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EFFECT OF HERBICIDES ON MICROBIOLOGICAL PROPERTIES OF SOIL

ABSTRACT: Microorganisms decompose herbicides and they may serve as bioindicators of soil changes following herbicide application. Certain microbial species may be used as bioherbicides. This study has shown that *Azotobacter* is most sensitive to herbicide application; it is, therefore, a reliable indicator of the biological value of soil. The numbers of this group of nitrogen-fixing bacteria decrease considerably in the period of 7—14 days after herbicide application. Simultaneously, the numbers of *Actinomycetes* and less so of fungi increase, indicating that these microorganisms use herbicides as sources of biogenous elements. Rate of herbicidal decomposition depends on the properties of the preparation applied, herbicide dose as well as on the physical and chemical soil properties, soil moisture and temperature, ground cover, agrotechnical measures applied and the resident microbial population.

KEY WORDS: microbes, herbicides, bioindicators, inoculants

INTRODUCTION

In modern agricultural production, herbicide application is a regular practice. While in developed countries weeds and pests reduce yields of agricultural crops from 15 to 20%, reductions soar to 50% in undeveloped regions (Dobrovoljskiy and Grishina, 1985). The problems caused by the increased application of herbicides call for multidisciplinary approach. Incorrect and indiscriminate application of herbicides affects negatively the health of humans, plants and animals. Particularly hazardous are the poorly degradable herbicides (triazins) whose persistence may lead to long-term accumulation.

Soil microorganisms are an important link in soil-plant-herbicide-fauna-man relationships. They take part in herbicide a) degradation, their activity, number and diversity may serve as b) bioindicators of changes in soil biological activity following herbicide application and, finally, some microbial species may be used as c) bioherbicides.

Herbicides become incorporated in soil directly, during plant treatment, and indirectly, via water or residues of plant and animal origin. After application, herbicides may evaporate (volatilize), may be washed away through surface run-off, may leach into deep soil strata and ground water, may be inactivated by plants, or may be adsorbed in soil in which case they become subject to chemical or microbiological degradation.

Herbicides are specific regarding their toxic level. However, the application of several chemicals may lead to synergy and development of toxic effects hazardous for humans and the ecosystem (Michaelidou et al., 2000). Herbicides may cause acute and genetic toxicity which are perilous for the biota inhabiting the ecosystem. The halflife of various herbicides ranges from 9 to 116 years. It means that in soil without microorganisms herbicide application would threaten all living things with unforeseeable consequences (Vrochinskiy and Makovskiy, 1979). The European Union has opted for sustainable agriculture, reduced pesticide use and monitoring of acute and genetic toxicity for the ecosystem (Petsikos-Panagiotarou, 2000). Rate of herbicide decomposition in soil depends on the properties of the preparation applied (Mishustin and Emtsev, 1987), herbicide dose (Schuster and Schröder, 1990; Milošević et al., 2001), physical and chemical soil properties (Willems et al., 1996; Miličić, 1987), humidity, temperature, plant cover, soil cultivation technique and the types of the soil microorganisms present (Barriuso and Houot, 1996; Govedarica et al., 1993, 2000; Willems et al., 1996; Milošević et al., 2000a, 2001).

HERBICIDE DEGRADATION BY MICROBES

Herbicide degradation in soil may be photochemical, chemical or microbial in nature. While photochemical decomposition predominates in air and water, only a small percentage of pesticides is decomposed in that way in soil. Chemical decomposition of herbicides in soil evolves through hydrolytic and non-hydrolytic transformations and oxidation. Microorganisms are efficient decomposers of aliphatic and hydroxyl compounds, but they decompose aromatic substances at a slower rate. The compounds that contain oxygen, sulfur or nitrogen in the ring are slowest to decompose (Janjić et al., 1996).

According to Lynch (1983), microbes degrade herbicides in the course of metabolic (when adaptation phenomena take place) and cometabolic processes. New compounds are formed from herbicide metabolites.

In general, herbicides affect microbes indirectly, causing physiological changes, increased enzymatic production or, when applied in high doses, death of susceptible groups of microorganisms (Cervelli et al., 1978). Soil microbiological population uses herbicides and their metabolites as sources of biogenous elements (Cook and Hutter, 1981; Radosevich et al., 1995). It has been noticed that certain groups of microorganisms (primary population) start to decompose herbicides a few days after their arrival. On the other hand, the so-called secondary population, which produces induced enzymes, decomposes herbicides while these are passing through a period of adaptation. Some

microbial groups are indifferent to herbicide application (Figure 1). Long-term application (19 years) of glyphosate reduces C biomass in soil, but ammonification and nitrification are increased compared with untreated soil (Hart and Brookes, 1996).

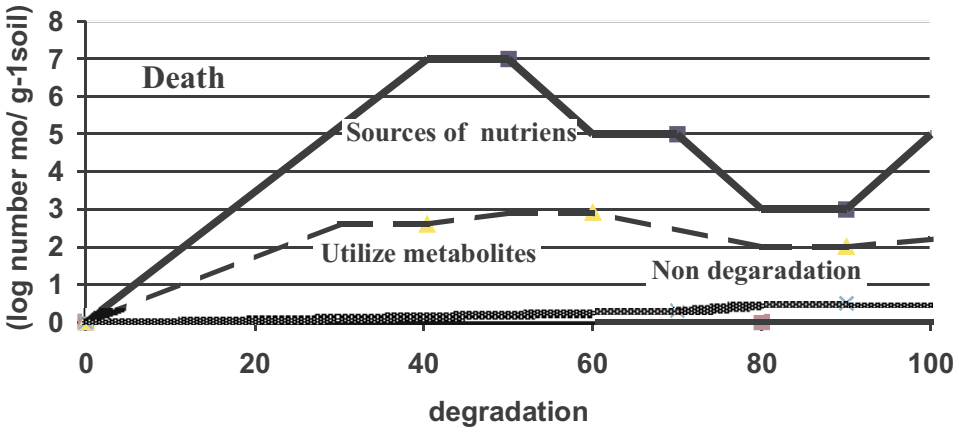


Fig. 1 — Effect of herbicides on soil microbiological population

Studies of numerous authors (Lynch, 1983; Radosevich et al., 1995; Milošević et al., 2001) show that herbicide-decomposing microorganisms belong to bacteria and fungi: *Arthrobacter, Pseudomonas, Bacillus, Actinomycetes, Mycoplana, Agrobacterium, Corynebacterium, Arthrobacter, Flavobacterium, Nocardia* and *Trichoderma*. Effect of herbicides on the composition and morphology of soil microbial population depends on the composition and dose of herbicides applied but also on the kind of microorganisms present (Mišković et al., 1983; Milošević et al., 2001). In general, herbicides affect soil microbes indirectly. Herbicides may be a source of nutrition for microbes (Cook and Hutter, 1981), in which case they significantly affect microbial growth and multiplication. However, herbicides also affect the microbes physiologically: a) by changing their biosynthetic mechanism (a change in the level of protein biosynthesis is reflected on the ratio of extracellular and intracellular enzymes); b) by affecting protein biosynthesis (induction or repression of synthesis of certain enzymes); c) by affecting the cellular membranes (changes in transport and excretion processes); d) by affecting plant growth regulators (transport of indolacetic acid, gibberellin synthesis and ethylene level); e) applied in high doses, they may kill microorganisms.

FACTORS AFFECTING MICROBIOLOGICAL DECOMPOSITION OF HERBICIDES

Rate of herbicide decomposition in the soil is influenced by the properties of the preparation applied, its dose as well as by the physical and chemical soil properties, soil moisture and temperature, plant cover, soil cultivation met-

hod and kinds of microorganisms present (Miličić, 1987; Schuster and Schröder, 1990; Radosevich et al., 1995; Milošević et al., 2001).

Herbicide properties

According to their structure, herbicides may be carbonic acids and their derivatives, aryloxyalkyl carbonic acids and their derivatives, carbamic acid derivatives, carbamide derivatives, thio- and dithiocarbamic acid derivatives, dipyridil derivatives, nitrophenols, nitroanilines or heterocyclic compounds comprising nitrogen in the ring (diazin and triazin). Toxicity, persistence and selectivity are important characteristics of herbicides (Konstantinović et al., 1998, 1999).

Table 1 — Period of herbicide degradation in soil (Konstantinović, 1999)

1—3 months	3—6 months	more than 12 months
Aminotriazol	Butan	Atrazine
Aziprotryne	Chlorbromuron	Bromacil
Carbetamide	Chloridazone	Chlortiamid
Chlorpropham	Chlortal-dimethyl	Dichlorbenil
Cyanazine	Chlortoluron	Lenacil
Dalapone	Cikloat	Methazone
Prometryn	Dinitramin	Metribuzin
Propahlor	EPTC	Napropamid
Propham	Etophumesat	Oksadiazon
Terbutrin	Izoproturone	Fenmedipham
2,4-D	Linuron	Pikloram
MCPA	Methamitron	Propyzamide
Dichloprop	Matolachlor	Simazine
	Mathobentiazuron	Terbacil
	Metabromuron	Trifluralin
	TCA	Imidazolinone
	Trietazin	Sulfonylurea

Regarding their degradation period, pesticides may be divided in two groups: a) residual, with long toxic action and b) contact, with short toxic action. A study of Barriuso and Houot (1996) showed that simazine mineralizes faster than atrazine. A hormone herbicide 2,4-D decomposes in soil very fast. It is decomposed by several microorganisms: *Mycoplana, Corynebacterium, Achromobacter, Rhizobium, Arthrobacter, Flavobacter* and some actinomycetes (Lynch, 1983).

Herbicides dose

Increase in herbicide dose tends to amplify its negative effect on microorganisms. High herbicide concentrations reduce the number of nodules in symbiotic nitrogen-fixing microoorganisms, nitrogenase activity microorganisms, dry matter in plants, lysis of bacteroids, and inhibition of ATP synthesis (Govedarica et al., 1993; Konstantinović et al., 1998; Milošević et al., 2001).

A dose of 1.6 l. ha^{-1} of dimethenamide (Frontier) caused larger reductions of the total number of microorganisms and azotobacters by 5—7% and 2—18%, respectively, than a dose of 1.4 l. ha^{-1} (Milošević et al., 2001a). A dose of 2 l. ha^{-1} of flumetsulam + trifluralin (Rival) caused a larger reductions of *Azotobacter* by about 2% than a dose of 1.7 l. ha^{-1} (up to 30 days) (Milošević et al., 2000a; Govedarica et al., 2001). Simultaneously, the larger dose of Rival increased the numbers of fungi and actinomycetes by 2—4% and 1%, respectively (Milošević et al., 2001a). Increased doses of dimethenamid (1.6 l. ha^{-1}) and metolachlor (1.7 l. ha^{-1}) caused larger reductions in the number of azotobacters than lower doses (Govedarica et al., 2001).

High doses of atrazine and alachlor (3 and 4 l·ha^{-1}, respectively) caused decreases in the total number of bacteria, ammonifiers and azotobacters and they reduced dehydrogenase activity (Konstantinović et al., 1999).

Under laboratory conditions, a normal dose of glyphosate inhibited DHA by 5—10% (3 weeks after herbicide application). A tenfold dose of glyphosate affected negatively the activity of this oxide-reducing enzyme by 5% (11 weeks after herbicide application) (Schuster and Schröder, 1990).

Physical and chemical soil properties

Pesticide adsorption or desorption depends on the physical and chemical soil properties. The process of adsorption depends on the concentration and solubility of herbicides in soil solution, ion exchange capacity, organic matter content, pH, moisture and temperature of soil, etc. Soils with heavy mechanical composition have a higher pesticide-adsorbing capacity than light (sandy) soil. Studies have offered different results regarding the rate of atrazine mineralization in soil. Atrazine mineralization is exceedingly low, which is an indication that this pesticide is very persistent. According to Willems et al. (1996), atrazine mineralization is slow and controlled by the amount of biomass and organic C, and it decreases with depth. Atrazine mineralization seems to be predominantly due to cometabolism rather than direct metabolism. Wolf and Martin (1975) stated that atrazine mineralization was 18% after 550 days, depending on physical and chemical soil properties. Klint et al. (1993) reported the value of 20% after 90 days. According to Willems et al. (1996), the amounts of 2,4-D mineralized in the soil layer between 1m and 1.5 m were generally high, exceeding on average four times the amounts mineralized in the topsoil. The authors hypothesized that the rapid mineraliza-

tion in deeper soil layers was probably due to complex interactions among microbial activity, microbial population structure, nutrition status and the physical and chemical properties of the soil.

Temperature and moisture

Increased moisture and temperature accelerate the degradation of atrazine and 2,4-D (Willems et al., 1996). The rate of atrazine mineralization is slow (< 2%), further decreasing in deeper soil layers, at low temperature and at low moisture content. Over a period of two and a half months, 10 to 80% of 2,4-D were mineralized, depending on the depth of soil profile.

Cultural practices

In well-tilled and loose soil that contains much oxygen, the rate of herbicide degradation is accelerated because of dynamic microbiological processes taking place in it. In uncultivated or excessively compacted soil, where anaerobic processes predominate, the rate of herbicide degradation is low. Methods of soil cultivation and herbicide application affected significantly the microbiological activity (Milošević et al., 1995, 1995a). Generally, chiseling and rototilling following glyphosate application tended to increase the numbers of soil microorganisms. Glyphosate application in bands reduced the number and enzymatic activity of soil microorganisms in relation to the broadcast application.

Application of NPK fertilizers reduced the degradation of the hormone herbicides 2,4-D and MCPA by 30—50%. Application of $CaCO_3$ considerably lowered the rate of herbicide degradation. No degradation could be registered 14 days after the application; on the 28th day, 2,4-D of MCPA were degraded by 29% and 45%, respectively (Burns, 1995).

Negligent use of heavy machines for soil tillage and sugarbeet transport under unfavorable weather conditions (rain and high soil moisture) may cause extensive soil structure deterioration and prolonged negative effects on subsequent crops (Milošević et al., 2001). Under unfavorable, i.e., anaerobic conditions, soil microorganisms produce metabolites which, synergizing with the applied herbicides, intensify their negative effects (Kosinkievicz, 1984a). Bacteria from the genus *Arthrobacter* sp., isolated from sugarbeet rhizosphere, produced phenolic compounds when exposed to soil and anaerobic conditions. These compounds intensified the phytotoxic effect of cycloate (Roneet) on the crop planted after sugarbeet (Kosinkievicz et al., 1984).

Plant cover

Plants play a role in pesticide decomposition. They incorporate pesticide via roots, stems and leaves. Pesticide dose and mode of application determine

how it is incorporated, by the aboveground plant parts or the roots (Kastori et al., 1996). Plant species affects the rate of mineralization of atrazine's triazine rings. Experiments of Barriuso and Houot (1996) showed that atrazine's triazine rings were mineralized faster in the soils under corn treated with this herbicide each year that in the untreated soils under wheat or grasses.

Milošević and Govedarica (2001) noted that prometryne application reduced some parameters of biological activity (total number of bacteria and the number of azotobacters) in the soil under soybean and sunflower. In the soil under soybean and sunflower, however, azotobacters could not be found 28 days after prometryne application while 45 days after the application its numbers reached up to 10.76×10^2 per one gram of soil.

Microbiological population

Pesticide decomposition is affected by the size and composition of microbiological population. Microorganisms are a highly heterogeneous group, including aerobes and anaerobes, heterotrophs and autotrophs or saprophytes, symbionts and parasites. Certain microbial species may decrease or increase the toxic action of herbicides. Most soil microorganisms are capable of decomposing herbicides, using them most frequently as sources of biogenous elements. Experiments have shown that microbes may use atrazine as a source of carbon (Radosevich et al., 1995) or nitrogen (Cook and Hutter, 1981). The hormone herbicide 2.4-D is rapidly decomposed in the soil, as much as 3.4×10^6 moles in a single day. Various microorganisms decompose 2.4-D: *Mycoplana, Corynebacterium, Achromobacter, Rhizobium, Arthrobacter, Flavobacter* and some actinomycetes (Lynch, 1983). Herbicides derived from the carbamic acid (phenmedipham, desmedipham) are decomposed microbiologically, chemically and photochemically. Among the fungi, *Rhizopus japanus, Aspergillus* ssp., *Penicillium* ssp. and *Metharizium anosoplie* are the most intensive decomposers. Among the bacteria, those are *Pseudomonas* sp. and *Bacillus* sp. According to Kosinkievicz (1984a), the phenolic compounds produced by *Pseudomonas acidovorans* may increase or decrease the phytotoxicity of lenacil (Venzar), the mode of action depending on herbicide concentration.

MICROBES: BIOINDICATORS AND INOCULANTS

The soil is a highly complex system. It is also dynamic, on account of microorganisms whose enzymes take part in most syntheses and decompositions. The number, enzymatic activity and biodiversity of microorganisms may serve as indicators of soil fertility, as well as indicators of all changes taking place in the soil as an ecological system (Milošević et al., 1997).

By means of adsorption, bioaccumulation and the production of metabolites, microorganisms are capable of mitigating or blocking the toxic action of herbicides (Janjić et al., 1996). Herbicides adsorb on cell surface, affecting

ion transport. They affect the metabolism inside the cell by binding to amino and sulfide groups. In the course of these processes, changes take place in the oxidoreduction level of soil and, depending on the chemical composition and dose of herbicide, the microorganism concerned may be killed. Bioaccumulation mitigates the toxic effect of herbicides.

Bioindicators

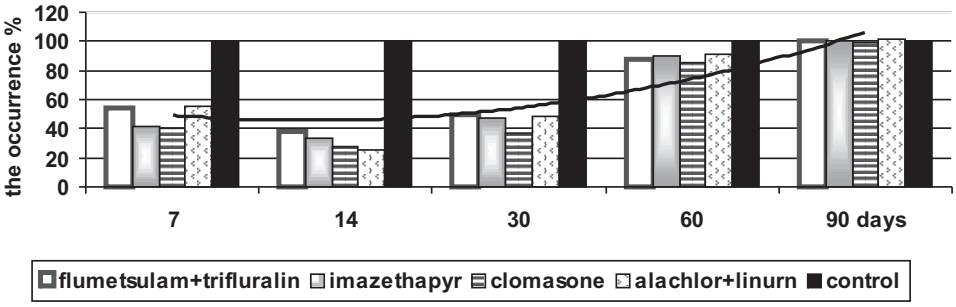
Application of pesticides and other chemicals used in agriculture affects the vital functions and population dynamics of soil microorganisms. Microorganisms are a heterogeneous group of organisms whose enzymatic systems comprise 60—90% of the total metabolic activity of the soil (Lee, 1994). Population size, enzymatic activity and biodiversity of certain systematic and physiological groups of microorganisms may serve as bioindicators of changes taking place in the soil following herbicide application (Milošević et al., 1995, 2001; Govedarica et al., 1993, 1995; Konstantinović et al., 1999). Results of our studies have shown that generally, herbicides tended to reduce the total number of soil microorganisms 7 to 30 days after application (Milošević et al., 2001).

Milošević et al. (1998, 2000a) reported that soybean treatment with flumetsulam + trifluralin (Rival), imazethapyr (Pivot), clomazone (Command) and alachlor + linuron (Linuron S-50) reduced the total number of microorganisms in the period of 14 days after application by 15 to 27% (Graph 1). After that period, the numbers of microorganisms in the treated variants reached the level of the control variant while on the 90th day after application the numbers of microorganisms in the variants with clomazone (Command) and alachlor + linuron (Linuron S-50) were increased in relation to the control (Milošević et al., 2000a).

Graph. 1 — Effect of herbicides on the total number of microorganisms

Nitrogen-fixing bacteria are important from the point of nitrogen balance in the soil and their reaction to herbicides is a good indicator of how effective an applied weed control action is.

Our studies have shown that *Azotobacter* is most sensitive to herbicide application (Milošević et al., 1995; 2000a, Milošević and Govedarica, 2000), and it may serve as a reliable indicator of the biological value of soil. Herbicide application reduced the number of azotobacters (Graph 2). The reduction was large in the period of 30 days after herbicide application. The largest rates of reduction occurred 14 days after application, 62% in the case of Rival and 78% in the case of Linuron S-50.



Graph. 2 — The effect of herbicides on the occurrence of *Azotobacter*

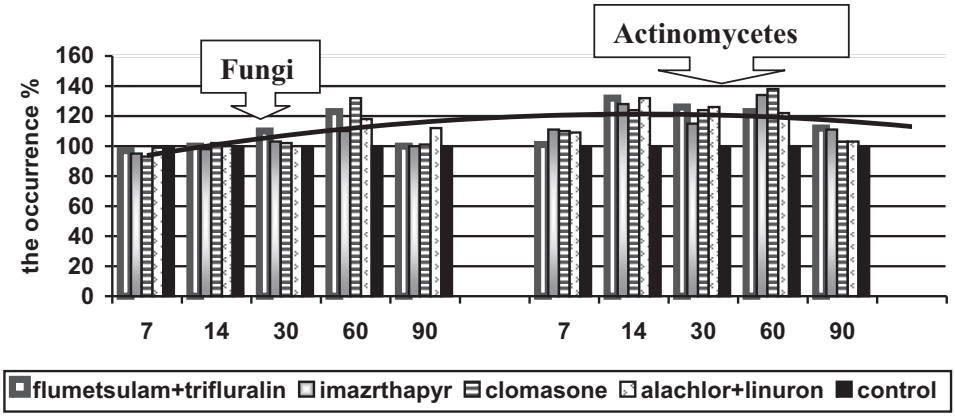
Milošević and Govedarica (2001) found that the negative effect of prometryne on the growth and development of azotobacters was higher in the soil under sunflower than in the soil under soybean. In the sunflower field, azotobacters could not be registered in the soil 28 days after prometryne application, but on the 45th day their number reached 10.76 x 10^2 per one gram of soil. According to Govedarica et al. (2001), the number of azotobacters was 2—3 times lower in a sugarbeet field treated with dimethenamide and metalochlor than in the untreated control variant.

Herbicide application also inhibits the symbiotic bacterium *Bradyrhizobium japonicum.* Nodulation rate is an indicator of symbiotic efficiency between soybean plant and the bacterium. Nodulation test shows the efficiency of nitrogen utilization in the process of biological nitrogen fixation. Herbicide application reduced the nodulation rate, i.e., the number of nodules formed on soybean roots, by 5—21% (Milošević et al., 2000a).

According to Cervelli et al., (1978), herbicides may kill sensitive microorganisms. However, herbicides may also be decomposed by enzymes produced by microorganisms, which subsequently use the metabolites as sources of biogenous elements (Cervelli et al., 1978; Milošević et al., 2001). It has been noticed that certain groups of microorganisms (the primary population) start to decompose herbicides a few days after their applications. However, the secondary population, which produces induced enzymes, starts to decompose herbicides after a period of adaptation.

Low concentrations of 2,4-dichlor-phenoxy-acetic-acid (2,4-D) promote the development of tumorous structures (p-nodules) on the roots of corn, wheat (Christainsen-Weniger, 1992; 1995; Christainsen-Weniger and Vanderleyden, 1994, cit. Christainsen-Weniger, 1997) and rice (Christainsen-Weniger, 1997). On inoculation, *Azo-*

spirillum brasilinse colonizes *p*-nodules. The nitrogenase activity of tumors inhabited by bacteria is significantly increased in comparison with the untreated control.



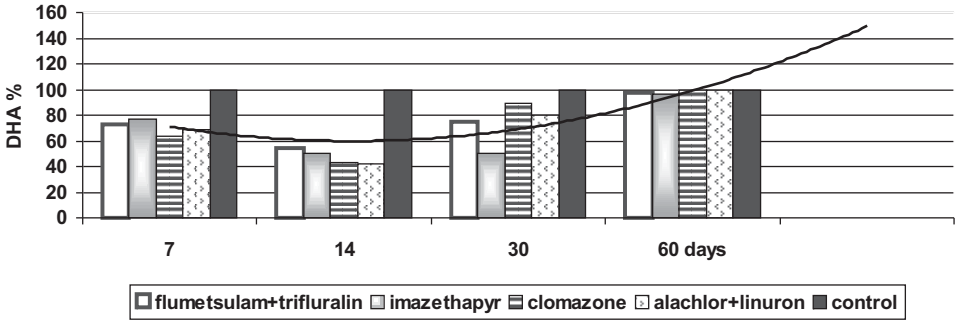
Graph. 3 — Effect of herbicides on fungi and actinomycetes

Generally, our studies have shown that herbicide application stimulated the growth and development of actinomycetes. Dimethenamide and metalochlor applied to sugarbeet (Govedarica et al., 2001), and prometryn applied to soybean and sunflower (Milošević and Govedarica, 2001) increased the number of actinomycetes. Only seven days after herbicide application, their numbers started to increase (Graph. 3). On the 14th day, the numbers of actinomycetes were increased by 35% in the treatments with Rival (flumetsulam+ trifluralin) and Linuron (alachlor+linuron). The application of imazethapyr and clomazone stimulated the development of actinomycetes, their number reaching the maximum value 60 days after herbicide application. The effect of herbicides on fungi is variable and it depends on the dose applied (Milošević et al., 2000; Milošević and Govedarica, 2001).

Enzymes are catalysts of biochemical processes in the soil and they take part in the cycling of carbon, nitrogen, phosphorus and sulfur. They play an important role in the maintenance of soil fertility and their activity may serve for the assessment of soil fertility (Skujinš, 1973; Govedarica et al., 1993; Milošević et al., 1997).

Being endocellular, oxido-reducing enzymes, dehydrogenases are a foundation of the enzymatic systems of all microorganisms (von Mersi and Schinner, 1991) and their role is essential in initiating the oxidation of organic matter in the soil, by transporting electrons or hydrogen from the substrate to the sink (Ross, 1971). DHA is a measure of microbial oxidative activity (Skujinš, 1973; Casida, 1977; Tabatabai, 1982; Trevors, 1984; Camiña et al., 1998), Many authors consider it as an indicator of soil biological activity (Lenhard, 1956; Thalmann, 1968; Skujinš, 1973; Milošević et al., 1993, 1996). Our previous studies showed that DHA is a good indicator of changes in oxido-reducing processes in the soil ta-

king place in consequence to herbicide application (Milošević et al., 1995, 1998, 2001; Govedarica et al., 1995, 2001).



Graph. 4 — Effect of herbicides on dehydrogenase activity

The tested herbicides (Graph 4) were significantly reducing dehydrogenase activity on the 7th, 14th and 30th day after application (Milošević et al., 2001). After that period, however, the oxido-reducing processes return to the level of the control variant.

According to Govedarica et al. (2001) metalochlor caused a larger reduction in dehydrogenase activity than dimethenamide. The application of a combination dimethenamide + chloridazon inhibited the oxido-reducing processes in the soil to a lesser extent than the application of individual herbicides. The values of DHA were on the level of the control variant 180 days after herbicide application. DHA was reduced after the application of prometryn (Milošević and Govedarica, 2001), but it was back on the level of the control variant 45 days after application.

DHA is a sensitive indicator of side-effects of herbicide application on non-target soil microorganisms (Schuster et al., 1987, cit. to Schuster and Schröder, 1990a). A field trial of Schuster and Schröder (1990a) showed a decreased dehydrogenase activity following the application of dichlorprop (13%) and glyphosate (5%). Under laboratory conditions, a normal dose of glyphosate stimulated DHA during the period of six weeks after application. A ten-fold dose of glyphosate, however, reduced the oxido-reducing processes by 5% to 25% (Schuster and Schröder, 1990).

According to Janjić et al. (1996) the amount of released CO_2 (as a measure of the intensity of soil microbiological activity) was reduced after the application of atrazine and cyanazine. Repeated atrazine applications caused a significant reduction in the intensity of soil respiration followed via the amount of released CO_2 (Džugeli, 1982., cit. Janjić et al., 1996).

Inoculants

Microorganisms may be added to soils contaminated with herbicides in order to mitigate negative residual effects on subsequent crops. Examining 16

herbicides, Burns (1995) selected two hormonal herbicides as indicators of microbiological degradation: 2(2,4-dichlorophenoxy) acetic acid (2,4-D) and 2-methyl-4-chlorphenoxyacetic acid (MCPA). Fourteen days after soil inoculation with bacteria isolated from contaminated soils, the rates of herbicide decomposition were 67—78% in the case of 2.4-D and 84—98% in the case of MCPA. The rate of decomposition depended on bacterial strain used for inoculation (Burns, 1995). In the non-inoculated soil, the rate of decomposition was 23—27%. However, 28 days after inoculation, the amounts of decomposed herbicides in the treated and untreated soils were equal. MCPA was decomposed faster that 2,4-D, 99% of it being decomposed 28 days after inoculation.

Weeds may be successfully controlled with preparations based on fungi (*Beauveria bassiana, Paeciloyces litacinus, Verticillum chlamydosporum*). It was found that *Sphacelotheca halepense* may be used as a mycoherbicide in the biocontrol of *Sorgum halepense* (Milanova and Karađova, 1997).

CONCLUSION

Microorganisms are constituting elements of the environment. Their abundance, enzymatic activity and biodiversity are good indicators of the balance in the agro-ecological system. To use microorganisms as indicators of changes in soil biological activity, it is necessary to find most reliable detection methods and most effective microbial strains. Inoculants based on certain microorganisms may be used as biopreparations in combination with or instead of chemical preparations. They are applicable in integrated weed management, which implies combining of cultural practices, biological and chemical methods of weed control. Microbiological research should be focused on isolating microbial strains which, on application, effectively decompose herbicides, irrespectively of the natural microbial population in the soil.

It is necessary to keep strengthening the scientific basis of modern agriculture because herbicides may be advantageously used only if their persistence, bioaccumulation and toxicity (acute and genetic) in agro-ecosystems are strictly controlled.

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УТИЦАЈ ХЕРБИЦИДА НА МИКРОБИОЛОШКА СВОЈСТВА ЗЕМЉИШТА

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Резиме

Микроорганизми земљишта су значајна карика у односу земљиште — биљка — хербициди — фауна/човек. Наиме, микроорганизми а) учествују у деградацији хербицида, б) њихова активност, бројност и разноврсност су биоиндикатори промена биолошке активности земљишта после примене ових хемијских једињења, и в) поједине врсте микроорганизама могу се применити као биохербициди.

Хербициди изазивају угинуће осетљивих група микроорганизама. Међутим, хербициди се могу разградити у земљишту ензимима микробних популација користећи ова хемијска једињења и добијене метаболите као изворе биогених елемената. Уочава се да поједине групе микроорганизама (примарна популација) разграђују хербициде већ после неколико дана након доспевања у земљиште. Међутим, тзв. секундарна популација која продукује подесне-индуковане ензиме разграђује ова хемијска једињења после периода адаптације.

Наша истраживања показују да је група микроба *Azotobacter* најосетљивија на примену хербицида, те може бити поуздан биоиндикатор биогености земљишта. Уочава се да је заступљеност ове групе азотофиксатора смањена у великом

проценту до 14. дана. Заступљеност *Actinomycetes* и у мањој мери гљива је повећана, што указује да ове групе микроорганизама користе хербициде као изворе биогених елемената.

Разградња хербицида у земљишту зависи од својства примењеног препарата, затим од количине (дозе) хербицида, али и од физичко-хемијских својстава земљишта (влажности, температуре, биљног покривача, обраде) и врсте микроорганизама.

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EFFECTS OF PESTICIDES ON *AZOTOBACTER CHROOCOCCUM*

ABSTRACT: Studied in this paper were the effects of three concentrations of four different pesticides (two herbicides — Ro-Neet and pyramin, one insecticide — lindan and one fungicide — mankogal) on the growth of pure cultures of three *Azotobacter chroococcum* strains. The lowest and highest concentrations used in the study were ten times lower and ten times higher than the concentration used in actual agricultural practice (in the field), respectively. The pesticides had different effects on the growth of the *Azotobacter chroococcum* strains. All three strains of *Azotobacter chroococcum* grew unimpeded regardless of the pyramin, Ro-Neet-a and lindan concentration, whereas all three mankogal concentrations caused growth inhibition to occur in the three strains, which can, therefore, be deemed highly susceptible to the fungicide mankogal.

KEY WORDS: *Azotobacter chroococcum*, fungicides, herbicides, insecticides

INTRODUCTION

In the last few decades, numerous soil microorganisms have been found to have a positive effect on plant development. Besides the well-known symbiotic nodular bacteria, free nitrogen fixers in the rhizosphere can also stimulate plant growth or reduce the damage causes by soil-borne plant pathogens (Kloepper et al., 1989). *Azotobacter* has been used as a potential nitrogenous fertilizer to increase suger beet yield (Martin, 1986, Steinberga et al., 1996, Mrkovački et al., 2001).

Whether pesticide treatments exert a significant inhibitory effect on root colonization by these PGPRs will be indicated by the impact on plant production, since the pesticide effects will be reduced.

Herbicide combinations control weeds and increase sugar beet yield to a significant degree. According to Glušac and Dražić (1982), Ro-Neet + Pyramin should be the first choice when selecting the herbicide combination for basic treatments preceding sugar beet sowing. Sugar beet producers often apply herbicides based on cycloate (Ro-Neet, Cycloate, Cycloherb) and chlori-

dazon (Pyramin FL, Pyramin turbo) prior to sowing with incorporation (Malidža and Glušac, 1999).

Some herbicides used in agriculture can be harmful to *Azotobacter chroococcum*. Glyphosate herbicides have been shown not only to inhibit the nitrogen fixation process in *Azotobacter chroococcum* but also to reduce the bacterium's respiration rate by 60% and hence preclude its positive effects (Santos and Flores, 1995).

Concerning the environmental importance of *A. chroococcum*, a study has been carried out with a popular herbicide called pendimethalin that established a connection between *A. chroococcum* and a transformational process that breaks down this herbicide into non-toxic products, thus demonstrating that the bacterium is important not only for agriculture but for the environment as well. Pendimethalin was efficiently degraded by *A. chroococcum* (45% after 10 days, 55% after 20 days) (Kole et al., 1994).

Insecticides affect soil microorganisms and their biochemical processes related to soil fertility and can stimulate *Azotobacter* growth (Das and Mukherjee, 1998).

The objective of the present study was to determine the effects of two herbicides, one insecticide and one fungicide on the growth of pure cultures of *Azotobacter chroococcum* under laboratory conditions.

MATERIALS AND METHODS

Used in this study were three highly effective strains of *A. chroococcum* (5,8 and 14) isolated from the rhizosphere of sugar beet at Rimski Šančevi. The strains had been taken from the collection of nitrogen-fixing microorganisms maintained at the Institute of Field and Vegetable Crops in Novi Sad (NSCNFB).

The strains were grown in a liquid Fjodor medium at pH = 7 and 30°C. Cell density in the inoculum was 10^{12} per ml of culture.

The lowest pesticide concentration in the study was ten times lower than the concentration used in field conditions (1). The other concentrations used were the field concentration itself (2) and a concentration ten times higher than that (3). The field concentrations per 300 l of water per hectare are 4 l for Ro-Neet and pyramin, 5 l for lindan, and 600—850 g/100 kg of seed for Mankogal.

Pesticide effects (Ro-Neet, Pyramin-herbicides; Lindan-insecticide and Mankogal-fungicide) were studied by the diffusion method in Petri dishes adding 0.1 ml of each concentration on a ∅ 10 mm paper disk. Prior to that, sterile disks were placed on the surface of the medium that had been inoculated with an *A. chroococcum* strain before being poured into the dishes. Five replicates were made for each of the pesticides. Each Petri dish contained one pesticide-free disk that was used as the control. Bacterial growth readings were recorded after 48 h of growth in a thermostat at 30°C.

RESULTS AND DISCUSSION

The study has shown that the herbicides and insecticides involved had no negative effect on the growth of *A. chroococcum* strains 5,8 and 14, i.e. no inhibition of growth was recorded. The fungicide, however, did have a negative effect on the growth of the strains regardless of the concentration.

Balajee and Mahadevan (1990) reported that the herbicide 2,4-D and its degradation products, p-chlorophenoxy-acetic acid and p-chlorophenol, were utilized by *A. croococcum* as the sole carbon source. Nitrogenase activity was stimulated by the chloroaromatics.

Martinez Toledo et al. (1991) studied the effects of the herbicide simazine on the biological activity of *A. chroococcum*. The herbicide was found to have no effect on *A. chroococcum* microbial growth on either the standard medium, dialysed soil medium or sterilized soil. Nevertheless, the presence of 50—300 mg of simazine per one milliliter of culture or one gram of soil did have a stimulating effect on *A. chroococcum* nitrogen fixation in non-sterile and sterile conditions alike. When microorganisms are grown in the presence of simazine, the cells have a higher ATP content than the control.

Quadruple herbicide doses had a slight inhibitory effect on an *Azotobacter* population in wheat (Singh et al., 1996), while the number of *Azotobacter* cells two and 14 weeks after herbicide application dropped as a result of the same (Piskorz, 1998).

Abul Kalam and Banerjee (1995) reported very slight inhibition of *A. vinelandii* growth after the application of a fungicide (tridemorph). Still, a study of the effects of five herbicides, two insecticides, and 12 fungicides on *A. chroococcum* growth and nitrogenase activity has shown fungicides, especially Captan and Ziram, to be the most toxic of all three types of pesticide (Vannini et al., 1992). The organophosphorous insecticides profenofos and chloropyrifos reduced the number of aerobic nitrogen fixers and significantly decreased nitrogen fixation (Martinez Toledo et al., 1992; Pozo et al., 1995), while 0.12 pmol/ml of supercypermetrine (the active ingredient) from the insecticide nerametrine EK-15 completely inhibited nitrogen fixation in *A. chroococcum* (Cernakova, 1993).

Results have shown that resistance to pesticides is a trait common to all strains of *A. chroococcum*. Reduced resistence with increased pesticide concentration was recorded in particular with Aldrina (Gupta et al., 1994).

A progressive increase of an *Azotobacter* sp. population until the third application of the insecticide carbofuran showed that the applications stimulated nitrogenase activity and the abundance of nitrogen-fixing bacteria in the rhizosphere of rice (Kanungo et. al., 1995).

The results of our study are in agreement with those cited above, i.e. we found the analyzed strains of *Azotobacter chroococcum* to be resistant to herbicides and insecticides and to show inhibition of growth as a result of fungicide application.

Table 1. — Growth of *A. chroococcum* strains as affected by different pesticide concentrations

Pesticide	Concentration	A. chroococcum strain 5	A. chroococcum strain 8	A. chroococcum strain 14
Pyramin	1	+	+	+
	2	+	+	+
	3	+	+	+
	ø	+	+	+
Ro-Neet	1	+	+	+
	2	+	+	+
	3	+	+	+
	ø	+	+	+
Lindan	1	+	+	+
	2	+	+	+
	3	+	+	+
	ø	+	+	+
Mankogal	1	—	—	—
	2	—	—	—
	3	—	—	—
	ø	+	+	+

1 — 10 x lower than conventional concentration
2 — conventional concentration (Ro-Neet and Pyramin-41/ha; Lindan 51/ha; Mankogal 600—850 g/100 kg)
3 — 10 x higher than conventional concentration
ø — control (pesticide-free disk)
+ positive culture growth around disk and — inhibited culture growth around disk

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УТИЦАЈ ПЕСТИЦИДА НА *AZOTOBACTER CHROOCOCCUM*

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Резиме

У раду је испитан ефекат три концентрације четири различита пестицида на раст чистих култура три соја *Azotobacter chroococcum*. Коришћена су два хербицида — Ro-Neet и Pyramin, један инсектицид — Линдан и један фунгицид — Манкогал. Најмања концентрација била је десет пута мања од оне која се користи у пракси (у пољу), а највећа десет пута већа. Добијени су различити ефекти пестицида на раст сојева *Azotobacter chroococcum*. Сва три соја несметано су расла на све три испитиване концентрације Pyramin-a, Ro-Neet-a и Линдана, док је Манкогал у све три испитиване концентрације изазвао инхибицију раста код сва три соја. Испитивани сојеви веома су осетљиви на фунгицид Манкогал.

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TRANSFERRING OF *SCLEROTINIA* RESISTANCE FROM WILD INTO CULTIVATED SUNFLOWER — COMBINING OF CONVENTIONAL AND LABORATORY TECHNIQUES

ABSTRACT: Five populations of each *H. molis*, *H. maximiliani*, *H. rigidus* and *H. tuberosus* were screened for resistance to stem form of *Sclerotinia*. On the basis of the results obtained by screening, nine crosses of resistant populations with either other wild species populations or with cultivated sunflower were made. As in some crosses a small quantity of seed was produced and the seeds germinated poorly, modified tissue culture methods were used to enhance germination and produce clones of interesting plants. These methods were found to be efficient both for seed germination and plant production and multiplication.

KEY WORDS: wild sunflower, *Sclerotinia* resistance, tissue culture

INTRODUCTION

White rot caused by the fungus *Sclerotinia sclerotiorum* is the major disease of sunflower (*Helianthus annuus* L.) in countries with a humid climate, while in countries with moderate climate, it causes the yield loss in rainy years (Škorić and Rajčan, 1992). This parasite usually attacks all parts of the plant: roots, stalks, leaves, flower buttons and heads (Zimmer and Hoes, 1978). There are no suitable cultural control methods (Lumsden, 1979) and no immune genotypes of cultivated sunflower have yet been found or developed.

Wild sunflowers constitute an important source of resistance against several major sunflower diseases including *Sclerotinia* (Georgieva-Todorova, 1993). In some cases, transfer of these traits into cultivated sunflower genome using conventional methods is difficult because of a high interspecific incompatibility. In sunflower, embryo rescue technique has been used to overcome this problem.

Micropropagation is interesting for wild sunflower species whose seeds germinate poorly. Imhoff et al. (1996) described a method for efficient propagation of three wild sunflower species using sterile rhizomes. Vasić et al. (2001) have used shoot tips and nodal segments for multiplication of *H. maximiliani*.

In this paper an integrated approach to the *Sclerotinia* resistance screening and breeding, combining conventional and modified tissue culture methods, is described.

MATERIAL AND METHODS

Wild sunflower accessions were grown in quarantine plot of Institute of Field and Vegetable Crops, Novi Sad.

Five populations of each *H. molis*, *H. maximiliani*, *H. rigidus* and *H. tuberosus* (Table 1) were screened for resistance to stem form of *Sclerotinia*. Four plants per population were artificially inoculated by incorporation of sclerotias in the stems in the phasis of butonisation. Wounds with sclerotia were covered with wet cotton and aluminium foil. Plot was regularly irrigated. Screening was done two weeks after full flowering, using the scale 1—5. Resistance was determined as percentage of healthy plants.

Plants of populations found to be resistant were crossed with either other wild sunflower populations or cultivated sunflower, using classical method.

Seeds of progenies of crosses were germinated in liquid MS medium (Murashige and Skoog, 1962) and planted in Jiffy pots. Well-grown plants were transferred into growth chamber. Further multiplication of plants was done by rooting side branches in sand, with watering with distilled water or water solutions of natural naphthenic acids isolated from lower fractions of "Velebit" oil.

RESULTS AND DISCUSSION

Two populations of *H. molis* were found to be 100% resistant to *Sclerotinia* attack on stem (Table 1). In all other tested species some highly tolerant populations were found, which confirms the notion that wild species could be valuable sources of if not resistance than tolerance to *Sclerotinia* (Škorić, 1988). In contrast to the results of Škorić and Rajčan (1992), population 1631 of *H. maximiliani* was only tolerant to stem *Sclerotinia* (Table 1). This could be explained by high variability that exists within the populations of wild sunflower species.

On the basis of the results obtained by screening, nine crosses of resistant populations with either other wild species populations or with cultivated sunflower were made (Table 2). Crosses with other wild species were done in cases where it was not possible to cross them with cultivated sunflower. Crosses were made with wild species crossable with cultivated sunflower with the hope that hybrids will be crossable with it as well.

Table 1. — Resistance of tested populations to artificial *Sclerotinia* infection on stem. Resistance is given as a percentage of healthy plants

Genotype	Resistance (%)	Genotype	Resistance (%)
mol 1530	25	max 2007	75
mol 1692	25	max 2010	75
mol x	100	max 34	75
mol 1298	100	max m	0
mol 285	50	max 1631	50
rig 2012	75	tub 6	0
rig 1696	25	tub 7	25
rig 1692	50	tub 1699	0
rig 1843	25	tub 675	50
rig 1844	0	tub 1702	75

Table 2. — Seeds and plant from interspecific crosses obtained by conventional and laboratory methods

Cross	Total seeds obtained	Number of germinated seeds	Percentage of regenerated plants	Percentage of plants obtained from side branches
CMS1-17A x max 2007	1	—	—	—
Ha-48 x tub 6	4	—	—	—
Ha-48 x tub 7	1	1	100%	100%
arg 1805 x max 1631	50	10	30%	100%
gig 1605 x max 1631	46	19	32%	—
tub 1700 x max 1631	42	10	30	—
max M x gig 2115	4	—	—	—
gro 1685 x molx	35	8	12%	—
mol 1298 x ann 2197	7	—	—	—

As in some crosses a small quantity of seed was produced (Table 2) and the seeds germinated poorly, modified tissue culture methods were used to enhance germination and produce clones of interesting plants.

Embryo culture in *in vitro* conditions can sometimes lead to decreased plant vigour (P e l l e t i e r et al., 1992). That is why, as suggested by P a u l and B a r t h o u (1994), for improvement of seed germination a non-sterile technique was used. Germination percentage varied from 80—100% (data not shown). Plants obtained were normal and vigorous. This is in accordance with the results obtained by P a u l and B a r t h o u (1994).

Micropropagation of interspecific progenies was done in non-sterile conditions as well. The sterile technique that was found to be efficient in propagation of *H. maximiliani* shoot tips (V a s i ć et al., 2001) was not efficient here. The same stands for growing branches in water or water solution of tested compounds. No matter which of the substances was used, no root formation was observed. The most efficient was growing branches in the sand. Rooting

was induced in all the variants, but the intensity of root formation was different among the variants.

The obtained results showed that wild sunflower species could be a potential source of genes for *Sclerotinia* resistance. Combination of classical crossing with embryo rescue and micropropagation was found to be a good method for obtention and multiplication of progenies of interesting interspecific crosses. Further studies on wild sunflower relatives regarding *Sclerotinia* resistance are in progress. The techniques described in this paper are going to be applied in the new interspecific crosses. All this will hopefully lead us to production of cultivated sunflower genotype with at least high tolerance to *Sclerotinia*.

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ПРЕНОШЕЊЕ ОТПОРНОСТИ ПРЕМА *SCLEROTINIA* ИЗ ДИВЉЕГ У ГАЈЕНИ СУНЦОКРЕТ — КОМБИНОВАЊЕ КОНВЕНЦИОНАЛНИХ И ЛАБОРАТОРИЈСКИХ ТЕХНИКА

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Резиме

Тестирано је по пет популација *H. molis, H. maximiliani, H. rigidus* и *H. tuberosus* на отпорност према *Sclerotinia* стабла. На основу добијених резултата извршено је девет укрштања отпорних популација са другим дивљим врстама или гајеним сунцокретом. Како је у неким случајевима добијена мала количина семена која су слабо клијала, коришћене су модификоване методе културе ткива да би се повећала клијавост и произвели клонови интересантних биљака. Ове методе су се показале ефикаснима и за наклијавање семена и за производњу и умножавање биљака.

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PHYTOGEOGRAPHICAL AND ECOLOGICAL CHARACTERISTICS OF THE VEGETATION ALLIANCE *THERO-SALICORNION* Br.-Bl. 33 EM. Tx. 50 GROWING ON CONTINENTAL SALT-AFFECTED SOILS (BANAT—YUGOSLAVIA)

ABSTRACT: Synecology of the communities of the alliance *Thero-Salicornion* Br.-Bl. 33 em. Tx. 50 growing on continental salt-affected soils (Banat, Yugoslavia) has been characterized on the basis of area type percentages and life form explanations, using methods of indicative geobotany.

KEYWORDS: continental salt-affected soils, the alliance *Thero-Salicornion*, area type percentages, life forms, ecological indices

INTRODUCTION

Communities of the alliance *Thero-Salicornion* Br.-Bl. 33 em. Tx. 50 may be encountered practically along the entire Mediterranean coast, as an azonal type of vegetation. On continental salt-affected soils of the Pannonian Plain, however, they are a part of the intrazonal vegetation in the region of climazonal vegetation of the alliances *Aceri tatarico-Quercion* Zolyomi et Jakucs 57 and *Festucion rupicolae* Soó (40) 64 (Soó, 1973).

Although removed from their original habitats by human influence, those communities have found extremely good conditions for successful development in saltworks along the Mediterranean coast. In the continental regions of the Pannonian Plain, however, they are at the stage of rapid retreat, and most of their surviving stands have a limited habitat.

The aim of this paper was to characterize the synecology of the alliance *Thero-Salicornion* communities on the continental salt-affected soils (Banat,

Yugoslavia) using methods of indicative geobotany and area types and life forms as diagnostic criteria.

INVESTIGATED AREA AND METHODS

The investigated area is located in the central part of Banat, near settlements Novi Bečej and Melenci, on the alluvion of the River Tisza (Figure 1).

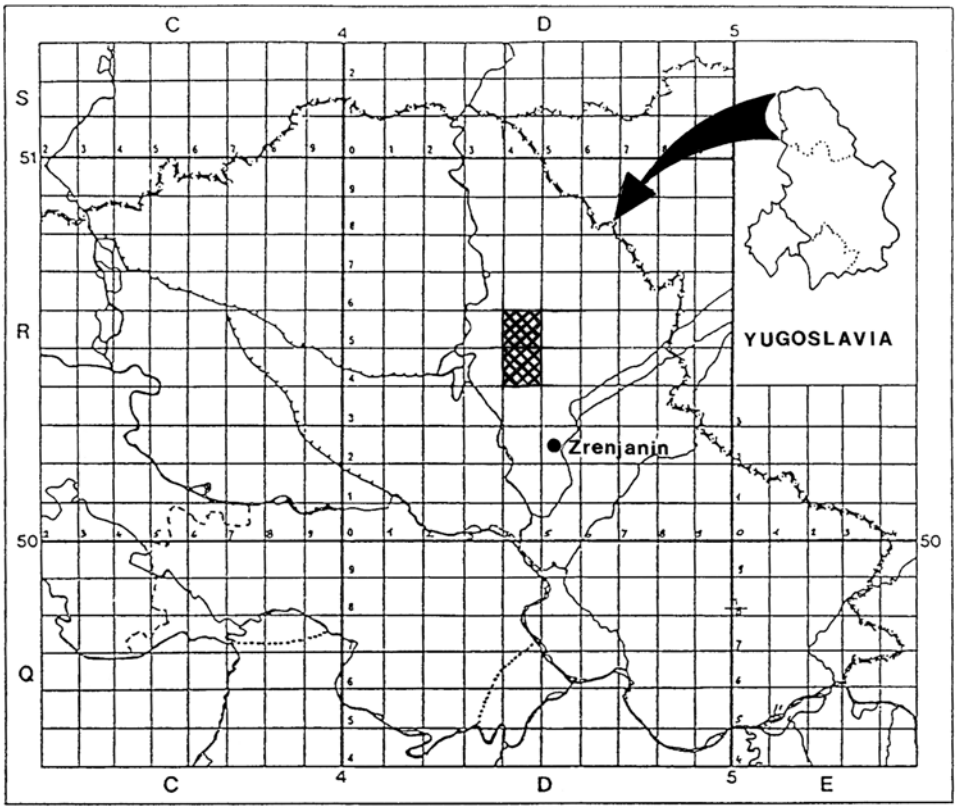

Figure 1. — Investigated area

The moderately continental climate of this area, influenced by the continental climate from the northeast, the Central European climate from the northwest, and the Mediterranean climate from the south, is alleviated by the presence of considerable bodies of water. The beginning of the vegetation period in these areas is characterized increases in precipitation and temperature, which are of crucial importance for plant growth. The precipitation, after reaching its maximum in June, starts an abrupt decrease. On the other hand, in the middle of the vegetation period, the temperature is relatively stable and its faster and more substantial decrease begins only after the intensive droughts in October.

Such relationship between precipitation and temperature results in the occurrence of semiarid or, in some years, arid periods unfavorable for the vegetation cover. These dry periods usually begin in July and continue till October (Figure 2) (K a t i ć et al., 1979).

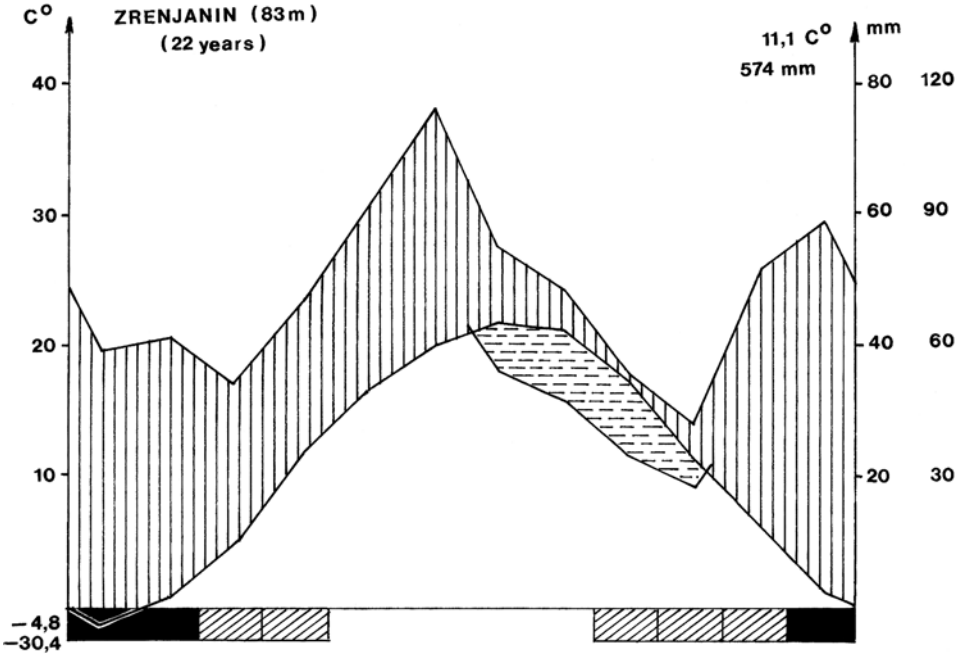

Figure 2. — Climate diagram after Walter for the meteorological station Zrenjanin

The swamps Slano Kopovo and Rusanda, formed by water accumulation in depressions, are located northeast of the town of Novi Bečej and northwest of the village of Melenci, in the zone of salt-affected pastures, on the solonchak-like solonetz and solonetz soils. Their strongly salinized margins, when exposed during semiarid and arid periods, become overgrown with the vegetation of the alliance *Thero-Salicornion*. Stands of the associations *Salicornieto-Suaedetum maritimae continentale* K n e ž e v i ć et B o ž a 88 and *Suaedetum maritimae* S o ó 27 develop along the banks of Slano Kopovo, stands of the associations *Suaedetum maritimae* S o ó 27, *Suaedetum pannonicae* (S o ó 27) W e n d e l . 43 and *Salsoletum sodae* S l a v n i ć (39) 48 along the banks of Rusanda (K n e ž e v i ć , B o ž a 1987, 1988).

In this study, the association *Salicornieto-Suaedetum maritimae continentale* from Slano Kopovo and the associations *Suaedetum maritimae*, *Suaedetum pannonicae* and *Salsoletum sodae* from Rusanda were analyzed from the phytogeographical and ecological points using floral elements after Gajić (G a j i ć , 1980), life forms after Raunkiaer (S o ó , 1966—1973), and ecological indices after Landolt (L a n d o l t , 1977, K n e ž e v i ć 1994).

The floristic composition of the associations, with the degree of presence and quantitative participation of their members, is given as an abridged comparative table (Table 1).

Table 1. — Comparative table of the investigated associations *Salicornieto-Suaedetum maritimae continentale* (I), *Suaedetum maritimae* (II), *Suaedetum pannonicae* (III) and *Salsoletum sodae* (IV)

Floristic element	Life form		I	II	III	IV
		Association character species				
Cosm.	T	*Salicornia europaea* L.	V +—2	—	—	—
Cosm.	T	*Suaeda maritima* L.	V 1—3	V 2—3	—	—
Pan. subend.	T	*Suaeda pannonica* Beck	—	—	V 2—4	II +
Eurasian	T	*Salsola soda* L.	—	II +	—	V 2—3
		Cypero — Spergularion				
Subsouthern. Siberian	T	*Crypsis aculeata* (L.) Aitt.	—	I +	—	—
		Festuco-Puccinellietea				
Sub-Pannon.	H	*Puccinellia limosa* (Schur) Holmb.	V +—3	V +—1	V 1—2	V 1—2
Pannonian	H	*Aster tripolium* L. var. *pannonicus* Jacq.	IV +—1	V +	IV +—1	IV +—1
Eurasian	T	*Atriplex litoralis* L.	II +	IV +	V +—2	V +—2
SubPontic-SubMediterr.	T	*Bupleurum tenuissimum* L.	I +	—	—	—
SubPontic-Central Asian	H	*Taraxacum serotinum* W. et K. subsp. *bessarabicum* (Horn.) H.-M.	I +	—	—	—
Pont.-Pannon	T	*Camphorosma annua* Pall.	—	—	I +	—
		Other species				
Cosm.	H	*Spergularia media* (L.) Presl.	II +—1	—	—	—
SubPon.-Sub Centr. Asian.-Sub. Mediterr.	T	*Heleochloa alopecuroides* (P. et M.) Host.	I +	—	—	—
European	T	*Lepidium ruderale* L.	I +	—	—	—
SubPontic.-SubMediterr.	T	*Lactuca salina* L.	I +	—	—	
Cosm.	H	*Bolboschoenus maritimus* (L.) Pall.	I +	I +	—	—
Cosm.	H	*Phragmiters communis* Trin.	—	II +	—	—
Eurasian	H	*Taraxacum officinale* Weber.	—	—	II +—1	—
Cosm.	T	*Polygonum aviculare* L.	—	—	I +	—
Eurasian	T	*Chenopodium urbicum* L.	—	—	II +	II +

I *Salicornieto-Suaedetum maritimae continentale*
II *Suaedetum maritimae*
III *Suaedetum pannonicae*
IV *Salsoletum sodae*

RESULTS AND DISCUSSION

The phytogeographical analyses of the investigated communities indicated the dominant role of the Pontic-Central Asian species, with the euhalophytes of the Pannonian floral element prevailing (from 16.66% in Ass. *Salicornieto-Suedetum maritimae continentale* to 50.0% in Ass. *Salsoletum sodae*) (Table 2). The analyses provided proof of the extent of adaptation of the investigated communities to the continental climate of the eastern arid regions, of their distribution limited to the Pannonian Plain and of their adaptation to a certain soil type. Even the widespread species (Euro-Asian, circumpolar and cosmopolitan) present in the floristic composition typically prefer saline sites. The other floral elements were represented by a single species, *Lepidium ruderale* L., a sub-Central European element, having minimal abundance in only one phytocoenological sample within the area types of the association *Salicornieto-Suaedetum maritimae continentale* (K n e ž e v i ć , B o ž a , 1988).

Table 2. — Area type percentages of the investigated associations *Salicornieto-Suaedetum maritimae continentale* (I), *Suaedetum maritimae* (II), *Suaedetum pannonicae* (III) and *Salsoletum sodae* (IV).

Floristic element	I (%)	II (%)	III (%)	IV (%)
Pontic — Central Asian	50	25	50	50
(Pannonian)	(16.66)	(25)	(37.5)	(50)
Eurasian	8.33	37.5	37.5	50
Circumpolar and cosmopolitan	33.34	37.5		
Central European	8.33			

There were no sub-Mediterranean species in the floristic composition of the stands of analyzed communities because they have not been able to penetrate the already formed communities of Pontic-Central Asian species which had inhabited the primary solonchak soils of Banat earlier, during the warm and arid boreal.

Study of life forms provides a reliable picture of habitat characteristics and its phytocoenological specificities. Concerning the analyzed communities, the study showed the dominance of therophytes, a significant participation of hemicryptophytes and absence of the other life forms, indicating the hemicryptophytic-therophytic character of these communities (Figure 3). Only in the association *Suedetum maritimae* was a proportional participation of therophytes and hemicryptophytes registered, obviously in consequence to increased humidity in the habitats of certain stands. However, most of the stands of this community had a strongly expressed therophytic character, the quantitative participation of the hemicryptophytes practically being insignificant (K n e ž e v i ć , B o ž a 1987, 1988). The increased quantitative and proportional participation of the therophytes in the floristic composition does not imply the spreading to the unoccupied space but the evolutionary adaptation of the investigated stands to high salinity, poor soil physical properties and considerable reduction of soil moisture towards the end of the vegetation period. In conse-

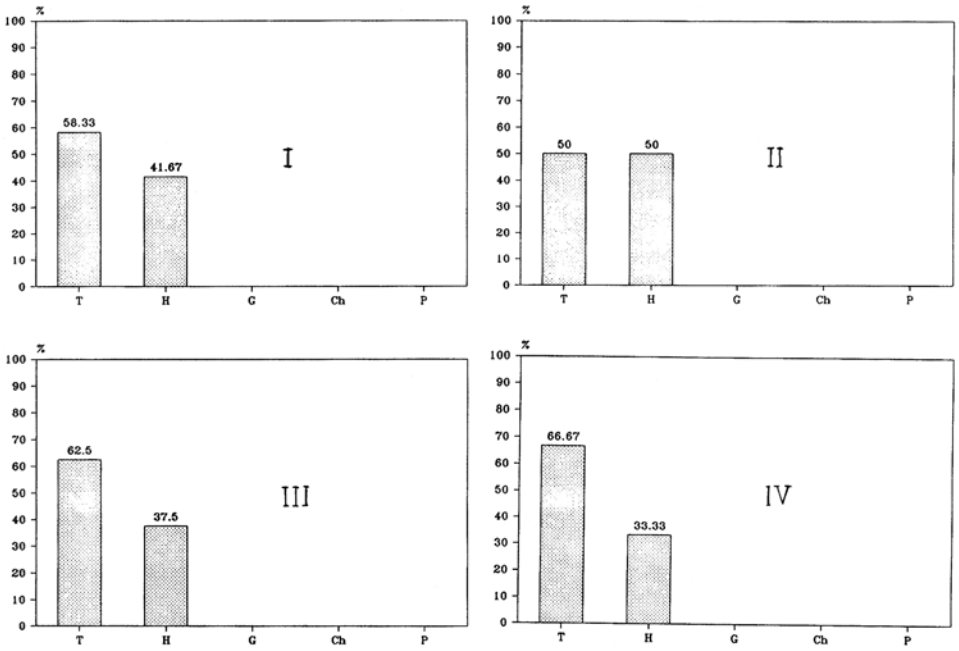

Figure 3. — Life forms of the associations *Salicornieto-Suaedetum maritimae continentale* (I), *Suaedetum maritimae* (II), *Suaedetum pannonicae* (III) and *Salsoletum sodae* (IV)

quence to such conditions, the investigated stands exhibited low species diversity and low plant coverage, which makes them similar to stands of the semi-desert character (K n e ž e v i ć , B o ž a , 1987, 1988).

On the basis of the average values of ecological indices, it was concluded that at the time of full vegetation the habitats of the investigated stands were semihumid (F-3.22 — Ass. *Salicornieto-Suaedetum maritimae continentale*; F-3.16 — Ass. *Suaedetum maritimae*) to semiarid (F-2.88 — Ass. *Suaedetum pannonicae*; F-2.68 — Ass. *Salsoletum sodae*) [Figure 4, (F)].

A specificity of the sites was a high salt content found in the rhizosphere layers. Therefore, the taxa bearing the ecological index "S —" were present in low percentages in the stands of the analyzed communities. In the stands of the association *Suaedetum maritimae*, these taxa were completely absent [Figure 4, (S)].

On the basis of the average values of soil chemical reaction, which varied from R-3.61 (Ass. *Suaedetum pannonicae*) to R-3.83 (Ass. *Salicornieto-Suaedetum maritimae continentale*), the analyzed sites were determined to have predominantly alkaline to strongly alkaline soils [Figure 4, (R)].

In addition to the poor floristic composition caused by extreme salinity and alkalinity, the stands of the alliance *Thero-Salicornion* were also characterized by low organic production. This low organic production was due to the paucity of nutrients at the analyzed sites, whose average contents varied from N-2.64 (Ass. *Suaedetum maritimae*) to N-2.79 (Ass. *Salicornieto-Suaedetum maritimae continentale*) [Figure 4, (N)].

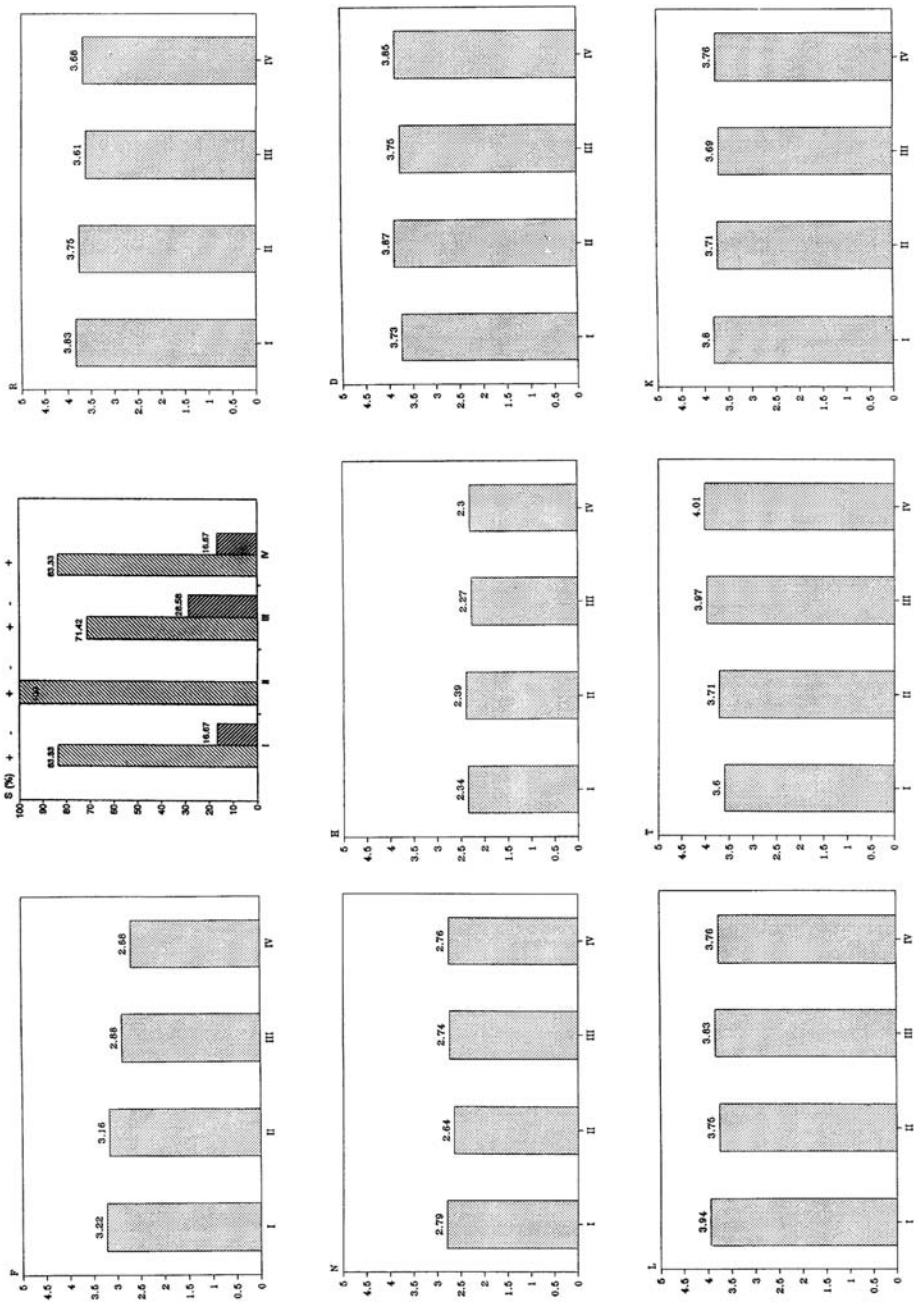

Figure 4. — Average values of ecological indices for soil moisture (F), soil salinity (S), soil chemical reaction (R), soil nutrients content (N), soil humus content (H), soil porosity /aeration/ (D), light (L), temperature (T) and continentality (K) in the associations: *Salicornieto-Suaedetum maritimae continentale* (I), *Suaedetum maritimae* (II), *Suaedetum pannonicae* (III) and *Salsoletum sodae* (IV).

The average ecological indices for humus content, ranging from H-2.27 (Ass. *Suaedetum pannonica*) to H-2.39 (Ass. *Suaedetum maritimae*), indicated that these stands developed on soils low in humus [Figure 4, (H)].

The mechanical composition of soil was not so unfavorable, on account of considerable sand contents, from D-3.73 (Ass. *Salicornieto-Suaedetum maritimae continentale*) to D-3.87 (Ass. *Suaedetum maritimae*) [Figure 4, (D)]. However, because of high salt contenta and intensive drought, only a limited number of succulent halophilous and haloxerophilous species was able to develop. Because of that the stands were characterized by the low coverage and by development in the conditions of considerably high light intensity. The values from L-3.67 (Ass. *Salsoletum sodae*) to L-3.95 (Ass. *Salicornieto-Suaedetum maritimae continentale*) are reliable indicators that most of the species were heliophilous [Figure 4, (L)].

Since the sites of the stands are inundated most of the time, they are characterized by a specific hydrothermic regimen. On the basis of the average values from T-3.60 (Ass. *Salicornieto-Suaedetum maritimae continentale*) to T-4.01 (Ass. *Salsoletum sodae*), it was concluded that during the period of most intensive vegetation development the sites provided a favorable thermic regimen [Figure 4, (T)].

Plants of continental regions were predominant in the analyzed stands. The narrow interval of average values from K-3.69 (Ass. *Suaedetum pannonicae*) to K-3.80 (Ass. *Salicornieto-Suaedetum maritimae continentale*) shows the adaptation of these plants to the high annual temperature variation, minimal air humidity and a relatively low precipitation [Figure 4, (K)].

CONCLUSION

The alliance *Thero-Salicornion* Br.-Bl. 33 em. Tx. 55 is represented on the continental salt-affected soils of Banat with the associations *Salicornieto-Suaedetum maritimae continentale* Knežević et Božа 88, *Suaedetum maritimae* Soó 27, *Suaedetum pannonicae* (Soó 27) Wendel. 43 and *Salsoletum sodae* Slavnić (39) 48. The domination of Pannonian euhalophytes among the phytogeographicaly characteristic Pontic-Central Asian species provides evidence of the habitats of these associations being limited to the area of the Pannonian Plain, of the dominant influence of the arid continental climate, and of their development under specific edaphic conditions.

The hemicryptophytic-therophytic character of these communities is a result of the evolutionary adaptation to the overgrowing of saline margins of swamps only after withdrawal of water, during semiarid and arid periods.

Because of a gradual drying of the soil, the sites are semihumid to semiarid at the time when the vegetation is fully formed. Because of a high salt content in the rhizosphere layer, the sites are predominantly alkaline in character and they permit almost exclusively the development of halophytes. Although their mechanical composition is basically not unfavorable, the organic production is low because of the low contents of nutrients and humus. The si-

tes are exposed to a high light intensity, in which heliophilous and therophytic species of arid continental regions are predominant.

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БИЉНОГЕОГРАФСКЕ И ЕКОЛОШКЕ КАРАКТЕРИСТИКЕ ВЕГЕТАЦИЈЕ СВЕЗЕ *THERO-SALICORNION* BR.-BL. 33 EM. TX. 50 СА КОНТИНЕНТАЛНИХ СЛАТИНА (БАНАТ — ЈУГОСЛАВИЈА)

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Резиме

Вегетација свезе *Thero-Salicornion* Br.-Bl. 33 em. Tx 55 заступљена је на континенталним слатинама Баната заједницама *Salicornieto-Suaedetum maritimae continentale* Knežević et Božа 88, *Suaedetum maritimae* Soó 27, *Suaedetum pannonicae* (Soó 27) Wendel. 43 i *Salsolaetum sodae* Slavnić (39) 48. Доминација панонских еухалофита међу биљногеографски карактеристичним понтско--централноазијским биљкама сведочи о ареалу заједница ограниченог на простору Панонске низије, доминантним утицајима аридне континенталне климе и развоју под специфичним едафским приликама.

Њихов хемикриптофито-терофитски карактер последица је еволуционе прилагођености обрастања заслањених обода бара тек након повлачења воде у полусушном и сушном периоду.

Услед постепеног пресушивања у доба потпуно формираног биљног покривача то су умерено влажна до умерено сува станишта. Због акумулације соли у

ризосферним слојевима имају претежно базан карактер и обрастају претежно халофитама. Иако у основи немају механички састав слабе су органске продукције због малог садржаја хранљивих материја и хумуса. Добро су осветљене површине на којима преовлађују хелиофилне и термофилне биљке сушних, континенталних предела.

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SELECTION OF BLACK POPLARS FOR WATER USE EFFICIENCY

ABSTRACT: Photosynthesis, transpiration, water use efficiency (WUE) and biomass production have been investigated in nine black poplar clones (section Aigeiros) in three field experiments. Eastern cottonwood clones (*Populus deltoides*) had the highest net photosynthesis and water use efficiency. European black poplar clones had the highest transpiration intensity. Correlation analysis showed that net photosynthesis was in a high positive correlation with biomass. Medium negative correlations existed between WUE and net photosynthesis, transpiration and biomass and WUE and biomass. The study showed a pronounced interclonal variability of the physiological and growth characters under study.

KEY WORDS: poplar, photosynthesis, selection, transpiration, WUE

INTRODUCTION

The genus *Populus* is widely distributed in Europe, North America, and Asia. Its broad range, ability of spontaneous and controlled intra- and inter-species hybridization, enabled the development of a large number of subspecies and transient forms, i.e., simple and complex hybrids. Poplars are distinguished by fast growth and easy vegetative propagation (Cain and Ormord, 1984). From the economic aspect, especially significant are poplars in the section *Aigeiros* which occupy small areas in Yugoslavia, but their share in felling volume and financial effects is great. Compared with other tree species of the temperate climate, poplars have the highest genetic potentials regarding fast growth and biomass production. According to some authors, they can produce great oven-dry biomass per hectare in a short time (Herpka, 1965). If we consider the forecast that the world will lack timber supply for mechanical and chemical processing, as well as for energy, the sudden interest in raising fast-growing tree species is not accidental. Therefore, the greatest number of poplar breeding programs has been directed to an increased utilization of their genetic potential and adaptive values. In this sense, the aim is to produce

cultivars (clones) characterized by superior growth and resistance to pests and diseases of leaf and stem.

Using hybridization and multiple selection methods, the Poplar Research Institute in Novi Sad has developed a number of poplar clones characterized by extremely fast growth and a series of desirable properties, the most important of which is the resistance to leaf and stem diseases. To create conditions for accelerating the procedure of producing fast-growing species, i.e., to make selection as early as possible and to initiate the construction of an ideotype, a program of long-term research of anatomic properties and physiological processes, i.e., structurally functional relations, was designed to understand the complex processes which result in superior growth (Orlović et all., 1998; 1999). The construction of an ideotype after Dickmann et all. (1994) is actually a method of summarizing the overall plant physiology. Recently, great attention has been drawn to the study of WUE, in order to detect genotypes that consume less water and are photosynthetically more efficient. In addition to poplars, this was also studied in provenances of the European silver fir (Larsen and Mekic, 1991). This paper presents the results of a study of variability of photosynthesis, transpiration, WUE and biomass production in black poplar clones grown in three field experiments.

MATERIAL AND METHOD

In spring 1995, three polyclonal field experiments with black poplar clones (section *Aigeiros* Duby) were established in the nursery of the Poplar Research Institute, after the block system with randomized treatments (clones). The experiments included clones characterized by fast growth throughout the rotation. Three clones (53/86, N1 and 54/86) belong taxonomically to the European black poplar (*Populus* nigra), 3 clones (Ostia, M1 and Robusta) are Euramerican poplars (*Populus x euramericana*), and 3 clones (PE 19/66, B-17 and S6-7) are eastern cottonwoods (*Populus deltoides*). The first experiment was established on humofluvisol, the second on a loamy form of fluvisol, the third on a sandy form of fluvisol (after classification by Skoric et all., 1985). The research was carried out during the vegetative growth period in the same year.

Net photosynthesis was determined polarographically by a Clark-type electrode. Very fine parts of leaves (up to 0.5 mm) without veins (Jones and Osmond, 1973) were cut for analysis. Leaf parts were placed into 1.5 ml of reaction medium consisting of 50 mmol HEPES (N-2-hydroxy-ethyl piperazine-N-2-ethane sulphonic acid), pH 7.6—7.8 and 1 mmol $NaHCO_3$, with constant temperature of 25°C. The process of photosynthesis was carried out under complete saturation with white light supplied by quartz-iodide lamp (Stankovic and Walker, 1977). Net photosynthesis was measured on the sections of fully formed and exposed leaves from 10 plants, in 4 blocks.

Transpiration intensity was determined in the laboratory by the gravimetric method. Vessel masses were measured together with the plants at 7 a.m. and 7 p.m. To determine the intensity of transpiration, samples of cut off leaf tops were immersed in water with paraffin oil on the surface, to prevent evap-

oration. Leaf area was then determined and transpiration intensity calculated in mg H_2O $m^{-2}s^{-1}$.

Water use efficiency was calculated as a ratio between the rate of transpiration and net photosynthesis.

In order to relate the variability of physiological parameters with growth parameters and to evaluate the possibility of their use in early selection for superior growth, the overall biomass was analyzed. Dry weight biomass was measured as an indicator of productivity, by drying the entire ramet. Samples were taken at the end of the first growing season. They consisted of 10 randomly selected ramets from each block and location. The ramets were dried at 105°C. The heating and weighing were repeated at 2-hr intervals until constant weight was obtained (B r o w n i n g , 1967).

The obtained data were processed by the following statistical parameters and statistical analyses: mean value, ANOVA, correlation and cluster analysis (Euclidean distance).

RESULTS

Net photosynthesis (Table 1) was most intensive in the clone PE 19/66 in the first and the second experiments and in the clone B-17 in the third experiment. The lowest net photosynthesis was in the European black poplar clones 54/86 in the first and second experiments and in the clone 53/86 in the third experiment. In the first experiment, transpiration rate ranged from 4.1667 (clone B-17) to 22.7778 mg H_2O $m^{-2}s^{-1}$ (clone Ostia). In the second and third experiments, transpiration was most intensive in the clone 53/86. The lowest transpiration rates were found in the clones M1 and PE 19/66. As determined by the ANOVA for net photosynthesis and transpiration, the differences among the clones as well as among the experiments were statistically highly significant.

Table 1. — Net photosynthesis and transpiration

Clone	Net photosynthesis mg CO_2 $m^{-2}s^{-1}$			Transpiration mg H_2O $m^{-2}s^{-1}$		
	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3
53/86	0.2961	0.3054	0.2314	18.0556	13.3333	15.2778
N1	0.3119	0.3392	0.2429	14.4444	13.0556	4.1667
54/86	0.2891	0.2816	0.2534	8.6111	8.0556	5.8333
Ostia	0.6164	0.5738	0.5751	22.7778	12.7778	6.3889
M1	0.8628	0.8219	0.5676	7.7778	3.3333	6.6667
Robusta	0.5905	0.5927	0.6054	6.9444	5.2778	7.7778
PE 19/66	1.0384	0.9530	0.5887	7.2222	4.1667	3.0556
B-17	0.6380	0.6956	0.6970	4.1667	4.7222	6.1111
S6-7	0.8954	0.7203	0.6833	10.0000	5.8333	6.1111
	F_{clone} 107.85***	F_{exp} 44.13***	$F_{cl\ x\ exp}$ 5.58***	F_{clone} 101.25***	F_{exp} 95.24***	$F_{cl\ x\ exp}$ 20.66***

The ratio between transpiration and net photosynthesis (water use efficiency) shows how many units of H_2O have to be transpired in order to fix one CO_2 unit (Larsen and Mekic, 1991). The highest WUE was in the clone B-17 (the first experiment), M1 (the second experiment) and PE 19/66 (the third experiment) (Table 2). Generally, the highest WUE was in the clones of eastern cottonwood. Due to different reactions of clones depending on soil types, clone x experiment interaction was also highly significant.

Table 2. — Water use efficiency (H_2O/CO_2)

Clone	Experiment 1	Experiment 2	Experiment 3
53/86	60.98	43.65	66.02
N1	46.31	38.49	17.15
54/86	29.78	28.60	23.02
Ostia	36.95	22.26	11.11
M1	9.01	4.05	11.74
Robusta	11.76	8.90	12.85
PE 19/66	6.95	4.37	5.19
B-17	6.53	6.78	8.76
S6-7	11.17	8.09	8.94
	F_{clone} 32.25***	F_{exp} 11.25***	$F_{clone\ x\ exp}$ 15.84***

The clones PE 19/66 in the first and the third experiment and B-17 in the second experiment had the greatest total oven-dry biomass. The lowest values were recorded in the clone 54/86 in the first and N1 in the second and third experiments (Table 3). Regarding this parameter, statistically highly significant differences existed among the clones and among the experiments. The interaction between clone and experiment was also statistically significant.

Table 3. — Oven-dry biomass (g)

Clone	Experiment 1	Experiment 2	Experiment 3
53/86	48.00	32.60	28.80
N1	54.11	29.60	17.50
54/86	47.00	32.60	29.10
Ostia	111.25	102.00	73.13
M1	144.50	133.05	102.43
Robusta	188.00	157.25	124.25
PE 19/66	301.50	221.50	179.50
B-17	268.50	227.52	173.00
S6-7	176.25	167.52	138.25
	F_{clone} 1273.17***	F_{exp} 1788.31***	$F_{clone\ x\ exp}$ 50.15***

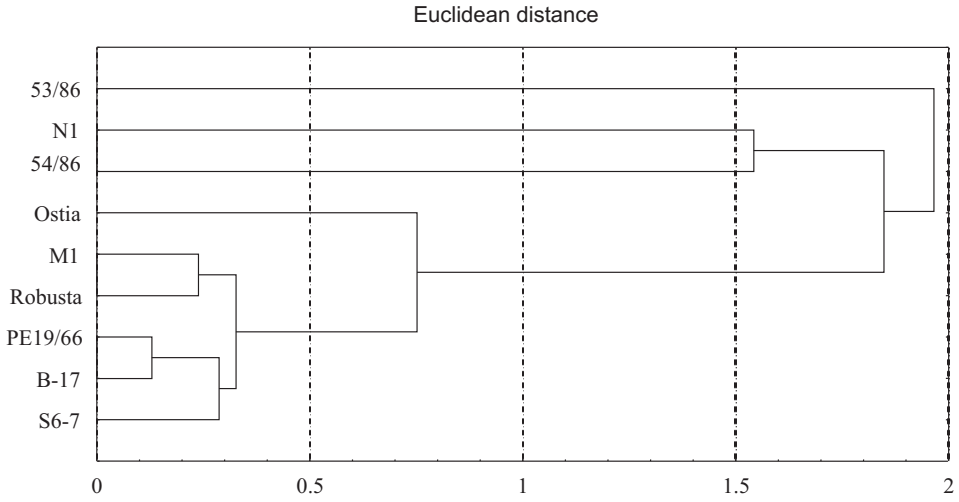
The coefficients of correlation among the studied characters show that net photosynthesis was in a high positive correlation with biomass. Medium negative correlation existed between WUE and net photosynthesis, transpiration

and biomass and WUE and biomass (Table 4). Transpiration rate was in a medium positive correlation with WUE.

Table 4. — Correlation coefficients

	Net photosynthesis	Transpiration	WUE	Biomass
Net photosynthesis		NS	—0.53**	0.85***
Transpiration			0.48*	—0.42*
WUE				—0.61***

Figure 1 — Dendrogram of cluster analysis



The dendrogram of the cluster analysis (Figure 1) shows that the clones of eastern cottonwood (PE 19/66, B-17 and S6-7) are in the first group, the clones M1 and Robusta (Euramerican poplar) in the second group, all of them grouped at small distances. The European black poplar clones (N1 and 54/86) are in the third group and they are grouped at a great distance. The clone 53/86, probably due to its high transpiration rate, is far from the other clones and it is grouped at the greatest distance.

DISCUSSION

The results of the research presented in this paper show a high positive correlation between net photosynthesis and biomass. In earlier poplar studies, net photosynthesis was positively correlated with growth elements only in some cases (Gordon and Promintz, 1976; Ceulemans and Impens, 1983; Isebrands et al., 1988; Ceulemans, 1990; Orlović et al., 1995). Compared with agricultural crops, positive correlation was much

more frequent for poplars. This is a consequence of the character of yield, which is vegetative for poplars and reproductive for agricultural crops (Ceulemans and Saugier, 1991). The studied clones differed in the amount of transpired water, i.e., in water use efficiency. Eastern cottonwood (*Populus deltoides*) had the greatest net photosynthesis while the European black poplar clones (*Populus nigra*) had a high intensity of transpiration. The high-yielding clones of eastern cottonwood and Euramerican poplar were superior in WUE. The results of the cluster analysis indicate that the studied properties were probably specific for the poplar species to which these clones belong, as announced by a previous research of several anatomical and physiological characters of poplar clones (Orlović et al., 1997). This study confirms the variability of the studied physiological characters and growth characters and a pronounced interclonal variability. Significant differences among the clones infer that these characters are genetically controlled to a high degree. The largest number of properties and parameters varied differently depending on the experiment (soil type), as proved by the statistically highly significant interaction clone x experiment. The results emphasize the need for further investigation which is significant for poplar selection under conditions of the changed water regimen in the soil, aiming at maximum utilization of poplar genetic potential for wood production.

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СЕЛЕКЦИЈА ЦРНИХ ТОПОЛА НА ЕФИКАСНОСТ КОРИШЋЕЊА ВОДЕ

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Резиме

У раду су приказани резултати истраживања фотосинтезе, транспирације, ефикасности коришћења воде (WUE) и биомасе девет клонова топола у три пољска огледа на различитим типовима земљишта (хумофлувисол, флувисол форма иловаста, флувисол форма песковита). Клонови америчке црне тополе (*Populus deltoides*) су имали највећу нето фотосинтезу и ефикасност коришћења воде. Резултати корелационе анализе су показали јаку корелацију између нето фотосинтезе и биомасе. Ефикасност коришћења воде и нето фотосинтеза, транспирација и биомаса и ефикасност коришћења воде и биомаса су били у негативној корелацији. Истраживање је показало високу интерклоналну варијабилност у погледу истраживаних физиолошких и параметара раста.

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GENOTYPIC VARIABILITY OF MORPHOLOGICAL CHARACTERISTICS OF ENGLISH OAK (*QUERCUS ROBUR* L.) ACORN

ABSTRACT: This paper deals with the acorn morphology (length, diameter and mass), analyzed in seventeen English oak genotypes (*Quercus robur* L.) from the English Oak Clonal Seed Orchard Banov Brod (Srem, Vojvodina). The highest values of acorn mass and length were measured in genotype 5. The largest diameters were measured in genotypes 6 and 21. Genotype 35 had the lowest acorn mass, length and diameter. The results from this study should serve as guidelines for the selection of trees yielding fruits possessing the desirable morphological characteristics.

KEY WORDS: acorn, morphology, *Quercus robur* L.

INTRODUCTION

Genus *Quercus*, represented by deciduous and evergreen trees and shrubs, belongs to the *Fagaceae* family. In our region, this genus is represented by several species. The English oak (*Quercus robur* L.) is one of the most important forest species. English oak forests are the most valuable forests in Europe from the economic aspect (Orlović et al., 2000). Owing to reckless timber harvesting in our country the former English oak sites were often left unforested or they were regenerated by other species (Erdeši, 1985). One of the methods of regeneration and restocking of partly naturally regenerated areas is to produce and use selected forest seed, produced in seed orchards (Littvay, 1999).

Oak fruit, acorn, contains a seed without an endosperm and an achlorophyllous embryo (Watson and Dallwitz, 2000). The size of English oak acorn depends on tree age and site characteristics, but its form, which is ovoid, is constant (Sárkány and Szalai, 1966). The size of a mature acorn is not constant and depends on the yield and other growth factors

(Maksimović et al., 1982). A direct correlation between acorn size and young seedling survival in stress conditions and a positive correlation of seedling size and acorn size are reported by Aizen and Woodcock (1996).

The cultivation of high quality oak and the intensified timber volume production are based on the production of superior seed. For this reason, in 1973, the best stands of *Quercus robur* L. were selected in the River Sava basin and designated as seed stands (Maksimović et al., 1982). Also, an English oak clonal seed orchard was established in the Sremska Mitrovica Forest Estate, from which the material for the analyses originates. This morphological study of acorn should produce guidelines for the selection of genotypes that yield fruits with desirable characteristics.

MATERIAL AND METHODS

Plant material was taken from the English Oak Clonal Seed Orchard Banov Brod, Forest Administration Višnjićevo, Forest Estate Sremska Mitrovica, State Enterprise "Srbijašume", Belgrade. The plantation is situated along the left bank of the River Sava, in the location "Šančine", the Banov Brod Forest, the village of Bosut. It was established by vegetative means, i.e. grafting. English oak seedlings of seed origin (acorn), aged from two to five years, were used as rootstock in the plantation establishment. Scions were taken from the trees (plus-trees) selected in the forests of East and West Srem and numbered from 1 to 85. The scions (shoots with three buds) were removed from plus-trees during the dormant period (early March) and kept until grafting (late April — early May). The scions for summer grafting were shoots with green bark whose buds were formed in May and June of the current year. In this way, the clonal seed orchard was established from 85 genotypes of English oak.

The following seventeen genotypes were selected for this study: 4, 5, 6, 16, 18, 20, 21, 22, 25, 28, 29, 30, 33, 35, 38, 40 and 85.

Acorn mass was determined by measuring the mass of 20 acorns and by calculating the average value.

Acorn length and diameter were measured using a slide gauge and expressed in cm.

The study results were processed statistically by the analysis of variance using the program MSTATC. The genotypes were compared using Duncan's test at $\alpha = 0.05$ significance level, with the values of the least significant digits — LSD. The means of the study parameters were ranked and marked with letters.

RESULTS AND DISCUSSION

The morphological study of the selected plant acorns indicates the presence of significant differences in both length and diameter (Table 1). Among the analyzed morphological characteristics, the least genotypic variability was observed for *acorn length* (CV = 5.23%). The highest average (32.3 mm), and

the highest critical values (31.0 mm, i.e. 35.0 mm) characterize the genotype 5. Genotype 16 had the smallest acorn length (23.8 mm) and the lowest critical values (20.7 mm, i.e. 26.0 mm). In general, the average size of the studied parameter in all genotypes was 27.2 mm. Compared with its length, *acorn diameter* had a somewhat higher variability among the studied genotypes (CV = 6.28%). The average minimum value for all plants was 14.4 mm, maximum 17.4 mm. Genotypes 21 and 6 had the largest diameters, 18.0 mm and 17.9 mm, respectively. The smallest acorn diameter occurred in genotype 35 (13.9 mm). The average diameter size for all studied plants was 15.9 mm. Several authors have attempted to differentiate individual oak species based on acorn size and form. However, Brookes and Wigston (1979) report that these parameters are not reliable discriminants, either for the differentiation between *Quercus petraea* and *Quercus robur*, or between these species and their hybrids.

Table 1. — *Quercus robur* acorn morphology, plantation "Banov Brod"

Genotype	Length (mm)			Diameter (mm)			Mass (g)		
	x	min	max	x	min	max	x	min	max
4	26.7^{d}	24.0	29.4	16.0^{def}	14.0	17.2	4.4^{d}	2.98	5.74
5	32.3^{a}	31.0	35.0	16.8^{bc}	15.0	18.3	6.1^{a}	4.54	7.32
6	25.3^{ef}	22.5	27.0	17.9^{a}	17.0	19.6	5.5^{bc}	3.76	7.15
16	23.8^{g}	20.7	26.0	16.2^{cde}	14.4	17.5	3.7^{fg}	2.43	5.31
18	26.0^{de}	24.3	27.6	14.9^{hi}	14.0	16.0	3.6^{g}	2.93	4.99
20	28.0^{c}	23.3	30.7	15.5^{fgh}	14.0	17.0	3.8^{efg}	2.29	4.97
21	28.5^{c}	26.3	29.9	18.0^{a}	15.3	19.5	5.8^{ab}	3.83	6.87
22	26.5^{d}	24.5	28.2	16.1^{def}	14.4	17.2	4.4^{d}	3.39	5.43
25	25.1^{ef}	23.9	27.0	15.7^{efg}	14.4	17.3	3.8^{efg}	3.30	4.98
28	29.8^{b}	26.3	31.8	16.5^{bcd}	14.6	18.0	5.5^{bc}	3.32	6.83
29	26.7^{d}	23.9	29.1	16.2^{cde}	14.1	17.8	4.3^{de}	2.83	5.24
30	26.5^{d}	24.3	30.0	15.5^{fgh}	13.8	17.1	4.2^{def}	3.18	5.47
33	26.8^{d}	24.2	29.3	15.0^{hi}	13.8	16.2	3.9^{defg}	3.10	4.91
35	24.6^{fg}	21.9	26.0	13.9^{j}	12.4	15.8	2.8^{h}	1.97	4.12
38	28.7^{c}	27.2	29.9	17.0^{b}	15.5	18.5	5.1^{c}	4.14	6.06
40	26.7^{d}	25.4	28.1	14.6^{i}	13.3	15.8	3.8^{efg}	3.09	4.72
85	30.7^{b}	28.8	32.6	15.2^{ghi}	14.2	16.2	4.2^{def}	3.30	5.16
Average	27.2	24.9	29.3	15.9	14.4	17.4	4.4	3.20	5.60
$LSD_{0.05}$	0.89	—	—	0.62	—	—	0.47	—	—
CV%	5.23	—	—	6.28	—	—	17.26	—	—

Kormanik et al. (1998) reported of the dependence of *Quercus rubra* L. seedling development on acorn size. Plant height, root collar diameter and seedling survival are significantly correlated with acorn mass. Miao (1995) provided data on the effect of acorn mass on seedling growth in *Quercus rubra*. The results show that the total biomass increased with the increase of initial acorn mass.

Acorn mass is the morphological characteristic with the highest variability (Table 1) among the studied plants. Disregarding the genotype, the values ranged between 3.2 and 5.6 g. The average acorn mass was 4.4 g. The values of individual genotypes varied between 3.6 g and 6.1 g, measured in genotypes 18 and 5, respectively. It was interesting to note that genotype 5, along with the highest acorn mass, also excelled all studied plants in acorn length.

The study of the above morphological characteristics of English oak acorn also included correlations (Table 2). Positive correlations were found between length and width and length and acorn mass. There was a very high correlation between width and acorn mass ($r^2 = 0.87$).

Table 2. — Correlation of acorn morphological features

Acorn morphology	Correlation coefficient
length — width	0.36
length — mass	0.66
width — mass	0.87

The shares of individual parts of the fruit in its total mass were also different (Table 3). On average, cotyledons amounted to about 87.5% of the fresh acorn mass, while pericarp and testa accounted for about 12.5%. The values of individual genotypes varied only up to four percent (from 86% to 90% for cotyledons, i.e. from 10% to 14% for the other part of the fruit). It is interesting that the percentage of individual parts of the fruit in its dry mass showed different tendencies compared with fresh mass. Namely, while the cotyledon percentage decreased, the percentage of pericarp and testa increased.

Table 3. — Percentage of individual fruit parts in its total mass

Genotype	Percentage in fresh mass (%)		Percentage in dry mass (%)	
	Cotyledon	Pericarp and testa	Cotyledon	Pericarp and testa
4	90	10	86	14
5	88	12	85	15
6	86	14	81	19
16	86	14	82	18
18	88	12	84	16
20	88	12	82	18
21	87	13	84	16
22	87	13	84	16
25	88	12	86	14
28	87	13	85	15
29	89	11	87	13
30	88	12	86	14
33	88	12	84	16
35	87	13	84	16
38	89	11	85	15

40	86	14	83	17
85	86	14	83	17
Average	87.5	12.5	84.2	15.8

CONCLUSION

The study of acorn morphological features in seventeen English oak genotypes from the English Oak Clonal Seed Orchard Banov Brod (Srem, Vojvodina) points to genotype 5 with the highest measured values of acorn mass and length. The largest diameters were measured in genotypes 6 and 21. The lowest acorn mass and sizes occurred in genotype 35.

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ГЕНОТИПСКА ВАРИЈАБИЛНОСТ МОРФОЛОШКИХ ОСОБИНА ЖИРА ХРАСТА ЛУЖЊАКА (*QUERCUS ROBUR* L.)

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Резиме

У раду су анализиране морфолошке особине (дужина, пречник и маса) жира код седамнаест генотипова храста лужњака (*Quercus robur* L.) из вегетативне семенске плантаже Банов Брод (Срем, Војводина). Највише вредности масе и дужине жира утврђене су код генотипа 5, највећи пречник код генотипова 6 и 21, док је генотип 35 имао најниже вредности масе, дужине и пречника жира. Добијени резултати треба да буду једна од смерница у одабирању оних генотипова који дају плодове са пожељним морфолошким карактеристикама.

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THE VARIABILITY OF LEAF ANATOMICAL CHARACTERISTICS OF *SOLANUM NIGRUM* L. (*SOLANALES*, *SOLANACEAE*) FROM DIFFERENT HABITATS

ABSTRACT: In Europe on the whole as well as in Yugoslavia, the most widespread weed species from the genus *Solanum* is *Solanum nigrum* L. Since this species inhabits different habitats, it developed several ways of adaptation to environmental conditions. The influence of ecological factors on plant organism and resulting plant adaptations are most evident in leaf morphology and anatomy. Therefore, the anatomical structure of leaves and leaf epidermal tissue of *S. nigrum* was analyzed and compared among plants that originated from different habitats, in order to determine leaf structural adaptations.

S. nigrum lamina has the mesomorphic structure with some xero-heliomorphic adaptations. The differences in stomata number, number of hairs, thickness of lamina, palisade and spongy tissue, as well as the size of mesophyll cells have been noticed. The highest values for most of the parameters have been recorded for the plants from cultivated soil. Largest variations of the examined characters were found for the leaves from ruderal habitats, where environmental conditions are most variable.

KEY WORDS: leaf adaptations, leaf anatomy, *Solanum nigrum*

INTRODUCTION

Within the family *Solanaceae*, the genus *Solanum* is richest in the number of species (T a k h t a j a n , 1997). Fifteen species of this genus are present in the flora of Europe (H a w k e s and E d m o n d s , 1972), nine in the flora of Serbia (S t j e p a n o v i c - V e s e l i c i c , 1974). Many of these species are crop weeds or weeds of ruderal habitats. The most widespread weed species in Europe and in Yugoslavia is *Solanum nigrum* L.

According to S o ó (1968), *S. nigrum* belongs to South-Eurasian (Mediterranean) floral element and today is a cosmopolitan species. It grows in different types of habitats, as weed in crops and in ruderal habitats, along the roads, fences and in neglected places (S t j e p a n o v i c - V e s e l i c i c , 1974).

Consequently, the species has developed several ways of adaptation to environmental conditions, mainly through phenotypic plasticity and variation of biomass production, which induced the occurrence of a large number of infraspecific taxa (Soó, 1968; Mahn and Lemme, 1989). Ecological indexes (humidity-3, soil acidity-3, amount of N in the soil-4, light-4, temperature-3) show that this species prefers neutral to weakly acid soils, rich in nutrients (Kojic et al., 1997). Although it can be found in xerophylous associations, it mostly inhabits light, mesophylous habitats. Considering temperature requirements, it belongs to mesothermic plants. It is a halophobic species which does not grow on salty soils (Landolt, 1977). The life form is therophyte (Landolt, 1977; Kojic et al., 1997).

Not many data concerning the leaf anatomy of *S. nigrum* can be found in literature. In describing some representatives of the family *Solanaceae* only main anatomical characteristics have been given (Metcalfe and Chalk, 1957). Rogers and Ogg (1981) studied morphological and anatomical characteristics of vegetative organs of some species belonging to *S. nigrum* complex (*S. nigrum, S. sarrachoides, S. americanum* and *S. ptycanthum*). They noticed that they varied in relation to environmental conditions. Stomata of these species are anomocytic to anisocytic, somewhat larger in *S. nigrum*, which can be explained by its polyploidy (Rogers and Ogg, 1981). Stomata number is two to three-fold higher on abaxial than on adaxial epidermis. Seithe and Anderson (1982) investigated hair morphology of some species from the genus *Solanum*, but not in *S. nigrum*.

It is well known that the leaf, compared with the other vegetative organs, is the best and fastest to react to changes in the environment. The influence of different ecological factors on plant organisms is best reflected on leaf morphological and anatomical structure. In this paper we analyze the anatomy of *S. nigrum* leaf and compare anatomical structure of leaves from different habitats, with the aim of determining their structural adaptations.

MATERIAL AND METHODS

Ten completely developed plants in flower were collected from each of the three habitats that differed in ecological conditions (light, humidity, temperature, soil composition). Sample 1 was collected from a ruderal habitat, exposed to direct sunlight, sample 2 from sandy soil of a riparian habitat, where plants were periodically exposed to sunlight, and sample 3 from a cultivated field, with soil rich in mineral elements, permanently exposed to direct sunlight.

Leaves from the middle part of the plants were separated for anatomic investigations. Epidermal tissue was analyzed on prints made after Wolf (1954). The type, number and size of stomata, along with the type and arrangement of hairs on adaxial and abaxial epidermis were determined using light microscope. Leaf cross-sections were made at ¼ leaf width and at the main vein, using a freezing microtome. Measurements included the thickness of lamina, mesophyll, palisade and spongy tissue, height and width of palisade cells and cells of adaxial and abaxial epidermis, and the height and width of

the main vein, main vein vascular bundle and vessels. All measurements were made in ten repeats. Data were statistically processed using STATISTICA for Windows program.

RESULTS AND DISCUSSION

Leaf epidermal characteristics

The analysis of epidermal prints has shown that the epidermal tissue consisted of cells with rugose anticlinal walls, especially abaxially (Figure 1). In-

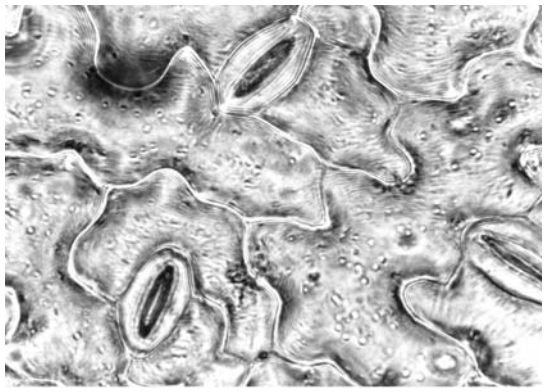

Figure 1 — Leaf epidermis, stomata of anisocytic type

dumentum, made of non-glandular and glandular hairs, was sparse on both epidermal sides. Hairs were more numerous along veins and on the abaxial lamina side. Non-glandular hairs were uniseriate, unbranched, conical, granular on the surface, consisting of two to three cells (Figure 2). The terminal cell

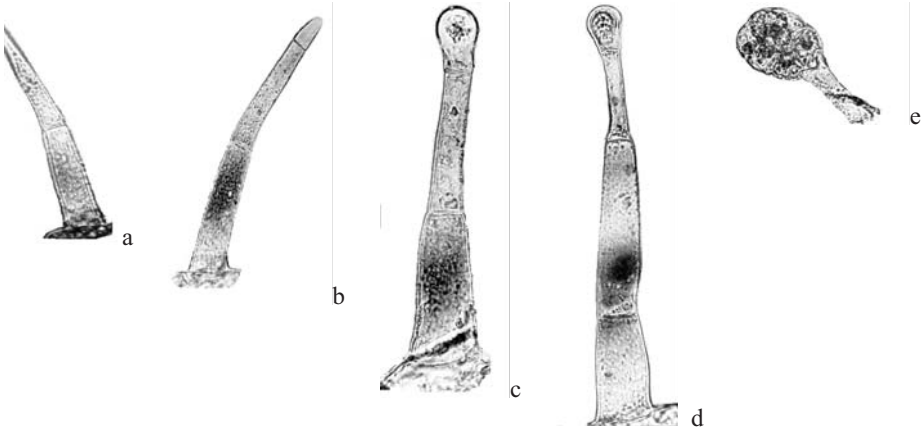

Figure 2 — Hair types: a-b — non-glandular hairs; c-e — glandular hairs

was narrower, with a sharp tip, while basal cells were much wider. These hairs differed in width, from wide to very narrow. Glandular hairs were not numerous. There were two types of glandular hairs. The hairs of one type consisted of a short, unicellular stalk and a multicellular secretory head, round to oval in shape (Figure 2). The hairs of second type had a longer stalk, made of two, three or four cells, and a unicellular secretory head at the top. The stalk cell under the head was narrow. It has been noticed that this cell dried faster than the other stalk cells. Stalk cells closer to the hair base were widened.

The hairs were more numerous on the leaves of sample 1, than on those of samples 2 and 3. This could be explained by the high level of habitat illumination, the greater number of hairs being an adaptation by which the plant could reduce transpiration and the warming of the leaf surface.

Leaves were amphistomatal, with stomata at the level of epidermal cells. The stomata were mostly anisocytic, sometimes anomocytic (Figure 1). Measurements have shown that they were larger and more numerous on the abaxial epidermis (Table 1). The leaves of sample 1 had the highest number of stomata on both epidermises, significantly higher than the leaves of samples 2 and 3. The lowest stomata number was recorded in the leaves of sample 2, coming from the plants that grew in the habitat periodically exposed to sunlight. Stomata were smallest on the leaves of sample 1, and largest on the leaves of sample 3. The values of abaxial epidermis stomata width have shown statistically significant difference among the three samples. The increased number of stomata of smaller size on the leaves of sample 1 appears to be an adaptation to higher temperatures, enabling the plant to regulate stomatal transpiration adequately.

Table 1. — Number (per mm^2 of lamina surface) and size (m) of stomata of *S. nigrum* leaves

		Sample 1	Sample 2	Sample 3
Number of stomata (adaxial epidermis)	$\overline{X} \pm S_{\bar{x}}$	131 ± 5	63 ± 3	64 ± 2
	σ	21,7	13,1	12,8
	min-max	92—174	37—94	44—87
	95 Pct	121—141	57—69	59—69
	CV	16,6	20,8	20,0
Number of stomata (abaxial epidermis)	$\overline{X} \pm S_{\bar{x}}$	181 ± 5	89 ± 2	116 ± 3
	σ	17,6	7,9	14,7
	min-max	155—216	81—109	94—144
	95 Pct	170—191	83—95	111—122
	CV	9,7	8,9	12,7
Stomata length (adaxial epidermis)	$\overline{X} \pm S_{\bar{x}}$	37,7 ± 0,7	39,0 ± 1,0	40,1 ± 0,7
	σ	4,0	5,7	3,6
	min-max	25,8—46,4	25,8—50,3	32,3—46,4
	95 Pct	36,2—39,2	36,8—41,1	38,7—41,4
	CV	10,7	14,5	8,9

Stomata width (adaxial epidermis)	$\bar{X} \pm S_{\bar{x}}$	21,2 ± 0,4	21,9 ± 0,6	23,3 ± 0,6
	σ	2,1	3,3	3,3
	min-max	16,8—25,8	15,5—28,4	15,5—29,7
	95 Pct	20,4—21,9	20,7—23,2	22,1—24,5
	CV	9,8	15,0	14,1
Stomata length (abaxial epidermis)	$\bar{X} \pm S_{\bar{x}}$	41,1 ± 1,0	38,1 ± 1,1	43,4 ± 1,2
	σ	5,3	6,2	6,7
	min-max	31,0—52,9	25,8—51,6	31,0—61,9
	95 Pct	39,1—43,1	35,8—40,3	40,9—45,9
	CV	12,9	16,2	15,5
Stomata width (abaxial epidermis)	$\bar{X} \pm S_{\bar{x}}$	26,9 ± 0,6	23,9 ± 0,7	29,7 ± 0,7
	σ	3,4	3,6	3,7
	min-max	21,9—36,1	16,8—31,0	23,2—38,7
	95 Pct	25,6—28,2	22,6—25,3	28,4—31,1
	CV	12,7	15,0	12,3

$\bar{X}$ — average value; $S_{\bar{x}}$ — the standard error of the average value; σ — standard deviation; min-max — minimum and maximum values; 95 Pct — limits of confidence 95%; CV — variation coefficients (%)

Mesophyll characteristics and leaf cells size

The lamina of *S. nigrum* was dorsiventral (Figure 3). The epidermal cells were much larger on the adaxial than on the abaxial side, covered with a thin cuticle. The mesophyll was divided to palisade and spongy tissue. Palisade tissue cells were large, elongated, arranged in a single layer. The spongy tissue was made of cells which were round or irregular in shape and arranged in 3—5 layers. Large intercellular spaces occurred among these cells. Some cells of the palisade and spongy tissues contain dark groups of crystals. The palisade and spongy tissues were equally thick, with the ratio of approximately 1:1.

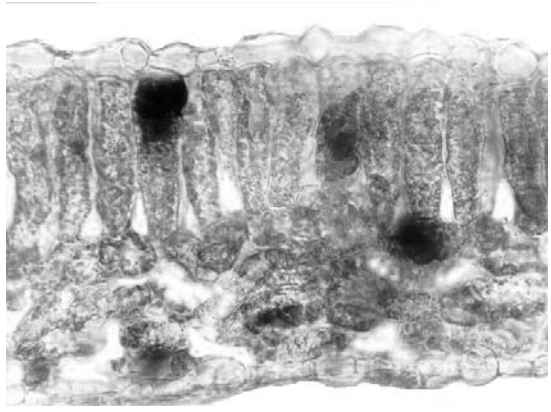

Figure 3 — Leaf cross-section at ¼ of leaf width

Vascular bundles, surrounded by a bundle sheath, were present in the mesophyll.

The main vein was prominent, especially on the abaxial side (Figure 4). A large bicolateral vascular bundle, with patches of poorly developed phloem

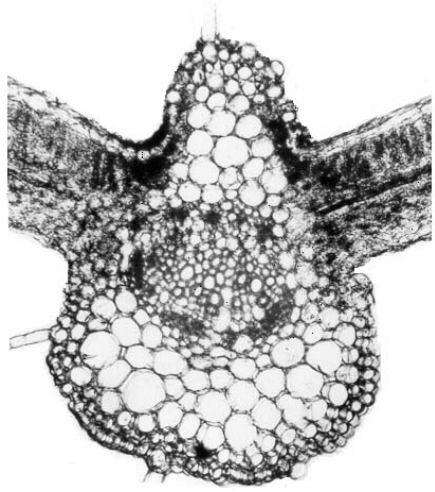

Figure 4 — Leaf cross-section at the main vein

and sclerenchyma tissue, was present in the main vein. Large vessels were noticed in the xylem. The main tissue of the main vein was made of parenchyma cells of different sizes, round in shape, with numerous small intercellular spaces among them. These cells did not contain chloroplasts.

Sample 1 — The average lamina thickness of the plants from this habitat was 265 m (Table 3). The palisade tissue was made of one, the spongy tissue of three layers of cells. The ratio of palisade and spongy tissue was 1.08. Groups of crystals in the mesophyll cells were most numerous in sample 1. This could be connected with soil properties of the ruderal habitat, which was rich in $CaCO_3$, P_2O_5 and K_2O. Data on soil characteristics are presented in Table 2 (K r s t i c et al., 1999) The cells of the adaxial epidermis were larger than those of the abaxial epidermis. The main vein was prominent and it contained a large vascular bundle.

Table 2. — Chemical properties of soil

Habitat	pH in KCl	pH in H_2O	$CaCO_3$ (%)	Humus (%)	Total N (%)	AL-P_2O_5 (mg/100 g)	AL-K_2O (mg/100 g)
1	8.06	8.35	12.69	1.22	0.081	40.73	23.50
2	7.98	8.21	15.17	0.93	0.061	12.40	7.50
3	7.46	8.05	2.78	3.46	0.228	16.73	20.50

According to the values of variation coefficients for the examined parameters, the greatest variability was recorded for the size of epidermal cells,

mesophyll thickness and the height of palisade tissue cells (Table 3). Variability was lowest for the size of vessels and the height of the main vein.

The plant laminas of sample 1 had mesomorphic structure, with presence of some xero-heliomorphic characteristics — more numerous stomata of smaller size, numerous non-glandular and glandular hairs on both epidermises, less cell layers of the spongy tissue.

Sample 2 — The laminas of these plants, 273 m thick on average, consisted of a single layer of palisade tissue and four to five layers of spongy tissue (Table 3). The ratio of palisade and spongy tissue was 1.10. The cells of the abaxial epidermis were larger than the same cells in the laminas from the other samples, their average height being 21.1 m and the average width 27.4 m. The main vein size, the size of the main vein vascular bundle and vessels, compared with the same values measured for the laminas of samples 1 and 3, were significantly lower (Table 3).

The values of variation coefficient showed that the greatest variability, compared with the other samples, is recorded for the thickness of palisade and spongy tissue, the main vein height and vessel size (Table 3). The size of epidermal cells and the width of the main vein vascular bundle varied to a lesser extent.

The plant laminas of sample 2 had the mesomorphic structure reflected in large epidermal cells (especially abaxialy), the smallest number of stomata, sparse hairs and the thinnest mesophyll. This structure could be connected with habitat conditions, where the plants were only periodically exposed to direct sunlight, while water was available in sufficient amounts.

Sample 3 — The average lamina thickness of the plants of sample 3 was 300 m (Table 3). The cells of the palisade tissue were arranged in a single layer, the cells of the spongy tissue in four. The ratio of these two tissues was 1.06. The cells of the adaxial epidermis were large, 31.2 m high and 46.4 m wide. The main vein was also large, with the largest vascular bundle (319 m high and 428 m width) compared with the other two samples. Large vessels were present in the xylem.

When compared with the other samples, the plant laminas of sample 3 showed smaller variability for most of the measured characters, especially for the thickness of lamina, mesophyll, palisade and spongy tissue (Table 3).

The plant laminas of sample 3 also had the mesomorphic structure. Highest values for most of the measured parameters were recorded for the laminas of this sample (Table 3). Statistically, these values were significantly higher than the values obtained for the other samples. This could be explained by the fact that the habitat from which these plants originated was a cultivated soil rich in mineral elements, especially nitrogen, which is the essential element for plant growth.

Table 3. — Leaf anatomical characteristics (m)

		Sample 1	Sample 2	Sample 3
Lamina thickness	$\bar{X} \pm S_{\bar{x}}$	265 ± 12	273 ± 11	300 ± 6
	σ	52,3	49,1	27,8
	min-max	163—326	194—357	265—367
	95 Pct	240—291	250,4—296,3	287—313
	CV	19,7	18,0	9,2
Mesophyll thickness	$\bar{X} \pm S_{\bar{x}}$	214 ± 10	211 ± 10	240 ± 6
	σ	43,5	44,9	26,6
	min-max	122—270	148—296	209—306
	95 Pct	193—235	190—232	228—253
	CV	20,4	23,3	11,1
Palisade tissue thickness	$\bar{X} \pm S_{\bar{x}}$	109 ± 4	112 ± 5	123 ± 3
	σ	22,8	24,6	12,8
	min-max	71—148	71—153	107—153
	95 Pct	101—118	102—122	117—129
	CV	20,9	22,0	10,4
Spongy tissue thickness	$\bar{X} \pm S_{\bar{x}}$	104 ± 4	103 ± 4,7	117 ± 4
	σ	23,4	24,3	18,9
	min-max	71—153	61—153	92—163
	95 Pct	95—112	934—113	108—126
	CV	22,6	23,5	16,2
The ratio of palisade and spongy tissue	$\bar{X} \pm S_{\bar{x}}$	1,08 ± 0,03	1,10 ± 0,04	1,06 ± 0,03
	σ	0,2	0,2	0,2
	min-max	0,8—1,4	0,8—1,6	0,8—1,4
	95 Pct	1,0—1,5	1,0—1,2	1,0—1,1
	CV	18,5	18,2	18,9
Palisade tissue cells height	$\bar{X} \pm S_{\bar{x}}$	107,7 ± 5,0	111,5 ± 17,8	123,1 ± 2,7
	σ	27,1	19,8	14,9
	min-max	65,5—151,2	80,1—148,7	93,2—153,7
	95 Pct	97,6—117,9	104,1—118,9	117,6—128,7
	CV	25,2	17,8	12,1
Palisade tissue cells width	$\bar{X} \pm S_{\bar{x}}$	25,7 ± 0,7	29,2 ± 1,1	24,2 ± 0,64
	σ	3,6	6,2	3,5
	min-max	17,6—32,8	15,1—44,1	17,6—30,2
	95 Pct	24,3—27,1	26,8—31,5	22,8—25,5
	CV	14,1	21,2	14,5
Adaxial epidermis cells height	$\bar{X} \pm S_{\bar{x}}$	31,1 ± 1,0	31,6 ± 1,0	31,2 ± 0,9
	σ	7,1	7,1	6,1
	min-max	20,2—55,4	22,7—58,0	20,2—50,4
	95 Pct	29,1—33,2	29,6—33,7	29,5—32,9
	CV	22,7	22,4	19,6

Adaxial epidermis cells width	$\overline{X} \pm S_{\bar{x}}$	42,3 ± 1,9	40,0 ± 1,4	46,4 ± 1,9
	σ	13,6	9,9	13,3
	min-max	21,4—85,7	24,0—63,0	25,2—85,7
	95 Pct	38,5—46,2	37,2—42,9	42,6—50,2
	CV	32,1	24,7	28,6
Abaxial epidermis cells height	$\overline{X} \pm S_{\bar{x}}$	19,9 ± 0,9	21,1 ± 0,7	18,8 ± 0,6
	σ	5,7	4,6	4,2
	min-max	12,6—42,8	12,6—30,2	12,6—28,9
	95 Pct	18,2—21,7	19,8—22,4	17,6—20,0
	CV	28,8	21,7	22,3
Abaxial epidermis cells width	$\overline{X} \pm S_{\bar{x}}$	24,5 ± 1,3	27,4 ± 1,3	22,0 ± 1,0
	σ	8,6	8,7	7,2
	min-max	12,6—55,4	12,6—45,4	12,6—47,9
	95 Pct	21,9—27,0	24,9—29,9	19,9—24,0
	CV	35,0	31,9	32,9
The main vein height	$\overline{X} \pm S_{\bar{x}}$	942 ± 34	800 ± 47	965 ± 40
	σ	106,0	149,8	125,9
	min-max	780—1118	572—988	780—1170
	95 Pct	867—1018	692—907	875—1055
	CV	11,3	18,7	13,05
The main vein width	$\overline{X} \pm S_{\bar{x}}$	766 ± 40	662 ± 27	828 ± 31
	σ	127,1	84,6	97,3
	min-max	650—1066	520—754	728—1001
	95 Pct	675—857	601—722	759—898
	CV	16,6	12,8	11,8
The main vein vascular bundle height	$\overline{X} \pm S_{\bar{x}}$	306 ± 15	251 ± 16	319 ± 16
	σ	47,1	49,6	51,7
	min-max	235—408	173—321	255—408
	95 Pct	272—340	216—287	282—356
	CV	15,4	19,7	16,2
The main vein vascular bundle width	$\overline{X} \pm S_{\bar{x}}$	394 ± 20	329 ± 16	428 ± 25
	σ	64,7	50,6	80,5
	min-max	342—561	255—377	342—556
	95 Pct	348—441	293—365	371—486
	CV	16,4	15,4	18,8
Vessel height	$\overline{X} \pm S_{\bar{x}}$	22,9 ± 0,7	21,2 ± 1,0	28,1 ± 1,0
	σ	3,7	5,7	5,7
	min-max	16,4—30,2	11,3—32,8	18,9—41,6
	95 Pct	21,6—24,3	19,0—23,3	26,0—30,3
	CV	16,1	26,9	20,1

Vessel width	$\overline{X} \pm S_{\bar{x}}$	21,0 ± 0,7	19,0 ± 0,9	24,8 ± 0,7
	σ	3,6	4,9	3,9
	min-max	12,6—30,2	12,6—32,8	17,6—32,8
	95 Pct	19,7—22,4	17,2—20,9	23,3—26,2
	CV	16,9	25,8	15,8

$\overline{X}$ — average value; $S_{\bar{x}}$ — the standard error of the average value; σ — standard deviation; min-max — minimum and maximum values; 95 Pct — limits of confidence 95%; CV — variation coefficient (%)

Table 4. — The significance of differences among the examined samples in the measured leaf characters

Samples	1—2	1—3	2—3
Number of stomata (adaxial epidermis)	*	*	ns
Number of stomata (abaxial epidermis)	*	*	*
Stomata length (adaxial epidermis)	ns	ns	ns
Stomata width (adaxial epidermis)	ns	*	ns
Stomata length (abaxial epidermis)	ns	ns	*
Stomata width (abaxial epidermis)	*	*	*
Lamina thickness	ns	*	ns
Mesophyll thickness	ns	*	*
Palisade tissue thickness	ns	*	ns
Spongy tissue thickness	ns	*	*
Palisade tissue cells height	ns	*	*
Palisade tissue cells width	*	ns	*
Adaxial epidermis cells height	ns	ns	ns
Adaxial epidermis cells width	ns	ns	*
Abaxial epidermis cells height	ns	ns	*
Abaxial epidermis cells width	ns	ns	*
The main vein height	*	ns	*
The main vein width	*	ns	*
The main vein vascular bundle height	*	ns	*
The main vein vascular bundle width	*	ns	*
Vessel height	ns	*	*
Vessel width	ns	*	*

* — the difference is significant for $\alpha = 0.01$; ns — the difference is not significant

The thickness of the tissues and the size of the measured cells have shown statistically significant differences between samples 2 and 3, while the differences were smaller between samples 1 and 3, which originated from habitats similar in illumination (Table 4). Samples 1 and 2 have shown statistically significant differences in the size of the measured characteristics of the main vein. Between samples 1 and 3, statistically significant differences were recorded for the thickness of lamina, mesophyll, palisade and spongy tissue, but not for the size of epidermal cells and characteristics of the main vein.

Among the measured characters, the values for the size of epidermal cells and the thickness of mesophyll, palisade and spongy tissue were most variable (Table 3). The smallest variability was recorded for the size of the main vein and main vein vascular bundle. Considerably higher variability was found in the laminas of the plants of sample 1, which originated from the ruderal habitat and sample 2, from the habitat with changeable exposure to sunlight, in comparison with laminas of sample 3. The variable habitat conditions in habitats of the plants of samples 1 and 2 resulted in the higher variability of the analyzed quantitative anatomic leaf characteristics.

Anatomic investigations of the leaf of *S. nigrum* point to the main, mesomorphic leaf structure, expressed through the presence of relatively large epidermal cells (especially adaxialy) with rugose anticlinal walls, rare indumentum, relatively small number of large stomata in the level of epidermal cells, thin cuticle, approximately the same thickness of palisade and spongy tissue, large cells of palisade tissue and loose mesophyll. The leaves of the plants of sample 1, that were exposed to direct sunlight during a whole day, had some xero-heliomorphic adaptations to the drier and lighter habitat conditions: more numerous hairs, more numerous stomata of smaller size on both epidermal sides, a fewer layers of cells of the spongy tissue, smaller intercellular spaces (mesophyll more compact) and smaller cell size. The highest values of cell size were recorded in the plants of sample 3, which originated from cultivated soil rich in mineral elements. The analysis showed that soil quality, along with the other ecological factors, has an important influence on the size of cells and tissues of the *S. nigrum* leaf.

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ВАРИЈАБИЛНОСТ АНАТОМСКИХ КАРАКТЕРИСТИКА ЛИСКЕ *SOLANUM NIGRUM* L. (*SOLANALES*, *SOLANACEAE*) СА РАЗЛИЧИТИХ СТАНИШТА

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Резиме

На подручју Европе и Југославије најраспрострањенија коровска врста из рода *Solanum* је *Solanum nigrum* L. Како ова врста насељава веома различита станишта, развила је неколико начина прилагођавања условима спољашње средине. Познато је да лист највише и најбрже реагује на утицаје спољашње средине, те се у његовој морфолошкој и анатомској грађи најбоље огледа утицај еколошких фактора на биљни организам. Стога је анализирана анатомска грађа лиске и покоричног ткива лиске ове врсте и упоређена је код биљака са различитих типова станишта, ради утврђивања њихових структурних адаптација.

Лиска *S. nigrum* је мезоморфне грађе, са извесним ксеро-хелиоморфним адаптацијама. Код листова биљака које потичу са различитих станишта установљене су разлике у броју стома на оба епидермиса, длакавости, дебљини лиске, палисадног и сунђерастог ткива, као и у величини ћелија мезофила. Највеће вредности за већину мерених параметара добијене су код биљака са обрадивог земљишта. Испитивани параметри су највише варирали код лиски биљака са рудералног станишта, где су и услови спољашње средине најваријабилнији.

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HETEROSIS IN CROSSES BETWEEN WHEAT GENOTYPES WITH DIFFERENT SPIKE ARCHITECTURE

ABSTRACT: In order to estimate hybrid vigor, wheat genotypes differing in spike architecture (normal, tetrastichon and branched) were crossed and the F_1 and F_2 generations analyzed for the number of fertile spikelets/spike, number of grains/spike and grain weight/spike. The parents used for crossing were Sava (normal spike), Forlani (normal spike), ZG T 171/1 (tetrastichon spike) and ZG 172 (branched spike). The F_1 and F_2 progenies, except those from the cross Sava x Forlani, had a lower number of fertile spikelets / spike compared with the better parent. In the crosses between genotypes with normal and branched spikes, the F_1 and F_2 progenies formed significantly fewer grains / spike. On the other hand, the F_1 of the crosses between genotypes with normal and tetrastichon spike showed a significant level of heterosis with respect to the number of grains / spike, particularly the cross Forlani x ZG T 171/1. In regard to grain weight / spike, significant heterosis was detected in all crosses except Sava x ZG 172.

The crosses between genotypes with normal and tetrastichon spikes that exhibited significant heterosis for two main yield components were most promising in the context of hybrid wheat development. Such crosses deserve further attention and investigation.

KEY WORDS: heterosis, spike architecture, yield components, wheat

INTRODUCTION

Research aimed at the development of F_1 hybrid wheat varieties proceeded intermittently over last 50 years. The interest in this topic seems to be gaining momentum again. So far, varieties with normal spikes have typically been used as parents in hybrid wheat development programs. The research presented in this paper explored the possibility of exploiting hybrid vigor in wheat by crossing wheat genotypes with different spike architectures. We crossed wheat varieties and lines differing in spike architecture as parents, and compared F_1 and F_2 performance with that of the better parent for three critical determinants of grain yield (number of fertile spikelets/spike, number of grains/spike and grain weight/spike) in order to see a) whether genotypes with branched and tetrastichon spike may successfully serve as parents of wheat hy-

brids and b) whether the F_1 and F_2 generations of such crosses could be used for commercial production.

MATERIALS AND METHODS

The experimental material was divided into three groups differing in spike architecture:

1. *Genotypes with normal spike:* a) Sava — a semidwarf (*Rht 8*), early (*Ppd 1*), winter wheat cultivar, derived from a cross Fortunato*2 / Red Coat at the Institute of Field and Vegetable Crops, Novi Sad (Yugoslavia) and b) Forlani — a tall Italian cultivar, susceptible to lodging but having a very productive spike (4—5 grains per spikelet), derived from a cross Villa Glori / Grano del Miracolo.

2. *Genotype with tetrastichon spike:* ZG-T-171/1 — a winter wheat line with 4-row (tetrastichon) spike, developed at the Institute for Breeding and Production of Field Crops (Croatia), from a cross Granata x Ranka.

3. *Genotype with branched spike:* ZG-172 — a tall winter wheat line with the branched spike developed at the Institute for Breeding and Production of Field Crops (Croatia), from a cross H 303 / Granatka // Granatka / Ranka.

The various spike types were crossed as follows:

a) **normal / branched** — (Sava x ZG-172 and Forlani x ZG-172)

b) **normal / tetrastichon** — (Sava x ZG-T-171/1 and Forlani x ZG-T--171/1)

c) **normal / normal** — (Sava x Forlani)

The F_1 and F_2 generations and their parents were grown in the 1992/93 season at the experiment field at the Institute of Field and Vegetable Crops, Novi Sad, Yugoslavia. The experiment was established in a randomized complete block design with three replicates. About 150 F_1 and 2000 F_2 plants were grown per combination. The sets of crosses and their parents were planted in rows 20 cm apart, with plants 6 cm apart within the row.

The spikes from the F_1 and F_2 generations were analyzed and compared with those of their contributing parents for the number of fertile spikelets / spike, number of grains / spike and grain weight / spike.

The F_2 progenies, except those from the cross Sava x Forlani, featured three or two types of spike structures e.g. normal, branched and (or) tetrastichon. The modes of inheritance for the spike architecture and the other traits have been presented in an earlier paper (D e n č i ć , 1988).

RESULTS AND DISCUSSION

Number of fertile spikelets / spike

In the crosses normal x branched spike, all F_1 and F_2 progenies showed a lower number of fertile spikelets / spike (from 50.2% to 64.7%) compared with the better parent (ZG 172). In the normal x tetrastichon crosses Sava x

ZG-T-171/1 and Forlani x ZG-T-171/1, all F_1 and F_2 progenies showed a lower number of fertile spikelets / spike (more than 30% below that of the better parent (Table1). Those results were expected since, in general, genotypes with branched and tetrastichon spikes have a large number of spikelets/spike. Furthermore, the results for the crosses between branched or tetrastichon x normal spikes showed that no heterotic effect could be achieved for the number of fertile spikelets/spike in either F_1 hybrids (which consisted of plants with normal spikes) or F_2 progenies (which consisted of plants with normal, tetrastichon and branched spikes). This may be explained by differences in the dispersion of dominant alleles for the number of fertile spikelets / spike of the parents, corresponding to the hypothesis of heterosis given by Jinks (1983).

Table 1. — Number of fertile spikelets and grains per spike and grain weight per spike in the crosses of wheat genotypes with different spike architectures

Parent and hybrid		No. of fertile spikelets / spike		No. of grains / spike		Grain weight / spike (g)	
		$\bar{x}$	(%)*	$\bar{x}$	(%)*	$\bar{x}$	(%)*
Sava (normal spike)		**17.8**		**47.0**		**1.46**	
Sava/ZG 172	F-1	16,9	35,3	50,5	71,0	1,92	81,7
	F-2	21,0	43,9	53,8	75,7	1,67	71,1
Forlani/ZG 172	F-1	19,8	41,4	63,0	88,6	2,75	117,0
	F-2	23,8	49,8	62,9	88,5	2,61	111.1
ZG 172 (branched spike)		**47.8**		**71.1**		**2.35**	
Sava/ZG-T-171/1	F-1	19,5	66,8	59,4	121,0	2,25	154,1
	F-2	18,5	63,4	50,9	103,7	1,90	130,1
Forlani/ZG-T-171/1	F-1	19,8	67,8	69,6	141,8	2,95	146,0
	F-2	17,7	60,6	50,4	102,6	2,72	134,7
ZGT-T-171/1 (tetrastichon spike)		**29.2**		**49.1**		**1.44**	
Sava/Forlani	F-1	17,4	97,7	58,0	121,1	2,53	125,2
	F-2	16,4	92,1	50,1	104,6	1,95	96,5
Forlani (normal spike)		**15.5**		**47.9**		**2.02**	
LSD 5%		*5,46*		*10,11*		*0,27*	
1%		*6,17*		*12,57*		*0,49*	

* — compared with better parent

Number of grains per spike

In the crosses Sava x ZG 172 and Forlani x ZG 172, the F_1 and F_2 progenies formed a significantly lower number of grains / spike than the better par-

ent, ZG 172 (Table 1). In contrast, in the crosses between genotypes with normal and tetrastichon spikes, pronounced and significant heterosis in F_1 was observed in both Sava x ZG-T-171/1 and Forlani x ZG-T-171/1 (21% and 41.8% respectively). In both crosses, the mean values for this trait in F_2 were lower but still heterotic. In the cross Sava x Forlani (both normal spikes), the F_1 hybrid was superior (21.1%) to the better parent (Forlani).

Grain weight / spike

The F_1 and F_2 plants of the cross Sava x ZG 172 achieved on average a significantly lower grain weight / spike than the better parent. In the cross Forlani x ZG 172 significant heterosis was observed in both F_1 and F_2 (17.0% and 11.1%, respectively) (Table 1).

In both crosses involving genotypes with normal and tetrastichon spikes, heterosis was highly pronounced in both F_1 and F_2 (Table 1), ranging from 30.1% up to 54.1% (Table 1).

In the F_1 plants of the cross Sava / Forlani, heterosis was demonstrated for both, the number of grains / spike and grain weight / spike, 21.1% and 25.2%, respectively (Table 1).

Most wheat crossing programs conducted in various countries have involved crosses between normal spike forms. Based on the results of this research, it seems that, concerning the development of hybrid wheat, the most promising crosses are those between genotypes with normal and tetrastichon spikes.

Potential heterosis of hybrid wheat must compensate for additional costs of F_1 seed production. Consequently, the required level of heterosis needs to be at least 6% (Pickett and Galwey, 1997). In this experiment, the levels of heterosis achieved for two of the main yield components in the crosses normal x tetrastichon spike were as high as 54.1%. Some open questions remain to be answered before a proper solution can be offered, such as the effect on the remaining yield component (number of spikes per unit area), as well as the testing of yield "*per se*" in regular field performance trials. Also, the low seeding rates used in the experiment should be reconsidered since some authors (Briggle et al., 1967a, b; Boland and Walcott, 1985) reported that heterosis in wheat increases with density. At any rate, we believe that crossing genotypes with normal and tetrastichon spike forms may be a good strategy for the production of hybrid wheat, as well as that this line of research deserves further attention and investigation.

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ХЕТЕРОЗИС У УКРШТАЊИМА ГЕНОТИПОВА ПШЕНИЦЕ СА РАЗЛИЧИТОМ АРХИТЕКТУРОМ КЛАСА

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Резиме

Са циљем процене хибридног вигора, т. ј. хетерозиса укрштени су генотипови пшенице са различитом архитектуром класа (нормални, тетрастихон и гранати), и у $Ф_1$ и $Ф_2$ генерацији су анализирани број плодних класића по класу, број зрна по класу и маса зрна по класу. Родитељи за укрштања су били сорта Сава (нормални клас), сорта Форлани (нормални клас), линија ЗГ Т 171/1 (тетрастихон клас) и линија ЗГ 172 (гранати клас). У $Ф_1$ и $Ф_2$ генерацији је, сем код укрштања Сава/Форлани, утврђен мањи просечан број плодних класића по класу у односу на бољег родитеља. У укрштањима генотипова са нормалним и гранатим класовима пшенице, у $Ф_1$ и $Ф_2$ генерацији је формиран значајно мањи број зрна по класу. Насупрот овим резултатима, у $Ф_1$ генерацији укрштања генотипова нормалних и тетрастихон класова утврђен је високозначајан позитиван хетерозис за својство број зрна по класу, и то нарочито у укрштању Форлани/ЗГ Т 171/1. За својство маса зрна по класу, значајан позитивни хетерозис је утврђен код свих укрштања, са изузетком комбинације Сава/ЗГ 172.

Потенцијално добра укрштања за производњу хибридног семена пшенице, а код којих је утврђен значајан хетерозис за две компоненте приноса, су укрштања између генотипова са нормалним и тетрастихон класовима, и зато овај тип укрштања заслужује додатну пажњу и даља истраживања.

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CLINICAL EFFECT OF IBUPROFEN AS AN ADJUNCT TO NON-SURGICAL PERIODONTAL DISEASE TREATMENT

ABSTRACT: Twenty-five patients with progresive periodontal disease entered this study in order to examine clinical effects of a non-steroidal anti-inflammatory drug — ibuprofen, used as an adjunct to non-surgical periodontal treatment. After scaling and root planing, patients were randomly assigned to either receive orally 200 mg of ibuprofen per day for one month (group A), or not receive the drug (group B). The obtained results show that the mechanical periodontal treatment brought to resolution the gingival inflammation with both group of patients. Although the mean values of the used indices were lower in group A than in group B, those differences were neither statistically nor clinically significant. We may conclude that systemic ibuprofen had no significant effect on plaque, gingival or bleeding index scores.

KEY WORDS: non-steroidal anti-inflammatory drugs, ibuprofen, periodontal disease

INTRODUCTION

Bacteria and bacterial products have been considered for a century to be of primary importance in the etiology of periodontal disease. Over the past two decades, however, it has become evident that their presence alone is not sufficient to clarify the pathophysiological mechanisms of periodontal tissue destruction.

More recently, several studies have emphasized the role of the host's immunoinflammatory responses during the destruction of periodontal tissues. Evidence suggests that arachidonic acid metabolites are implicated as leading biochemical mediators in the periodontal tissue destruction. Arachidonic acid is a polyunsaturated fatty acid that is liberated from membrane phospholipids of the cells involved in inflammatory reaction. By enzyme cyclooxigenase free arachidonic acid is oxydized to prostanoides, which include prostaglandins, prostacyclin and tromboxane, metabolites with potent biological activities. Recent data support the concept that one of the distinguishing host response mechanisms which are associated with periodontal disease progression is the local formation and secretion of prostaglandin E_2 (PGE_2) that has proinflammatory properties and can stimulate bone resorption. Studies have shown positive cor-

relation of PGE_2 levels within the periodontal tissues and within gingival crevicular fluid to the clinical expression of periodontal disease. Furthermore, examination of healthy and diseased human periodontal tissues suggests that local production of arachidonic acid metabolites and PGE_2 in particular is closely associated with periodontal status and it appears to reflect the disease activity.

Inhibition of PGE_2 synthesis can be achieved by using one of the three major pharmacological approaches. The first approach is to stabilize cell membrane, suppress the cellular degranulation and reduce the level of free arahidonic acid by exploiting the biochemical properties of steroids. The second approach is to prevent the oxidation of arachidonic acid and the subsequent hydrolysis to form PGE_2 by using antioxidants. The third approach is the direct inhibition of the enzyme cyclooxigenase through the action of non-steroidal anti-inflammatory drugs.

The purpose of this study was to investigate clinical effects of ibuprofen, one of the non-steroidal anti-inflammatory drugs, applied as an adjunct to conservative periodontal disease therapy.

MATERIAL AND METHODS

Twenty-five patients, fourteen males and eleven females, suffering from periodontal disease entered this study. All of them received mechanical periodontal treatment — dental plaque and supra and subgingival calculus removal. After thorough scaling and root planing, patients were randomly assigned to either receive orally 200 mg of ibuprofen per day for one month (group A), or not receive the drug (group B). Oral hygiene instructions were given to both group of patients and they were motivated for oral hygiene. Plaque index (Green-Vermillion), gingivitis index (Ramfjord) and gingival bleeding index (Cowell) were used to assess the periodontal status of the patients. The criteria for scoring were as follows:

Plaque index:

0 = no plaque and calculus on tooth surfaces
1 = plaque and calculus on the gingival third of tooth surface
2 = plaque and calculus on the middle third of tooth surface
3 = plaque and calculus on the incisal third of tooth surface

Gingivitis index:

0 = absence of signs of inflammation
1 = mild to moderate inflammation, not extending around the tooth
2 = mild to moderately severe inflammation, extending all around the tooth
3 = severe gingivitis with tendency to bleeding and ulceration

Gingival bleeding index:

0 = no bleeding on probing
1 = bleeding within 30 sec. after probing

2 = bleeding immediately after probing
3 = spontaneous bleeding

Patients were examined, and data were collected at the baseline, and then once each of the four consecutive weeks after the mechanical treatment.

RESULTS

The obtained results showed that mechanical periodontal treatment brought to resolution the gingival inflammation with both group of patients. The mean values of periodontal indices used in this study remained significantly low throughout the examination period but with constant increase. Although the mean values of plaque, gingivitis and gingival bleeding index were lower in group A than in group B, the differences were not statistically significant. The reason for that could be a small number of examinees but, in our opinion, those differences were not clinically significant either. The reason could also be the low dose of the drug, or it could be the low anti-inflammatory effect of the systemically administrated ibuprofen on gingival tissues. Nevertheless, it can be stated that in our study, no clinical effect of systemic ibuprofen was observed.

Table 1. — Gingival bleeding index

Week of examination	Group A	Group B	p
0	1.41	1.41	$p > 0.05$
I	0.75	0.82	$p > 0.05$
II	0.71	0.78	$p > 0.05$
III	0.78	0.81	$p > 0.05$
IV	0.82	0.89	$p > 0.05$

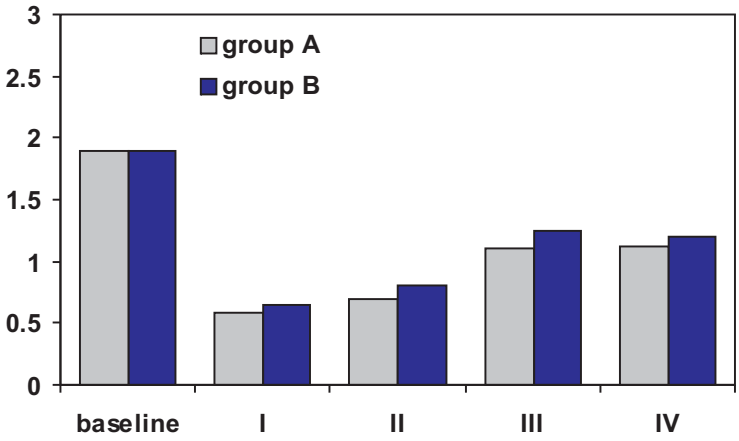

Figure 1. — **Plaque index** — the bars represent mean values for each week of the examination period

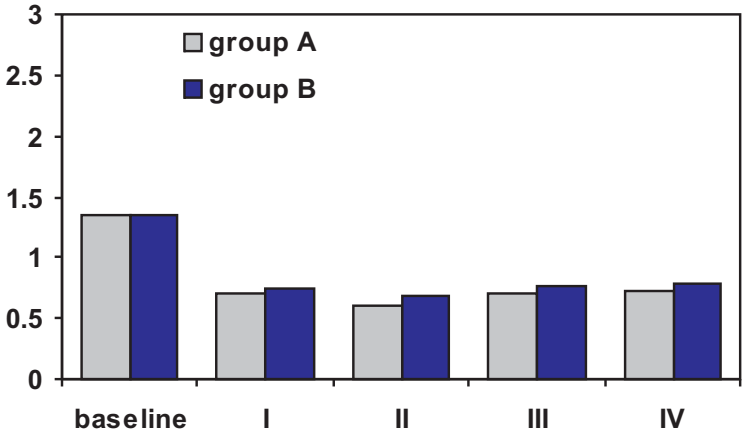

Figure 2. — **Gingivitis index** — the bars represent mean values for each week of the examination period

DISCUSSION

A number of studies, involving both humans and animals, have been conducted to investigate the effects of non-steroidal anti-inflammatory drugs on periodontal disease progression. The results of most of these studies have shown beneficial effects of these drugs on gingival crevicular fluid levels of PGE_2, and on bone loss as well.

Investigating the effects of different non-steroidal anti-inflammatory drugs on experimental periodontitis in beagle dogs, Offenbacher et al. (1992) reported a significant decrease in the gingival crevicular fluid levels of PGE_2 in all non-steroidal anti-inflammatory drug-treated animal groups [5]. Abramson et al. (1992) analyzed the clinical and biochemical effects of systemic flurbiprofen on gingivitis in humans. They concluded that gingival crevicular fluid levels of PGE_2 were significantly decreased in flurbiprofen-treated patients when compared with the placebo group. One week after drug administration was discontinued, PGE_2 levels returned to baseline levels [1]. Heasman et al. (1993), who also investigated effects of systemic flurbiprofen on experimental gingivitis in 21 patients, reported similar results [2]. Jeffcoat et al. (1988) reported of significantly lower bone resorption in 15 patients who received 50 mg flurbiprofen for two months. Several other investigators reported significant bone gain in non-steroidal anti-inflammatory drug treated group of patients [3].

At the same time, studies to correlate these positive effects of non-steroidal anti-inflammatory drugs on gingival crevicular fluid levels of PGE_2 and on bone loss, with clinical effects such as plaque scores, gingival and bleeding index scores, often had contradictory results. While Haesman and coworkers, for example, stated that the reduction of gingival crevicular fluid levels of PGE_2 coincidences with clinically reduced gingival bleeding scores, several other investigators reported opposite results. Vogel et al. (1984) investigated the

effects of systemic sulindac on experimental gingivitis in 18 male dental students and concluded that there was no significant effect on gingival crevicular flow and bleeding index [6]. Johanson et al. (1990) investigated the effects of naproxen, another non-steroidal anti-inflammatory drug, on gingival inflammation. The results of their study showed that the drug had no significant effect on plaque, gingival and bleeding index scores. A significant effect of naproxen was only seen in resolution of gingivitis after plaque was removed [4].

The results of our study are close to these findings. It seems that, clinically, non-steroidal anti-inflammatory drugs do not lead to a potent anti-inflammatory effect. Rather, they appear to stabilize the existing periodontal condition and diminish or inhibit the rate of disease progression.

CONCLUSION

The results of this study showed no clinical benefit of systemically administrated ibuprofen used as an adjunct to non-surgical periodontal therapy.

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КЛИНИЧКИ ЕФЕКТИ ПРИМЕНЕ ИБУПРОФЕНА У КОНЗЕРВАТИВНОЈ ТЕРАПИЈИ ПАРОДОНТАЛНЕ БОЛЕСТИ

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Резиме

Бактерије и бактеријски продукти се већ дуги низ година означавају као главни узрочници пародонталне болести. Међутим, данас је јасно да и други механизми, пре свега имуни одговор домаћина, играју велику улогу у деструкцији пародонталних ткива. Истраживања су показала да арахидонска киселина и њени метаболити, а пре свих простагландин Е2, имају знатан инфламаторни потенцијал и да доводе до ресорпције алвеоларне кости. Резултати многих студија показују да примена нестероидних антиинфламаторних лекова доводи до смањења нивоа простагландина E_2 у гингивалној течности и до смањења коштане ресорпције.

Ово истраживање предузето је са циљем да се испита утицај ибупрофена, једног од нестероидних антиинфламаторних лекова, као допуне конзервативној терапији пародонталне болести, на клинички налаз на пародонцијуму. Двадесет петоро пацијената је након конзервативне терапије подељено у две групе. Пацијенти групе А узимали су свакодневно током месец дана 200 mg ибупрофена, док пацијенти групе Б нису примали никакву медикацију. Резултати су показали да је код пацијената обе групе, након уклањања супра- и суб-гингивалних наслага и обраде пародонталних џепова, дошло до значајног смањења инфламације гингиве. Између, пак, средњих вредности плак индекса, гингивалног индекса и индекса крварења гингиве пацијената две испитиване групе није било статистички а, по нашем мишљењу, ни клинички значајних разлика. Стога закључујемо да ибупрофен није имао значајнији позитиван утицај на клинички налаз на пародонцијуму код ових пацијената.

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THE INFLUENCE OF SEX ON THE OLFACTORY FUNCTION IN HEALTHY SUBJECTS

ABSTRACT: The sense of smell is the least examined of all senses. The significance of the organs of smell is in their influence on the mental state as well as on the vegetative, visceral and sexual functions. The objective of this experiment was to define the influence of sex on the olfactory function. It was performed on 120 subjects (60 females and 60 males) divided into three age groups (20—30; 31—40; 41—50 years of age). The experiment was carried out by the Fortunato-Niccolini olfactometric method using six odorous experimental substances: A — anethol, PH — phenyl-ethyl-alcohol, C — citral, M — menthol, V — vanillin and P — pyridine, the thresholds of perception (TP) and identification (TI) being defined for each odorous substance. The examined females had slightly lower thresholds of perception (TP) and identification (TI) in relation to the males of the same age group. However, the differences were not statistically significant except for the group of subjects between 41 and 50 years of age where the females, being in the pre-menopause, had significantly better olfactory functions. The results can be explained by the weakening of the olfactory power as a result of ageing in both sexes, however, the females still experienced the protective role of sex hormones.

KEY WORDS: sex factors, smell

INTRODUCTION

The sense of smell is the least examined of all senses, which is the consequence of the fact that membranes of smell are located deep in the nostrils and thus unreachable to researchers. Besides, sensibility of smell is a subjective phenomenon that is not easy to examine in animals. Furthermore, the sense of smell is, to a certain extent, undeveloped in humans in comparison with the sense of smell in some animals, so that the results obtained for experimental

animals are not entirely applicable for humans. The sense of smell in humans is associated with their mental moods, much more than any other sense. With its numerous connections to the limbic system and reticular formation, the olfactory system influences the regulation of numerous vegetative functions, visceral functions and sexual behavior. It plays a role in creating emotions and adjusting visceral and vegetative responses to particular emotional states. Also, it is the connection between higher cortical functions and the endocrine system. The orbitofrontal cortex has the crucial role in the processing of complex olfactory information data, especially the ability to discriminate smells (J o v i ć , 1998). The objective of this paper was to define standards for the threshold of perception (TP) and the threshold of identification (TI) for the examination of the response to odorous experimental substances in healthy subjects divided according to sex and age, and to identify the influence of sex and age on the olfactory function.

MATERIALS AND METHOD

The work has been performed at the Clinical Center of Novi Sad, Department for Diseases of Ear, Nose and Throat. The examination included 120 subjects who were divided according to sex (sixty females and sixty males). Both groups were divided according to age into three subgroups of twenty subjects (20—30, 31—40, 41—50 years of age). The groups consisted of volunteers and patients of the Clinic, nonsmokers with no acute or chronic rhinological disorders. The subjects were not supposed to have been exposed to harmful factors influencing the organ of smell at their work places (oil, chemical and leather industries). The Fortunato-Niccolini olfactometer apparatus consisted of six cylindrical bottles, each with the capacity of 530 ccm, in which we placed the odorous experimental substances (anethol — A, phenyl-ethyl-alcohol — ph, citral — C, menthol — M, vanillin — V, pyridine — P). The amount of substance was 30 ccm, and the remaining 500 ccm were air.

Each experiment started with the insufflation of 5 ccm of the odorous air into the nose of the subject. If the subject was not able to smell the odor, or was able to smell it but not to differentiate it, the procedure was repeated with larger amounts of air (10, 15, 20 ccm). In that way we defined the thresholds of perception (TP) and identification (TI) for a particular odorous substance. The procedure was repeated for all six odorous experimental substances.

RESULTS

The obtained results are shown in tables:

Table 1 — The threshold of perception (TP) and the threshold of identification (TI) in the female subjects aged between 20 and 30

Substance	xp [cm^3]	xi [cm^3]
A	9.00	11.00
PH	5.00	5.25

C	5.50	9.25
M	6.75	9.50
V	6.00	7.75
P	5.00	5.00
Σ	6.21	7.96

Legend:
Xp (cm^3) — the average values of TP of the examined odorous substances in ccm^3 of air.
Xi (cm^3) — the average values of TI of the examined odorous substances in ccm^3 of air.
A — anethol, PH — phenyl-ethyl-alcochol, C — citral, M — menthol, V — vanillin, P — pyridine

By using the t-test with the error degree of 5%, we arrived at the conclusion that the values of TP were statistically lower than the values of TI, in this group of subjects.

Table 2 — The threshold of perception (TP) and the threshold of identification (TI) in the male subjects aged between 20 and 30

Substance	xp [cm^3]	xi [cm^3]
A	10.00	12.50
PH	5.25	7.50
C	7.50	9.50
M	8.00	11.00
V	7.00	10.50
P	5.00	5.00
Σ	7.13	9.33

See legend in Table 1.

The values of TP were significantly lower in relation to the values of TI ($p < 0.01$), in this group of subjects.

Table 3 — The threshold of perception (TP) and the threshold of identification (TI) in the female subjects aged between 31 and 40

Substance	xp [cm^3]	xi [cm^3]
A	8.50	11.00
PH	5.25	7.50
C	7.25	10.00
M	7.00	10.50
V	7.50	11.75
P	5.25	5.25
Σ	6.79	9.33

See legend in Table 1.

The values of TP were statistically significantly lower than the values of TI with 99% of reliability, in this group of subjects.

Table 4 — The threshold of perception (TP) and the threshold of identification (TI) in the male subjects aged between 31 and 40

Substance	xp [cm^3]	xi [cm^3]
A	10.50	12.00
PH	5.75	8.25
C	8.25	10.75
M	7.75	11.75
V	8.25	12.00
P	5.25	6.00
Σ	7.63	10.13

See legend in Table 1.

The values of TP were statistically significantly lower than the values of TI with 99% of reliability, in this group of subjects.

Table 5 — The threshold of perception (TP) and the threshold of identification (TI) in the female subjects aged between 41 and 50

Substance	xp [cm^3]	xi [cm^3]
A	10.50	14.25
PH	8.00	9.75
C	10.75	14.00
M	10.25	15.50
V	10.50	14.75
P	6.25	8.50
Σ	9.38	12.79

See legend in Table 1.

The values of TP were significantly lower in relation to the values of TI ($p < 0.01$), in this group of subjects.

Table 6 — The threshold of perception (TP) and the threshold of identification (TI) in the male subjects aged between 41 and 50

Substance	xp [cm^3]	xi [cm^3]
A	12.00	16.00
PH	8.50	12.75
C	12.25	15.50
M	12.75	17.75
V	12.50	16.50
P	8.00	10.25
Σ	11.00	14.79

See legend in Table 1.

By using the t-test with the error degree of 1%, we arrived at the conclusion that the values of TP were statistically lower than the values of TI, in this group of subjects.

The thresholds of perception (TP) and identification (TI) of the experimental odorous substances were lower in the females in the first two age groups, with statistical significance ($p < 0.05$) in favor of the females between 41 and 50 years of age in relation to the males of the same age.

DISCUSSION

Analyzing the olfactory function in relation to sex in the group within the age range between 20 and 30, we noted slightly higher thresholds of perception (TP) and identification (TI) of the odorous experimental substances in the males, but without statistical significance in the differences (Tables 1 and 2; Savović, 2001). In the group within the age range between 31 and 40, we also noted slightly higher thresholds of perception (TP) and identification (TI) of odorous experimental substances in the males, again without statistical significance (Tables 3 and 4).

In the group within the age range between 41 and 50, we noted a weaker olfactory function in the males, which was statistically significant at the reliability level of 95% but not at the level of 99% (Tables 5 and 6). The statistically significant difference in the olfactory function in the males and females aged between 41 and 50 can be explained by a decrease in the olfactory perception after the age of 40 in both sexes. However, most of the examined females have not experienced the post-menopausal period yet, so their better olfactory ability can be attributed to the protective function of sex hormones.

While analyzing and defining the obtained results, we noticed that the available literature provided different data on the influence of sex on the olfactory function.

Having examined a group of 146 subjects divided according to age and sex, Kobal et al. (1996) found a statistically significantly better olfactory function in females, with the reliability of 99%.

Having examined a group of 572 subjects aged between 5 and 90 and divided according to the age and sex, Broich et al. (2000) obtained the following result: in the group comprising 58% of females and 42% of males, the olfactory function was significantly lower in the latter. Davies et al. (1999) and Petrulis et al. (1999) also found significantly better olfactory function in females.

Hvastia and Zanuttini (1997), examining a group of 20 subjects (10 males and 10 females), found that the females experienced a better olfactory function than the males.

Contrary to the above-mentioned researchers, Simola et al. (1998) and Kalmey et al. (1998) found no difference in the olfactory function between the sexes.

Hornung and Leopold (1999) proposed that differences in the olfactory function in males and females might originate from anatomic differences of nasal cavities between the sexes.

Gangestad and Thornhill (1998) found significant differences in the olfactory function between males and the females with regular periods, while they could not find such difference between males and the females with irregular periods.

CONCLUSION

The females examined for the perception of the odorous substances had slightly lower thresholds of perception (TP) and identification (TI) in relation to the males of the same age group. However, the established differences were not statistically significant except for the group of subjects aged between 41 and 50 where the females, being in the pre-menopause, exhibited a significantly better olfactory function. These results can be explained by the weakening of the olfactory power in consequence to ageing in both sexes, however, the females still experiencing the protective role of sex hormones.

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УТИЦАЈ ПОЛА НА МИРИСНУ ФУНКЦИЈУ КОД ЗДРАВИХ ИСПИТАНИКА

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Резиме

Осећај мириса је најслабије проучен од свих других осећаја. Важност органа мириса за човека огледа се између осталог и у томе што највише од свих осетних органа утиче на душевно расположење, вегетативне, висцералне и сексуалне функције. Циљ овог рада је био да се утврди утицај пола на мирисну функцију. У раду је обухваћено 120 испитаника (60 жена и 60 мушкараца) разврстаних у три старосне групе (20—30 г., 31—40 г., 41—50 г.). Испитивање је урађено олфактометријском методом по Fortunato-Niccolini-ју са шест испитиваних мирисних материја A — anethol, PH — phenyl-ethyl-alcochol, C — citral, M — menthol, V — vanilline, P — pyridin, при чему су одређени прагови перцепције (ТП) и прагови идентификације (ТИ) за сваку испитивану мирисну материју. Жене имају нешто ниже прагове перцепције (ПП) и идентификације (ПИ) испитиваних мирисних материја у односу на мушкарце исте животне доби, али без статистичке значајности, осим у групи испитаника 41—50 г. где жене имају знатно бољу мирисну функцију, што се може објаснити падом мирисне способности с годинама у оба пола, али код жена те животне доби још увек постоји довољан ниво естрогена који протективно утиче на мирисну функцију.

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4.1. Figures are of two kinds: black-and-white photographs and drawings. Photographs must have good contrast; line drawings must be neatly drawn,

boldly in black ink on good quality white tracing paper. In addition to the usual line-drawn graphs, also treat metabolic schemes, complicated formulas, and large or complex tables as figures.

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6. Units, names, formulas, and abbreviations.

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1.1. Зборник за природне науке прима оригиналне радове и прегледне чланке као и кратка саопштења из свих научних области које обухвата назив часописа. Прегледни радови се објављују само на позив редакције. Радови који су већ објављени или су послати за објављивање у другом часопису не могу бити прихваћени.

1.2. Прихватају се рукописи писани на енглеском језику. Језик мора бити исправан у погледу граматике и стила. Аутори треба да предају рукопис у три примерка (оригинал и две копије). Аутори чији матерњи језик није енглески такође треба да приложе и копију рада на изворном језику.

1.3. По примању рукописа, аутори ће добити редни број свога рада. Тај број треба наводити у даљој преписци. Редакција ће обавестити ауторе о приспећу рукописа и мишљењу рецензената у року од три месеца од пријема. Сваки рад рецензирају најмање два рецензента. Ако рад не буде прихваћен, рукопис се не враћа аутору.

1.4. Рукописе за објављивање треба слати на адресу редакције Зборника за природне науке, Ул. Матице српске 1, 21000 Нови Сад, Југославија.

2. Припрема рукописа

2.1. Рукописи се куцају са двоструким проредом у свим деловима текста (укључујући литературу, табеле итд.), на папиру формата А4. Све маргине треба да буду широке 2,5 сантиметра.

2.2. Рукопис треба поделити на: Сажетак, Кључне речи, Увод, Материјал и/или методе, Резултати испитивања, Расправа, Литература, Сажетак на српско-хрватском језику, Захвалност.

2.3. Назив рада треба да буде информативан, али не дужи од десет речи.

2.4. Кључне речи треба да указују на целокупну проблематику истраживања. Треба их навести абецедним редом и одвојити зарезима. Кључне речи не треба да пређу сто словних знакова.

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2.7. Податке о финансијској помоћи, саветима и другим врстама помоћи треба навести на крају рада, под насловом Захвалност.

2.8. Радови не смеју бити дужи од 12 куцаних страна, укључујући литературу, табеле, легенде и слике.

3. Литература

3.1. Литературу треба ограничити на неопходан број навода.

3.2. Литературне наводе треба сложити абецедним редом, на следећи начин:

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б. Чланци из књига

Аутор ED, Аутор SI, Аутор BB (1991) Назив цитираног дела књиге. У: A. Blom, B. Lindau, Eds., Назив књиге, Ed 3, Vol 2, Издавач, Град, 242—255.

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Аутор VA (1989) Назив тезе. Докторска дисертација. Универзитет, Град.

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Аутор AE (1987) Назив књиге, Издавачи, Град, 237.

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Навод „у штампи" треба да се односи само на прихваћене радове; навести име часописа у којем ће рад бити објављен.

3.3. Имена часописа треба скраћивати према „Bibliographic Guide for Authors and Editors" (BIOSIS, Chemical Abstracts Service and Engineerings Index, Inc., 1974).

3.4. Референце у тексту треба да укључе презиме аутора и годину издања. Ако има два аутора, треба навести обојицу, а у случају три или више аутора треба навести првог аутора и назначити „et al.".

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.

4. Илустрације

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-

личке шеме, компликоване формуле и велике или компликоване табеле такође треба третирати као слике.

4.2. Сва слова, бројке и симболи треба да буду довољно велики у оригиналу, тако да после смањивања не буду мањи од 1,5 mm. Текст на сликама и графиконима такође треба исписати тушем.

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.

7. Кратка саопштења

7.1. Зборник за природне науке нуди могућност објављивања кратких саопштења о свим научним областима обухваћеним називом часописа.

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 о приближном времену објављивања бити обавештен у писаној форми.

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.