the parasite *Pleurogenoides medians* isolated from *Rana ridibunda, R. kl. esculenta* and *R. lessonae* from Obedska bara.

This paper is the first report on this problem from the region of Yugoslavia.

A significant diversity in body shape was found.

The highest values of the measured characters, except for egg size, were obtained with the sample of marsh frog, the smallest with the sample of pool frog.

Highly significant differences were established in all measured characters.

The highest variability was observed in the dimensions of suckers and ovary, while the length of eggs was the most stable characteristic.

The host species has a large influence on the development of trematodes.

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УТИЦАЈ ВРСТЕ ДОМАЋИНА НА МОРФОЛОШКУ ВАРИЈАБИЛНОСТ ВРСТЕ *PLEUROGENOIDES MEDIANS* (OLSON, 1876) TRAVASSOS, 1921 (*TREMATODA: LECITHODENDRIIDAE*)

Естер Ј. Поповић, Оливера Н. Бјелић-Чабрило, Ружица М. Мићић
Институт за биологију, ПМФ, Универзитет у Новом Саду,
Трг Доситеја Обрадовића 2, 21000 Нови Сад, Југославија

Резиме

Трематода *Pleurogenoides medians* је чест паразит водоземаца и извесних врста гуштера. Значај варијабилности морфолошких карактера трематода није довољно проучаван и на подручју Југославије Поповић et al. (1995) су указали на снажан утицај станишта.

Анализом морфолошких карактера 90 адултних примерака паразита *P. medians* изолованих из интестинума врста *Rana ridibunda, R. kl. esculenta* и *R. lessonae* Обедске баре, аутори су уочили разлике у облику и димензијама тела и установили велик утицај врсте домаћина на развиће трематода. Највеће вредности, сем за димензије јаја, констатоване су за паразите велике зелене жабе, а најниже међу трематодама које су инфестирале примерке мале водене жабе. Утврђене су високо сигнификантне разлике за све мерене карактере. Димензије пијавки и јајника су најваријабилнији карактери, док је дужина јаја била најпостојанија.

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Author CD, Author DC (1990) Title of article. *Plant and Soil* 135: 102—134.

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Author VA (1989) *Title of thesis*. PhD Thesis. University, City.

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Author AE (1987) *Title of the book*. Publishers, City, pp 237.

e. No authors or editors

Title of booklet, pamphlet, etc. (1989) Publisher of company, City.

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6.3. Biological names in Latin should be italicized (underlined).

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3.5. Ако се наводе два или више радова истог аутора, објављених у истој години, потребно је у тексту и списку литературе ставити a, b, c итд. иза године објављивања.

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4.1. За илустрације могу се користити црно беле фотографије и цртежи. Фотографије треба да имају добар контраст а цртежи треба да буду цртани тушем, на папиру доброг квалитета. Осим графикона, метабо-

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4.3. Илустрације треба приложити уз рад а не уметнуте у текст. По могућности, легенде треба назначити на илустрацијама.

4.4. Места илустрација треба означити на левој маргини, арапским бројевима.

4.5. Свака илустрација треба да има текст који објашњава садржај прилога. Текст за илустрације треба куцати на посебној страни.

5. Табеле

5.1. Табеле треба куцати на одвојеним странама (једна табела по страни) и приложити их на крају рада.

5.2. Табеле се означавају арапским бројевима.

5.3. Свака табела треба да почне насловом који објашњава њен садржај.

5.4. Напомене треба куцати одмах испод саме табеле.

5.5. Места табела у тексту треба означити на левој маргини.

6. Јединице, имена, формуле и скраћенице

6.1. Треба користити SI ознаке количина и јединица (SI Systeme International d'Unit's), изузетно се могу користити и друге званично прихваћене јединице.

6.2. Моларну концентрацију треба означити са M и подвући.

6.3. Биолошка имена на латинском треба подвући.

6.4. Хемијске структурне формуле и једначине треба нацртати (не исписивати или куцати), и припремити за фотографску репродукцију.

6.5. Прихватају се само стандардне скраћенице. При коришћењу специјалних скраћеница, пун термин треба навести приликом првог спомињања, а скраћеницу додати под наводним знацима.

6.6. Математички изрази треба да буду написани тако да се користи најмањи број редова, али да се сачува читљивост, нпр. 2/3 уместо 2:3, exp (-ab) уместо a-ab, итд.

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7.2. Величина кратког саопштења је ограничена на 4 куцане стране, укључујући све илустрације.

7.3. Кратко саопштење се пише по упутствима за припрему рада нормалне дужине, сем што у литератури треба изоставити наслове рада.

8. Обавештавање аутора

8.1. Када рукопис буде прихваћен, аутор ће бити писмено обавештен о приближном времену објављивања.

8.3. Исправљање текста припремљеног за штампу треба ограничити на штампарске грешке. Значајне промене текста ће се наплаћивати. Кориговани текст треба вратити уредништву у најкраћем могућем року.

8.3. Аутори добијају 50 бесплатних примерака сепарата. Ако аутор жели већи број сепарата може их наручити код издавача уз надокнаду.

9. КОПИЈА РАДА НА ДИСКЕТИ

После прихватања рада потребно је доставити дискету са коначном верзијом рада. Дискета треба да садржи текст рада, табеле и слике (прилоге) на DD или HD дискети од 3,5 инча. Приложите и једну копију одштампаног рада ради лакше обраде табела и слика. Молимо да обраду текста вршите програмом Word for Windows (било која верзија). Приликом копирања рада на дискету, придржавајте се следеће процедуре: File> Save as>Options>Embed True Type fonts>ok>Save.