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MAIN MYCOTOXIN CONCERNS IN EUROPE: MYCORED AND ISM EFFORTS TO HARMONIZE STRATEGIES FOR THEIR REDUCTION IN FOOD AND FEED CHAIN**

ABSTRACT: There is an urgent need to study in Europe the plant exposure to mycotoxin risk due to the identification of new toxigenic species, the continuous evolution of species profile on the food crops and climate changes that influence the quality of level of toxigenic fungi colonization of plant hosts. In particular, *Fusarium* and *Aspergillus* problem in Europe has enormous importance; recent epidemics in wheat in some areas of Northern and Central Europe and in grape in southern Europe have brought this problem into focus again. This concern has driven many efforts at EU level aimed to harmonize strategies for **myco**toxin**red**uction in food and feed chain. This is the target of a large collaborative project of four-year duration (MYCORED as acronym), that was approved within the European FP7- *Food, Agriculture, and Biotechnologies* Work Programmes (www.mycored.eu). MYCORED aims to develop strategic solutions for reducing mycotoxin contamination in major crops. Novel methodologies, efficient handling procedures and information/dissemination, and educational strategies are considered in a context of multidisciplinary integration of know-how and technology to reduce mycotoxin exposure worldwide. The direct involvement of ICPC countries (Argentina, Egypt, Russia, South Africa) and international organizations (CIMMYT, IITA) together with strong scientific alliances with international experts and national and international societies for mycotoxicology is a strong point of the project through sharing experiences and resources from several past/ongoing mycotoxin projects in a global context.

Similarly, the International Society for Mycotoxicology (ISM) (http://www.mycotoxsociety.org) aims at increasing scientific knowledge concerning biology, chemistry and any sciences/disciplines related to mycotoxins and toxigenic fungi, through membership networking, scientific meetings, symposia, discussions, technical courses and publications.

In this context, it would be extremely important that MYCORED and ISM develop a network of cooperation-interaction with the whole scientific community in order to contribute to the efforts for harmonizing both research and legislation on mycotoxins.

KEY WORDS: *Aspergillus, Fusarium*, ISM, mycotoxin reduction, MYCORED

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INTRODUCTION

Mycotoxins (B e n n e t t et al., 2003) are secondary metabolites produced by toxigenic fungi that contaminate food, feed chain, and represent a risk for human and animal health. They are responsible for many different toxic effects including the induction of cancer, and digestive, blood, kidney and nerve defects. The mycotoxin problem is particularly relevant for human health in tropical areas, such as Sub-Saharan Africa, where crops are quite susceptible to contamination by the carcinogenic aflatoxins and fumonisins. Research is making strong efforts to improve knowledge and reduction of mycotoxin contamination. Mycotoxin reduction targets have been identified by some international food organizations (e.g. FAO, CIMMYT, EFSA, IITA, SAFE consortium), EU reports and relevant food industry representatives (V a n E g m o n d et al., 2007; P i ñ e i r o, 2004).

In Europe, the main concern is related to *Fusarium* and *Aspergillus* diseases that have assumed a great relevance for both health and economical implications focused by the recent epidemics in wheat in some area of Northern and Central Europe and in grape in Southern Europe (B a t t i l a n i et al., 2006; M a g a n, 2006; L o g r i e c o, 2001; L o g r i e c o et al., 2002).

The MYCORED is a large collaborative project of four-year duration that has been approved within the European FP7- *Food, Agriculture and Biotechnologies* Work Programmes; it aims to develop novel and strategic solutions for reducing both pre- and post-harvest contamination in selected feed and food chains (Table 1).

Novel methodologies, efficient handling procedures and information, dissemination and educational strategies are considered in a context of multidisciplinary integration of know-how and technology to reduce mycotoxins exposure worldwide.

MYCOTOXINS

Within the project, the knowledge and reduction of aflatoxins, trichothecenes, fumonisins, and ochratoxin A have been considered the most relevant issue to be addressed (Fig. 1).

Aflatoxin B1 is produced by many species of *Aspergillus*, most notably *A. flavus* and *A. parasiticus*; it is a proven carcinogen for humans (C a s t e g n a r o and W i l d, 1995), immunotoxic, and it causes stunted growth in children and growth retardation in animals. High-level of aflatoxin exposure produces acute hepatic necrosis and later it can result in cirrhosis, and/or carcinoma of the liver.

Fumonisins are reported as neurotoxic and possible carcinogens and are associated with several mycotoxicoses, including equine leukoencephalomalacia, porcine pulmonary edema, and experimental kidney and liver cancer in rats (H o w a r d et al., 2001).

Trichothecenes are immunotoxic compounds produced by various species of *Fusarium*; they act as potent protein synthesis inhibitors and cause multiorgan effects including emesis and diarrhea, weight loss, nervous disorders, cardiovascular alterations, immunodepression, hemostatic derangements, skin toxicity, decreased reproductive capacity, and bone marrow damage (U e n o, 1989).

Ochratoxin A, a toxin produced by *A. ochraceus*, *A. carbonarius* and *Penicillium verrucosum*, is one of the most abundant food-contaminating mycotoxin in the world. It is a nephrotoxin (J u n g and E n d o u, 1989), possibly carcinogenic for humans and associated to Balkan Endemic Nephropathy (JECFA, 2001).

It is also expected that combination of mycotoxins would have at least an additive, if not synergistic relevant effect. These toxins are the primary sources of both yield losses and increase of management costs worldwide.

Main food chains affected by mycotoxins

Wheat and maize have the major mycotoxin safety concerns worldwide. The research on wheat has been much more intensive, while Maize is being treated from a toxicologically point of view as significant as wheat too. The primary mycotoxins that contaminate maize are trichothecenes, zearalenone and ochratoxin A, while maize is mainly contaminated by fumonisins and aflatoxins. *Fusarium* head blight (FHB) is one of the most serious diseases affecting wheat and barley worldwide and it is associated with the infection of several *Fusarium* species (most frequently *F. graminearum, F. culmorum, F. avenaceum, F. poae*) (B o t t a l i c o and P e r r o n e, 2002). Though the disease results in reduced seed quality and yield, the most serious problem related to this disease is the mycotoxin contamination of the harvest.

Fusarium ear rot (Fig.2) is diseases that affects maize and render stored grain unsuitable for human and livestock consumption. Its primary causal agent is *F. verticillioides* (W h i t e, 1999), but *F. subglutinans* and *F. proliferatum* are also associated with the disease that can result in mycotoxin contamination of maize grain.

Although in Europe there are no data on the economic costs of mycotoxins, in Hungary the direct and indirect losses were estimated in a wheat epidemic (1998) to 100 million Euro. In the United States alone, the mean economic annual costs of farmer gate cereal crop losses due to aflatoxins, fumonisins and trichothecenes are estimated to be $ 932 million (CAST report, 2003).

Grape and wine – Ochratoxin A addresses especially raisin, sultanas, and the grape-wine chain. The main ochratoxigenic species isolated from grapes are *A. carbonarius* and *Aspergillus* species belonging to the *Nigri* section (A s t o r e c a et al., 2010).

Grapes and their processed products are very important in Europe where about 47% of worlds vineyards (4.8 million of ha) are found and 75% of the world's wine (190 million of hl is produced). Data on the economic impact of ochratoxin A in grapes and wine are not available or are not in the public do-

main because their release could cause a collapse in the wine market, which is a high value product but not considered as essential.

Dried fruits and nuts present high risk for aflatoxin contamination, with high social and economic impacts resulting from stringent EU limits. A study carried out by the JECFA/WHO (Codex Alimentarius Commission, April 2007, CX/CF 071/9) estimated that the effect of applying to "ready-to-eat" almonds, hazelnuts, and pistachios the EU limits for aflatoxins (4 ppb) has an economic impact on rejection of about 9 million Euros worldwide.

MYCORED PROJECT

The project prefigures the participation of 26 beneficiaries worldwide. It has been developed on the outcome of several European projects on mycotoxins by supporting, stimulating, and facilitating education and cooperation with countries having major mycotoxin concerns related to international trade and human health. The direct involvement of ICPC countries (Argentina, Egypt, Russia, South Africa) and international organizations (FAO, CIMMYT, and IITA) with focus on developing countries in Africa, South-Central America, and Asia together with Strategic Alliances with major public research institutions in the United States (three USDA Centers and four Universities), Australia, South Africa and Malaysia, strengthen the value of the project through sharing experiences and resources coming from a number of past and ongoing mycotoxin projects at a global level.

An External Advisory Board, composed of scientists from concerned disciplines and related initiatives, industrialists of the agrifood sector, food regulatory bodies and representatives of European Technology Platform (ETP) Food for Life, is vital for the technical discussion of the whole consortium, providing an external perspective on the project, advise the consortium issues of knowledge transfer and exploitation, recommend effective actions to reach the goals of the project.

Work Programme

The work programme is organized in 10 work packages (WP), each one with specific targets and objectives, defined by deliverables and milestones that focus on the pre-harvest and post-harvest phases of the food and feed chains (Fig. 3).

WP1: Optimization of plant resistance and fungicide use with the aim to promote the mycotoxin resistance cultivar/genotype registration at European level, to improve knowledge in plant-host interaction and using fungicides in a rationale way for prevention and reduction of mycotoxins in maize and in wheat;

WP2: Biocontrol reducing mycotoxins in cropping systems with the aim to prevent pre-harvest mycotoxin accumulation in crops by using antagonist microorganisms towards mycotoxin producing fungi;

WP3: Modelling and development of Decision Support System with the aim to predict mycotoxin risk levels at real time associated with different crops in different geographic areas and years and generating data for good risk management and rationalise products logistics post-harvest;

WP4: Novel post-harvest and storage handling practices with the aim to develop innovative and novel strategies for reducing mycotoxins by post-harvest and storage handling;

WP5: Novel application of food processing technologies with the aim to develop innovative and novel strategies for reducing mycotoxins in food processing;

WP6: Advanced technology for diagnostics, quantitative detection and novel approaches to control toxigenic fungi with the aim to detect and quantify toxigenic fungi and to generate new information and novel systems to control and reduce mycotoxin biosynthesis in food commodities;

WP7: Advanced analytical tools for rapid multi-toxins detection of mycotoxins and relevant biomarkers with the aim to perform the analyses required in WP1-6 including metabolite profiling of certain wheat and maize plants, the selection of suitable biomarkers and to improve the performance of quantitative methods of with respect to speed and accuracy as a basis for the reduction of mycotoxins in food and feed chain;

WP8: Information, education & dissemination with the aim of promoting the use of MYCORED solutions and of increasing know-how and awareness;

WP9: General management with the aim of effectively co-ordinating vertical and horizontal activities;

WP10: Demonstrating the creation of an *ambient intelligence* (AmI), strategies post-harvest and the collection of physical parameters;

A global effort

A range of multidisciplinary activities, including plant pathology, mycology, chemistry, biology, food/feed technology, physics, is required for a collaborative effort across the EU, various ICPC countries, international organization, and solid alliances bringing together expertise and resources from several actors in order to build up a solid framework for collaboration.

MycoRedconsortium brings together the experience of strategic institutions for mycotoxin research from countries in which mycotoxin food/feed contamination has a major direct importance on human and animal health as well as in the economy. Nine different EU countries (Italy, France, Germany, UK, Spain, Austria, Denmark, The Netherlands, Hungary) plus Turkey and Russia are presented in the project, an example of the mobilisation of the resources of several countries in a coordinated way, thus contributing to the "European Research Area" concept. In an attempt to gather enough critical mass and expertise, the project consortium is also composed of five international organizations (CIMMYT, IITA, FAO and INC), one technological research centre from Egypt and one university from Argentina, four companies

and a consistent number of strategic alliances. Finally an External Advisory Board and Users Club are also devised to broaden this point of contact with agrofood/feed companies and other related actors fostering a two ways dialog, in order to keep the project application oriented.

A multidisciplinary effort, food/feed companies, bio and chemical and electronic and microsystems oriented research institutes and companies, can be identified within the consortium.

Expected impact

The main targets pointed by MYCORED will have direct impact on the health of European citizens and animals at global level, on the safety of raw materials and final products in the food and feed chain, on the economy of agro-food industry and rural areas, through the improvement of the communication, information and education of consumers and agro-food chain operators.

Through the objective-driven approach of this project, a strong potential socio-economic impact will be addressed through the significant reduction of mycotoxin contamination in food and feed chain in Europe and for some targets worldwide.

A significant decrease of the number of acute and chronic pathologies in Europe and in ICPC Countries due to the consumption of mycotoxin-contaminated products will have a direct effect on the reduction of the economic costs for the human and animal health care systems.

A decrease of the costs of rejection of contaminated row materials (especially dried fruits and cereals) and products will have a positive impact for farmers, food/feed producers and retailers. Over the past year, mycotoxin regulations in the European Union continued to rapidly change: failure to comply with the latest standards can have major repercussions for exporters and importers, ranging from costly retesting and reprocessing to impounded or rejected shipments.

An economical saving will be achieved by improving the capability of reducing safety problems due to mycotoxin contamination starting from an early stage in the production chain (pre-, post- harvest and processing).

The application of various very innovative technical solutions proposed in MycoRedwill increase the safety of feed and foodstuff through a full and effective interconnection and communication of sensing systems and decision making bodies, advancing the corrective actions for a better storage/shipment and production process.

The establishment of new opportunities for the industry to use the research results will increase the European Agrofood industries competitiveness.

Promotion of new Spin-off SMEs in Europe and ICPC countries will look at the agrofood application markets as a huge potential business area. Training activities carried out under MycoRed will help in satisfying the new demands of skilled employees in food safety.

Users

MYCORED aims to increase mycotoxin awareness in five users communities: for consumers, increasing food safety for humans and feed safety for animals; for agro-food producers, providing tools useful in pre- and post-harvest solutions to mitigate mycotoxin in plant products and in the subsequent food/feed chains; for food/feed industries, reducing mycotoxin contamination by improved handling procedures during storage and processing, (bio)control measures against toxigenic fungi and apply new detection kits; for policy makers and legislators, providing information and know-how for evaluating mycotoxin risks, including possible outcomes of climate change and increased international trade; for the scientific community, increasing advanced knowledge on mycotoxins improving global communication, international networking and dissemination of innovative research results.

CONCLUSION AND PERSPECTIVES

MycoRed will have a huge economic impact both for the Agrofood international community (farmers, animal and plant breeders, etc.) as well as for European agro-food industries even if there are still no European financial analyses quantifying the improvements that strategies proposed by MycoRed can provide in mycotoxins reduction. It is the aim of MycoRed project to collaborate by carrying out a study for identifying further areas on which new technologies and improved handling solutions will have more safety and economic impact.

These objectives require the development of a severe effective control system that evaluate compliance in food safety and quality, animal health, animal welfare, animal nutrition and plant health sectors with EU standards and within the EU and Third Countries in relation to their exports to the EU. They will be developed through relations with European Food Safety Authority (EFSA) and international relation between Third Countries and the institution involved in MycoRed project.

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Tab 1. – List of plants and related mycotoxins selected for this project

Plant	**Chain**	**Toxin**	**Fungal genera**
Maize	Food/feed	Fumonisins Aflatoxins	*Fusarium* *Aspergillus*
Wheat	Food/feed	Trichothecenes Ochratoxin A	*Fusarium* *Penicillium*
Grape (including raisins and sultans)	Food*	Ochratoxin A	*Aspergillus*
Dried fruits (Peanuts, figs, pistachios, almonds)	Food	Aflatoxins	*Aspergillus*

* wine

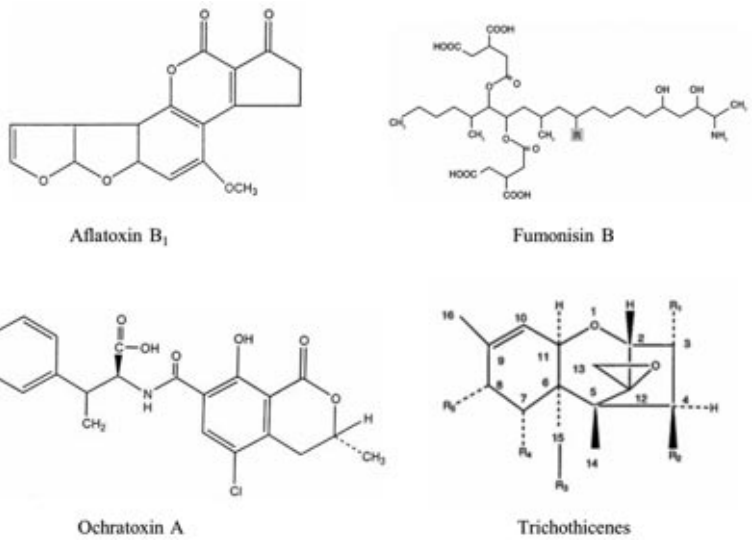

Fig. 1. – Mycotoxins selected for the project

Fig. 2. – Fusarium ear rot

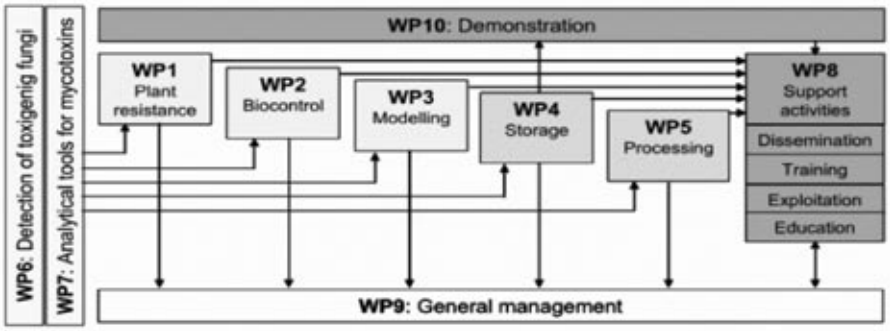

Fig. 3. – Work programme of Mycored project

ЗНАЧАЈ МИКОТОКСИНА У ЕВРОПИ: УЛОГА MYCORED-A И ISM-A У УСКЛАЂИВАЊУ СТРАТЕГИЈА РЕДУКЦИЈЕ МИКОТОКСИНА У ЛАНЦУ ИСХРАНЕ

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Резиме

У Европи се појавила прека потреба за изучавањем и праћењем изложености биљака ризику од контаминације микотоскинима због појаве нових токсигених врста плесни, константне промене њиховог профила у усевима и утицаја климатских промена на ниво колонизације биљака плеснима. У скорије време изузетно велике проблеме су изазвале *Fusarium* и *Aspergillus* врсте у Европи, где скорашња епидемија на пшеници у неким деловима Северне и Централне Европе и на грожђу у Јужној Европи указују на ову проблематику. На нивоу Европске уније улажу се велики напори ка решавању проблема везаних за редукцију микотоксина (**myco**toxin**red**uction) у ланцу исхране. Ово је уједно и циљ велике сарадње у оквиру четворогодишњег пројекта, MYCORED као акроним, који је одобрен од стране европског FP7 – „Храна, Пољопривреда и Биотехнологије" радног програма (www.mycored.eu).

MYCORED има намеру да развије стратегијска решења за смањење контаминације већине усева микотоксинима. Нове методологије, ефикасно управљање процесима, информацијама / дисиминација података и стратегије образовања се изучавају у контексту мултидисциплинарне интеграције искуства и технологије, у циљу смањења изложености микотоксинима широм света. Директан ангажман ICPC земаља (Аргентина, Египат, Русија и Јужна Африка) и интернационалних организација (CIMMYT, IITA), заједно са снажном научном подршком интернационалних експерата и интернационалних удружења за микотоксикологију, је ослонац овог пројекта јер долази до размене искустава и средстава добијених у оквиру завршених и актуелних пројеката на глобалном нивоу.

Интернационално удружење за Микотоксикологију (ISM) (http://www.mycotox-society.org), слично као и наведени пројекти, има за циљ повећање научних сазнања из биологије, хемије и других научних дисциплина које су везане и за микотоксине и токсигене плесни, путем умрежавања чланова, научних скупова, симпозијума, дискусија, стручних курсева и публикација.

У складу са досад наведеним, од изузетне је важности да MYCORED и ISM развију мрежу сарадње-интеракције са целокупном научном заједницом у циљу усклађивања истраживања и законодавства везаних за микотоскине.

КЉУЧНЕ РЕЧИ: *Aspergillus*, *Fusarium*, ISM, MYCORED, редукција микотоксина

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DETERMINATION OF THE SPECIES AMONG MITIS GROUP OF GENUS *STREPTOCOCCUS* USING A MOLECULAR IDENTIFICATION KEY

ABSTRACT: We analyzed the gene order conservation among the species of genus *Streptococcus* and based on this we developed a molecular identification key. The identification key was not made up based on the genes' amino acid or nucleotide sequences but on the gene order of certain genome parts, and in this order the genes were marked with their symbols. First, we created a key to determine the groups within the genus *Streptococcus* using conserved synteny blocks in genomes. According to this, within those species that have a fully known genome sequence, the mitis group can be divided into two subgroups: mitis I where (*S. pneumoniae*, *S mitis* and *S. oralis*) and mitis II (*S. gordonii*, *S. sanguinis* and *S. parasanguinis*). Then we made an identification key to determine the species within the mitis subgroups. For determination of the species we chose genome parts which contain such a gene that is found in all species but the genome parts around this special gene are different in various species. Three genome parts were sufficient to determine the species correctly. These sequences were around the following genes: Xaa-pro dipeptidyl-peptidase (x-prolyl-dipeptidyl aminopeptidase) – *pepX*, a leucyl-tRNA synthetase – *leuS* and a 50S ribosomal protein L13 and 30S ribosomal protein S9 – *rplM* and *rpsI* (these are always found beside one another).

KEY WORDS: bacterial systematic, genus *Streptococcus*, molecular identification key, the mitis group

INTRODUCTION

The members of the genus *Streptococcus* are clinically important species and these organisms are often isolated from human clinical specimens (T o n g et al., 2003). They are subdivided into six major clusters or species groups: the mitis, anginosus, salivarius, mutans, bovis, and pyogenic groups (K a w a m u r a et al., 1995). Within these clusters, the mitis group contained *Streptococcus pneumoniae,* an important human pathogen and most closely related oral streptococcal species *Streptococcus mitis* and *Streptococcus oralis*. The members of this group are difficult to identify correctly. They show 40%–60%

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DNA–DNA similarity values with each other and clinical strains strongly hybridized to both *S. mitis* and *S. oralis* and sometimes to *S. pneumoniae*, which led to misidentification (A r b i q u e et al., 2003). The 16S rRNS gene sequence analysis, although the most recent global prokaryotic classification is primarily based on it, cannot differentiate exactly these species, because some of them share greater then 95% 16S rRNS sequence homology (R o s e l l ó - - M o r a, 2005). Recently, a new method, multilocus sequence analysis (MLSA) is being used in identification of bacterial species and strains (B a l d w i n et al., 2005; B i s h o p et al., 2009). However, the taxonomy of this group is still problematic.

Since 1995, when the first sequence of an entire bacterial genome that of *Haemophilus influenzae* Rd. was published, the total number of publicly available genome sequence has grown rapidly (C o e n y e et al., 2005). The analyses of sequenced bacterial genomes show that the level of gene order conservation is high when organisms are phylogenetically closely related, but conservation is lost rapidly. Only a few operons, typically coding for physically interacting proteins, are conserved in all or most of the genomes (T a m a m e s, 2001).

Here we report a new approach in microbial systematic based on the gene order of bacterial genomes. Analyzing the genomes we tried to find such genome parts that we could use to separate the groups within the genus *Streptococcus*. Then, to identify the species we searched for those fragments which are unique for one species. Due to the importance of the mitis group of *Streptococcus*, and also to the difficulties with the species identification, first we constructed the molecular identification key for this group. The key allows the correct identification of these species.

MATERIALS AND METHODS

The genomic sequences and sequence annotation for 11 *Streptococcus* species, (among them 6 species belonging to mitis group) which were published at the time of this study (September 2011), were obtained from NCBI's FTP site at http://www.ncbi.nlm.nih.gov/Genomes.

The sequences of the proteins encoded in complete genomes were extracted from the Genome division of the Entrez retrieval system. Protein homologous was detected by BLAST similarity searches (A l t s c h u l e et al., 1997). For two proteins to be considered as homologous, their sequence identity must be at least 75%, and the expected value (E-value) must be less then 10^{-5}.

The genome comparison and synteny block determination was made by SynteBase/SynteView (L e m o i n e et al., 2008) and by Microbe browser (G a t t i k e r et al., 2009). SynteBase/SynteView is available at http://www.synteview.u-psud.fr and the Microbe browser at http://microbe.vital-it.ch/. Comparison of protein homologous is also performed by Microbe browser, too.

RESULTS AND DISCUSSION

The mitis group currently includes the important pathogen *S. pneumoniae* and 12 other species, *S. australis*, *S. cristatus* (formerly *S. crista*), *S. gordonii*, *S. infantis*, *S. mitis*, *S. oligofermentans*, *S. oralis*, *S. parasanguinis* (formerly *S. parasanguis*), *S. peroris*, *S. pseudopneumoniae*, *S. sanguinis* (formerly *S. sanguis*), and *S. sinensis*. Out of these there is a full genome sequence of 6 species on the NCBI's FTP site: *S. pneumoniae*, *S. mitis*, *S. oralis*, *S. gordonii*, *S. sanguinis* and *S. parasanguinis*. In case of *S. pneumoniae* the complete genome of many strains is sequenced, so we chose a reference strain according to the literature and we used that in the comparisons. In case of *S. pneumoniae* it is the strain R6.

The comparison analysis of the eukaryotic and prokaryotic genomes has shown that the gene order of certain genes is more or less the same of each species of the genus (L a r k i n et al., 2009). Using this we formed an identification key which follows the classical identifications but it contains molecular features. The characters we used for forming the key have not the DNA or protein sequences of the genes but their symbols. These are most likely symbols of 3 or 4 characters. As it happened that the same gene had several different symbols, we chose the one that is used for the given gene in the *E. coli* to be the referring one. For indicating the malolactic enzyme (NAD-dependent malic enzyme, EC 1.1.1.38) at the *S. sanguinis* the symbol *mleS* is used; in the *E. coli* this gene is indicated with the symbol of *sfcA*, so we also used this one. If there was no an adequate symbol in *E. coli* for a certain gene, we used the original symbol that was given with that species.

In the genomes there are many genes whose function is unknown and therefore there is no symbol for such a gene. Those unknown genes whose sequence is the same in all the species (they probably code the same protein) were identified in the key with the number of the amino acids of the protein coded by the gene (in case of the *S. pneumoniae* it is the number that shows how much amino acid the protein has in the strain R6). To check and prove that it was the same gene a comparison of the protein sequences was performed using the BLASTP program. Those genes that code various proteins in various species and they were not present at each species, were marked with x. 0-3x in the key means that in certain species there is no other gene between two identical genes, while in other species there could be 1, 2, or 3 genes as well. If a gene (known or unknown) is in parentheses it means that it is not present in all the species of the given group.

Developing the identification key we had the hypothesis that in certain species the genes were in a certain order. Accordingly, a species was determined by the order of its genes in genome. Similar species (that belong to the same genus) also contained such genome parts where the order of the genes was the same. Since there are 6 groups within the *Streptococcus* genus differentiated due to morphological, physiological, biochemical, and other characteristics, it was presumed that these differences were also present on the genome level. The analysis of the genomes showed that there were such synteny blocks whose

order of the genes was characteristic only to one or the other *Streptococcus* group. Based on this result we constructed a key that identified which group a species belonged to if we know its genome.

The identification key for determining the groups within the *Streptococcus* genus:

Genus *Streptococcus*

1a 1: murD murG ftsQ ftsA ftsZ ylmE ylmF (ylmG) ylmH DivIVA (0-1x) ileS
2: iscS-2 thiI (2-4x) rplU (0-1x) rpmA **2**
1b 1: murD murG ftsQ in one locus and ftsZ ylmE ylmF (ylmG) ylmH DivIVA (0-1x) ileS in another one
2: iscS-2 thiI in one and rplU x rpmA in another locus . . . . . **mitis group I. (*S. pneumoniae, S. mitis, S. oralis*)**
2a. 1: nrdD (0-1x) 519 (0-5x) 101 139 89 (0-3x) spxA recA
2: gidB (0-4x) lemA htpX **3**
2b. 1: nrdD and recA are not near each other
2: gidB lemA in one and htpX in another locus . . . . **salivarius group, *S. suis***
3a 1: folC folE folP folB folK murB potA potB potC potD
2: clpX 56 folA thyA mvaS mvaA . . . . . **pyogenic group, mutans group, bovis group**
3b 1: murB potA potB potC potD
2: clpX (0-1x) folA thyA in one and mvaS mvaA in another locus . . . . . **mitis group II (*S. gordonii, S. sanguinis, S. parasanguinis*)**

From the representatives of the anginosus group we had no genome sequence available therefore this group was not included in the key. The *S. suis* is not in any of the groups according to literature so it was separately defined (K a w a m u r a et al., 1995).

The mitis group was divided into two subgroups here. The reason for this was the great difference between the genomes of the two groups. The representatives of the mitis group I had a very different gene sequence from the sequences of all other *Streptococcus* species; therefore we had to separate this group. These results were equal with the measured level of 16S rRNS sequence homology among the *Streptococcus* species (K a w a m u r a et al., 1995). Those species which belong to the mitis group I according to the gene order found in the synteny blocks exhibited more than 99% 16S rRNS sequence homology with each other, while between the members of the mitis group I and II the sequence homology was less then 98%.

For the accurate determination of the species we chose 3 genome parts taking into consideration the following:

1. If possible, there should be a great difference in gene order in these parts of genomes among the species and
2. The parts should contain such a gene which was found in almost all the other species.

Three parts were sufficient enough to determine a species with full accuracy. The three genes selected were the following:

1. Xaa-pro dipeptidyl-peptidase (x-prolyl-dipeptidyl aminopeptidase, EC = 3.4.14.11) – *pepX*
2. leucyl-tRNA synthetase (EC=6.1.1.4) – *leuS*
3. 50S ribosomal protein L13 and 30S ribosomal protein S9 – *rplM rpsI* (these are always one beside the other)

Although the full genome of many strain in the *S. pneumoniae* species is known, there are still minor differences among them. Certain genes are not found in the genomes of every strain. These genes, which appear in certain strain only, were put in parentheses in the key.

Below is the species identification key for mitis groups.

Species identification: *Mitis* group I

Streptococcus pneumoniae

1. hsdM hsdS hsdR (66) argR pepX dnaE pfkA pykF (85) 290 (127) (55 62) 103 238 271
2. chbA chbB chbC pflF talC gldA leuS (280) 232 139 ruvB 191 uppS cdsA eep proS bglA glmS
3. folP sulB folE sulD 123 rplM rpsI (0-10x) (121 bglA 79 113 bglG 104 168 448) 58 xylS basA hysA kdgA kdgK

Streptococcus mitis

1. dnaG rpoD 109 cpoA 441 58 44 obg 52 argR pepX 4138 dnaE pfkA pykF ldh gyrA srtA
2. rpoA rplQ 119 445 498 gpmB leuS 288 280 151 139 231 138 tnpC 30 30 ruvB 187 tnpC tnpA uppS cdsA epp pros 459 glmS
3. 257 264 89 406 rpsI rplM tnpA natB natA pheT 124 pheS

Streptococcus oralis

1. dnaG rpoD 109 cpoA 441 62 44 obg 54 argR pepX dnaE pfkA pykF murA 183 425 map
2. glmS 137 bglA proS eep cdsA uppS 194 ruvB 138 231 leuS 346 363 sfuB 233 445 88 rplQ rpoA
3. pheS pheT 317 399 rplM rpsI 315 54 170 amiA aliB cysQ cps4A cpsB cps4C cps4D cpsE

Species identification: *Mitis* group II

Streptococcus gordonii

1. rimJ gcp 231 110 248 222 246 391 180 586 119 135 pepX 283 426 cppA 311 mvk mvaD mvaK2
2 deoR 321 chbA chbB chbC pflF talc gldA 187 leuS 90 119 498 203 202 210 210 204 210 adhB

3. cysS 134 lrp 376 615 320 trmH 214 degV rplM rpsI 384 172 93 279 110 64 cadD

Streptococcus sanguinis

1. ubiE 245 391 164 ugpQ 99 125 pepX 301 427 cppA 311 mvk mvaD mvaK2
2. deoR 159 chbA chbB chbC pflF talC gldA 187 leuS 381 275 324 85 34 cynR 116 sfcA
3. cysS 134 lrp 376 582 320 trmH 173 degV rplM rpsI 73 202 78 131 92 copA cadD

Streptococcus parasanguinis

1. gla 390 lrgA lrgB 99 524 dnaQ 164 pepX cppA 320 mvk mvaD mvaK2
2. deoR 322 chbA chbB chbC pflF talC gldA dacA aliA leuS 137 pdxH 277 254 149 284 242 proW pdxT bglA glmS
3. cysS 132 lrp 376 malL 1073 320 rlmB 170 287 rplM rpsI int 87 195 204 249 104 104 176 642 arsR

As it is seen from the key, the gene sequence of the *S. mitis* and *S. oralis* within the mitis group I is very similar. This is in correspondence with the earlier observations that the two species are difficult to distinguish from each other due to a high degree of similarity (Bishop et al., 2009). There was a bigger difference between these two and the *S. pneumoniae*. In the first section (the surrounding the *pepX* gene) from *dnaG* to *pykF* the sequence was almost identical between the two genomes and after the *pykF* they showed an important difference. In the surrounding of the *leuS* gene there was only a slight difference between *S. mitis* and *S. oralis*, but the sequence from *leuS* to *gluS* was the same as in the *S. pneumoniae*. In the third section, the part after the *rpsI* gene was completely different between *S. oralis* and *S. mitis*. Such a big similarity among the genomes made it necessary to use three gene sequences in order to determine a species accurately.

The chosen genome parts were completely different between mitis group II and the mitis group I. In the mitis group II there was a similarity between the gene sequences of the *S. gordonii* and *S. sanguinis* (around the *rplM* and *rpsI* genes, and also in the sections between *leuS* and *pflC*, and from *pepX* to *mvaD*). However, using three parts it was possible to determine the species with accuracy. The *S. parasanguinis* showed greater difference from the genome of the previous two species.

CONCLUSION

The full genome sequences in the database made it possible to develop such an identification key that allowed easier identification of the bacterium isolates. The methods used so far cannot give an unambiguous classification for

the bacterial systems (Konstantinidis and Tiedje, 2007). Presently, the most accepted method is the identification based on 16S RNA. However, on the level of the species, when the sequence homology of the 16S RNA is above 95%, misclassification often occurs (Kunin, 2005). Using this identification key, with having the surrounding of three genes (*pepX*, *leuS* and *rplM*) given, makes it possible to accurately determine a species. Its disadvantage is that, in order to function, it needs a full genome sequence or genome parts with many genes but presently it is possible only for about 1000 species. The number of completely sequenced genomes is increasing day by day and it is only the question of time when we will have the full genome sequences of all the species. The new data will enable to make the key more accurate and help the compilation of a complete bacterial taxonomy.

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МОЛЕКУЛАРНИ ИДЕНТИФИКАЦИОНИ КЉУЧ ЗА ОДРЕЂИВАЊЕ ВРСТА МИТИС ГРУПЕ РОДА *STREPTOCOCCUS*

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Резиме

Род *Streptococcus* обухвата веома значајне клиничке врсте као што је *S. pneumoniae*. Детерминација ових врста и данас представља потешкоћу, иако се у њиховој идентификацији користе и молекуларне методе као што је ДНК-ДНК хибридизација или 16С рРНК ген анализа. У овом раду представљамо нову методу за егзактно одређивање бактеријских врста. Она се базира на редоследу гена у њиховом геному и на основу тога је сачињен један молекуларни кључ за идентификацију врста. Род *Streptococcus* обухвата 6 група (митис, ангиносус, саливариус, мутанс, бовис и пиогена) и направљен је кључ за њихову детерминацију. Овај кључ обухвата секвенце генома које су сличне у појединим групама. Затим, због важности митис групе, израђен је кључ за детерминацију припадника ове групе. Приликом израде кључа за идентификацију врста, изабрали смо такве секвенце генома које се налазе поред гена, који се могу наћи у геному сваке врсте, али се редослед гена у овим секвенцама разликује у довољној мери да се могу користити у тачној идентификацији. Тако смо изабрали секвенце око три следећа гена: Хаа-pro дипептидил-пептидаза (х-пролил-дипептидил аминопептидаза) – *pepX*, леуцил-тРНА синтетаза – *leuS* и 50S рибосомални протеин L13 и 30S рибосомални протеин С9 – *rplM* и *rpsI*. Ове три секвенце генома су биле довољне за тачну детерминацију врста.

КЉУЧНЕ РЕЧИ: митис група, молекуларни идентификациони кључ, род *Streptococcus*, систематика бактерија

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DETERMINATION OF THE ANTIOXIDANT ACTIVITY OF *FICUS CARICA* AQUEOUS EXTRACT

ABSTRACT: The aim of this study was to examine the antioxidant activity of water extracts from fig leaf. Water extracts were prepared according to traditional medicine. The antioxidant activity of the extracts was spectrophotometrically determined. Using the potassium permanganate colorimetric method it was found that the water extract that was maintained at the refrigerator had lower antioxidant activity than extract that was maintained at the room temperature.

KEY WORDS: antioxidant activity, extract, *Ficus carica* L., flavonoids

INTRODUCTION

The therapeutic benefit of plants was often attributed to their antioxidant properties. Some plant extracts are believed to have strong antioxidant effects.

Ficus carica L. (Moraceae) is commonly used herbal remedy in traditional medicine. Traditionally, fig has been used to treat constipation, bronchitis, hyperlipidemia, eczema, psoriasis, vitiligo, and diabetes. The leaf decoction of fig is taken as a remedy for diabetes and calcification in the kidney and liver. However, the antioxidant activity and citotoxicity against various cancer cell lines reported in fig are potentially promising in its future therapeutic uses (W a n g et al., 1996; K i k u z a k i et al., 1993).

Nowadays, it is a trend to search for natural products to replace the synthetic ones in different domains, especially food and medicine. *Ficus carica* leaves are known to be reach in flavonoids (L e u n g et al., 1996) and this is why we have studied their antioxidant properties versus a synthetic, water-soluble antioxidant, the ascorbic acid.

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MATERIAL AND METHODS

Material: Potassium permanganate and sulfuric acid were purchased from "Reactivul" Bucureşti, the ascorbic acid from Merk.

Apparatus: Spectrophotometer SPEKOL 10 (Carl Zeiss. Jena) fitted with 30 ml quartz vats and magnetic stirrer.

Plant material: Leaves of *Ficus carica* were collected in October 2010 and were dried at 20°C in a dark place. Four g of the dried sample were chopped into small parts and then extracted with 60 ml water for 70 h at 10°C, followed by filtration. The final volume of the extract was 40 ml.

Antioxidant properties screening by the potassium permanganate assay:

The method is based on the redox reactions between the antioxidant sample and the potassium permanganate in sulfuric acid media, leading to the discoloration of the last one.

Variable amounts of 1/10 diluted samples (v = 0.2 ml or 0.6 ml – depending on the intensity of the antioxidant activity) were introduced in a 30 ml quartz vat containing an oxidative mixture formed from: 1.5 ml potassium permanganate 0.01M; 3.5 ml sulfuric acid 2M and (20-v) ml distilled water. That moment was considered the zero time. The spectrophotometer signal (mV) was then registered at 530 nm until constant value. The variation of the potassium permanganate concentration was afterwards determined based on a calibration curve previously done (E x a r c h o u et al., 2002; S z a b o et al., 2005; C a c i g, 2007).

The extract E1 was kept in a refrigerator (10°C) and the extract E2 was stored at room temperature (20°C), and the analyses were done after 1, 2, 4, 18, and 40 days from their getting.

RESULTS AND DISCUSSION

A calibration curve was done by preparing a series of six solutions with different concentrations of potassium permanganate and registering the electronic signal (mV) for each of them. The obtained graphic and the corresponding equation are presented in Figure 1.

Based on this, the potassium permanganate concentration (x) can be determined from the signal value (y) with the formula:

$$x = \frac{y - 0{,}929}{381328} \qquad (1)$$

The shape of the curves representing the variation of the potassium permanganate concentration for the studied samples depends on the extracts (E1 or E2).

Figure 2 shows the comparative curves for the extracts (E1 and E2), first day after their getting. These curves showed that the E1 extracts induced more conspicuous decrease of the potassium permanganate concentration, meaning that their antioxidant activity was higher. This is why for the further experiments only 0.2 ml of E1 extracts were added.

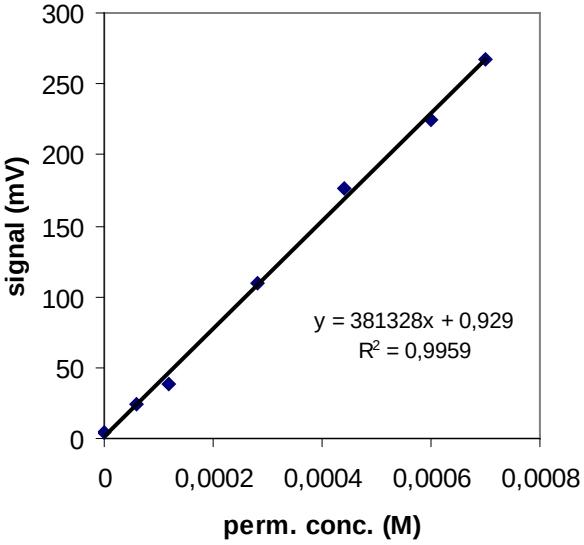

Fig. 1. – Calibration curve for the potassium permanganate in the oxidative mixture

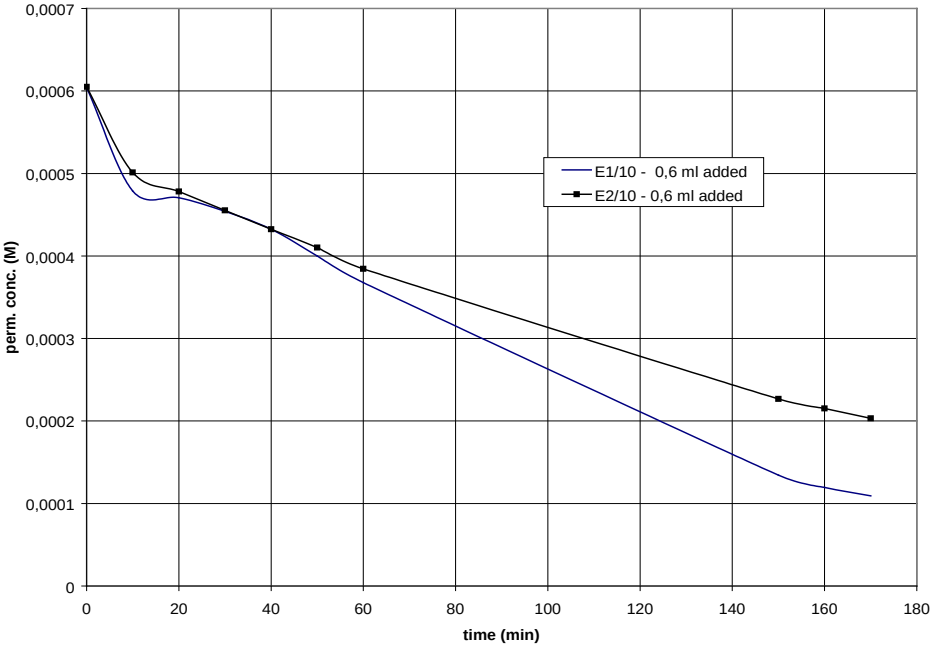

Fig. 2. – Variation of the potassium permanganate concentration after adding 0.6 ml (E1) – 10 times diluted and extract (E2) – 10 times diluted, first day from their getting

Figures 3 and 4 show the comparative evolution on the extracts on day 2, 4, 18, and 40 from their getting.

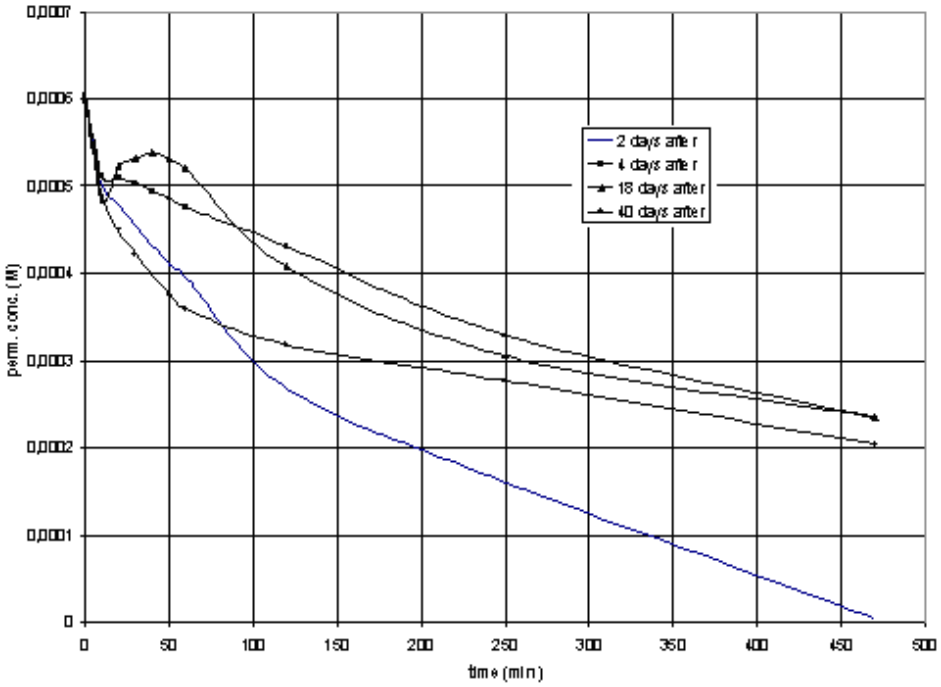

Fig. 3. – Variation of the potassium permanganate concentration after adding 0.2 ml of (E1) – 10 times diluted, on day 2, 4, 18, and 40 from their getting

Most curves showed a decrease in the potassium permanganate concentration, as expected. Still in some cases the rapid decrease was followed by a false increase, due to the formation of MnO_2 particles, which afterwards precipitated.

Probably during the first phase, corresponding to the rapid decrease of the concentration, there was a reaction of the substances with higher antioxidant activity which were able to reduce Mn (VII) to Mn (II). After that, reacted the substances with lower antioxidant activity which reduced Mn (VII) only up to Mn (IV) – MnO_2 leading to the precipitate formation. Later, a portion of the MnO_2 started to precipitate and another portion was probably dissolved by the sulfuric acid from the media and causing the absorption to decrease again.

In addition, the curves showed that antioxidant activity decreased during the first 18 days. However, on day 40 of the analysis a conspicuous increase of the antioxidant activity was noticed probably related to the fermentation processes. For the E1 extract, the antioxidant activity seemed to be higher than at the beginning.

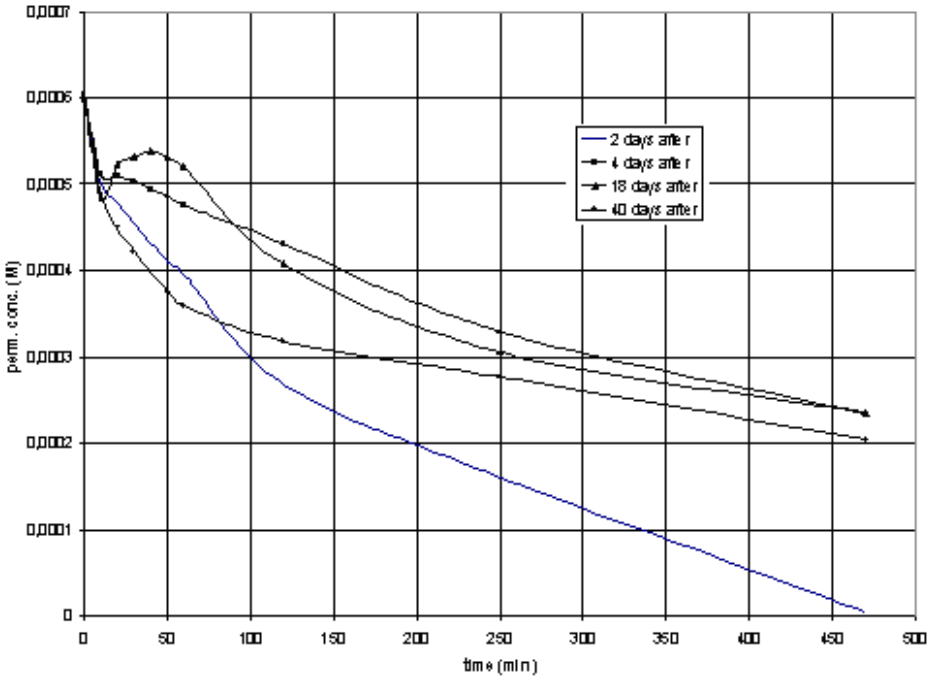

Fig. 4. – Variation of the potassium permanganate concentration after adding 0.6 ml (E2) – 10 times diluted, on day 2, 4, 18, and 40 from their getting.

In order to quantitatively compare the antioxidant activities, we proposed the following formula:

$$A_{50} = \frac{t_{standard}}{t_{plant\,sample}} \cdot \frac{C_{standard}}{m_{plant}} \cdot \frac{V_{standard}}{V_{plant\,sample}} Dil \cdot \frac{V_{extract}}{100} \qquad (2)$$

where: A_{50} – antioxidant activity expresses as a function of the time until the sample induces a decrease of the permanganate concentration up to half, reported to a standard (mmol equivalent standard / g)

$t_{plant\ sample}$ – the time until the sample induces a decrease of the permanganate concentration up to half (min)

$t_{standard}$ – the time until the standard (ascorbic acid) induces a decrease of the permanganate concentration up to half (min)

$C_{standard}$ – standard (ascorbic acid) concentration (mmol/ml)

m_{plant} – mass (g) of the plant sample submitted to extraction

$V_{plant\ sample}$ – volume of the plant extract submitted to the analysis

$V_{standard}$ – volume of the standard submitted to the analysis

Dil – dilution

$V_{extract}$ – volume (ml) of the obtained extract

The A_{50} values variation can be noticed from Tab. 1.

Tab. 1. – A_{50} values for the studied extracts

Sample	Day 2	Day 4	Day 18	Day 40
E1	0.00011	0.000097	0.000076	0.00032
E2	0.0001	0.000043	0.00005	0.000076

CONCLUSION

The spectrophotometric method we propose allowed the quantitative evaluation of the antioxidant activity for the plant extracts.

In our study, E2 extracts shoed higher antioxidant activity than E1 extracts. Fermentative processes seemed to modify the antioxidant activity of the E2 extracts.

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ОДРЕЂИВАЊЕ АНТИОКСИДАТИВНЕ АКТИВНОСТИ ВОДЕНИХ ЕКСТРАКТА *FICUS CARICA*

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Резиме

У раду је испитивана антиоксидативна активност воденог екстракта из лишћа *Ficus carica* који се често употребљава у традиционалној медицини. Листови *Ficus carica* су познати као изузетно богати флавоноидима.

Водени екстракти су чувани на собној температури од 20° C (E2), у хладњаку на температури од 10° C (E1), Антиоксидативна активност екстраката је одређена спектрофотометријски мерењем на таласној дужини од 530 nm. Резултати испитивања показују да екстракт са собне температуре има већу антиоксидативну вредност од екстраката чуваних на нижим температурама. Резултати испитивања показују да екстракти E2 имају већу антиоксидативну активност него екстракт E1 зато што ферментација има велик утицај у овом случају.

КЉУЧНЕ РЕЧИ: антиоксидативна активност, екстракт, *Ficus carica* L., флавоноиди

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MORPHOLOGICAL VARIABILITY OF POPULATIONS OF *DACTYLORHIZA MACULATA* (L.) SOÓ AND *D. MAJALIS* (REICHENB.) P. F. HUNT ET SUMMERHAYES (ORCHIDALES, ORCHIDACEAE) FROM STARA PLANINA MOUNTAIN (SERBIA)

ABSTRACT: Comparative morphological taxonomic analysis was conducted on two populations of *Dactylorhiza maculata* (L.) Soó 1962 and one population of *D. majalis* (Reichenb.) P. F. Hunt et Summerhayes 1965 from the area of Stara planina mountain in order to determine new characters that could be used to distinguish these two species and characters that could eventually be used for defining infraspecific taxa within these species. Taxonomic studies have included processing of quantitative and qualitative characters. A total of 60 characters were analyzed, out of which 34 quantitative and 26 qualitative. Analysis of quantitative and qualitative characters included 90 specimens – 60 of *D. maculata* and 30 of *D. majalis*. Based on the results obtained by multivariate statistical analysis and literature data, we selected sets of taxonomic characters that allow a clear differentiation of infraspecific forms within the species *D. maculata*, which is characterized by intensive variability on the selected localities on Stara planina.

KEY WORDS: *Dactylorhiza maculata*, *D. majalis*, infraspecific variability, multivariate statistics

INTRODUCTION

Genus *Dactylorhiza* (Orchidaceae, Orchidoideae) is taxonomically problematic genus in which many species show variability within and between populations. Also, within this genus is common interspecific hybridization where hybrids are generally of high fertility and sometimes they outnumbered the parental populations (S o ó, 1980). Many problematic taxa of this genus are alotetraploids, believed to be the results of multiple hybridization between two

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broadly defined parental species *D. fuchsii* (Druce) Soó and *D. incarnata* (L.) Soó (Heslop-Harrison, 1968; Hedren, 2002, Devos et al., 2003). Numerous evidence suggests that this complex is a dynamic system of polyploidy speciation and extinction in which polyploids evolve continuously from the same set of broadly defined parental lines (Hedren, 2003). Last morphometric research showed that *Dactylorhiza* alotetraploids often have morphological characters that are generally intermediate between *D. incarnata* and *D. fuchsii* (Gathoye and Tyteca, 1993).

Species from this genus are present in temperate and north Eurasia, northern Africa, eastern (Ontario), and western (Alaska) North America, with its center of diversity in Europe and southwest Asia (Strid and Tan, 1991). Number of species (12-75) recognized by different authors varies significantly (Pedersen, 1998; Bateman and Denholm, 2003). In flora of Serbia, there are 6 species in genus *Dactylorhiza* (Diklić, 1976).

Presence of transitional characteristics between clearly defined taxa indicates parallel directions in phenotypic radiation, i.e. models of phenotypic variability induced by changes in environmental conditions. Such a phenomenon of parallel more or less variable characteristics is common within a species and between related species. As large mountain ranges show significant level of habitats diversity, it is also expected a higher degree of differentiations between distant populations of same species, and a similar form of plasticity in phylogenetically close species. These types of changes are regularly followed by taxonomic infraspecific differentiation within species and detection of known and description of new infraspecific forms. In this light, we set the directions of research of morphological differentiations of populations of *D. majalis* and *D. maculata* originating from the western part of Stara Planina Mountain in Serbia.

MATERIALS AND METHODS

Plants material was collected from three sites in the area of Stara Planina Mountain. A total of 90 plants were collected: Babin Zub (30 plants), Dojčino vrelo (30 plants) and Krvave bare (30 plants) (Table 1).

Tab. 1 – Sampling sites

Taxon	Locality	GPS coordinates
Dactylorhiza maculata (L.) Soó, *Nom. Nov. Gen. Dactylorhiza 7* (1962)	Krvave bare (mac krb)	N43 19.744 E22 47.883
Dactylorhiza maculata (L.) Soó, *Nom. Nov. Gen. Dactylorhiza 7* (1962)	Dojčino vrelo (mac doj)	N43 22.000 E22 36.299
Dactylorhiza majalis (Reichenb.) P. F. Hunt & Summerhayes, *Watsonia* 6: 130 (1965)	Babin Zub (maj bab)	N43 22.387 E22 37.449

Material was dried and prepared with standard method and is kept in the herbarium of the Department of Biology and Ecology, Faculty of Sciences in Novi Sad (BUNS). During the processing of materials, dried specimens were labeled with standard OTU labels (Original Taxonomy Units). Prior to morphological and morphometric investigation, there was a rehydration of material by thermal treatment in ethanol solution, after which material was set in the glycerol substrate and thus analyzed. In each analysis, we reject the maximum number of plants that did not meet the criteria. The measurements were performed with precision caliper gauge (precision 0.01 mm) at temperature of 25°C.

The data obtained by measurement were statistically analyzed using the computer program STATISTICA for Windows 10.0. Statistic analyzes included descriptive and discriminate statistics and multivariate analysis (discriminant and correspondent analysis). As a part of multivariate analysis, we tested statistical significance by univariate and multivariate analysis (Quick, 1997).

Morphological taxonomic studies have included the processing of quantitative and qualitative character, which had previously been selected from literature (Soó, 1973; Buttler, 1996; Pignatti, 1982; Йорданов, 1964; Pancă and Beldie, 1972; Soó, 1980; Diklić, 1976; Hayek, 1933; Shipunov and Bateman, 2005). A total of 60 characters was analyzed, out of which 34 quantitative and 26 qualitative (Table 2). Amongst quantitative characters, we analyzed 25 morphometric, 2 meristic and 7 characters that represent indexes calculated based on proper morphometric characters.

RESULTS AND DISCUSSION

Statistical analysis of quantitative characters

We observed and statistically analyzed 7 morphometric indexes on 73 plants (*D. majalis* – 24, *D. maculata* – 49 plants). In addition, statistical analysis was performed for 25 morphometric characters and included 67 plants (*D. majalis* – 23, *D. maculata* – 44 plants). Based on measurement data, we calculated mean value (x), standard deviation (σ) and coefficient of variation (CV %) (Table 3). Coefficients of variation for the same character for all three populations are grouped in three ways: (1) CV% for all three populations are in the same zone of variability; (2) CV% for two populations of *D. maculata* are in the same zone of variability, population of *D. majalis* differ; and (3) CV% for population of *D. maculata* from Dojčino vrelo and *D. majalis* population are in the same zone of variability, population of *D. maculata* from Krvave bare differ. Only in two cases, coefficients of variation values are in different zones of variability for all three populations.

Tab. 2 – Analyzed characters

Organ	Quantitative characters	Qualitative characters	Number of characters states
Stem	Height Height to inflorescence Width	Edge Margin	
Leaf	Number (len) Length Width Index l/w	Hairiness Lowermost leaf shape (lls) Uppermost leaf shape (uls) Lowermost leaf apex shape (lla) Uppermost leaf apex shape (ula) Leaf arrangement (lar) Leaf spots presence (lsp) Presence of spots on the uppermost leaf (psu) Presence of spots on the lowermost leaf (psl) Position of the widest part of leaf Lowermost leaf margin (llm) Uppermost leaf margin (ulm)	2 3 4 2 3 3 3 3 3 2
Inflores-cence	Flowers number (fln) Inflorescence length	Inflorescence shape (ins) Inflorescence compactness (inc)	2 2
Flower	Lowermost bract width Lowermost bract length Index l/w of lowermost bract Uppermost bract width Uppermost bract length Index l/w of uppermost bract Flower length Index length br/fl Ovary length Spur length Index length sp/ov Labellum length Labellum width Index l/w of labellum Length llll 2-3 Length rlll 2-3 Width llll 4-3 Width rlll 4-3 Length mll Length A (same as labellum length) Length B Length C 2A/(B+C) Length llll 4-2 Length rlll 4-2	Lowermost bract shape (lbs) Uppermost bract shape (ubs) Spur shape (sps) Labellum lobes shape (los) Labellum median lobe shape (lms) Labellum median lobe apex shape (lma) Right lateral labellum lobe shape (srl) Left lateral labellum lobe shape (sll) Margin of right lateral labellum lobe (mrl) Margin of left lateral labellum lobe (mll)	3 4 2 3 5 4 7 7 5 5

l – length; w – width; br – bract; fl – flower; sp – spur; ov – ovary; llll – left lateral labellum lobe; rlll – right lateral labellum lobe; mll – median labellum lobe; lll – lateral labellum lobe; A – length from bursicula to the top of median labellum lobe; B – length from bursicula to the top of lateral labellum lobe; C – length from bursicula to separation point of lateral and median labellum lobes; 2A/(B+C) – labellum index; * numbers 4-2, 4-3, 2-3 represent labellum measurement points

Tab. 3 – Basic statistical parameters of quantitative characters of examined populations

	D. majalis – Babin Zub				*D. maculata* – Dojčino vrelo				*D. maculata* – Krvave bare			
	N	x	σ	CV(%)	N	x	σ	CV(%)	N	x	σ	CV(%)
Vegetative region												
Stem height	23	394.99	72.06	18.24	22	300.66	59.75	19.87	22	221.25	31.64	14.30
Height to inflorescence	23	307.17	55.87	18.19	22	233.54	47.18	20.20	22	174.87	26.61	15.22
Stem width	23	3.87	1.07	27.64	22	4.47	0.95	21.17	22	4.16	0.56	13.53
Leaf length	23	127.14	39.72	31.24	22	128.89	32.66	25.34	22	114.56	20.18	17.62
Leaf width	23	26.88	8.78	32.65	22	24.96	6.61	26.49	22	23.02	5.11	22.18
w of lowermost bract	23	3.32	0.95	28.66	22	7.49	1.94	25.91	22	5.57	1.41	25.38
l of lowermost bract	23	24.00	6.77	28.20	22	29.18	7.75	26.57	22	22.09	5.76	26.10
w of uppermost bract	23	1.48	0.52	35.16	22	3.20	0.57	17.75	22	3.04	0.56	18.52
l of uppermost bract	23	7.46	1.71	22.87	22	10.14	2.31	22.83	22	10.17	1.79	17.56
l/w of leaf	24	4.85	1.22	25.15	25	5.54	1.97	35.60	24	5.11	1.55	30.27
l/w of lowermost bract	24	7.48	2.05	27.41	25	4.03	0.78	19.48	24	4.08	0.63	15.48
l/w of uppermost bract	24	5.44	1.43	26.29	25	3.21	0.63	19.50	24	3.40	0.46	13.49
l of bract/l of flower	24	1.74	0.43	24.71	25	2.91	0.70	24.01	24	2.05	0.62	30.30
Generative region												
Inflorescence length	23	87.82	31.06	35.37	22	67.12	21.53	32.08	22	46.38	11.67	25.15
Flower length	23	13.80	2.02	14.67	22	9.87	1.08	10.93	22	11.01	1.01	9.15
Ovary length	23	10.86	1.62	14.95	22	12.78	2.02	15.82	22	11.52	1.47	12.78
Spur length	23	9.01	1.35	15.01	22	5.13	0.87	16.91	22	5.84	0.82	14.04
Labellum length	23	8.21	1.28	15.64	22	9.15	1.07	11.68	22	9.12	1.11	12.19
Labellum width	23	9.52	1.45	15.21	22	11.93	1.46	12.26	22	11.42	1.54	13.46
l llll 3-2	23	2.91	1.15	39.51	22	5.48	1.75	32.01	22	4.71	1.28	27.19
l rlll 3-2	23	2.91	1.28	44.06	22	5.83	1.71	29.29	22	4.49	1.21	26.93
w llll 4-3	23	3.24	0.72	22.18	22	1.81	1.40	77.14	22	1.59	0.70	43.95
w rlll 4-3	23	2.87	0.60	21.02	22	1.61	0.75	46.48	22	1.48	0.58	39.11
l mll	23	4.84	0.78	16.19	22	2.21	0.92	41.87	22	2.43	0.73	30.03
Length A	23	8.21	1.28	15.64	22	9.15	1.07	11.68	22	9.12	1.11	12.19
Length B	23	4.47	1.30	29.04	22	7.62	1.16	15.22	22	7.30	1.20	16.41
Length C	23	3.23	1.04	32.38	22	6.88	1.14	16.51	22	6.60	1.07	16.23
l rlll 4-2	23	3.43	0.64	18.76	22	6.37	1.45	22.81	22	5.10	1.03	20.28
l llll 4-2	23	3.48	0.62	17.76	22	6.25	1.86	29.71	22	5.41	1.07	19.78
l sp/ov	24	0.84	0.15	17.86	25	0.41	0.08	19.66	24	0.52	0.09	17.87
l/w of labellum	24	0.86	0.15	17.44	25	0.78	0.09	11.08	24	0.79	0.09	10.81
2A/(B+C)	24	2.35	0.74	31.49	25	1.29	0.17	13.58	24	1.32	0.13	9.92

	CV% are in different zones of variability for all three populations
	CV% for all three populations are in the same zone of variability
	CV% for two populations of *D. maculata* are in the same zone of variability, population of *D. majalis* differ
	CV% for population of *D. maculata* from Dojčino vrelo and *D. majalis* population are in the same zone of variability, population of *D. maculata* from Krvave bare differ

L – length; w – width; sp – spur; ov – ovary; llll – left latetal labellum lobe; rlll – right lateral labellum lobe; mll – median labellum lobe; lll – lateral labellum lobe; A – length from bursicula to the top of median labellum lobe; B – length from bursicula to the top of lateral labellum lobe; C – length from bursicula to separation point of lateral and median labellum lobes; 2A/(B+C) – labellum index; * all values are in mm

Variance analysis

To determine differences in the variability of analyzed quantitative characters we applied univariate analysis of variance (ANOVA). There is a statistically significant difference in variability of 12 characters between two populations of *D. maculata*. In case of both analyzed species there is statistically significant difference in variability of all characters except 4 (stem width, leaf length and width, and index leaf length/leaf width). Defined set of variable character between the two species does not belong to a group of taxonomically valuable characters in interspecific differentiation. However, significant variability between populations of *D. maculata* provide solid base for further analysis and selection of those characters that contribute to infraspecific separation.

To determine the differences between populations in relation to total morphological variability of the sample, we applied multivariate analysis of variance (MANOVA). Results indicate statistically significant differences in variability of analyzed quantitative characters. These results confirmed significant interpopulation variability of morphological characters within *D. maculata*, which was detected by univariate analysis.

Discriminant analysis of quantitative characters

To identify quantitative characters that are major contributors in discrimination of groups, we used discriminant analysis. Along the first discriminant axis these are following characters: height (of stem) to inflorescence, stem width, leaf length, leaf width, left lateral labellum lobe length 3-2 (llll 3-2), right lateral labellum lobe length 3-2 (rlll 3-2), median labellum lobe length (mll), left lateral labellum lobe length 4-2 (llll 4-2) and index spur length/ ovary length (sp/ov). On the second axis, major discriminant contributors are height (of stem) to inflorescence, length A, right lateral labellum lobe length 4-2 (rlll 4-2) and index bract length/flower length (br/fl).

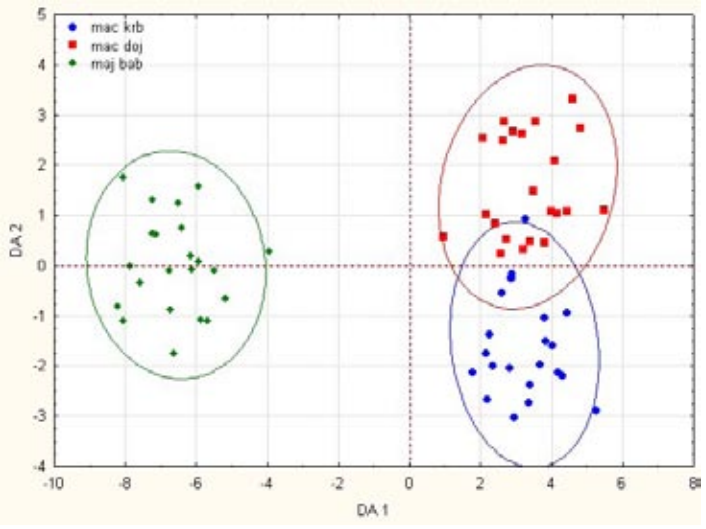
In the area of first and second discriminant axis, based on the analyzed morphometric characters and their indexes, both populations of *D. maculata* are on one and population of *D. majalis* is on the other side of the first axis (Fig. 1). Based on morphometric characters, there is separation of individuals of two populations of *D. maculata* along the second axis (Figure 2A). If we observed results obtained for morphometric indexes (Figure 1B), specimens from all three populations are dispersed along the second axis, and they cannot be well separated.

If we consider distribution of populations in area of first and second discriminant axis and values of discriminant characters, we can conclude that the population of *D. majalis* is characterized by longer and thinner stem, wider leaves, longer median labellum lobe, and a larger index spur length/ovary length. Populations of *D. maculata* are characterized primarily by stem length, where it is longer within the population from Dojčino vrelo. Although we observed differences in the variability of morphomet-

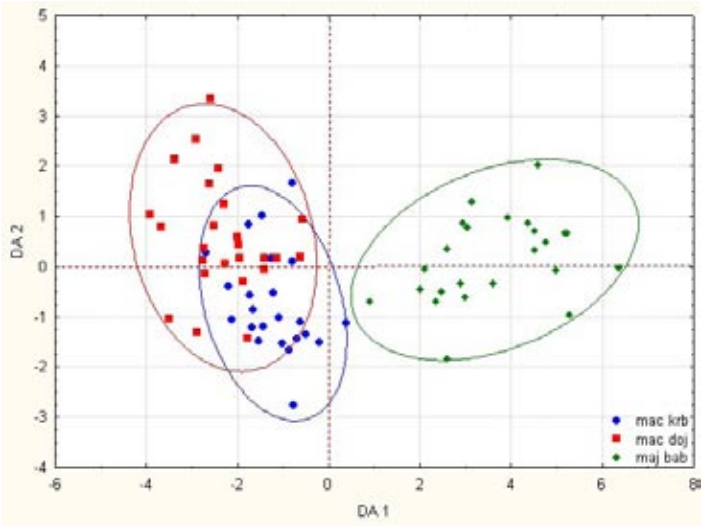
ric characters between analyzed populations, they were insufficient for the definitive separation of studied populations into appropriate infraspecific categories.

Correspondence analysis of qualitative characters

We examined 28 qualitative characters of vegetative and generative regions on 77 plants (*D. majalis* – 23, *D. maculata* – 54 plants) from three



A



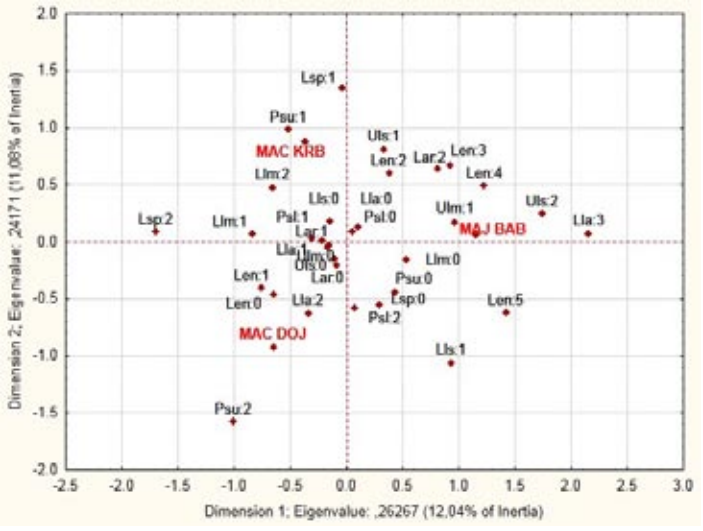
B

Fig. 1 – Positions of analyzed specimens in the area of the first and the second discriminant axis (A – morphometric characters, B – morphometric indexes)

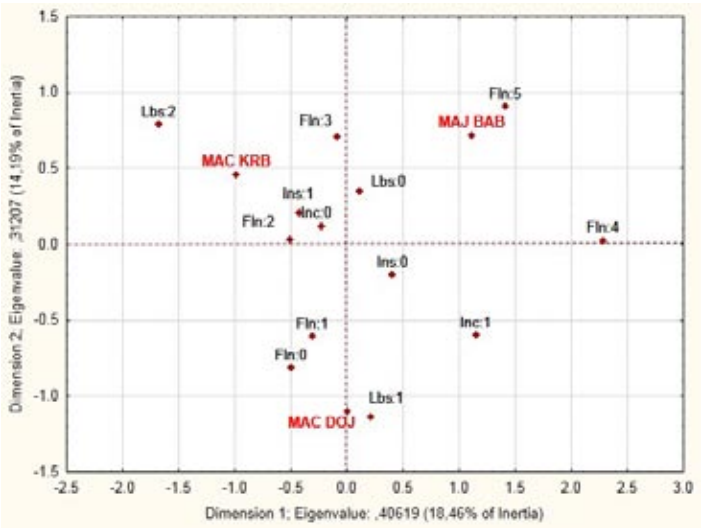
localities. Further analysis was based on 24 characters for the species *D. maculata* and on 22 in the analysis that included both species together. List of qualitative characters and number of characters states are given in Tab. 2. In analysis of qualitative characters variability first we coded characters states and than calculated states frequencies. Finally, the frequencies of characters states were processed by correspondent analysis. In correspondent analysis we also included meristic characters (leaves and flowers number) that had been previously translated into non-parametric data by forming the numerical range. Three analysis were performed, one for every region of plant – leaf, flower and inflorescence.

The positions of leaf characters states in the area of I and II correspondence axis shows a clear separation between populations of two different species along I correspondence axis. Two populations of *D. maculata* are separated by II correspondence axis, where the population from Krvave bare is located in positive and population from Dojčino vrelo in negative zone of II axis. Qualitative characters that contribute to the separation of *D. majalis* from other species are lanceolate form of upper leaves, wavy margin of the lower and upper leaves, and the presence of black spots across the whole surface of leaves, oval, narrowed into thorn apex of lowermost leaf and 7 leaves on stem (Fig. 2A). Population of *D. maculate* from Dojčino vrelo is characterized by acuminate lowermost leaf apex shape, 3 or 4 leaves on the stem, and black spots sporadically present on the surface of upper leaves. Population from Krvave bare is characterized by entire and wavy lowermost leaf margin and absence of spots on upper leaves (Fig. 2A).

In relation to inflorescence characters, (Fig. 2B) populations are separated in a similar way as in previous analysis. Characteristic of *D. majalis* population are 25-29 flowers (noted in 4 plants) and more than 30 flowers in inflorescence (in 11 plants) and elongated-lanceolate acuminate bracts.



A



B

Fig. 2 – Positions of characters states and populations centroids in area of I and II correspondence axis (A – leaf; B – inflorescens)

Populations of *D. maculata* are clearly separated by a few characters – population from Krvave bare is characterized by broad, cylindrical, dense inflorescence with many flowers (15 to 24) and elongated egg-shaped acuminate bracts, while the population from Dojčino vrelo is characterized by loose inflorescence with fewer flowers (1-14) and elongated-lanceolate bracts with obtuse apex shape (Fig. 2B).

In relation to flower characters, there is a separation of population from Dojčino vrelo and *D. majalis* population on the positive side of I and negative side of II correspondence axis, while the population from Krvave bare is on the negative side of I and positive side of II axis (Fig. 3). Population of *D. majalis* is characterized by wide baggy spur, while the populations of *D. maculata* are related with sets of labellum characters (Fig. 3). Population of *D. maculata* from Krvave bare can be described by following characters: trapezoidal (frequency (f) = 0.037) and semicircular (f 0.296) form of the right lateral labellum lobe, broad-triangular (f 0.555) and arc form of median labellum lobe, obtuse (f 0.444), acuminate (0.037) and concave (f 0.037) form of median labellum apex, equal lateral labellum lobes (f 0.815), short and wide baggy spur (f 0.926) and semicircular left lateral labellum lobe shape (f 0.296). Population of *D. maculata* from Dojčino vrelo is characterized by following characters (Fig. 3): 3 unequal labellum lobes (f 0,333), oval median labellum apex shape (f 0.630), irregularly-triangular (f 0.148), triangular (f 0.370) and broad-oval (0.185) median labellum lobe shape, left lateral labellum lobe with oval (f 0.074) and acuminate (0.037) margins, right lateral labellum lobe with waved (f 0.704) and acuminate (f 0.074) margins, trapezoidal (f 0.444), trapezoidal directed upward (f 0.037) and in a form of ¼ circle (f 0.037) form of the left lateral labellum

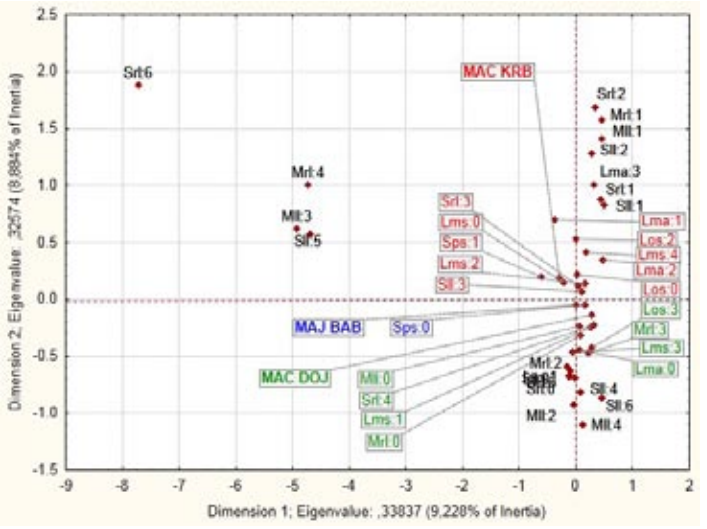


Fig. 3 – Positions of flower characters states and populations centroids in area of I and II correspondence axis

lobe, trapezoidal (f 0.630), semicircular (f 0.296) and trapezoidal directed upward (f 0.074) form of the right lateral labellum lobe.

Based on the obtained data and differential characters from literature (S o ó, 1973) we can proposed infraspecific ranking for population of *D. maculata* from Krvave bare as *maculata* variety, which is characterized by three more or less equal labellum lobes, a features that occurs in studied population with high frequency (0.815) and with median labellum lobe which is more or less triangular (broadly triangular 0.555, triangular 0.148 and irregular triangular 0.222). If we also consider index bract length/flower length (Tab. 3) plants from this population can be classified as form *bracteosa* (Harz) Soó that is characterized by bracts that are longer than flowers (index 2.05 ± 0.62). Plants from Dojčino vrelo population have three unequal labellum lobes (0.333) upon which this population could be classified as *palustris* (Cam.) Soó variety, but this is very questionable due to the relatively low frequency of this character. Form of plants from this population is also questionable, but we noted that flowers had intense fragrance that could point to f. *fragrans* (Harz) Soó.

As a result of analysis carried out on examined *D. maculata* populations, a key for infraspecific differentiation within this species was formed:

1. Labellum lobes are unequal . . . . . var. *palustris* (Cam.) S o ó 1960
1. Labellum lobes more or less equal 2
2. Bracts and flowers equal length or bracts slightly shorter than flowers . . . . . var. *maculata* f. *maculata*
2. Bracts longer than flowers . . var. *maculata* f. *bracteosa* (Harz) S o ó 1960

CONCLUSION

Based on the results obtained by statistical analysis and literature data, the population from Krvave bare can be defined as *Dactylorhiza maculata* subsp. *maculata* var. *maculata* f. *bracteosa* (Harz) S o ó 1960, and the population from Dojčino vrelo as – *Dactylorhiza maculata* subsp. *maculata* var. *palustris* (Cam.) S o ó 1960 f. *fragrans* (Harz) S o ó 1960. Infraspecific sensibility for *D. majalis* population was not determined since this study included only one population of this species. On the other hand results of statistical analysis for these populations have shown the existence of sets of differential characters between these two species. *D. majalis* is characterized by longer and thinner stem, wider leaves, longer median labellum lobe, bigger index spur length/ovary length, lanceolate shape of leaves, more than 25 flowers in inflorescence and wide baggy spur. Populations of *D. maculata* are distinguished primarily by length of stem, where it is longer in the population from Dojčino vrelo. In addition, there are sets of labellum characters for each of *D. maculata* population. As a result of analysis carried out on examined *D. maculata* populations a key for infraspecific differentiation within this species was formed.

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МОРФОЛОШКА ВАРИЈАБИЛНОСТ ПОПУЛАЦИЈА ВРСТА *DACTYLORHIZA MACULATA* (L.) SOÓ I *D. MAJALIS* (REICHENB.) P. F. HUNT ET SUMMERHAYES (ORCHIDALES, ORCHIDACEAE) СА СТАРА ПЛАНИНЕ (СРБИЈА)

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Резиме

Упоредна морфолошка таксономска анализа спроведена је на две популације врсте *Dactylorhiza maculata* (L.) Soó и једне популације врсте *D. majalis* (Reichenb.) P. F. Hunt et Summerhayes са подручја Старе планине ради утврђивања нових карактера који би се могли користити за разликовање ове две врсте као и карактера који би се, евентуално, могли искористити за дефинисање инфраспецијских таксона унутар ових врста. Таксономска морфолошка истраживања су обухватала обраду квантитативних и квалитативних карактера. Анализирано је укупно 60 карактера, од тога 34 квантитативна и 26 квалитативних на 90 јединки. На основу резултата мултиваријантне статистике и података из литературе, издвојени су сетови таксономских карактера који омогућавају јасну диференцијацију инфраспецијских облика унутар врсте *D. maculata* која се на изабраним локалитетима на Старој планини карактерише интезивнијом варијабилношћу.

КЉУЧНЕ РЕЧИ: *Dactylorhiza maculata*, *D. majalis*, инфраспецијска варијабилност, мултиваријантна статистика

ACKNOWLEDGMENT

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SOME EXTINCT PLANT TAXA ON THE TERRITORY OF NOVI SAD AND THEIR VULNERABILITY STATUS IN VOJVODINA AND SERBIA

ABSTRACT: Natural habitats on the territory of Novi Sad are almost fully destroyed today, as well as their characteristic plant taxa. The reason for disappearance of natural habitats is the development of suburban communities, which is an irreversible process. Plant taxa, specific for wet, salty, and sandy ecosystems grew on those habitats twenty years ago and earlier. This paper presents the overview of 9 taxa (*Suaeda maritima* subsp. *maritima, Androsace elongata* subsp. *elongata, Cirsium boujartii* subsp. *boujartii, Aster sedifolius* subsp. *canus, Blackstonia perfoliata* subsp. *serotina, Plantago maritima* subsp. *maritima, Salvia nutans, Allium angulosum,* and *Typha schuttleworthii*). These taxa presented integral parts of autochthonous flora of Novi Sad. Since some of these taxa were found in the field 21 years ago and some even 93 years ago, they are extinct from the flora of Novi Sad.

KEY WORDS: extinction, flora, habitat, Novi Sad

INTRODUCTION

The development of city quarters and communities, and increased human activity led to large and irreversible changes to the structure of the flora and fauna of urban and suburban areas. It resulted in disappearance or critical endangerment of the taxa, which formed the characteristic plant texture and were characterized by specific ecology of steppes, salty, and wet habitats before the process of urbanization.

Natural habitats are literally extinct and they are replaced by the vegetation dominated with synanthropic species of dense texture.

The territory of Novi Sad, especially its northwestern part, today's city quarters Detelinara and Novo Naselje, was characterized by habitats such as

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natural salty habitats, salty sands, fragments of steppes, and wet habitats in the past. Most of them have disappeared and some of the taxa characteristic to the above-mentioned habitats are extinct or became very rare.

This paper describes nine autochthonous taxa extinct from the flora of Novi Sad. Those plants are *Suaeda maritima* (L.) D u m o r t 1827 subsp. *pannonica* (G. Beck) S o ó ex P. W. B a l l 1964, *Androsace elongata* L. 1753 subsp. *elongata*, *Cirsium boujartii* (Pill. et Mitterp.) S c h u l t z - B. 1856 subsp. *boujartii*, *Aster sedifolius* L. 1753 subsp. *canus* (W. et K.) M e r x m. 1974, *Blackstonia perfoliata* (L.) H u d s o n 1762 subsp. *Serotina* (Koch ex Reichenb.) V o l l m a n 1914, *Plantago maritima* L. 1753 subsp. *maritima, Salvia nutans* L. 1753, *Allium angulosum* L. 1753, and *Typha schuttlewortii* K o c h et S o n d e r in K o c h 1844.

Suaeda maritima subsp. *pannonica* is a Pannonian endemic plant (B o ž a , 1999), which grows in very salty and wet habitats and characterizes the alliance *Thero-Salicornion* Tx. 55 (S o ó, 1970).

Androsace elongata subsp. *elongata* is subspecies characteristic for steppes and sandy habitats (S o ó, 1970), belonging to Pontic floristic element (Stevanović, !). It belongs to alliance *Festucion rupicolae* Soó (29) 40 corr. Soó 64 (S o ó, 1970).

Cirsium boujartii subsp. *boujartii* is a plant of ruderal habitats, distributed in Hungary (C s i k y et al., 2005) and Romania (B u t o r a c, 1999), and it is a member of the alliance *Onopordion* Br.-Bl. 26 (S o ó, 1970). The habitats of this plant are destroyed by the spread of suburban communities.

Aster sedifolius subsp. *canus* is a plant growing in wet thickets, salty marshes (S o ó, 1970). It belongs to Pontic-Pannonian floristic element (Stevanović, !) and is an edificator of the alliance *Festucion rupicolae* Soó (29) 40 corr. Soó 64 (S o ó, 1970).

Blackstonia perfoliata subsp. *serotina* is a plant growing in wet, sandy and salty habitats (S o ó, 1966), and belongs to Central-Eastern Mediterranean--Submediterranean-West Pontic-Pannonian floristic element (B u d a k, 1999) and it is an edificator of the alliance *Nanocyperion flavescentis* W. Koch 26 (S o ó, 1966).

Plantago maritima subsp. *maritima* grows in wet salty habitats characterizing the vegetation class *Festuco-Puccinellietea* Soó 68 (S o ó, 1968). In a wider sense, it belongs to Euro-Asian floristic element (Stevanović, !).

Salvia nutans is a plant of loess heaths belonging to Pontic-Pannonian floristic element and it is considered a post-glacial, steppe relict (S o ó, 1968). It characterizes the alliances *Festucion rupicolae* Soó, (29) 40, corr. Soó 64 and *Danthonio-Stipion stenophyllae* Soó, 47, corr. 71 (S o ó, 1968).

Allium angulosum is a plant of occasionally flooded meadows characterizing the alliance *Molinion coeruleae* W. Koch 26 and belongs to Euro-Asian floristic element (S o ó, 1973).

Typha schuttlewothii is a taxon of Middle-European floristic element characteristic for the alliance *Phragmition communis* W. Koch 26, emend. Soó 47 (S o ó, 1973). In Serbia, ass. *Typhaetum schuttleworthii* B. Jovanović 82 (J o v a n o v i ć, 1982) is recorded.

MATERIAL AND METHODS

Nine floristically and phytogeographically important plants, extinct from the flora of Novi Sad, are presented in phylogenetic classification (Takhtajan, 1997) in this paper. For each plant, the following data are defined: general distribution and distribution in Serbia according to the *Red Book of Flora of Serbia 1* (Josifović, M. ed. 1970-1976; Diklić, N. ed. 1986; Stevanović, V. ed. 1999), and the distribution in Vojvodina based on literature data and personal field research. The distribution is presented on UTM maps 10x10 km. Floristic elements are presented according to Soó (Soó, 1966, 1968, 1970, 1973), the *Red Book of Flora of Serbia* 1 (Stevanović, V. ed. 1999) and suggestions of the reviewer (Stevanović, !). The nomenclature is taken from the *Flora Europaea* (Tutin et al., eds., 1968, 1972, 1976, 1980; Tutin et al., eds., 1993).

For each presented taxon, the first and the last result in Novi Sad and its surroundings is given, as well as the type of the habitat and the category of endangerment in Serbia. Localities in Novi Sad are presented on the map of Novi Sad (Geoplan and Grad Novi Sad, 1995, proportion 1:10.000).

RESULTS AND DISCUSSION

Fam.: Chenopodiaceae, Subfam.: Salsoloideae, Tribus: Suaedeae

Suaeda maritima (L.) Dumort 1827 subsp. *pannonica* (G. Веск) Soó ex P. W. Ball 1964

General distribution of this Pannonian endemic species (Bža, 1999) is eastern and central Europe (Ball, Akeroyd, 1993), Austria, Hungary, Moravia in the Czech Republic, Serbia, and Dobruja in Romania (Soó, 1970). *Suaeda maritima* subsp. *pannonica* is a typical halophyte, which formed special, very dense alliances *Suaedetum pannonicae* Knežević, Boža 87 (Knežević, Bža, 1987) near Melenci.3

In Serbia, it is present in northern, i.e. Pannonian part. Today, it can be stated with certainty that it grows only near Melenci, on the bank of the lake Rusanda, bog Okanj, as well as in Slano Kopovo near Novi Bečej, which is also its south border of its distribution in Serbia. It has disappeared from the localities south of Stari Bečej and near Sombor, while the data about the presence of this species near Senta and in Subotičko-Horgoška Sand are not certain.

Concerning its endangerment, it belongs to category of critically endangered taxa whose population is over 250 mature individual plants (Bža, 1999).

The only data about the presence of this species in Novi Sad is from 1974 without stating the precise locality (Obradović, Budak, 1974). This species is extinct from the territory of Novi Sad (Fig. 1. ●., 2.)

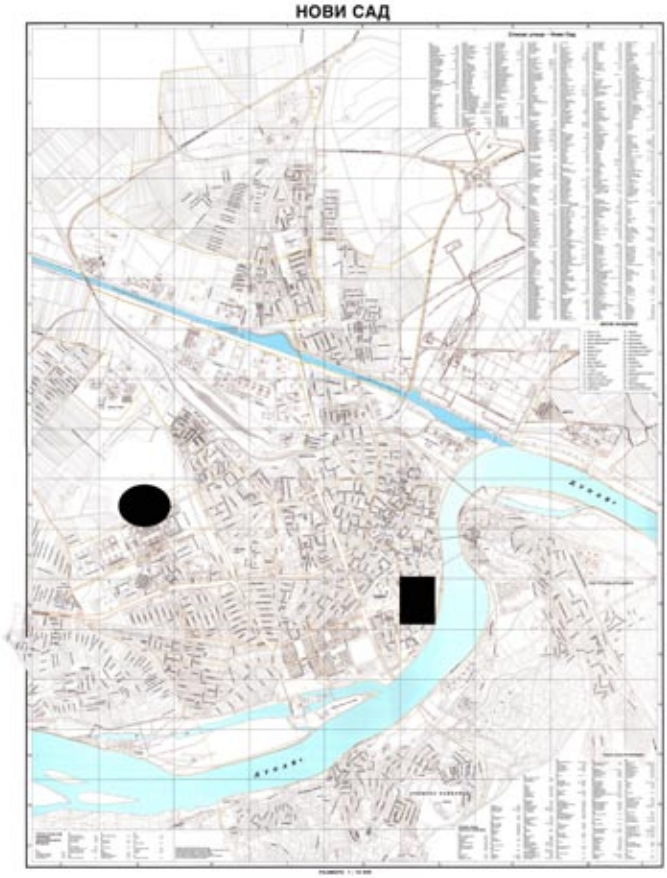

Fig. 1. – Localities at the territory of Novi Sad from which the species *Suaeda maritima* subsp. *pannonica* ● and *Androsace elongata* subsp. *elongata* ■ are extinct.

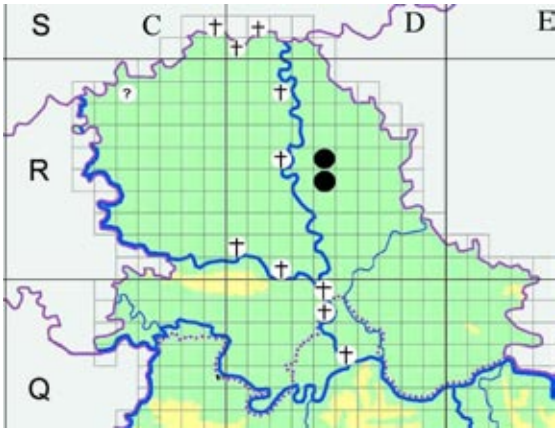

Fig. 2. – Distribution of *Suaeda maritima* subsp. *pannonica* in Vojvodina (B o ž a , 1999) corr.

Fam.: Primulaceae, Tribus: Androsaceae
Androsace elongata L. 1753 subsp. *elongata*

This subspecies is characterized by Pontic type of distribution, with areal radiation towards middle Europe, where it populates relict steppes (Stevanović, !). However, one part of the areal in the Pannonian Plain is disjoint (S o ó, 1970). Its distribution covers the middle Europe, central and southern parts of Russia, Caucasus, and southern Siberia (Stevanović, !). In Europe, it grows from middle Germany, across Poland towards east to the Ural (F e r g u s o n, 1972).

It is a typical plant of steppes and is present on dry pastures, plain slopes, and in sandy and salty thickets (S o ó, 1970).

In Serbia, it grows in eastern and southeastern parts, from Vrška Čuka across Niš and Bela Palanka to Vranje (N i k o l i ć, 1972). In Novi Sad, it was present on the northern border of its distribution in Serbia.

The only locality on which this plant was present in Novi Sad are grasslands near the University where it was found only once in 1988 (B o ž a, B u d a k, 1990/91). *Androsace elongata* subsp. *elongata* is extinct f r o m the territory of Vojvodina (Fig. 1. ■, 3.).

Fig. 1. – Localities at the territory of Novi Sad from which *Suaeda maritima* subsp. *pannonica* ● and *Androsace elongata* subsp. *elongata* ■ are extinct

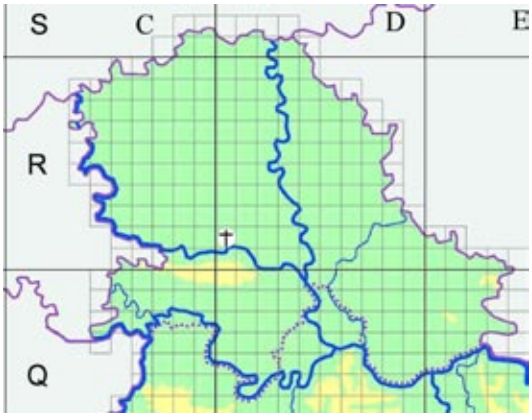

Fig. 3. – Distribution of *Androsace elongata* subsp. *elongata* in Vojvodina (B o ž a, B u d a k, 1990/91)

Fam.: Asteraceae, Subfam.: Cichorioidae, Tribus: Carduuseae
Cirsium boujartii (Pill. et Mitterp.) S c h u l t z - B. 1856 subsp. *boujartii*

This subspecies grows only in Romania (W e r n e r, 1976) and Hungary (C s i k y et al., 2005) today. It belongs to Pannonian floristic element (B u t o r a c, 1999). For Serbia, it is recorded in Deliblato Sand in 1914 (W a g n e r, 1914) and near Novi Sad (J á v o r k a, 1925). The data for Novi Sad are later cited by O b r a d o v i ć (1966) and O b r a d o v i ć, B u t o r a c (1975). There are no data on this subspecies for Novi Sad after 1925.

C. boujartii subsp. *boujartii* has disappeared from Deliblato Sand and from the territory of Novi Sad, which was its only locality in Serbia, so it is extinct from the flora of Serbia (B u t o r a c, 1999). On the aforementioned localities, it was present also on the southwestern border of its areal (Fig. 4. ●, 5.)

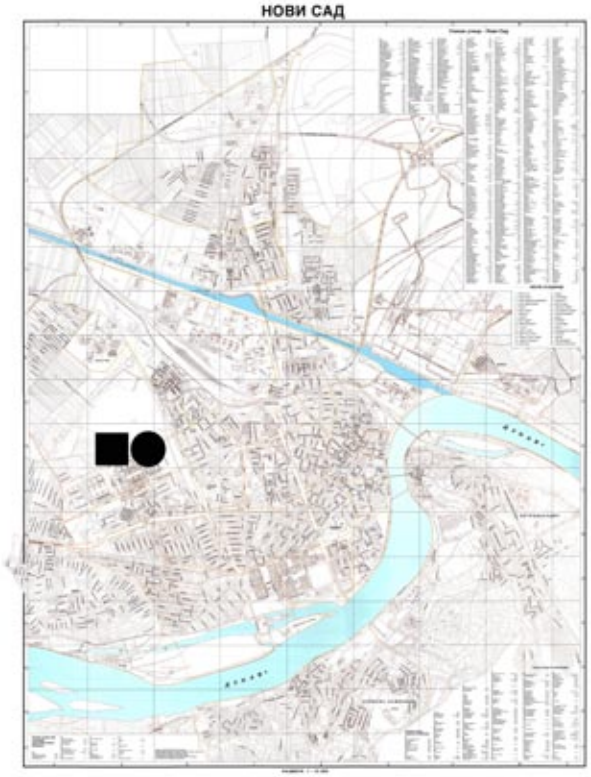

Fig. 4. – Localities at the territory of Novi Sad from which *Cirsium boujartii* subsp. *boujartii* ● and *Aster sedifolius* subsp. *canus* ■ are extinct

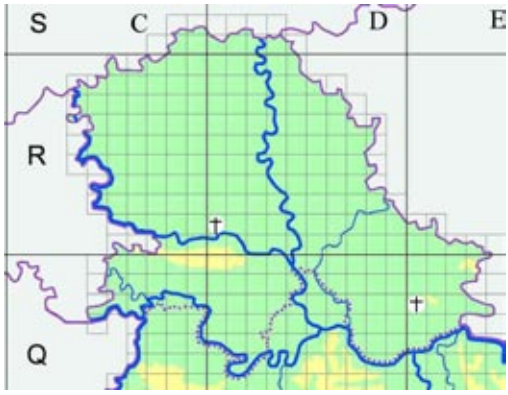

Fig. 5. – Distribution of *Cirsium boujartii* subsp. *boujartii* in Vojvodina (B u t o r a c, 1999)

Fam.: Asteraceae, Subfam.: Asteriodeae, Tribus: Astereae
Aster sedifolius L. 1753 subsp. *canus* (Waldst. et Kit.) M e r x m. 1974

The general distribution of this taxon covers Austria, Hungary, Romania, Serbia and Bulgaria (G a j i ć, 1975). The subspecies belongs to Panonnian--Pontic floristic element (Stevanović, !).

According to Gajić, in Serbia, this plant grows only in Vojvodina, where it is often found on salty habitats (G a j i ć, 1975). According to our research, this taxon is present on salty habitats along large rivers.

For the territory of Novi Sad, it was firstly recorded by Tatar in 1939 (T a t á r, 1939). There are no later data for the territory of Novi Sad. The taxon is found near Novi Sad (Veternik, Futog) in 1974 (O b r a d o v i ć, B u d a k, 1974). However, the aforementioned habitats were destroyed by the process of urbanization and this subspecies were not recorded there in the last 30 years.

This taxon is endangered (Fig. 4. ■, 6.) in Serbia (Vojvodina) today.

Fig. 4. – Localities at the territory of Novi Sad from which *Cirsium boujartii* subsp. *boujartii* ● and *Aster sedifolius* subsp. *canus* ■ are extinct

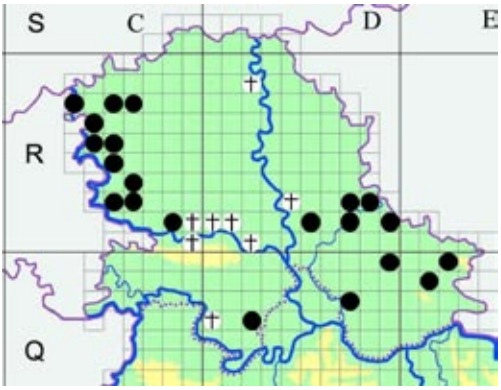

Fig. 6. – Distribution of *Aster sedifolius* subsp. *canus* in Vojvodina (B r d a r, 2000) corr.

Fam.: Gentianaceae, Tribus: Gentianeae
Blackstonia perfoliata (L.) H u d s o n 1762 subsp. *serotina* (Koch ex Reichenb.) V o l l m a n 1914

General distribution of this species covers the Mediterranean area (J o v a n o v i ć - D u n j i ć, 1973) and it belongs to central-eastern Mediterranean--Sub-Mediterranean-West Pontic-Pannonian floristic element (B u d a k, 1999). In Europe, it grows in southern and central parts, in the north to Netherlands (T u t i n, 1972). It populates muddy habitats, swamps, rarely salty marshes and sandy plains (S o ó, 1966).

Its distribution in Serbia covers Bačka, Banat and western Serbia near Ljubovija (B u d a k, 1999; T o m o v i ć et al., 2009).

The first data for Novi Sad and its surroundings were recorded by Z o r k ó c z y (1896), K u p c s o k (1915) and P r o d á n (1916), later J a n j a -

t o v i ć et al. (1980) and O b r a d o v i ć (1981). For the last time it was found on salty habitats near Novi Sad in 1985.

It has disappeared from the surroundings of Novi Sad; it is very rare in Vojvodina and belongs to the group of endangered taxa (T o m o v i ć et al., 2009). (Fig. 7. ●, 8.)

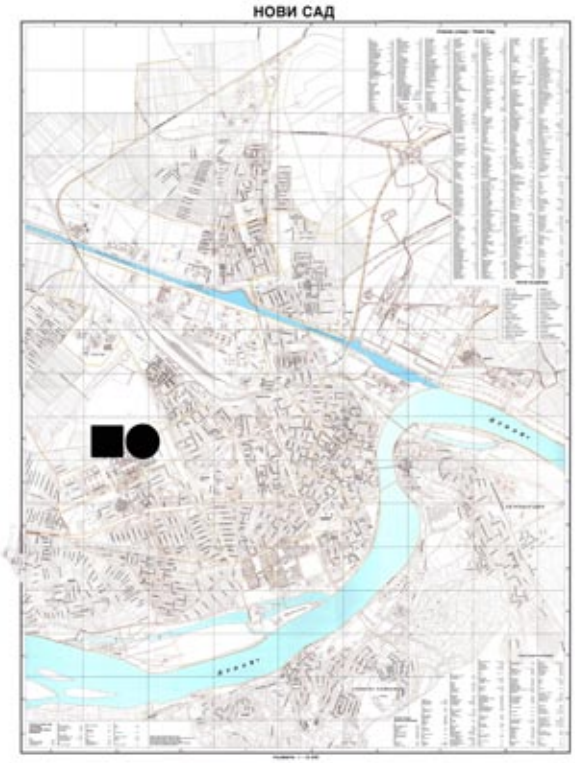

Fig. 7. – Localities at the territory of Novi Sad from which *Blackstonia perfoliata* subsp. *serotina* ● and *Plantago maritima* subsp. *maritima* ■ are extinct

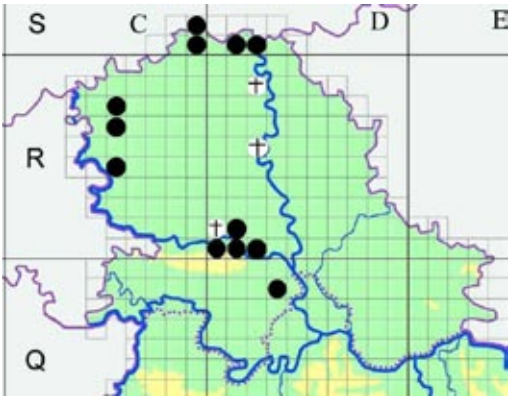

Fig. 8. – Distribution of *Blackstonia perfoliata* subsp. *serotina* in Vojvodina

Fam.: Plantaginaceae
Plantago maritima L. 1753 subsp. *maritima*

P. maritima represents the aggregate that is constituted of proportionally large number of subspecies distributed in different parts of the northern hemisphere. The populations from Serbia (Vojvodina) belong to typical subspecies *maritima*. General distribution *P. maritima* subsp. *maritima* covers almost entire Europe, towards the East to Middle Asia and Mongolia; it is also present in North and South America (J a n k o v i ć, G a j i ć, 1974). In wider sense, it belongs to Euro-Asian floristic element (Stevanović, !).

It is present in larger part of Europe, from the North of Scandinavia and Baltic and Atlantic coastline, to Spain, northern Italy and Pannonian Plain in the South. It is absent from the Balkan peninsula except in several localities on the Adriatic coastline (Stevanović, !). *Plantago maritima* subsp. *maritima* grows on salty localities with the largest concentration of salt, as well as on muddy, salty habitats (S o ó, 1968).

In Serbia, it grows only in certain parts of the Pannonian region in Bačka and Banat. The first data for the territory of Novi Sad is from 1915 (K u p c s o k, 1915); for the last time it was recorded on salty habitats together with *Blackstonia perfoliata* subsp. *Serotina* in 1985. In this locality, it was present on one of the southern borders of distribution in Serbia.

Plantago maritima subsp. *maritima* belongs to the category of endangered or vulnerable species in Serbia, which has disappeared from the territory of Novi Sad (Novo Naselje) (Fig. 7. ■, 9.)

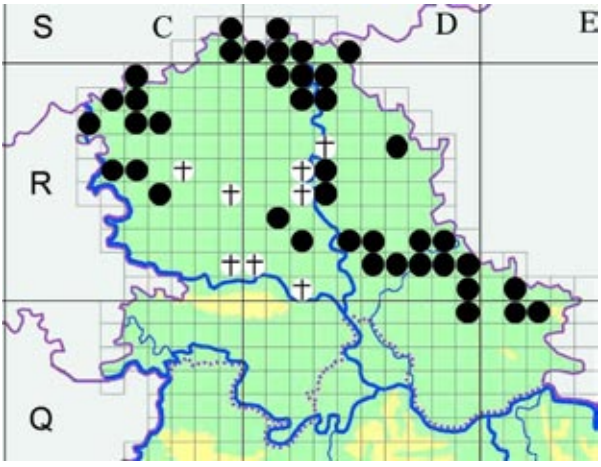

Fig. – 9. Distribution of *Plantago maritima* subsp. *maritima* in Vojvodina (A p r o, 2001) corr.

Fam.: Lamiaceae, Subfam.: Nepetoideae, Tribus: Salvieae
Salvia nutans L. 1753

General distribution of this species covers the Pannonian and Vlaška plains, Dobruja, Bessarabia, Crimea and steppes in Ukraine to the northern Sub-Caucasus (B u d a k, 1999). It belongs to Pontic-Pannonian floristic element. In the Pannonian Plain, it is considered a postglacial steppe relict (S o ó, 1968). It grows on loess plains and steppes (S o ó, 1968).

In Serbia, it was present only in Vojvodina (D i k l i ć, 1974), in Bačka and Srem. The first data for the territory of Novi Sad were recorded by Zorkóczy (Z o r k ó c z y, 1896) on the localities between Novi Sad and Futog. Kupcsok (K u p c s o k, 1915) stated that it grows between Novi Sad and Kisač. These are the only data for the territory of Novi Sad.

Salvia nutans is extinct from the flora of Novi Sad where it was on the southern border of its distribution in Serbia. In the *Red Book of Flora of Serbia – 1*, it was described as an extinct taxon (B u d a k, 1999). (Fig. 10. ●, 11.)

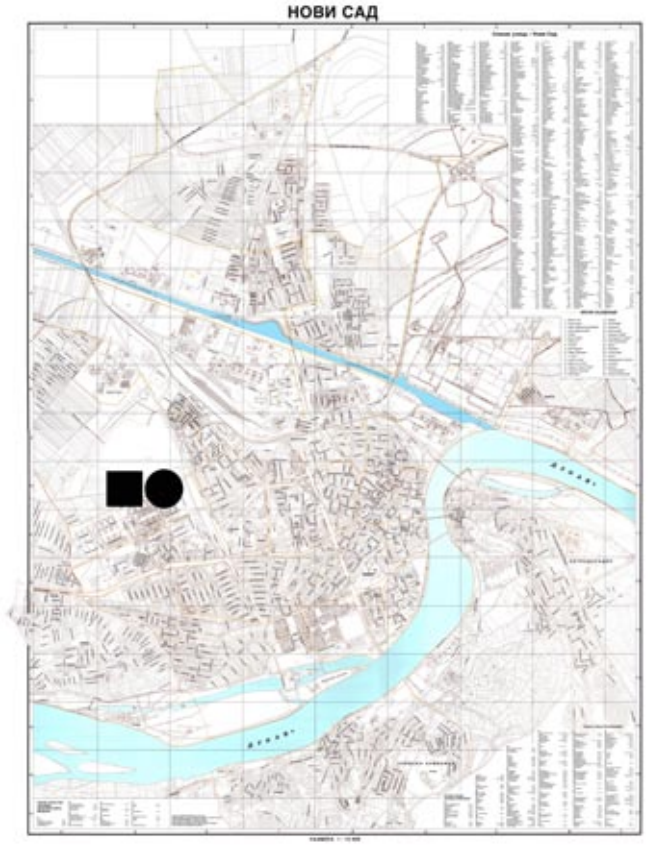

Fig. 10. – Localities at the territory of Novi Sad from which the species *Salvia nutans* ● and *Allium angulosum* ■ are extinct

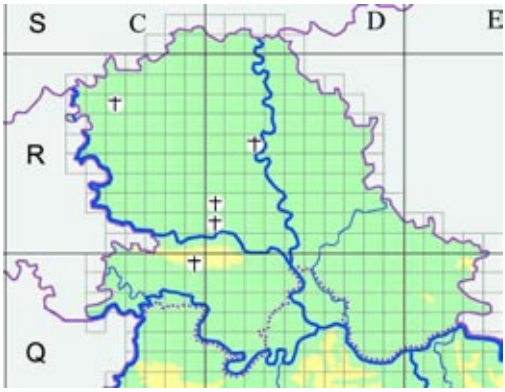

Fig. 11. – Distribution of *Salvia nutans* in Vojvodina (B u d a k, 1999)

Fam. Alliaceae, Subfam.: Alliaoideae, Tribus: Allieae
Allium angulosum L. 1753

General distribution of *Allium angulosum* covers middle and southern Europe from eastern France and northern Italy across Poland in the east (S t e a r n, 1980), across the Balkan Peninsula and the European part of the Russia to Siberia (В ъ л е в, А с е н о в, 1964). It belongs to Euro-Asian floristic element (S o ó, 1973). It grows on muddy plains and swamps on wet hay meadows, sandy marshes, and flooded meadows (S o ó, 1973).

It grows in Vojvodina, Mačva and Šumadija as well as on two localities in eastern, i.e. southeastern Serbia (A n a č k o v, 2003; 2009).

It is rare in Vojvodina. The first data for Novi Sad is from 1980 (J a n j a t o v i ć et. al., 1980) from sandy and salty habitats, where it could not be found later. It has disappeared from the territory of Novi Sad, Novo Naselje city quarter. Most probably, it belongs to the category of vulnerable species because of the vulnerability and appearance of invasive weeds on the habitats. (Fig. 10. ■, 12.)

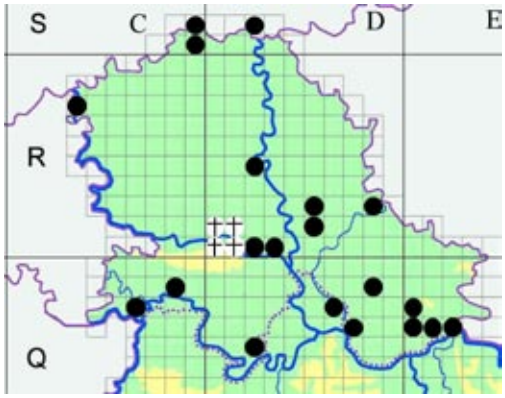

Fig. 12. – Distribution of *Allium angulosum* in Vojvodina (A n a č k o v, 2003; 2009) corr.

Fam: Typhaceae
Typha schuttleworthii K o c h et S o n d e r in K o c h 1844

General distribution of this plant covers southern and eastern parts of middle Europe, from eastern France and northern Italy (C o o k, 1980), to Balkan Peninsula (J o v a n o v i ć, 1986), the Alps and the Carpathian Mountains (S o ó, 1973). It belongs to middle European floristic element (S o ó, 1973). This species grows in fresh waters and on muddy habitats (S o ó, 1973).

For Serbia, there are very few data; it is recorded in eastern (T o m o v i ć et. al., 2009), southeastern, southwestern and central Serbia as well as in Novi Sad. It was recorded in Novi Sad by Fritsch in 1909 for the first time (R a n đ e l o v i ć, 1999), and later Javorka (J á v o r k a, 1925) whose data were cited by O b r a d o v i ć, 1966; O b r a d o v i ć, B u t o r a c, 1975; J o v a n o v i ć 1986.

Typha schuttleworthii has disappeared from the flora of Novi Sad and Vojvodina, where it grew in swamp vegetation. In the *Red Book of Flora of Serbia* 1, it is stated as critically endangered (R a n đ e l o v i ć, 1999), i.e. endangered species (T o m o v i ć et. al., 2009). (Fig. 13, 14.)

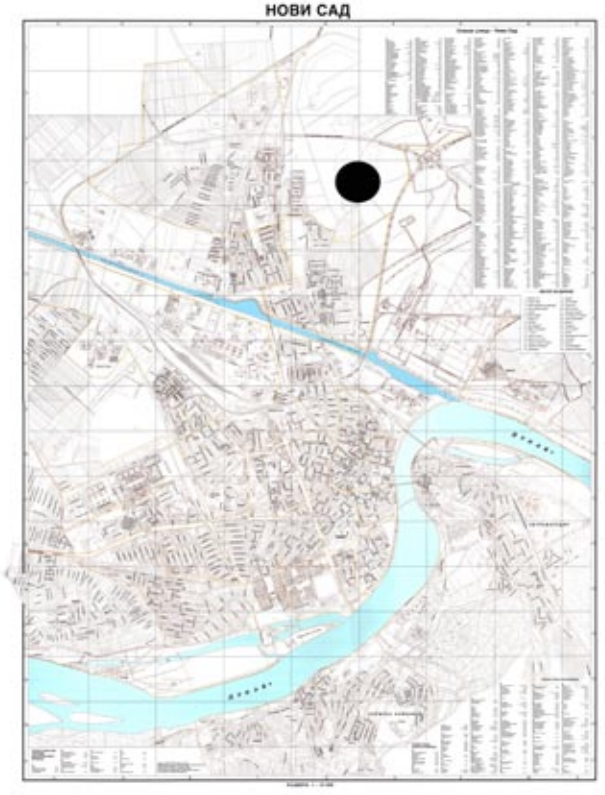

Fig. 13. – Locality at the territory of Novi Sad from which the species *Typha schuttleworthii* ● is extinct

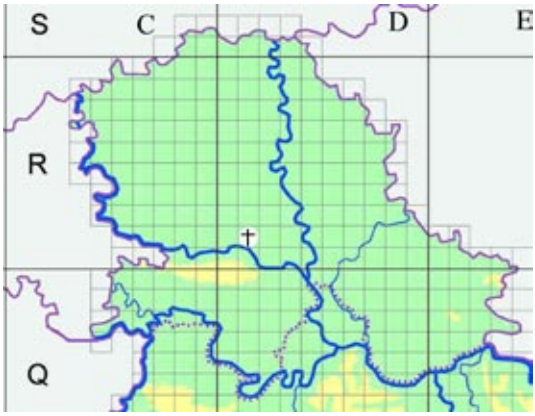

Fig. 14. – Distribution of *Typha schuttleworthii* in Vojvodina (R a n đ e l o v i ć, 1999)

CONCLUSION

This paper describes 9 autochthonous taxa, which disappeared from the flora of Novi Sad.

Suaeda maritima subsp. *Pannonica* is a Pannonian endemic plant, characterizing the alliance *Thero-Salicornion* Tx. 55. In Serbia, it is considered as a much-endangered taxon. The only data regarding this plant for Novi Sad is from 1974.

Androsace elongata subsp. *elongata* belongs to Pontic floristic element in the alliance *Festucion rupicolae* Soó (29) 40 corr. Soó 64. It was found in Novi Sad only once in 1988. It has disappeared from the flora of Vojvodina.

Cirsium boujartii subsp. *boujartii* belongs to Pannonian floristic element, and it is distributed in Romania and Hungary. It creates groups of the alliance *Onopordion* Br.-Bl. 26. The only data for Novi Sad is from 1925. It is extinct from the flora of Serbia.

Aster sedifolius subsp. *canus* is a taxon of Pannonian-Pontic floristic element and it is an edificator of the alliance *Festucion rupicolae* Soó (29) 40 corr. Soó 64. It was recorded for the first time at the territory of Novi Sad in 1939 and it was found in 1974 for the last time. Today it is an endangered taxon in Serbia (Vojvodina).

Blackstonia perfoliata subsp. *serotina* is of Central-Eastern Mediterranean--Sub-Mediterranean-Western Pontic-Pannonian floristic element, and it is an edificator of the alliance *Nanocyperion flavescentis* W. Koch 26. The first data for Novi Sad were from 1896, 1915, and 1916, and in 1985, it was found for the last time. In Vojvodina, it is rare and belongs to the group of endangered taxa.

Plantago maritima subsp. *maritima* characterizes the vegetation of the class *Festuco-Puccinellietea* Soó 68, and in wider sense, it belongs to Euro-Asian floristic element. The first data for Novi Sad were from 1915, and for the last

time it was recorded in 1985. It grows only in Bačka and Banat. In Serbia, it belongs to the group of endangered or vulnerable subspecies.

Salvia nutans belongs to Pontic-Pannonian floristic element and steppe, postglacial relicts. It is the characteristic species of the alliance *Festucion rupicolae* Soó (29) 40 corr. Soó 64 и *Danthonio-Stipion stenophyllae* Soó 47. corr. 71. The only data for Novi Sad and its territory were from 1896 and 1915. This species is extinct from the flora of Serbia.

Allium angulosum is the species from the alliance *Molinion coeruleae* W. Koch 26 and belongs to Euro-Asian floristic element. The first data for Novi Sad were from 1980, where it could not be found later. In Serbia, it belongs to vulnerable species.

Typha schuttleworthii is middle European floristic element and is characteristic for the alliance *Phragmition communis* W. Koch. 26 emend. Soó 47. The first data for Novi Sad were from 1909, and the last data were from 1925. In Serbia, it is an endangered species.

Abbreviation: corr. – corrected map of distribution

(Stevanović, !) additions and suggestions of Professor Vladimir Stevanović, PhD, corresponding member of SASA

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НЕКИ ИШЧЕЗЛИ БИЉНИ ТАКСОНИ СА ПОДРУЧЈА НОВОГ САДА И ЊИХОВ СТАТУС УГРОЖЕНОСТИ У ВОЈВОДИНИ И СРБИЈИ

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Резиме

На територији града Новог Сада данас су скоро потпуно уништена природна станишта, као и биљни таксони који су их карактерисали. Разлог нестанка природних станишта је ширење приградских насеља, што је незаустављив процес. На тим стаништима су пре двадесет и више година расли биљни таксони специфични за влажне, слатинске и пешчарске екосистеме. У овом раду је приказано девет таксона (*Suaeda maritima* subsp. *maritima, Androsace elongata* subsp. *elongata, Cirsium boujartii* subsp. *boujartii, Aster sedifolius* subsp. *canus, Blackstonia perfoliata* subsp. *serotina, Plantago maritima* subsp. *maritima, Salvia nutans, Allium angulosum* и *Typha schuttleworthii*), који су у прошлости чинили саставни део аутохтоне флоре Новог Сада. Будући да ови таксони нису нађени на терену дуже од 21 године, а неки чак 93 године, нестали су из флоре околине Новог Сада.

КЉУЧНЕ РЕЧИ: флора, ишчезавање, Нови Сад, станиште

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HERBARIUM BUNS – PLANT GALL COLLECTION

ABSTRACT: The plant gall collection is part of the Herbarium BUNS collection, University of Novi Sad. Collection began with the formation in 1976, and as a unique type of collection in Serbia, it has existed for 35 years. Today's collection *Herbarium cecidologicum* is made of two units – Plant gall collection (dried specimens) and database, and includes 438 data: 294 data for dried specimens and 144 collected literature data about the distribution plant galls.

Galls collection has multiple significances: assessment biodiversity (diversity of plants and diversity of causers), estimate the population status of certain causers taxa, primarily invertebrates, monitoring the spread of pests, which usually occurs in population of the cultivated species. Collecting data in one database, providing data on new plant gall species and distribution on both challengers as well as host plants is one of the main tasks of this collection.

KEY WORDS: causer, gall, plant collection, plant-hosts

INTRODUCTION

Natural history collections are valuable source for biodiversity studies. They provide a unique ability to review data for a long time. On the data stored in museum, collections are based on contemporary research: the loss and fragmentation of habitats, biological invasion, and the consequences of global climate changes (S u a r e z and T s u t s u i, 2004).

With its, mostly unusual structure, plant galls attracted the attention of naturalists from ancient times. However, plant galls should be distinguished from distortion of plant tissues, resulting from the feeding of certain groups of insects, when as part of "healing" damaged tissue leads to its thickening or twisting of leaves, so the caterpillar could be fed within them. Plant galls represent a kind of association between the two organisms, in which the gall causers is a parasite, and a plant whose tissue forms gall is a host. The definition that most defines the galls is: *A gall is an abnormal growth produced by a plant or other host under the influence of another organism. It involves enlargement*

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and/or proliferation of host cells and provides both shelter and food or nutrients for the invading organism (R e d f e r n and S h i r l e y, 2002).

Herbarium BUNS is the official botanical collections at the University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, registered in the Index herbariorum (H o l m g r e n and H o l m g r e n, 2003), and it is equivalent to the museum's collection, with the base at the University. Within the herbarium, there are 14 separated collections, one being a Plant gall collection (*Herbarium cecidologicum*), which is the result of collecting materials for floristic researches. This collection stands out, since plant gall collections are often part of gall causers' collections. Because of the great diversity of gall causers species (causers can be bacteria, algae, fungi and animals) the existence of separate collections, due to the type of causer is justified, and the largest collection of plant galls are part of entomological collections. In Serbia, the most important is the collection of Duška Simova-Tošić, deposited in the Natural History Museum in Belgrade.

Plant gall collection in herbarium BUNS is formed in 1976, when the first plant galls were collected. After that, there has been continuity in the collecting of the galls in the field and the literature data. Galls have been collected mainly in Vojvodina (Serbia) because of the intensive field research in the Pannonian part of Serbia during the last four decades. By 1976, there was no collection of galls at the former Institute of Biology (present Department of biology and ecology), which was why they were dried and deposited as part of the herbarium collections.

The most important collector is Dr. Pal Boža, who collected approximately 90% of the material in the collection. Beside him, there are many researchers that are known for their work, such as Dragiša Savić, Predrag Radišić, Dr. Goran Anačkov, and Ranko Perić. Nowadays, the collection of material is expanded to the Balkan Peninsula and other regions where scientific expeditions are organized. Research of plant galls has several goals: updating knowledge of diversity and distribution of the galls, recording new species whose causers are invasive and/or potentially invasive species in the studied area, and that are developed in an economically important cultivated species. In accordance to the principles of sustainable development, for already existing dried material of plant galls, presently only data on the distribution are collected with additional photo documentation. All of these data are included in the general herbarium database.

MATERIAL AND METHODS

Plant gall collection is organized in two separate systems: a collection of dried specimens and an electronic database.

The galls collection consists of dried specimens, collected in the period from 1976-2010, during regular field research. Galls, whose causers are animals, were collected at different life stages and therefore there are no larval forms for all specimens in the collection. Plant galls were identified by indirect methods – based on the determination of the host plant.

The plant material was determined by standard keys for the determination of the study area (Javorka, 1925; Josifović, 1971-1977; Sarić, 1986; 1992). Nomenclature of plant taxa has been aligned to the contemporary monographs and checklists (Greuter, 1984; Greuter, 1986; http://ww2.bgbm.org/EuroPlusMed/; http://plantnet rbgsyd.nsw.gov.au/iopi/iopihome.htm), the systematic of plant species is given according to the phylogenetic system (Takhtajan, 2009). Plant material with galls was dried in dark, dry place and then deposited in cardboard boxes with lids, measuring 5x8x12 cm and 5x8x19 cm. The collection undergoes periodic treatment at low temperatures when the material is stored in a cold chamber at the temperature of -16°C, for 20 days and then treated with insecticides.

Plant gall causers were identified by using the keys for the determination of the gall (Moesz, 1938; Ambrus, 1970, 1974; Redfern and Shirley, 2002; Bijkerk, 2011), which provide an overview of the plant galls, by identifying host plant, morphology and the part of plant that is infected (Bože, 1983a). For plant galls that are found with larvae, the larvae are preserved in 50% alcohol solution and deposited along with the plant material. Systematic of gall causers is given according to the phylogenetic system (*Fauna Europaea* (2011); Garrity, 2005).

Electronic database of plant gall collection in herbarium BUNS is a central part of the herbarium. It is based on data collection, literature data (Moesz, 1938; Ambrus, 1970, 1974; Simova-Tosic, 1965; Radoičić, 1994), and personal communications. Where possible, data for each specimen is connected with photorecord(s), as part of the database. The objectives of the database are:

- Recording information about the plant galls, their host plants and causers
- Reconciliation of the known data for the galls, their hosts, and causers
- Quickly and efficiently searching the collection
- A basis for interdisciplinary research of flora and fauna, flora and bacteriology

RESULTS AND DISCUSSION

Plant gall collection (*Herbarium cecidologicum*) is a special collection of herbarium BUNS and it is built from two units – a collection of dried specimens and electronic database. In the collection are deposited 294 dried specimens, organized chronologically, and electronic database contains information for the stored specimens, the data from literature and personal communications. The database consists of 438 row data, and it can be searched by the host plants, causers, regions, sites, and the date of collecting according to the source. In the collection, database (including photo documentation) and the book of evidence galls are recorded with numbers from 70000001 to 70000438. In this paper, plant gall causers are presented. All other information is available on the website presentation of Herbarium BUNS [http://www.dbe.uns.ac.rs/o_depart-manu/laboratorije/herbarijum].

Aceria artemisiae (Canestrini 1891)
Aceria campestricola (Frauenfeld 1865)
Aceria cerrea (Nalepa 1898)
Aceria erinea (Nalepa 1891)
Aceria fraxinivora (Nalepa 1909)
Aceria macrochela (Nalepa 1891)
Aceria macrocheluserinae (Trotter 1902)
Aceria salviae (Nalepa 1891)
Agrobacterium tumefaciens Smith & Townsend 1907
Andricus caliciformis (Giraud 1859)
Andricus caputmedusae (Hartig 1843)
Andricus conglomeratus (Giraud 1859)
Andricus coriarius (Hartig 1843)
Andricus coronatus (Giraud 1859)
Andricus corruptrix (Schlechtendal 1870)
Andricus curvator (Hartig 1840)
Andricus foecundatrix (Hartig 1840)
Andricus gemmeus (Giraud 1859)
Andricus hungaricus (Hartig 1843)
Andricus kollari (Hartig 1843)
Andricus lignicolus (Hartig 1840)
Andricus lucidus (Hartig 1843)
Andricus multiplicatus (Giraud 1859)
Andricus quercuscalicis (Burgsdorff 1783)
Andricus quercustozae (Bosc 1792)
Andricus solitarius (Fonscolombe 1832)
Andricus testaceipes (Hartig 1840)
Andricus truncicola (Giraud 1859)
Andricus vindobonensis (Mullner 1901)
Anthocoptes octocinctus (Nalepa 1894)
Aphelonyx cerricola (Giraud 1859)
Apiomyia bergenstammi (Wachtl 1882)
Asphondylia cytisi (Frauenfeld 1873)
Asphondylia melanopus (Kieffer 1890)
Asphondylia miki (Wachtl 1880)
Asphondylia ononidis (Loew 1873)
Augasma aeratella (Zeller 1839)
Biorhiza pallida (Olivier 1791)
Brachycaudus helichrysi (Kaltenbach 1843)
Brachycolus cerastii (Kaltenbach 1846)
Cecidophyes galii (Karpelles 1884)
Ceutorhynchus constrictus (Marsham 1802)
Colopha compressa (Koch 1856)

Contarinia coryli (Koltenbach 1859)
Contarinia craccae (Kieffer 1901)
Contarinia loti (De Geer 1776)
Contarinia medicaginis (Kieffer 1895)
Contarinia melanocera (Kieffer 1904)
Contarinia subulifex (Kieffer 1897)
Contarinia tiliarum (Kieffer 1890)
Craneiobia corni (Giraud 1863)
Cryptosiphum artemisiae (Buckton 1879)
Cynips agama (Hartig 1843)
Cynips longiventris (Hartig 1840)
Cynips quercus (Fourcroy 1785)
Cynips quercusfolii (Linnaeus 1758)
Dasineura affinis (Kieffer 1886)
Dasineura bayeri (Rubsaamen 1914)
Dasineura crataegi (Winnertz 1853)
Dasineura fraxinea (Kieffer 1907)
Dasineura szepligetii (Kieffer 1909)
Dasineura urticae (Pessir 1840)
Dasyneura marginemtorquens (Bremi 1847)
Dasyneura potentillae (Wachtl 1885)
Dasyneura viciae (Kieffer 1888)
Dichrona gallarum (Rubsaamen 1899)
Diodaulus linariae (Winnertz 1853)
Diplolepis rosae (Linnaeus 1758)
Diplolepis mayri (Schlechtendal 1877)
Diplolepis nervosa (Curtis 1838)
Diplolepis spinosissimae (Giraud 1859)
Dorytomus taeniatus (Fabricius 1781)
Drisina glutinosa (Giard 1893)
Dryomyia circinans (Giraud 1861)
Dysaphis crataegi (Kaltenbach 1843)
Epitrimerus trilobus (Nalepa 1891)
Eriophyes pyri (Pagenstecher 1857)
Eriophyes viburni (Nalepa 1889)
Eriopyes tiliae (Pagenstecher 1857.)
Euura testaceipes (Brischke 1883)
Geocrypta galii (Loew 1850)
Hadrobremia longiventris (Kieffer 1909)
Hartigiola annulipes (Hartig 1839)
Jaapiella genisticola (Loew 1877)
Jaapiella veronicae (Vallot 1827)
Janetia cerris (Kollar 1896)
Janetia nervicola (Kieffer 1909)
Janetia pustularis (Kieffer 1909)

Janetiella oenephila (Haimhoffen 1875)
Lasioptera rubi (Schrank 1803)
Lipara lucens (Meigen 1830)
Liposthenes glechomae (Linneaus 1758)
Liriomyza urophorina (Mik 1894)
Loewiola centaureae (Loew 1875)
Mecinus collaris (Germar 1821)
Mikiola fagi (Hartig 1839)
Neuroterus minutulus (Giraud 1859)
Neuroterus numismalis (Fourcroy 1785)
Neuroterus quercusbaccarum (Linnaeus 1758)
Neuroterus saliens (Kollar 1857)
Ortezia urticae (Linnaeus 1758)
Pemphigus borealis (Tullgren 1909)
Pemphigus protospirae (Lichtenstein 1885)
Pemphigus spyrothecae (Passerini 1860)
Phorodon humuli (Schrank 1801)
Phyllocoptes goniothorax (Nalepa 1889)
Phytoptus tetrarinchus (Nalepa 1890)
Pontania pedunculi (Hartig 1837)
Pontania vesicator (Bremi-Wolf 1849)
Pontania viminalis (Linnaeus 1758)
Prociphilus bumeliae (Schrank 1801)
Psyllopsis fraxini (Linnaeus 1758)
Rabdophaga terminalis (Loew 1850)
Rhopalomyia baccarum (Wachtl 1883)
Roepkea marchali (Borner 1931)
Sacchiphantes abietis (Linnaeus 1758)
Schizomyia galiorum (Kieffer 1889)
Semiaphis anthrisci (Kaltenbach 1843)
Sibinia femoralis (Germar 1824)
Spurgia esulae (Gagne 1990)
Synophrus politus (Hartig 1843)
Tetramesa hordei (Harris 1830)
Thecabius affinis (Kaltenbach 1843)
Trigonaspis megaptera (Panzer 1801)
Wachtliella persicariae (Linnaeus 1767)
Zygiobia carpini (Loew 1874)

The collection contains the recorded data on the distribution of 227 plant gall species or causers, belonging to 65 genera, and 17 families. These galls are formed on the 132 plant species, belonging to 78 genera, and 41 families. For the Pannonian part of Serbia 377 data have been recorded and for the Balkan 61 data have been recorded.

More different plant galls occur on the herbaceous plant than on woody and bushy plants. Analyzing plant genera as hosts for different gall causers species, two plant genera stands out and they are: *Quercus* L. (oak, 61 species of galls) and *Salix* L. (willow, 30 species of galls). These species are the building constituents of natural habitats and anthropogenic, landscaped areas (parks, alleys). In the group of herbaceous plants galls, the most of galls were recorded for the genus *Artemisia* L. (mugwort, eight species of galls). Plant galls on the woody species are most commonly developed on the leaves. In herbaceous plants, galls are developed in equal measure on the flowers (inflorescence) and leaves. Of biological importance is to emphasize that out of 410 analyzed plant galls, 77 are developed on the generative organs; disproportion of the population of the plant host and causers could have negative impact, both on the causers and the host plants populations; recording the related causer species that are nonindigenous, could have a negative impact for the generative reproduction of host plants and thereby weaken the genetic potential of the population.

Plant gall, whose causer is *Diplolepis rosae* (L i n n a e u s, 1758) has the largest distribution and it can be assumed to be distributed along the entire real of *Rosa canina* L. 1753 and its related species. The species of the genus *Tilia* L., *Ulmus* L., *Vitis* L. and *Rubus* L. are often gall hosts as well, primarily galls occurs on the leaves and branches. The trees of these species in certain localities bear plant galls in almost all leaves.

Species *Agrobacterium tumefaciens* S m i t h & T o w n s e n d 1907, is a frequent causer of galls in woody dicots, and in the database collection it was recorded for only two host species.

For all other galls, which are deposited in the herbarium, causers are animals, belonging to the classes: Arachnida (C u v i e r, 1912) and Insecta (L i n n a e u s, 1758). The most of the causers belong to genera *Eriophyes* S i e b o l d, 1851 (Eriophyidae, Arachnida) and *Andricus* H a r t i g, 1840 (Cynipidae, Insecta).

CONCLUSION

There is no doubt that this coevolutional solution and the degree of adaptation of organisms to survive, is a unique natural phenomenon. However, the weak interest of researchers for the collection of such materials has resulted in small number of plant gall collections both in Serbia and in general. Most of the scientific collections do not have a plant galls or they are not adequately processed, classified, and as such become available to scientific, professional and general public.

Collection of Herbarium BUNS is one of the few scientific collections having this type of natural materials in its depots. In terms of knowledge of species and the coevolutional relationship of plants and gall causers, this collection highly contributes to the general knowledge of biodiversity.

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ХЕРБАРИЈУМ BUNS – КОЛЕКЦИЈА ГАЛА

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Резиме

Савремена истраживања биодиверзитета најчешће почивају на до сада објављеним подацима у литератури и подацима који су депоновани у биолошким колекцијама. Због тога је основни циљ и задатак свих јавних колекција да подаци о ексикатима буду добро организовани у електронске базе података, које имају могућност једноставног и брзог проналажења различитих типова података и информација.

Хербаријум BUNS је званична ботаничка збирка Универзитета у Новом Саду и у њој је депоновано око 110.000 ексиката, организованих у 14 колекција. Једну од колекција чини и збирка гала (шишки, цецидија), које су сакупљане током флористичких истраживања, а након тога и детерминисане и сачуване као *Herbarium cecidologicum*. Ова колекција, са пратећом базом података, представља непроцењив извор информација о галама у Србији, биљкама – домаћинима и изазивачима, односно о њиховој дистрибуцији. Збирку чине 294 ексиката и преко 100 литературних навода и усмених саопштења о дистрибуцији гала на подручју Србије.

КЉУЧНЕ РЕЧИ: биљка-домаћин, биљне колекције, гале, узрочник

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CHARACTERIZATION OF SAND BOILS WITH GRADING ENTROPY

ABSTRACT: Grain size and grain distribution by size are dominant factors determining soil behaviour. The shape and position of a grain distribution curve contain implicit information about the propensity of sand boiling or piping at flood conditions. The author used 1040-grain distribution curves taken from 12 sand boil locations to study the relationship between sand boils, hydraulic soil failures and entropy. The results have justified the hypotheses and indicated some fairly important details for practical consideration. Calculating grain distribution entropy is not "magic" with mathematics: it simply helps put the expected behaviour of soils into a different perspective and promotes orientation for classifying soils according to a new parameter related to grain movement.

KEY WORDS: dike failure, entropy, grain size distribution, sand boil

INTRODUCTION

Boil formation and sand boiling failures are relatively infrequent loss events in the history of dyke breaches in Hungary. Boils normally form in intersections with old riverbeds in locations where one can observe the intercalation of grainier soil, which is different from its environment (N a g y , 2000). Past experience suggests that only a small percentage of boils actually terminated in disaster (N a g y , 2004), the threat has been localised most of the time (presumably due to efficient flood control measures, e.g. the boil at Csongrád in Photo 1). A series of detailed subsoil studies (N a g y , 1993) performed along 4200 km of Hungarian flood control dykes between 1984 and 1996 revealed that the number of shorter or longer sections with soil failure potential is so large (over 1500) that efficient protection can only be expected at locations where the phenomena associated with boil formation are visible.

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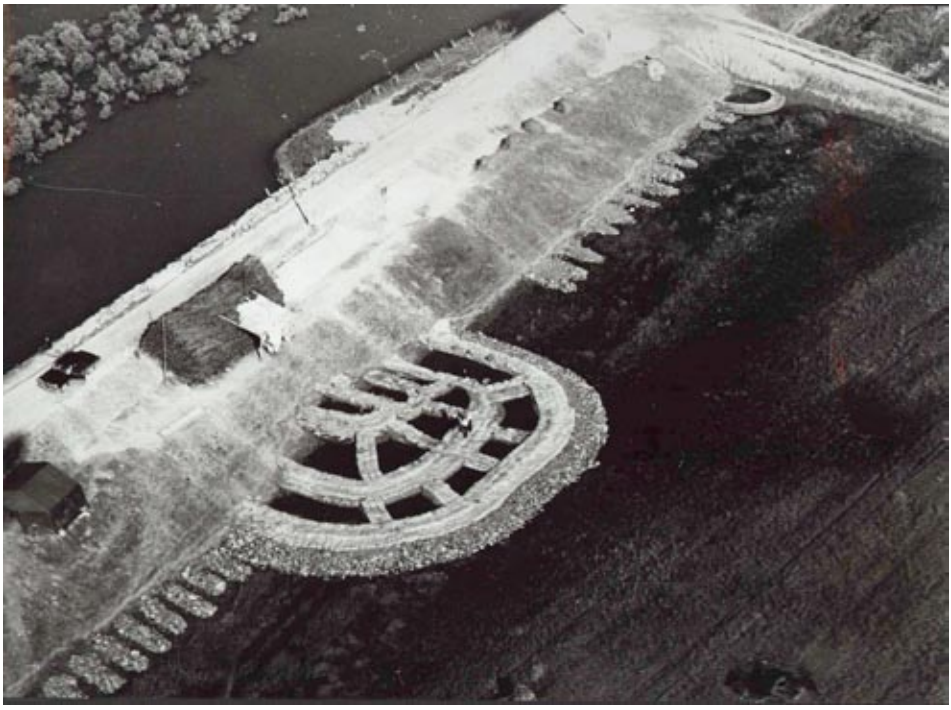

Photo 1. – The boil at Csongrád

Grain size and grain distribution by size are dominant factors determining soil behaviour. The distribution of soil grains by size has been studied for a long time and grainy soils are named based on information provided by grain distribution curves. The direct of use the various parameters of a grain distribution curve, such as grain sizes associated with percentage values, the coefficient of irregularity, etc. is mostly limited to judging compatibility and various water and grain movement phenomena. Indeed, these quantities provide a relatively good characterization of soils with grain distribution curves showing calm, regular shapes or a narrower diameter range, but the rules derived from these relationships lose validity as soon as grain distribution is irregular, i.e. the rules have no general validity. Taking the grain distribution curve fully into account would obviously improve the accuracy of rules based on grain distribution, such as the calculation of permeability.

THE DEFINITION OF ENTROPY

Entropy is a term used to describe the degree of disorder of a statistical population in the theory of probability. It is easy to calculate it for the grain distribution of soils. The distribution by size of grains in a soil is determined

by fractioning with a set of screens or a sedimentation experiment or frequently by using both methods simultaneously.

When soils are screened, each frequency value will reflect the proportion of the total screened volume that belongs to a screened fraction. Frequencies are added up based on the fractions falling through the screens that correspond to fraction boundaries and by doing so we get a grain distribution curve (L ő r i n c z, 1986, 1993)

The closer the aperture size of the screens in a stack, i.e. the narrower the fractions are, the greater the accuracy of the ordinates of a grain distribution curve will be. As there is not much practical use of narrowing fractions down, the following aperture sizes in a screen set are becoming quite standard in Hungary:

$$d\ (mm) = (0.063), 0.125, 0.25, 0.5, 1, 2, 4, 8, \ldots,$$

That is to say, the aperture size of the next screen in a set is twice the aperture size of the previous screen.

The calculation method introduced to determine the grain distribution entropy of soils is based on the assumption that the screen set described above has been used; and in case another screen set was used for the purposes of identifying grain distribution, the grain distribution curve itself will have to be split at the fraction boundaries and then calculate the frequency of the resulting fractions.

As each fraction covers a different width diameter range, the C number of elementary cells in a fraction will vary in case z elementary cell width is identical (L ő r i n c z, 1986, 1993). To ensure that subsequent calculations suitable for determining the grain distribution entropy of even colloid state substances (i.e. that they have positive entropy) we need to select an extremely small z elemental cell width. The following elemental cell width was applied:

$$z = 2^{-17}\ mm$$

It is worth mentioning that elemental cell width was set at $z = 2^{-22}$ mm in other application areas of grain distribution entropy mainly to ensure the comprehensive use and generalization of the term (L ő r i n c z et al., 2005, 2008). The value selected for this study ($z = 2^{-17}$) is suitable for the purpose as we examined the behaviour of natural soils.

INTRINSIC ENTROPY

The mass of grains in the elementary cells of a fraction with uniform distribution will be equal, i.e. the frequency of every elementary cell is identical (L ő r i n c z, 1986, 1993):

$$\alpha = \frac{1}{C}$$

where C is the number of elementary cells in a fraction. The intrinsic entropy of fraction *i* is:

$$S_{0i} = -\frac{1}{\ln 2} Ci \left(\frac{1}{Ci} \ln \frac{1}{Ci} \right)$$

hence:

$$S_{0i} = \frac{\ln Ci}{\ln 2} \qquad (1)$$

To illustrate the above, let us calculate the intrinsic entropy of a few screened fractions.

- the diameter range of the 1-2 mm fraction is 1 mm.
- the number of elementary cells in this fraction is:

$$C = \frac{1mm}{2^{-17} mm} = 2^{17} \text{ cells}$$

- intrinsic entropy:

$$S_0^{1-2} = \frac{\ln 2^{17}}{\ln 2} = \frac{17 \ln 2}{\ln 2} = 17$$

The intrinsic entropy of the 2-4 mm fraction is:

$$C = \frac{2mm}{2^{-17} mm} = 2 * 2^{18}$$

$$S_0^{2-4} = 18$$

and so on. The difference between the intrinsic entropy values of subsequent fractions is 1. That is due to doubling the width of subsequent fractions and, concurrently, the radix of the logarithm is also 2.

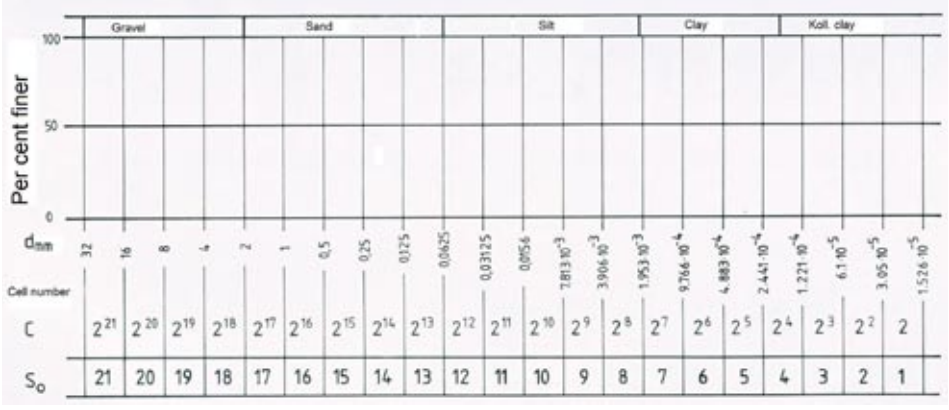

Fig. 1. – The intrinsic entropy S_0 and cell numbers C_i of the fractions for the recommended and applied elementary cell width *z*

THE ENTROPY OF SOILS SPLIT INTO FRACTIONS

Screens are used to split the soil into fractions or the grain distribution curve derived from a sedimentation experiment is "fractioned off", while distribution within fraction boundaries is assumed to be uniform. The frequency of fraction *i* is represented as x_i.

$$\sum_{l=1}^{n} x_1 = 1$$

Hence respective fraction frequencies are:

$$x_1, x_2, \ldots, x_n$$

The number of elementary cells in fraction *i* is C_i. In the case of uniform internal distribution in the fractions, the intrinsic frequency of these cells is:

$$\alpha_1 = \frac{x_1}{C_1}, \alpha_2 = \frac{x_2}{C_2}, \ldots, \alpha_n = \frac{x_n}{C_n}$$

Entropy value is:

$$S = -\frac{1}{\ln 2}\sum_{i=1}^{n} x_i \ln x_i + \frac{1}{\ln 2}\sum_{i=1}^{n} x_i \ln Ci \qquad (2)$$

A comparison of the first addend with formula (3) reveals that it is nothing else but the ΔS increment of entropy growth resulting from mixing the fractions.

Let us select element *i* from the second addend of the formula:

$$x_i \frac{\ln C_i}{\ln 2} = x_i S_{0i}, \text{ see formula (1)}$$

Hence, this is the product of multiplying the intrinsic entropy of fraction *i* with the frequency of the same fraction. Accordingly, the second addend of expression (2) can be rearranged as follows:

$$S_0 = \sum_{i=1}^{n} x_i S_{0i}$$

where x_i= the frequency of fraction *i*,
S_{0i}= the intrinsic entropy of fraction *i*.

We call this entropy S_0 of the soil before mixing base-entropy.

ΔS INCREMENT OF ENTROPY

We can say based on the entire formula (2) that soil grain distribution entropy equals the sum of the base-entropy before mixing and the incremental entropy growth that results from mixing (I m r e et. al., 2008):

$$S = S_0 + \Delta S$$

Based on the definition, incremental entropy growth ΔS is:

$$\Delta S = -\frac{1}{\text{h } 2}\sum_{i=1}^{n} x_i \text{ h } x_i \qquad (3)$$

When two systems are mixed, the grain distribution entropy of the resulting mixture will remain unchanged or will increase. If a soil is mixed with itself, the grain distribution entropy of the "mixture" will not change, whilst mixing different soil types will always result in entropy growth. Entropy growth reaches its maximum at 50-50% mixing ratio.

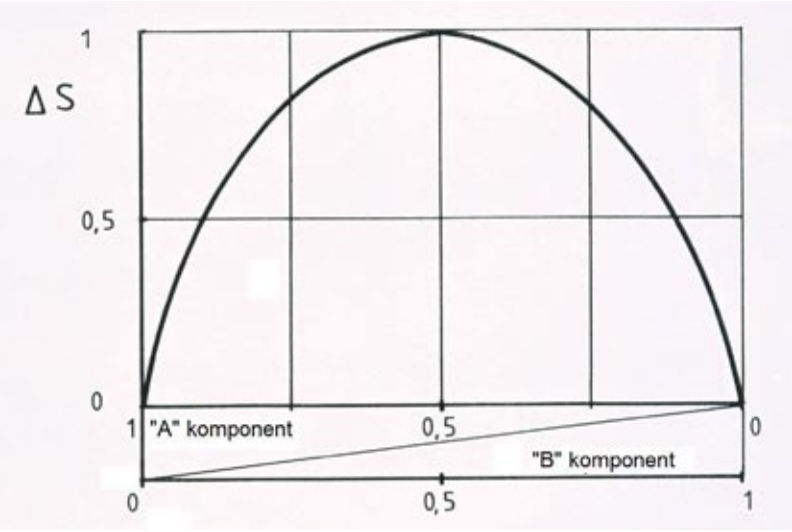

Fig. 2. – The increments of entropy growth as a function of the ratio of mixing

ΔS CHANGE OF ENTROPY GROWTH

As Fig. 2 suggests, the entropy of a mixture containing two components, both with zero entropy, will change in line with the ratio of mixing and will peak when the ratio of the two components is the same. Increasing the number of components will result in an absolute increase of incremental entropy growth (or mixing entropy). The incremental entropy growth of mixtures con-

taining an arbitrary number of components will reach a maximum when the frequency of the fractions is identical, i.e. with F as the number of fractions, then in case:

$$x_1 = x_2 = ... = x_F = \frac{1}{F}$$

Using these frequencies, the incremental value is:

$$\Delta S_{\max} = -\frac{1}{\ln 2} F\left(\frac{1}{F} \ln \frac{1}{F}\right)$$

hence

$$\Delta S_{\max} = \frac{\ln F}{\ln 2}.$$

The maximum incremental entropy growth for a given width range is obtained when fraction frequencies are identical. Maximum entropy growth depends on the fraction number F.

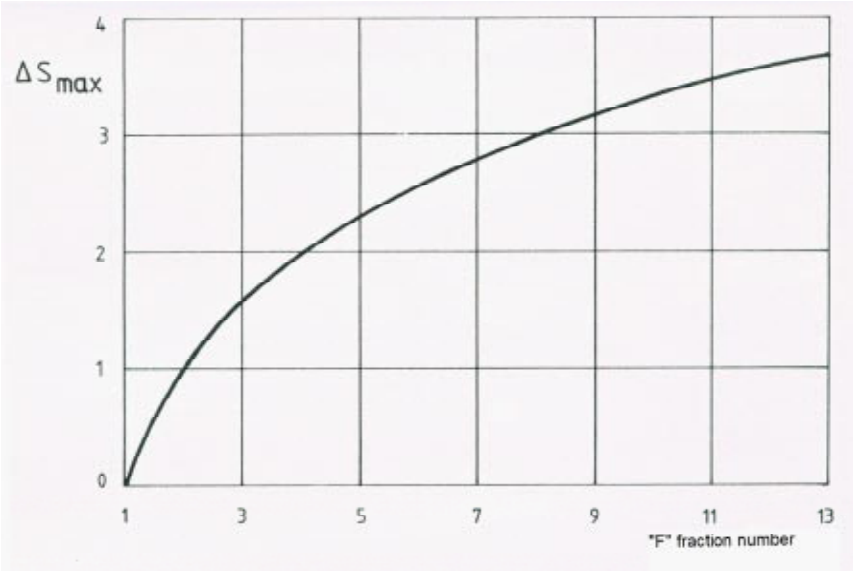

Fig. 3. – The effect of the number of fractions in a mixture on the maximum value of mixing entropy

Based on the findings of a large number of laboratory mixing experiments we can safely say that the soil with S_0 fundamental entropy at close to 2/3 of the range will provide the most compact skeleton from among the possible infinite number of mixtures of different grain distribution within a range of grain width (L ő r i n c z, 1986, 1993), i.e.

$$S_0 = S_{0\min} + 2/3\left(S_{0\max} - S_{0\min}\right)$$

where S_{0max}= the intrinsic entropy of the coarsest soil fraction, and
S_{0min}= the intrinsic entropy of the finest soil fraction.

APPLYING GRAIN DISTRIBUTION ENTROPY TO TRANSIENT PHENOMENA

If grains can be washed out of a soil, grain distribution will be altered and the original conditions of grain distribution will not be upheld any longer. Only soils that withstand suffusion are suitable for the purpose of soil filtration, and designs rely on their grain distribution (L ő r i n c z et al., 2004, 2005).

Demixing may occur during filter placement, which may lead to a complete modification of the parameters taken into account during screening tests. Earlier experience (L ő r i n c z, 1986, 1993) suggests that soils with grain distribution curves that cover ranges of 4 or fewer fractions either continuously or incompletely are not susceptible to suffusion because the coarsest fraction of the range of 4 fractions can be used as the filter of the finest fraction. It is obvious that no mixture of these fractions, regardless of proportion, lends itself to suffusion. The propensity to suffusion can be studied based on the grain distribution curve of soils covering ranges wider than 5 fractions. Including $\Delta S/\ln F$ values, Fig. 4 helps evaluate internal grain movements (ΔS stands for entropy growth, F represents the number of all fractions potentially included in a grain distribution range), which can be calculated with this equation:

$$A = \frac{S_0 - S_{0\min}}{S_{0\max} - S_{0\min}}$$

depending on normalised entropy. The curve shows symmetry with the axis $A = 0.5$, curve data include the $\Delta S/\ln F$ values of what are known as distributions with "optimal" entropy.

In Fig. 4, the ranges labelled "sand boil", "stable" and "suffusion" have been identified based on a large number of experiments (L ő r i n c z, 1986). Each point in the figure represents a shape of a grain distribution curve, i.e. the figure is the representation of an infinite number of parallel grain distribution curves. Points **A**, **B**, **C** and **D** are highlighted in the figure:

- Point **D** represents a 1/3-2/3 mixture of only two (the finest and the coarsest) of fractions of a range covering 5 fractions with the following coordinates: $A = 2/3$, $\Delta S/\ln F = 0.571$.
- Point **A** stands for grain distribution curves containing any number of fractions, but each fraction is identically filled (see Fig. 4).
- Point **B** corresponds to soils with 2/3 grain distribution.

- **C** shows the point of grain distribution at which entropy is at its maximum, the coordinates in the present representation are: A=0.79, $\Delta S/\mathrm{In}F$=1.167, with larger fraction numbers the point shifts towards A=1 on the curve.

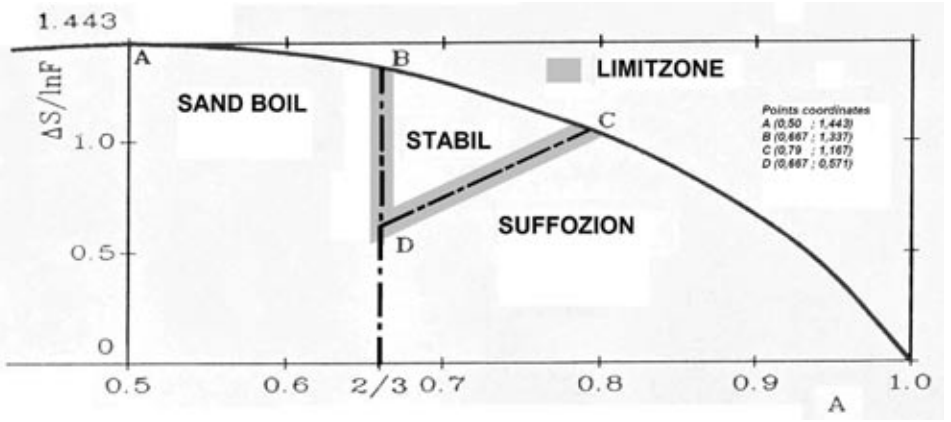

Fig. 4. – Stands for grain distribution curves containing any number of fractions

Experience suggests that fine grains form a matrix before point **B** in case A < 2/3, they "lift to disaggregate" the coarse skeleton and the further away the point is from A = 2/3 in the direction of A = 0 the more intensive, intensive the floatation of coarse grains in the matrix.

If scouring occurs in soils with the propensity to boil, the value of **A** converges towards 0.5 in the ranges affected by scouring. Scouring with hydraulic failure shows the same features regardless of the original value of **A**!

The transition from the matrix of fine grains to coarse grained skeleton starts above A = 2/3. The transition is very sharp in mixtures of two fractions and fractions missing, if **A** is only slightly larger than 2/3, a rough skeleton is formed and fine grains can freely move or are scoured away.

Transition occurs within the **B-C-D** range in soils with continuous grain distribution. In terms of grain movement, this range may be called safe as fine grains do not form a matrix any longer and no coarse skeleton builds up yet.

Grain distributions beyond point **C** and those on the right hand side of the **CD** line show a skeleton formed by coarse grains with the majority of fine grains allowed to move freely, hence grain movement and suffusion will begin among proper circumstances. Normally, relatively small quantities get scoured away, although larger volumes may also get dislodged from soils with missing size fractions.

It is validly observed both as regards the sand boil and the suffusion ranges that the soils in the vicinity of the top boundary curve, which represents the soil types with optimum entropy, are safer, and the threat of scouring increases as we move further away from the boundary curve and the lines at which ranges terminate.

In the case of hydraulic soil failure, i.e. when soils are completely remixed and restructured, the point representing the soil moves towards a position where A = 0.5 regardless of its former position. Changes are of different character with samples taken from below the waterline. In this case, if the sampling device is not closed, fine grains are washed away while the sample is lifted and grain distribution will converge from a position near A = 0.5 towards A = 2/3 (L ő r i n c z, 1986, 1993).

PRACTICAL EXPERIENCES

We have used data from past events (controlled boils and boil related soil failures) for the purpose of a practical study of the propensity of sand boil formation with the help of grain distribution entropy. Data collection was broad based and we processed over 120 calculations from 104 grain distribution curves plotted for 12 locations (Tab. 1). Of the soil samples from the 12 test locations, the ones marked No. 4, originating from the dyke failure at Hosszúfok, were from a location without a sand boil as the failure of the dyke was caused by dispersive soil.

Tab. 1. – From among the levy failures

No.	place	**Year**	**soil samples**
1.	At Kutyatanya slice gate	1987	5
2.	Bölcske-Madocsai dike 79+415 section	1965	17
3.	Surány dike failure (photo 2, figure 5)	1991	11
4.	Hosszúfok dike failure	1980	4
5.	Mályvád reservoir 4+550 section	1980	5
6.	Maros great piping	1970	8
7.	At Karaszi-fok slice gate	1986	14
8.	Mosoni-Duna 9+250 section	1991	14
9.	Duna 23+700 section	1965	14
10.	VITUKI sand boil model soil	1974	1
11.	Dunafalva dike failure	1954	7
12.	Dunakiliti dike failure	1954	4

From among the levy failures listed in Tab. 1, the failure at Surány (Photo 2) is used to illustrate the results of our calculations. The breach occurred before midnight on August 5, 1991 most probably through hydraulic soil failure. The grain distribution curves of several soil mechanical drill holes lowered during the study of the circumstances of the breach are available (e.g. Fig. 5). Although a scour hole formed at the location of the dyke breach and no sample could be obtained from the failed section (it had been washed away), we had to assume that the undamaged parts of the dyke and the soils samples taken there represented local conditions accurately.

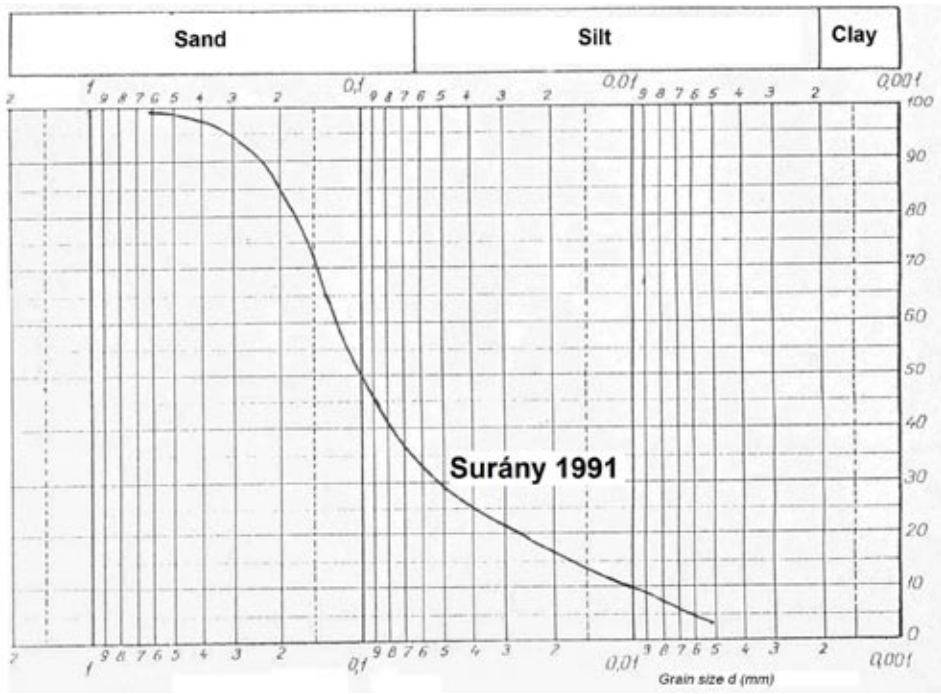

Fig. 5. – The grain distribution curves of several soils

The grain distribution curves indicate that poorly graduated, finely grained soils classified as "stable" or "boil hazard" can be found below the stable silt, sand with sand meal and silt with sand meal and sand soils near the surface. The shape of the grain distribution curve of soils posing a "boil hazard" is mostly regular, and these soils contain 3-4 fractions when analysed for the purposes of an entropy test. The grain distribution curve indicates that these soils pose an erosion hazard, have a low boundary gradient of critical hydraulic failure, and also pose subsoil failure hazard (Fig. 6).

The large sand boil of the Maros River also included a layer that had the propensity to boil based on grain distribution entropy. It is well known, though, that the large sand boil of the Maros River was described as "flow across the dyke via a rotten tree root." However, the washout of grains was also observed, hence flood fighters rightly engaged in boil prevention. The definition of a sand boil is important from this perspective. For the purposes of fighting floods, it is practical to connect the concept of a sand boil with the washout of grains, or in other words, it is grain washout that differentiates a boil from a flow across the dyke. This corresponds to the practical guide of fighting floods, which requires protection against a boil whenever grain washout is observed on the protected side.

Photo 2. – The failure at Surány

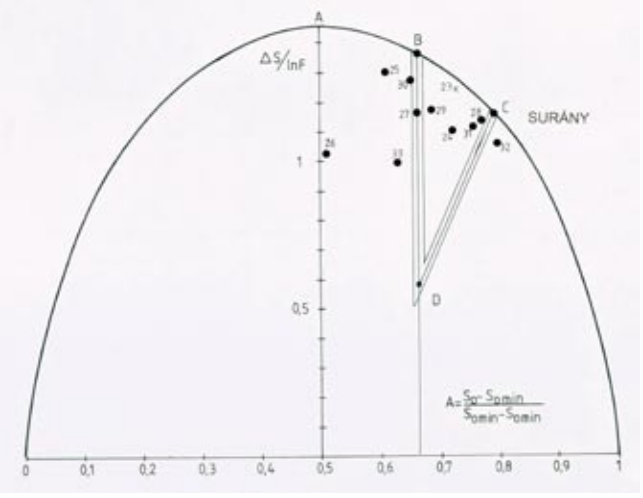

Fig. 6. – A low boundary gradient of critical hydraulic failure

In summary of the analysis of entropy in practice we can assert that, except for a single instance (the samples of the disperse soil of the *Hosszúfok* dyke failure, mentioned above) all of the analysed locations contained a layer of soil

that had the propensity to boil. Simultaneously all of the samples taken from the *Moson branch of the Danube, Dunafalva and Dunakiliti*, i.e. from the upstream sections of the Danube in Hungary belonged to the range of soils that have the propensity to boil. Mention must be made of the fact that this result is the same that we know from experience: most of the sand boils were recorded in that area.

SUMMARY

As regards the evaluation of various transient phenomena, the recognition that grain distribution curves contain the information needed for a "geometric" description of this set of occurrences is generally accepted. However, all entropy values, each of the points in Fig. 4 characterize a grain distribution curve of a certain shape and represent an infinite number of parallel grain distribution curves, since the calculation does not differentiate between clay and shingle with identical curve shapes.

Grain distribution entropy identifies soil types that have low entropy (a steep grain distribution curve) in quantifiable form and therefore pose the greatest boil hazard. That requires accurate grain distribution curves, though. A grain distribution analysis, however, is not capable of matching the accuracy required by mathematics in the range of silt-clay grains; hence valid boil hazard estimates are only possible when grain analysis relies on screening. Locations for which the measured values of a grain distribution curve are uncertain do not lend themselves to an accurate calculation of entropy. Grain distribution curves defined with or without chemicals may show significant differences, which will also influence entropy substantially (L ő r i n c z, N a g y, 1995, 2010).

Soil entropy values may be calculated from grain distribution curves. An entropy value characterises a grain distribution curve of a certain shape, i.e. a single entropy value represents an infinite number of parallel grain distribution curves. However, a grain distribution curve cannot be derived from an entropy value. As grain distribution curves contain more data than entropy values, it would be practical to retrace the information gathered from entropy studies into grain distribution curves. This could be done, for instance, with the help of a single distinguished point or value of grain distribution curves. Experience with the description of sand boil formation could be an important factor in selecting this point.

At present, knowledge concerning the process of sand boils formation and the internal and external factors leading on to soil failure is limited. We cannot define parameters of the criteria of sand boil formation, we cannot unambiguously determine in advance the location and the water level at which disaster is to be expected and we cannot recommend any other form of prevention but increasing the length of travel of the flow or closing the tube of the boil when flood levels keep increasing. Nevertheless, this series of tests is another step taken towards the full understanding of sand boil behaviour and the process

of sand boil related soil failure. Although the studies relating to boil formation were intensively pursued in the seventies, they stopped completely in the eighties in Hungary. Now, after the turn of the millennium, it would be practical to revive our classified knowledge and apply our new level of awareness to increasing our understanding of sand boil related soil failures (L ő r i n c z, N a g y, 1995, 2010). That would be necessary because recurring floods (see Photos 1, 2 and 3) force us to face this problem repeatedly.

Photo 3. – The lowest amount of energy to get restructured though boiling

Grain distribution entropy is a tool (at present for researchers only) that helps single out soils that have the propensity to form boils. This tool also helps identify the types of soil that require the smallest hydraulic gradient, the lowest amount of energy to get restructured through boiling. Bear in mind that all soils can be washed away provided the hydraulic gradient is sufficiently large and energy is sufficiently powerful.

Based on the above recommendations are formulated for continuing the research:

- When studying grain distribution, we frequently pay little attention to sampling itself. This paper also wished to highlight the reliability of grain distribution results. As the effectiveness of entropy tests depends fundamentally on the reliability of grain distribution data, it would be practical to learn more about the reliability of grain distribution curves from the perspective of sampling and screening-wet mechanical analysis, but the

degree to which the results might help understand field processes is a question.

- At present, grain distribution entropy values are only suitable for determining the geometric potential of boil formation. Laboratory test findings would be needed to gain an understanding of the hydraulic conditions of this group of occurrences to create common ground for discussions about sand boil formation.
- Detailed studies would be needed about the substances washed out from sand boils along with comparisons to subsoil grain distribution curves. That could provide unquestionable evidence about whether a site is subject to suffusion or a boil. The data available for studying substances washed out of boils based on experience from previous floods are limited. It would therefore be useful to determine with the help of grain distribution entropy whether it is a single fraction that gets washed out of a soil or grain movement affects a complete layer.

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ОДРЕЂИВАЊЕ ПРОВИРНЕ ВОДЕ СА ГРАДИЈЕНСКОМ ЕНТРОПИЈОМ

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Резиме

У карпатском басену често долази до оштећења одбрамбених насипа услед провирања насипа и пролома тла насипа. Провирање насипа се најчешће јавља у пресеку мртвог корита, тамо где се различите структуре земљишта инфилтрирају једна у другу. Досадашња искуства показују да само мали проценат провирања доводи до катастрофе; најчешће се успешно локализују (вероватно као резултат ефикасне одбране).

Обрађени су подаци практичног испитивања на насипима склоним провирању уз помоћ криве гранулометријскоj састава. За истраживање су коришћени подаци који су узети са 11 угледних места; извршено је више од 112 прорачуна, на месту провирања. Ентропију земљишта можемо одредити помоћу криве гранулометријског састава. Квантитативним испитивањем састава тла (на основу криве гранулометријског састава) добијају се земљишта са ниском ентропијом, осетљива на провирање. Стога морамо имати тачну криву гранулометријског састава. У случају испитивања муља – глине крива расподеле се не може одредити са математичком тачношћу. Процена могућности провирања у већини случајева се може применити са пробраним подацима добијених испитивањем састава.

Крива гранулометријског састава је такав инструмент – тренутно још увек само у рукама истраживача – који помаже у препознавању тла склоног провирању. Уз помоћ криве гранулометријског састава могу се одредити она тла код којих је реструктурализацијом провирања на најмањи хидраулички градијент потребна најмања енергија. Не треба заборавити да се највећим хидрауличким градијентом и одговарајућом великом енергијом свако земљиште може испрати.

На основу добијених резултата, при сваком испитивању, само са једним изузетком било каквог слоја земљишта, који анализом криве расподеле зрнаца приказује тенденцију провирања земљишта. У опсегу земљишта погодних за провирање испране су обале Дунава, Дунафелдвар и од неколико редних бројева „Дунакилити”. На овим местима је дошло до прелома бране.

Крива расподеле зрна скреће пажњу на следеће чињенице:

- У случају анализе криве гранулометријског састава важан је избор места за узимање узорака. Пошто ефикасност ентропијског истраживање у основи зависи од поузданости испитаних добијених података, поуздани узорци се узимају са места где се препознају процеси који су се одвијали.
- Крива расподеле зрна за сада је могућа за геометријско одређивање настајања провира. За њихово појављивање треба узети у обзир и хидрауличке услове и заједно анализирати појаву провира и криве расподеле зрна.
- Потребно је детаљно испитати материјале испране провирањем, у поређењу са кривом расподеле зрна из слојева земљишта. То би био јасан доказ да ли се код провирања одвија суфозија или се испира један део земљишта.

КЉУЧНЕ РЕЧИ: провирне воде, ентропија, распоред зрна по величини, провирање насипа

INSTRUCTION TO AUTHORS
(www.maticasrpska.org.rs)

This version of Instruction to Authors is valid starting from the year 2012 and the journal number 122

1. General remarks

1.1. Matica Srpska Proceedings for Natural Sciences (short title: Proc. Sci. Matica Srpska) publishes manuscripts and review articles as well as brief communications from all scientific fields as referred to in the title of the journal. Review articles are published only when solicited by the editorial board of the journal. Manuscripts that have already been published *in extenso* or in parts or have been submitted for publication to other journal will not be accepted. The journal issues two numbers per year.

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1.3. Upon the reception of the manuscript, the author shall be assigned with a manuscript code, which has to be referred to in any further correspondence. The authors will be notified about the manuscript reception within seven days and about the reviewers' opinion within two months from submission. All submitted manuscripts are reviewed and proofread.

2. Planning and preparing of the manuscript

2.1. Type the manuscripts electronically on A4 (21 x 29.5 cm) format with 2.5 cm margins, first line indent, and 1.5 line spacing. When writing the text, the authors should use *Times New Roman* size 12 font and when writing the abstract, key words, summary, and footnotes use font size 10.

2.2. First name, middle initial and last name should be given for all authors of the manuscript and their institutional affiliations, institution name, and mailing address. In complex organizations, a full hierarchy should be mentioned (e.g. University of Novi Sad, Faculty of Sciences – Department of Biology and Ecology). The institution of employment of each author should be stated below the author's name. The position and academic degrees should not be cited. If there is more than one author, indicate separately institutional affiliation for each of the authors. Put the name and mailing address (postal or e-mail address) of the author responsible for correspondence at the bottom of the first page. If there is more than one author, write the address of only one author, usually the first one.

2.3. Structure the text of the original articles into Abstract, Key Words, Introduction, Material or Methods, or Material and Methods, Results or Results and Discussion, Discussion, Conclusion, References, Summary and Key Words in Serbian language, and Acknowledgement (if there is one). Original articles should not be longer than 10 pages, including the references, tables, legends, and figures.

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6. Units, names, abbreviations, and formulas

6.1. SI units of measurement (Système international d'unités) should be used but when necessary use other officially accepted units.

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6.4. Chemical formulas and complex equations should be drawn and prepared for photographic reproduction.

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7.2. A caption with the explanation should be put below each figure.

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