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Konstantin Viktorovich Sudakov

P. K. Anokhin, Research Institute of Normal Physiology
Russian Academy of Medical Sciences, Moscow, Russian Federation

SYSTEM QUANTA AS DISCRETE UNITS OF BEHAVIOR

ABSTRACT: System quanta are specific operators in the dynamic activity of the organism's functional systems discovered by P. K. Anokhin. By the term "system quantum" of life activities we propose to understand discrete system processes proceeding from any arising need up to its satisfactions.

Every system quantum of behavior presents a self-regulating organization including the initial need, motivation, system architectonics as described above, intermediate and final results and continuous evaluation of the achieved results by dynamic reconstruction of acceptor of action's result. System quanta may be organized at different levels of life activity. These levels extend in their hierarchy from genes and functional systems of metabolic and autonomic level to zoo-social populations of animals and human communities.

Every system quantum is also characterized by information properties. Information properties of every system quantum are closely related to the initial need and its satisfaction.

KEY WORDS: system quantum, functional systems

INTRODUCTION AND GENERAL DEFINITION OF SYSTEM QUANTA

In 1979 I suggested a hypothesis about systemic quantification of the behavior. In order to make a distinction with the commonly accepted term "quantum" in physics, we entitled the proposed unit of behavior as "system quantum". System quanta are specific operators in the dynamic activity of the organism's functional systems discovered by P. K. Anokhin (3, 19, 21).

By the term "system quantum" of life activities we propose to understand discrete system processes proceeding from any arising need up to its satisfactions.

System quanta are disclosed externally behaviorally and by the results of satisfaction of organisms needs. Inside the organism (internally) the system quanta — their structure and dynamic — are realized through physic-chemical and information processes of system architecture of functional systems. The

brain mechanisms of the functional systems include the following successively changing each other stage: afferent synthesis, decision-making, prediction and estimation of required results-acceptor of action's result, metabolic and autonomic behavioral reactions of an organism (3).

According to our concept all continuum of behavioral and psychic activity in humans and animals is split into separate resulting system quanta, which provide the satisfaction of leading requirements of living beings.

Every system quantum of behavior presents a self-regulating organization including the initial need, motivation, system architectonics as described above, intermediate and final results and continuous evaluation of the achieved results by dynamic reconstruction of acceptor of action's result (Fig. 1).

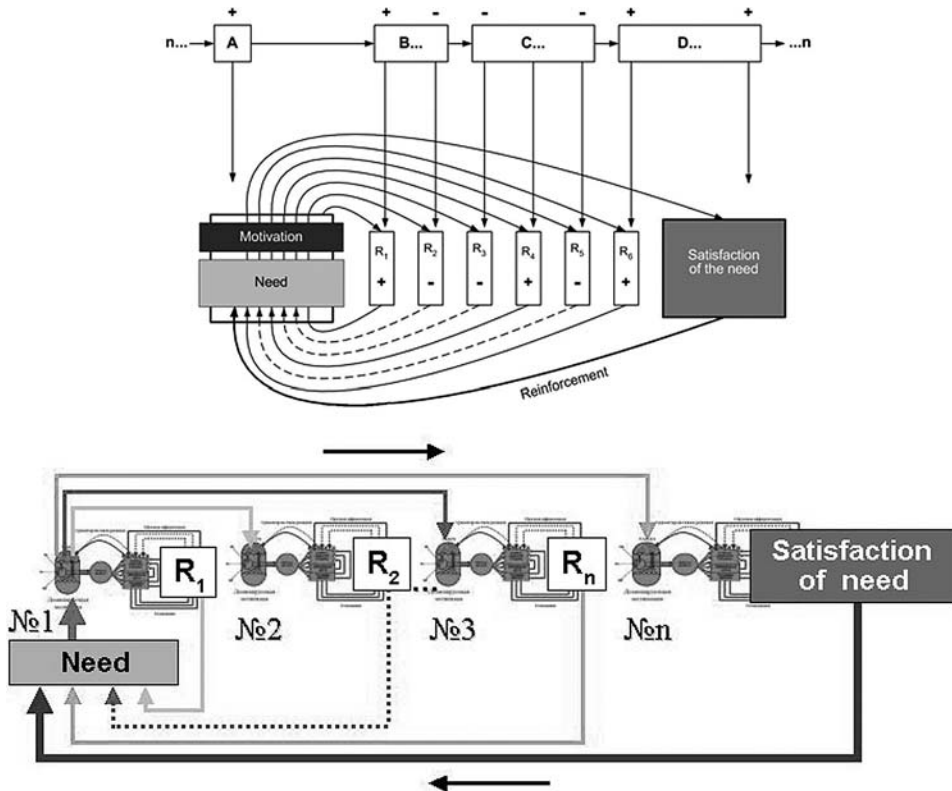


Fig. 1 — Dynamic organization of behavioral system quantum.
Above: system quantum of behavior; below: stage reconstruction of behavioral.

In development of central architectonics of system quanta of behavior and psychic activity under influence of reverse afferentation from parameters of the achieved results and signals from the acceptor of action's result to afferent synthesis, a dynamic reconstruction of system architectonics of a behavioral act takes place, which in the long run provides the achievement by a subject of the adaptive result — satisfaction of the initial need.

System quanta may be organized at different levels of life activity. These levels extend in their hierarchy from genes and functional systems of metabolic and autonomic level to zoo-social populations of animals and human communities.

Needs of organisms also arise at different levels of life activity. They include metabolic (biological) needs of the required nutrients, oxygen, optimal temperature, osmotic pressure, pH etc. Zoo-social needs determine the formation of system quanta of behavior of groups of animals. Social needs of people form system quanta of their social activity. With these system quanta biological and social needs are satisfied after the achievement of biologically or socially significant results. A detailed classification of needs is given in (10).

One can consider as examples the internal system quanta satisfying an optimal level of nutrients in an organism, of osmotic pressure, of a level of products of a metabolism, of body temperature, etc.

System quanta of behavioral level have an external active link of self-control due to interaction with the environment. Functional systems and corresponding biological motivations compose such system quanta and determine purposeful behavioral activity.

At a zoo-social population level, system quanta and functional systems composing them differ to some extent. Separate individuals with a set of their own homeostatic and behavioral functional systems represent components of these system quanta. Cumulative activity of individuals united in system quanta produce the end results of activity of functional systems at this level. Some of these individuals perform specific functions (leaders, executors, watchmen, etc.).

In human populations system quanta are built by social needs of people and are directed to their satisfaction. At that every social organization performs its specific functions.

System quanta of behavior can be formed sometimes entirely based on genetically determined mechanisms. The so-called instinctive behavior is produced in that case. In the organization of system quanta of individually acquired behavior the mechanisms of learning and memory play an important role.

We distinguish successive and hierarchic quantization of behavior. Under successive quantization the satisfaction of one need is followed in time by the appearance of the other need and so on. Under hierarchic quantization the result of the action (satisfaction of the need) of one system quantum is delayed in time to the future and in order to achieve the result it is necessary to perform a number of successive system quanta and hierarchically changing each other.

GENERAL SYSTEM QUANTA CHARACTERISTICS

Specific physical and chemical processes determine metabolic need and its satisfaction and in this way form energy basis of any system quantum of a living organism. Besides, every system quantum is also characterized by information properties.

Information properties of every system quantum are closely related to the initial need and its satisfaction.

It is possible to postulate information equivalent of the need and its satisfaction. Information equivalent of need is formed in all cases when a deviation occurs of the result from its value that is optimal for life maintenance. The information of the need is preserved at all levels of system quanta regardless of the change of physical and chemical processes representing the need. Different nervous and humoral processes, excitation of special nervous centers and formation of behavior are processes that also include all significant information for the need. Two specific main processes — the information on need and its satisfaction — are compared on special brain structures, which constitute an information screen. These screens are based on processes of imprinting and corresponding structures of acceptor of action results and include outstripping replication of information on the need on the structures of acceptor of action results.

The subjective core of any system quanta is reflected in these information screens. Estimation of the information on the need and its satisfaction is intrinsic to all life activities from information molecules to integrative processes: irritability, emotional sensations and verbal language in human mental activity. They also take part in different stages of evolutionary development of living beings.

A leading information signal in behavioral system quanta is emotion. Negative emotions accompany the needs, positive emotions accompany the satisfaction.

Under repeated and sometimes single satisfaction of the need positive emotions begin to be foreseen in advance even when the need arises.

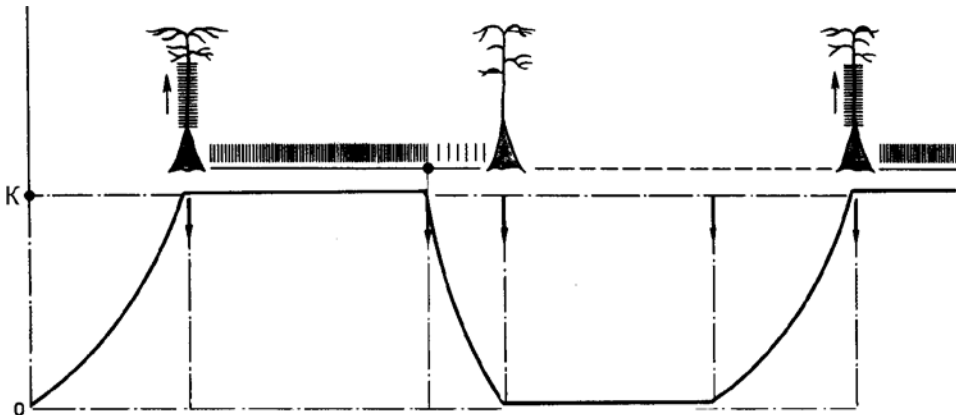


Fig. 2 — Trigger mechanism of excitation of neuron in the motivation center of hypothalamus. It is shown, that previously silent hypothalamic neuron starts to generate pulses only when its excitability achieves a critical level (K) under the influence of an increasing humoral need. Neuron pulses stop when its excitability decreases to an initial level when the need is satisfied. Pulse activity of neuron arises again at the subsequent formation of need and increasing of excitability of neuron up to a critical level.

Trigger mechanisms. The activity of system quantum originates after excitability of elements forming it achieves certain critical level (Fig. 2). Activity of system quanta proceeds until the initial need is satisfied.

The most investigated are the trigger mechanisms of system quanta of behavior. Biological motivations originating on the basis of these system quanta are built on the trigger principle (20).

The wave and particle properties of system quantum. Any system quantum may be characterized by the properties of a particle or a wave. Thus system quantum can be considered as a discrete unit (“particle”) of a continuum of a life span. On the other hand, the trigger mechanism determines wave properties of any system quantum.

Torsion mechanism in system quanta activity. The torsion mechanisms are known to be produced by the rotator spin moments of interacting particles. Spin moment corresponds to the direction of the spin, which can change its direction when new information influences molecular environment of the particles.

Two opposite tendencies continually operate in every system quantum. One is revealed at the increase of the value of the result, the other one — at its decrease. The first acts to decrease the value of the result to a normal level, the other — to increase it.

When initial need is formed all components of the organism, united by a dominating motivation, start to organize behavioral system quantum, directed to the search of the substances, satisfying the need. At the achievement of the required result the reinforcement occurs, the motivation disappears, the activity is reduced and subjects quite often get relaxed and may fall asleep.

Holographic principle in organization of system quanta. As is well known, in optics, a holographic principle was discovered by Dennis Gabor. Creating a hologram, a light wave is normally split by a special prism into two waves. One is a supporting wave, and the other, a subject wave, reflected from the object to be photographed. To reconstruct a hologram, only a supporting beam is sufficient.

Taking into consideration the analogy with a physical hologram one can consider the need as an information reference (supportive) wave. On the basis of motivation that wave forms an acceptor of action results performing the prediction of required result. Multi channel reverse afferentation from achieved results may be represented as a signal (subject) wave (22) (Fig. 3).

Dominating motivation is a basic factor for brain hologram retrieval (23). Dominating motivation retrieves memory traces (engrams) from acceptor of action’s result containing the properties of the needed result and specific conditional stimuli and pathways previously contributing or inhibiting the achievement of adaptive results useful for the organism and satisfying the organism’s vital needs.

Various indicators of general herd needs operate as a reference (supportive) wave in zoo populations. The signal (subject) wave in this case is determined by signals from the achieved results of activity important to the herd.

Social environment play a decisive role in development of social needs of humans. These needs include absent in animals human motivations, such as

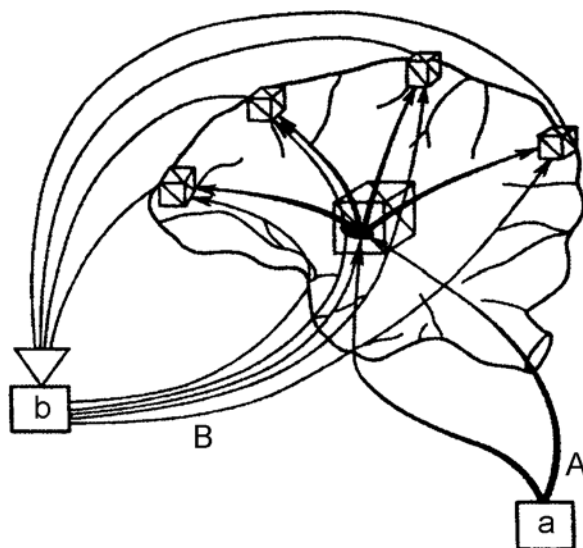


Fig. 3 — The scheme showing analogies to holographic principle of mechanisms of formation and interaction of dominating motivation and a reinforcement on structures of a brain. A — “reference” nervous and humoral indicators of the need; B — the “signal” from the reinforcement; a — metabolic need; b — reinforcement. Afferentation about metabolic need acts in analogy to a physical “reference” wave. Afferentation about parameters of result acts as a “signal” wave.

aspiration for the general or special education, working activity, creativity and even self-sacrifice in the name of public interests etc. Social needs of individuals substantially change their biological needs, socially tinting them and play the role of a supportive wave.

A signal (subject) wave is provided by the information from receptors signaling the parameters of events in social environment.

The accumulated individual and public knowledge, laws, morals, ethics forms a reference wave in social populations of people. A signal wave arises from individual and public work of institutes, governmental organs, industrial and agricultural production establishments, etc.

Information waves corresponding to the need and its satisfaction are distributed to structures of the brain in the certain time sequence. The conditions for their interference on brain structures and for construction of holograms are created in such a way. Thus a brain holographic screen of the information is formed.

These brain holographic screens are constructed on the structures of the acceptor of action results of different functional systems. Molecular engrams are built on the acceptor of action results with the properties of reinforcing events. The information from the parameters of basic needs and the results achieved comes exactly to these engrams.

The holographic information screens in the brain structures are presented by plasmatic membranes and liquid crystals — molecules of DNA and RNA of glial and neural cells. They compose acceptors of action results of various functional systems. Information models of the reality are continually built with

the structures and functions of the brain in advance of overt behavior, due to information signals about needs and their satisfaction.

Prediction of the required results and their assessment in system quanta of behavior. Prediction of properties of the required results is realized on information screens at all mentioned levels of system quanta. Prediction reactions in the brain are related to the corresponding activities of the acceptors of action results of different functional systems. Processes of afferent synthesis activate the engrams of an acceptor of action results, outstripping the real events, at brain holographic screens. These brain holographic screens predict future results and continually control both various needs of the organism, and their satisfaction. The inherited neuron structures and mechanisms as well as the acquired mechanisms of learning and memory underlie the prediction of metabolic, homeostatic and behavioral results.

Programming of the properties of the required results can be carried out rigidly, for example, in system quanta of instinctive and autonomic activity, or flexibly in cases of the skills acquired in the individual life. The acceptor of action results is continually enriched with information from parameters of the achieved results and ways of their achievement.

System quanta of human and animal behavior are organized through interaction of a dominating motivation with reinforcement, satisfying or, on the contrary, not satisfying initial needs of the organism. An acceptor of action results is the main point of interaction of motivation and reinforcement in central organization of system quanta. It programs the properties of required results. The continued estimation of parameters of results achieved by the organism is performed by acceptor of action results with the reverse afferentation.

NEUROPHYSIOLOGICAL AND NEUROCHEMICAL MECHANISMS OF LEADING MOTIVATION

The experiments (12, 22) testify to the fact that biological motivations in animals, caused by various metabolic needs such as hunger, thirst, fear, aggression, sex and so on are formed on the background of ascending activating influences of hypothalamic centers on other brain structures: limbic ones, thalamus, brain stem reticular formation including brain cortex and its frontal structures, in particular. Those influences are distinctly seen on animals' electroencephalograms under urethane anesthesia.

In a complicated cortical-subcortical architectonics of biological motivations a leading, pacemaker role belongs to motivation-genic centers of hypothalamus. Destruction of these structures completely eliminates their activating influence on other brain structures and corresponding biological motivations (16, 12).

Social motivations in humans are built by pacemakers localized not in hypothalamus but in other brain structures including frontal brain cortex (5).

It was found that in the process of forming dominating biological motivations cortical neurons in their turn influence the pacemaker center of hypothalamus (specific descending activating and inhibitory influences). As a result

ascending and descending influences under dominating motivations develop into dynamic cortico-subcortical reverberating interactions that define the energy of dominating motivations. On that ground a retrieval of memory traces by dominating motivation occurs. In mechanisms of ascending activating influences of hypothalamic structures on other brain regions different neurotransmitters and neuropeptides participate in various integrative correlations. Dynamic integration of chemical mechanisms of various brain structures on different stages of animals' resultant activity was revealed as well (1, 14, 15).

Oligopeptides stimulating and inhibiting biological motivations were also discovered.

It was shown that oxytocin microinjection into lateral hypothalamus in fed rabbits stimulated an additional food intake. β -lipotropin injection into lateral ventricles in fed animals caused activation of instrumental food-searching behavior and additional food intake. The same effect was observed under ACTH₁₀₋₁₄ and MSH₄₋₇ intracerebroventricular injections.

Pentagastrin intra-abdominal injection activated feeding behavior in fed rabbits while microiontophoretic application to neurons of lateral hypothalamus in fed rabbits caused a pattern of pulse-to-pulse activity typical for hunger state; ACTH₅₋₈ application activated self-stimulation in rabbits. Bradykinin injection into cerebral lateral ventricles facilitated defense reactions in rabbits under electrical stimulation of ventromedial hypothalamus.

Additional intra-cerebral lateral ventricular injection of angiotensin II, arginine-vasopressin, delta-sleep-inducing peptide, substance P, lei- and met-enkephalins, β -endorphin suppressed defense and feeding reactions and self-stimulation caused by electrical stimulation of ventromedial and lateral hypothalamus.

It was shown that under cerebral lateral ventricular injection of anti-serum to β -endorphin, angiotensin II and delta-sleep-inducing peptide double-stage effects were observed: for the first hours — a suppression of biological motivations and their facilitation on the next day (11, 23).

Motivations are formed by the principle of dominant (25). In every given moment of time a dominating social or biological motivation, caused by the need, the most significant for survival or adaptation, governs the brain. The rest subdominant motivations support or inhibit the dominant one. When the leading motivation is satisfied, sub-dominant motivations become dominating hierarchically.

Special experiments showed that dominating motivation significantly changed convergent and discriminating properties of brain neurons increased their sensibility to neurotransmitters, neuropeptides and other biologically active substances. Neuron sensitivity to reinforcing factors satisfying the initial needs significantly widens. Besides, dominating motivation increases sensitivity of the corresponding peripheral receptors (4, 7).

Different dominating biological motivations (hunger, thirst and fear) are distinctly reflected in the character of distribution of dominating inter-spike intervals of single unit activity in various brain structures. Most of the neurons of various brain structures in rabbits that are in need of food, water or avoidance of danger generate a burst like impulse activity. Typical inter-spike in-

tervals can be registered in these neurons. After a 24-hours food deprivation, for example, neurons with burst activity in sensomotor, visual, insular cortex, n. caudatus, dorsal hippocampus, lateral hypothalamus and reticular formation of midbrain have a dominating bimodal distribution of inter-spike intervals within 1—20 and 150 ms. After a 24-hours water deprivation in structures of dorsal hippocampus, septum, n. caudatus, lateral and supraoptical nuclei of hypothalamus on the background of neurons activity also two modes of inter-spike intervals dominate, but within 25 and 150 ms.

Under electrical stimulation of “fear center” of ventromedial hypothalamus in immobilized animals neurons of sensomotor and visual cortex show domination of inter-spike intervals within 45 and 150 ms (26).

Under the influence of dominating motivations the activation of *c-fos* and *c-jun* early gene expression in brain structures was revealed (2).

It is significant that neurons involved into dominating biological motivation despite their generalized localization in brain structures make up only a certain number of common neuron populations of brain structures. The part of neurons involved in dominating biological motivation is significantly higher in brain stem and gradually decreases in direction to the cerebral cortex. Cortical neurons in their adaptive behavior fulfill other not less significant functions: they react on conditioned stimuli, location and migration of reinforcing external agents and so on (9). Unlike the structures involved in architecture of genetically determinate biological motivation the most of the brain cortical neurons fulfill plastic adaptive functions of learning and estimation of the factors of the environment by subjects.

The experiments mentioned above testify to the fact that dominating motivations change the properties of brain structures and respective peripheral receptors making them selectively perceive and interact with factors that satisfy the needs underlying these motivations. As a result under the effect of a dominating motivation a peculiar informational constellation of neurons of different brain structures is formed able to perceive reinforcing influences.

SYSTEM MECHANISMS OF REINFORCEMENT: INTERACTION OF MOTIVATION AND REINFORCEMENT OF BRAIN STRUCTURES

From functional system theory position the processes of reinforcement include: the impact of different parameters of behavioral results on the receptors of the organism, which experiences a definite need and their comparison by reverse afferentation with the acceptor of action's result.

Acceptor of action's result of every functional system presents a mosaic architectonics, widely spread along different cortical and subcortical brain structures.

In line with the hypothesis, proposed by P. K. Anokhin, a structural basis of acceptor of action's result in functional systems defining various behavioral acts in humans and animals consists of interneurons of different brain structures on which copies of effector excitations of cortical pyramidal neurons are spread along pyramidal tract collaterals (Fig. 4).

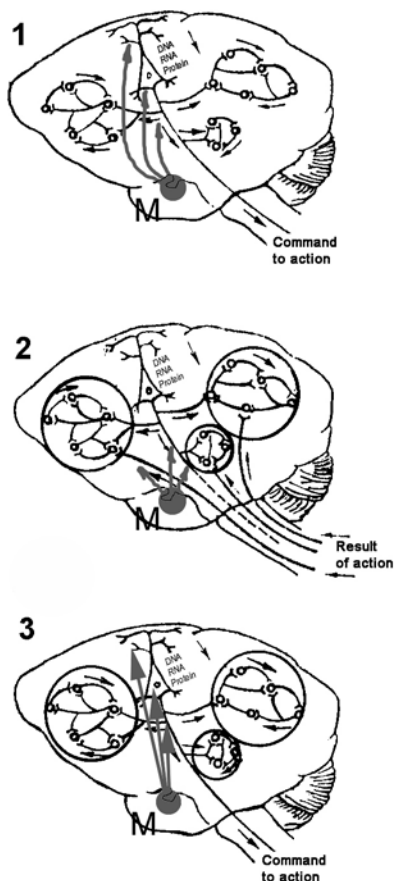


Fig. 4 — Multilevel architectonics of acceptor of action's results in brain

1. Under the influence of a dominant motivation (M) effector stimuli of pyramidal neurons are spread on axons of a pyramidal tract. At the same time copies of these stimulations through collaterals of a pyramidal tract are spread on intercalary neurons of different brain structures which are cyclically interacted.
2. Stimulations from the parameters of the achieved results are "imprinted" on intercalary neurons which constitute the acceptor of action's result.
3. Motivation in advance stimulates structures of acceptor of action's results earlier formed by afferentation from different parameters of the achieved results.

Due to cyclic interactions between interneurons associated into acceptor of action's result, excitations in these neurons by reverberation are able to remain for a long time and to continuously evaluate the reverse afferentation coming to them from different parameters of the results achieved by the subjects. The confirmation of the spread of pyramidal tract stimuli on interneurons was found in special experiments where reactions of interneurons of different brain structures in response to antidromic stimulation of the central end of pyramidal tract cut in the olive of medulla were investigated.

Under antidromic stimulation of the pyramidal tract responses of neurons were registered in somato-sensory, visual cortex and dorsal hippocampus. The same neurons vividly reacted on stimuli of different sensory and biological modality and on stimulation of motivation-genic centers of hypothalamus. All that shows that on the brain inter-neurons that constitute the acceptor of action's result activated by a dominant motivation, sensory information coming from various organism's receptors excited by relevant parameters of reinforcing influences, can be "imprinted".

In the processes of successive change of system quanta a dynamic architectonics of acceptor of action's result is formed, reflecting on informational grounds the chain of actions and results leading to the satisfaction of the relevant need.

Every parameter of the reinforcing effect leaves its own specific information trace in the corresponding visual, taste, auditory, tactile etc. projection brain zones, determining in this way a generalized in various brain structures architectonics of acceptor of action's result. At that if genetic components of acceptors of action's result of different functional systems are conservative the architectonics of the acceptors of action's results dynamically changes during individual's life span according to the changes in the parameters of the reinforcing influences. Every reinforcement, as a part of a many-sided activity leaves its own information imprint on the acceptor of action's result structures of a corresponding functional system.

Under stable conditions and the same types of reinforcement the "imprints of reality" on the structures of relevant acceptors of actions result from stereotypes able to maintain for a long time and sometimes the whole individual's life. In changing life conditions "imprints of reality" are unstable and quickly change each other. "Imprints of reality" on the structures of acceptors of action result in different functional systems direct human or animal behavior to the optimal satisfaction of the vital needs playing the role of a certain vector of behavior.

As the central units of many functional systems of homeostatic and behavioral level converge on the brain structures, a generalized acceptor of action's result is formed on which every given moment anticipatory (forthcoming) reactions of a dominating functional system are revealed.

Reinforcing stimuli are addressed just to the brain neurons which initially are involved into dominating motivation and in their discharge activity reflect a specific for the given motivation character of inter-spike intervals distribution.

Reinforcement (food and water intake, danger avoidance) significantly changes brain neuron activity involved into the initial motivation. At that, neuron burst-like activity changes into a regular one with domination of only one inter-spike interval (13, 17, 26) (Fig. 5).

It has been found that under reinforcement an increased *c-fos* and *c-jun* early genes expression, observed in motivations and accompanying them searching activity, decreases, and expression of late genes determining processes of cell differentiation, adherence and memory, appears (2).

A series of experiments shows that the animals' behavior conditioned by early gene expression under electrical stimulation of hypothalamus motivation-genic centers is not blocked by protein synthesis inhibitors such as cycloheximide, puromycin, actinomycin D and others. However, under training when behavior initiated by motivation ends with the achievement of the needed results that is the formation of functional systems, the mentioned protein synthesis inhibitors block this behavior.

Pentagastrin on the background of protein synthesis inhibitors restores a feeding behavior, bradykinin — a defensive one, ACTG₄₋₁₀ — a self-stimula-

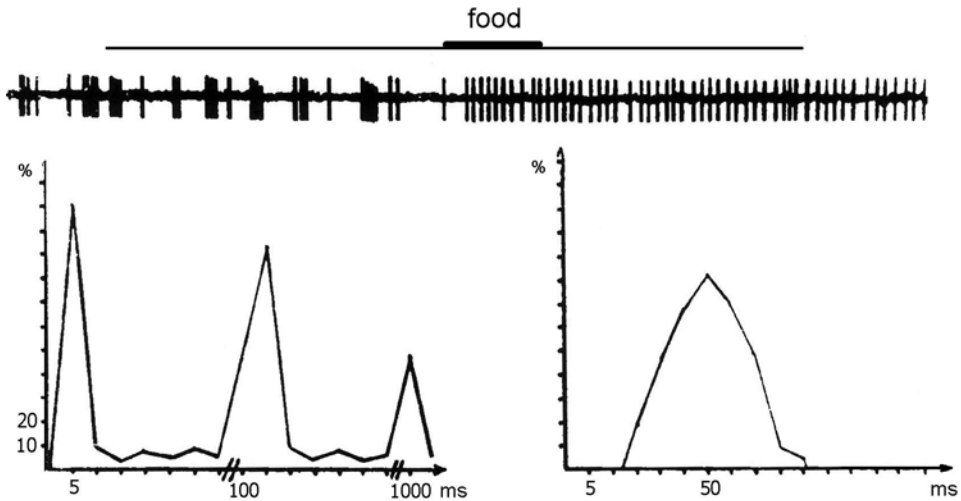


Fig. 5 — Changes of neuron impulse activity of sensorimotor cortex in hungry rabbit after food intake

The moment of food intake is shown by bold line. 3 peaks of interspike intervals in pattern of a neuron activity of a hungry rabbit distribution of interspike intervals of a neuron after a rabbit's feeding. On X—line — the value of interspike intervals (ms). On Y-line — percentage of the intervals from the general number of the examined intervals.

ting behavior. At these responses of single neurons to stimulation of brain motivation-genic centers are restored as well (18).

Inhibitors of DNK synthesis — azidothymidine and amidothymidine also suppress a trained behavior blocking selectively behavioral molecular engrams and anticipation of useful adaptive results by animals.

So under reinforcement on the structures of acceptors of action's results of system quanta of behavior molecular engrams are formed.

Imprinting mechanism of acceptor of action's result. As known, K. Lorenz was the first to discover imprinting phenomenon in newborn birds (6). He showed that subjects, that newborns saw first, in particular, the parents, were imprinted in their memory and defined their reaction to follow these subjects. But K. Lorenz and his followers in particular N. Tinbergen (24) did not answer the question: "What are the intimate mechanisms of imprinting and how long does this imprinting mechanism last in ontogenesis of living beings"?

Imprinting phenomenon has got a new understanding from the theory of functional systems point of view.

In 1978 we proposed an imprinting hypothesis of constructing acceptor of action's result.

According to the imprinting hypothesis of forming acceptor of action's result under the influence of the results of behavior their different parameters by reverse afferentation are imprinted as molecular engrams on the respective structures of acceptor of action's result. In that way acceptor of action's result in every functional system is formed and becomes enriched during the whole

individual's life by previous reinforcements and imprints of properties of parameters of the reinforcing effects on the structure of a dominating motivation.

The experiments of V. A. Pravdivtzev (8) confirmed the statement. While registering the impulse activity of inter-neurons in brain visual cortex the author elaborated in cat's conditioned-reflex reaction on combination of electrical stimulation of central end of cut pyramidal tract with electro-skin stimulation. These experiments showed that after 8—10 such tests the neurons began to respond to the conditioned stimulus of electrical stimulation of pyramidal tract by reaction typical for electro-skin stimulation.

As shown above the interaction of reverse afferentation from the parameters of the achieved behavioral results with the initial dominating motivation takes place.

The reinforcement forms significant memory traces — engrams of properties of parameters of reinforcing results. Every time at the next occurrence of similar need these engrams are exited in anticipation of future end results and act as a directing component of the corresponding behavior. The parameters of the achieved results are compared with the predicted features of future results — by acceptor of action's result and are evaluated by reverse afferentation. The estimation of parameters of intermediate and end results by the acceptor of action's result allows the correction of the future behavior when necessary.

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СИСТЕМ КВАНТУМИ КАО ДИСКРЕТНЕ ЈЕДИНИЦЕ ПОНАШАЊА

Константин Викторович Судаков

Истраживачки институт нормалне физиологије им. П. К. Анохина
Руска Академија медицинских наука, Москва, Руска Федерација

Резиме

Систем квантуми су специфични оператори у динамичкој активности функционалних система организма, које је открио П. К. Анохин. Под појмом „систем квантум” животне активности подразумевамо дискретне системске процесе који потичу од растуће потребе и трају до задовољења те потребе.

Сваки систем квантум понашања представља саморегулативну организацију која у себи садржи иницијалну потребу, мотивацију, архитектонику система, међурезултате и финалне резултате, као и континуирану евалуацију постигнутих резултата динамичком реконструкцијом акцептора резултата дејства. Систем квантуми могу да се организују на различитим нивоима животних активности. Ови нивои, у зависности од њихове хијерархије, протежу се од гена и функционалних система метаболичког и аутономног нивоа, све до зоо-социјалних популација животиња и људских заједница.

Сваки систем квантум такође се карактерише информацијама. Информације сваког систем квантума у тесној су вези са иницијалном потребом и задовољењем те потребе.

*Sanja T. Kerešić^{1, 2}, Đorđe R. Malenčić¹,
Milan T. Popović¹, Marija M. Kraljević-Balalić¹,
Jegor A. Miladinović², Aleksandar D. Ilić²*

¹ Faculty of Agriculture, University of Novi Sad
Trg D. Obradovića 8, 21000 Novi Sad, Serbia

² Institute for Field and Vegetable Crops
M. Gorkog 30, 21000 Novi Sad, Serbia

NITROGEN METABOLISM ENZYMES, SOLUBLE PROTEIN AND FREE PROLINE CONTENT IN SOYBEAN GENOTYPES AND THEIR F1 HYBRIDS

ABSTRACT: Nitrate reductase and glutamine synthetase activity, as well as free proline and soluble protein content were measured in eight soybean parent genotypes and six F1 hybrids. The aim of this study was to determine variability and the mode of inheritance for these parameters, and point out the genotypes of interest for future breeding programs. Analysed genotypes and their F1 hybrids expressed significant differences in activities of nitrate reductase and glutamine synthetase enzymes, as well as in soluble proteins and free proline contents. Since mode of inheritance for all investigated traits was in most cases dominance or heterosis, it can be concluded that these parameters are under control of dominant genes. The obtained results suggest that genotypes with favorable traits, such as variety Linda, line 1511, and F1 hybrids (Linda x LN92-7369) and (Balkan x BL-8), could be of interest as a good starting material for further breeding programs.

KEY WORDS: Nitrate reductase, glutamine synthetase, free proline, soluble protein, inheritance, soybean

INTRODUCTION

The assimilation and metabolism of inorganic nitrogen in plants is a complex process involving a series of enzymes. Nitrate is reduced to NH_4^+ by the reaction of nitrate reductase (NR) and nitrite reductase (NiR). The conversion of NH_4^+ into glutamate proceeds *via* two pathways. In the GS/GOGAT pathway, NH_4^+ is incorporated into glutamine by glutamine synthetase (GS), which is then converted with 2-oxoglutarate (2-OG) to glutamate by glutamate synthase (GOGAT). Glutamate dehydrogenase (GDH) catalyzes the incorporation of NH_4^+ into glutamate by reversible reductive amination of 2-OG (Inokuchi and Okada, 2001; Cruz et al., 2004).

The cytosolic NR is the first enzyme in the pathway of nitrate assimilation, and its activity is highly regulated. Sufficient NR activity is a prerequisite for optimal utilization of soil N. NR has a major role in incorporation of N for plant yields under field conditions and it is widely known to be substrate inducible (Bergareche and Simon, 1988).

GS is the key enzyme responsible for the assimilation and re-assimilation of ammonia. In higher plants GS is one of the major enzymes responsible for the assimilation of ammonium absorbed from the growth medium, generated by nitrate reduction or re-assimilated after release of endogenous NH_4^+ by ammonium-evolving processes such as photorespiration (Lea and Milfin, 1974; Fentem et al., 1983; Claussen and Lenz, 1999).

Glutamine is preferred as an N source in various biosynthetic reactions, to amino acids, purine and pyrimidine nucleotides, and other N-containing metabolites (Rao and Venkateswelu, 1986). Although NH_4^+ is the immediate substrate of GS its effect on GS is still a matter of debate. Genes of cytosolic GS 1 were found to be NO_3^- , but not NH_4^+ , inducible in some plant species, while in some other the GS 1 promoter was NH_4^+ -inducible. Similar discrepancies are reported for chloroplastic GS 2. Conflicting results are also reported on the effect of NH_4^+ on GS activity that has been found to be stimulatory or inhibitory. Accumulation of GS product glutamine acts as one of the signals for NR inactivation.

Prolonged water shortages affect practically all metabolic processes and often result in severe reductions in plant productivity. One of most common responses in plants undergoing water deficit is production and accumulation of amino acid proline in its free form. A strong correlation has been established between increased cellular proline levels and the capacity to survive water and high salinity stress as well, in barley (Singh and Paleg, 1972), maize (Pinter et al., 1979) and sugar beet (Štajner et al., 1995).

In higher plants, proline can be synthesized by two possible pathways using either glutamate or ornithine as precursor (Armengaud et al., 2004). Regulation of the glutamate pathway is well documented and has been shown to be the predominant pathway in response to osmotic stress.

The aim of this study was to determine the mode of inheritance for the activity of nitrogen assimilating enzymes, NR and GS, and free proline and soluble protein content in soybean parent genotypes and their F1 hybrids.

MATERIAL AND METHODS

Plot area utilized for this experiment was located on the experimental fields at the Institute of Field and Vegetable Crops in Novi Sad. The experimental design was a randomized, complete block with four replications.

Biochemical analyses were performed at the Biochemical laboratory of the Faculty of Agriculture in Novi Sad. Eight soybean genotypes and six of their F1 hybrids were chosen, and plant leaves were used for the analysis. The samples were taken in R1 period, at the beginning of plant flowering.

The activity of NR and GS was determined according to C o o m b s and H a l l (1982). NR activity was expressed in $\mu\text{M NO}_2^- \text{ g}^{-1}$ fresh matter h^{-1} , and GS activity was calculated from the hydroxamate content produced and expressed in $\mu\text{M } \gamma\text{-GH } \text{g}^{-1}$ fresh matter h^{-1} . The activity of the enzymes was monitored spectrophotometrically at 540 nm.

Soluble proteins were extracted from soybean laves with 0.1 M phosphate buffer, pH 7, and the protein content was determined according to L o w r y et al. (1951), using bovine serum albumin as standard, and expressed as mg proteins g^{-1} fresh matter.

Free proline content was determined according to B a t e s et al. (1973), and expressed as $\mu\text{g proline } \text{g}^{-1}$ fresh matter.

Each measurement was performed in triplicate.

Statistical evaluation was performed using software Statistica, Version 7.0. The experiments were repeated three times, and differences between genotypes were determined using LSD test for 0.05 significance level. The mode of inheritance was determined compared to mean parents value, for 0.05 significance level (Š t a j n e r et al., 1993, K o b i l j s k i and D e n č i ć, 1999).

RESULTS AND DISCUSSION

The analysed genotypes and their F1 hybrids differed significantly in enzyme activities, as well as in soluble proteins and free proline content (Tab. 1).

The highest activity of NR was recorded in line 1511, while the lowest was in Lori. Mode of inheritance for this trait was dominance, either positive or negative, in all analysed crosses (Tab. 1).

NR gene expression and activity can be influenced by many factors, such as light and temperature (J i a n g and H u l l, 1998). In this work, these environmental factors should have no significant effects on comparison of NR activity because they were essentially the same for all experimental groups. The level and the distribution of activity of NR between the different plant organs, however, vary among species apparently due to their natural habitat and are also dependent upon the development stage of the plant (G h o s h et al., 2004).

The highest NR activities were expressed in lines 1511 and 1581/99, both grown in genotype collection for their high seed protein levels. Obtained results support the positive correlation previously established between NR activity and protein level in soybean seed (M i l a d i n o v i ć et al., 1996). As the values for the NR activity could serve as an indicator of the nitrogen state in plants and a biochemical criterion in the selection of protein rich cultivars, we can assume that F1 hybrid (1499/99 x 1511), with its high NR activity, will also have high protein content, and recommend it for future studies.

Tab. 1 — Average values and mode of inheritance for NR and GS activity and soluble protein and free proline content in soybean parent genotypes and F1 hybrids

PARENTS AND F1 HYBRIDS	NITRATE REDUCTASE ($\mu\text{mol NO}_2^- \text{ g}^{-1} \text{ h}^{-1}$)	GLUTHAMINE SYNTHETASE ($\mu\text{M } \gamma\text{-GH } \text{ g}^{-1} \text{ h}^{-1}$)	SOLUBLE PROTEINS (mg protein g^{-1} fresh matter)	PROLINE CONTENT ($\mu\text{g proline } \text{g}^{-1}$ fresh matter)
LN92-7369	0.182	59.53	14.19	0.522
1581/99	0.939	62.51	15.92	0.286
1511	1.352	40.68	16.33	0.306
1499/99	0.491	49.61	15.92	0.375
Lori	0.074	58.05	16.66	0.468
Linda	0.159	90.05	18.21	0.442
Balkan	0.848	44.15	15.06	0.266
BL-8	0.753	59.04	16.12	0.404
LN92-7369x 1581/99	0.272 ^{d-}	57.30 ^{d+}	16.06 ^{d+}	0.313 ^{d-}
1499/99 x 1581/99	0.654 ^{d-}	70.45 ^{h+}	17.16 ^{h+}	0.322 ⁱ
1499/99 x 1511	1.013 ^{d+}	49.61 ^{d+}	14.11 ^{h-}	0.316 ^{d-}
Lori x LN92-7369	0.184 ^{d+}	57.80	17.85 ^{h+}	0.276 ^{h-}
Linda x LN92-7369	0.165 ^{d+}	154.73 ^{h+}	17.88 ^{d+}	0.531 ^{d+}
Balkan x BL-8	0.846 ^{d+}	156.03 ^{h+}	18.84 ^{h+}	0.571 ^{h+}

i = intermediate inheritance
d = dominant inheritance
h = heterosis occurred

The variety Linda had the highest, and the line 1511 the lowest GS activity. In F1 hybrids, the cross (Linda x LN92-7369) had the highest, and (1499/99 x 1511) the lowest GS activity. The mode of inheritance for this trait was positive dominance or heterosis (Tab. 1).

The content of soluble proteins in leaves of soybean genotypes investigated also varied significantly. Variety Linda had the highest, and the line LN92-7369 the lowest protein content among parents, while in F1 generation the highest level of soluble proteins showed F1 hybrid (Balkan x BL-8), and the lowest (1499/99 x 1511). The mode of inheritance was dominance, or, in the most cases, heterosis (Tab.1).

The free proline content was the highest in parent genotypes LN92-7369, Lori, and Linda, and the lowest was in Balkan and 1581/99 genotypes. In F1 generation, however, the highest proline content had (Linda x LN92-7369) and (Balkan x BL-8) hybrids, which expressed dominant and heterotic mode of inheritance, potentially and could be used in further breeding programs as potentially drought resistant lines.

CONCLUSION

Our study showed that analysed genotypes and their F1 hybrids expressed significant differences in NR and GS enzyme activities, as well as in soluble proteins and free proline content. Since mode of inheritance for all investigated traits was in the most cases dominance or heterosis, it can be concluded that these parameters are under control of dominant genes. The obtained re-

sults suggest that genotypes with favorable traits, such as variety Linda, line 1511, and F1 hybrids (Linda x LN92-7369) and (Balkan x BL-8), could be used in breeding drought more tolerant genotypes with higher nitrogen-fixing capacity and high protein content.

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АКТИВНОСТ ЕНЗИМА МЕТАБОЛИЗМА АЗОТА,
САДРЖАЈ РАСТВОРЉИВИХ ПРОТЕИНА И ПРОЛИНА
У ГЕНОТИПОВИМА СОЈЕ И ЊИХОВИМ F1 ХИБРИДИМА

Сања Т. Керечи^{1, 2}, Борђе Р. Маленчић¹, Милан Т. Поповић¹, Марија М.
Краљевић-Балалић¹, Јегор А. Миладиновић¹, Александар Д. Илић²

¹ Пољопривредни факултет, Универзитет у Новом Саду,
Трг Д. Обрадовића 8, 21000 Нови Сад, Србија

² Институт за ратарство и повртарство, М. Горког 30,
21000 Нови Сад, Србија

Резиме

У раду је одређена активност ензима метаболизма азота нитрат редуктазе и глутамин синтетазе, као и садржај растворљивих протеина и слободног пролина у осам родитељских генотипова и шест F1 хибрида. Циљ истраживања је био да се утврде варијабилност наведених генотипова за одабране параметре и начин њиховог наслеђивања. Установљено је постојање статистички значајних разлика између генотипова, како у погледу ензимске активности, тако и у количини растворљивих протеина и слободног пролина. Будући да су се ова својства наслеђивала претежно доминантно, уз појаву хетерозиса, може се претпоставити да су под контролом гена са доминантним дејством. Као генотипови са повољним особинама издвојили су се родитељска сорта Linda и линија 1511, и хибриди (Linda x LN7369) и (Balkan x BL-8), који би могли послужити у узгоју генотипова соје повећане толерантности на сушу са повећаним капацитетом за усвајање азота и садржај протеина.

Rudolf R. Kastori^{1, 2}, *Radovan Z. Marinković*²,
*Petar Đ. Sekulić*², *Ivana V. Maksimović*^{1, 2},
*Mira M. Pucarević*¹

¹ Faculty of Agriculture, Trg Dositeja Obradovića 8, Novi Sad, Serbia

² Institute of Field and Vegetable Crops, Maksima Gorkog 30, Novi Sad, Serbia

GENETIC SPECIFICITY OF MAGNESIUM NUTRITION IN SUNFLOWER*

ABSTRACT: Magnesium content was analysed in five of the most grown sunflower hybrids in Serbia, as well as in different populations of wild sunflower species: *Helianthus agrophyllus* (5), *Helianthus annuus* (4), *Helianthus neglectus* (3), *Helianthus petiolaris* (5), *Helianthus tuberosus* (5). Magnesium content in the populations of wild sunflower species ranged from 317 to 824 mg/100 g DW. The highest magnesium content was found in *Helianthus petiolaris* and the lowest in *Helianthus tuberosus*.

Different populations within each species differed significantly in magnesium content. The highest variation coefficient was found in *Helianthus tuberosus* and the lowest in *Helianthus petiolaris*.

Magnesium concentration in hybrids was significantly different as well, and in two years it was in average between 575 and 813 mg/g DW.

The results suggest that genetic variability between magnesium concentrations in wild species and hybrids of sunflower is very high. This should be taken into consideration when requirements for mineral nutrition are analysed, as well as when wild species are included in breeding programs.

KEY WORDS: sunflower, hybrids, wild sunflower species, magnesium content

INTRODUCTION

Sunflower mineral nutrition plays an important part in the effective utilization of sunflower yield potential and oil quality. Numerous research results indicate that various plant species and their genotypes are specific with respect to nutritional requirements (E p s t e i n, 1972; K l i m a š e v s k i, 1974; S a r i ć, 1981). Their specificity refers to the temporal dynamics of nutrient uptake,

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plant sensitivity to nutrient shortage or excess, nutrient distribution in plants, etc. (Kastori, 1983). The knowledge of specific crop requirements for nutrients is important both theoretically and practically. It allows farmers to optimize plant mineral nutrition, which in turn makes room for maximum utilization of genetic yield potential of various crops and their genotypes.

Magnesium concentration in plants is under the influence of many biotic and abiotic factors such as: phase in ontogeny (Robinson, 1970, 1975), leaf part (Krstić et al., 1988), plant species (Kiss et al., 2005), the number of plants per surface area, fertilization and crop rotation (Kovačević, 1985; Kastori et al., 2004), growing sites (Kiss et al., 2001). According to the results presented in the above mentioned papers, it can be concluded that different plant species and genotypes differ in magnesium accumulation depending on ecological conditions and applied technology of production, which is important when their specific needs for magnesium nutrition are considered.

In the breeding of sunflower and the other crops, “distant hybridization” or crossing with wild relatives allowed the development of new genotypes resistant to unfavorable biotic (diseases, insect pests) and abiotic (e.g. shortage or excess of nutrients, drought, high concentration of salts) factors. To facilitate the use of wild relatives, it is advisable to know their biological properties including specific aspects of their mineral nutrition. Sunflower (*Helianthus* sp.) has numerous wild relatives which differ significantly by their morphological and other traits (Rogers et al., 1982).

Because sunflower is behind the other crops with respect to the available knowledge of genetic requirements for magnesium in its cultivated and wild varieties, we decided to study these problems.

MATERIALS AND METHODS

The plants were grown on weakly calcareous Chernozem of good physical and chemical properties (Tab. 1). At flowering, completely developed upper leaves, which are physiologically the most active, were taken for the analyses (Ćupina i Sakač, 1989). Magnesium content was analysed in five of the most grown sunflower hybrids in Serbia, as well as in different populations of wild sunflower species from Northern America: *Helianthus neglectus* (3), *Helianthus petiolaris* (5), *Helianthus agrophyllus* (5) *Helianthus tuberosus* (5) and *Helianthus annuus* (5). Magnesium concentration was determined by ICP. The data were statistically processed by analysis of variance, calculation of the least significant difference (LSD) and coefficient of variation using Statistica 7 Computer program.

Tab. 1 — Basic soil properties

Depth (cm)	pH		CaCO ₃ %	Humus %	Total N (%)	AL-P ₂ O ₅ mg/100 g	AL-K ₂ O
	In KCl	In H ₂ O					
0—30	7.19	8.22	2.76	1.96	0.154	15.0	24.1
30—60	7.37	8.26	4.56	1.95	0.142	6.6	20.0

RESULTS

Magnesium concentration in different wild sunflower species and different populations of each species are shown in the Figs. 1—5. The results suggest that analyzed wild sunflower populations differ significantly not only in their morphological features (Rogers et al., 1982), but also in their chemical composition, namely magnesium concentration. These results are in accordance with those of Krstić and Sarić (1987) who also found significant differences in chemical composition between different wild sunflower species with respect to nitrogen, phosphorus and potassium content. The highest magnesium concentration was found in *Helianthus petiolaris*, and the lowest in *Helianthus tuberosus* (Tab. 2). Different populations within each species differed significantly in magnesium content (Figs. 1—5). Magnesium concentration in analysed populations of *Helianthus neglectus* ranged between 624 and 809, in *Helianthus petiolaris* between 684 and 824, in *Helianthus agrophyllus* from 361 to 452, in *Helianthus annuus* between 542 and 694 and in *Helianthus tuberosus* between 317 and 374 mg Mg/100 g DW. The highest variation coefficient between populations within the same species was found in *Helianthus tuberosus* (4.7 mg Mg/100 g DW), the species that had the lowest magnesium concentration, while and the lowest variation coefficient was in *Helianthus petiolaris* (2.4 mg Mg/100 g DW), species that had the highest magnesium content. Magnesium content was analysed in five of the most grown sunflower hybrids in Serbia. In the two year study period magnesium concentration in hybrids varied from 575 in NS-H-111 to 813 mg Mg/100 g DW in NS-H-Krajišnik (Tab. 3).

Between the two years of study, average magnesium concentration in hybrids was not significantly different. In hybrids, average magnesium concentration was much higher than in wild species (695 compared to 561 mg/100 g DW), suggesting that during the breeding process magnesium utilization by plants was improved and its accumulation increased. Differences in magnesium concentration in sunflower hybrids were discovered earlier (Gachon, 1972).

The results suggest that genetic variability between Mg concentrations in wild species and hybrids of sunflower is very high. This should be taken into consideration when requirements for mineral nutrition are analysed, as well as when wild species are included in breeding programs.

Tab. 2 — Average magnesium concentration in wild sunflower species (mg Mg/100 g DW)

Species	Mg concentration	Coef. of variation
<i>Helianthus agrophyllus</i>	408 ^C	4.50
<i>Helianthus annuus</i>	591 ^B	3.24
<i>Helianthus petiolaris</i>	741 ^A	2.42
<i>Helianthus neglectus</i>	727 ^A	4.06
<i>Helianthus tuberosus</i>	339 ^D	4.72
Average	561.2	3.79
LSD _{α = 0.05}		57.1

Tab. 3 — Magnesium concentration in sunflower hybrids in two years of study (mg Mg/100 g DW)

Hybrids	Year		Average
	2005	2006	
NS-H-111	599 ^D	552 ^D	575
NS-H-45	729 ^C	696 ^C	713
NS-H-Bačvanin	743 ^{BC}	704 ^C	723
NS-H-Velja	714 ^C	591 ^D	653
NS-H-Krajišnik	806 ^{AB}	820 ^A	813
Average	718	672	695
LSD _{α = 0.05}	67.5		

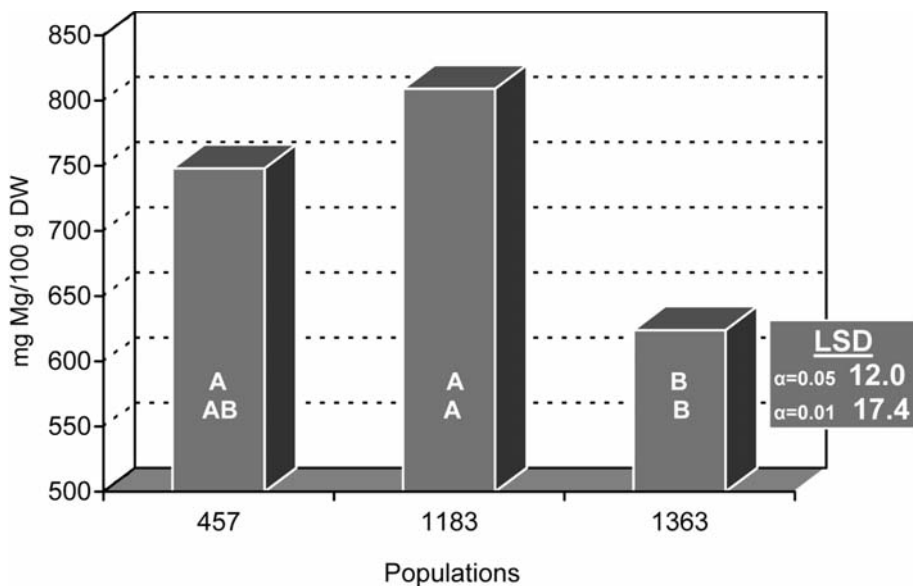


Fig. 1 — Magnesium concentration in different populations of *Helianthus neglectus*

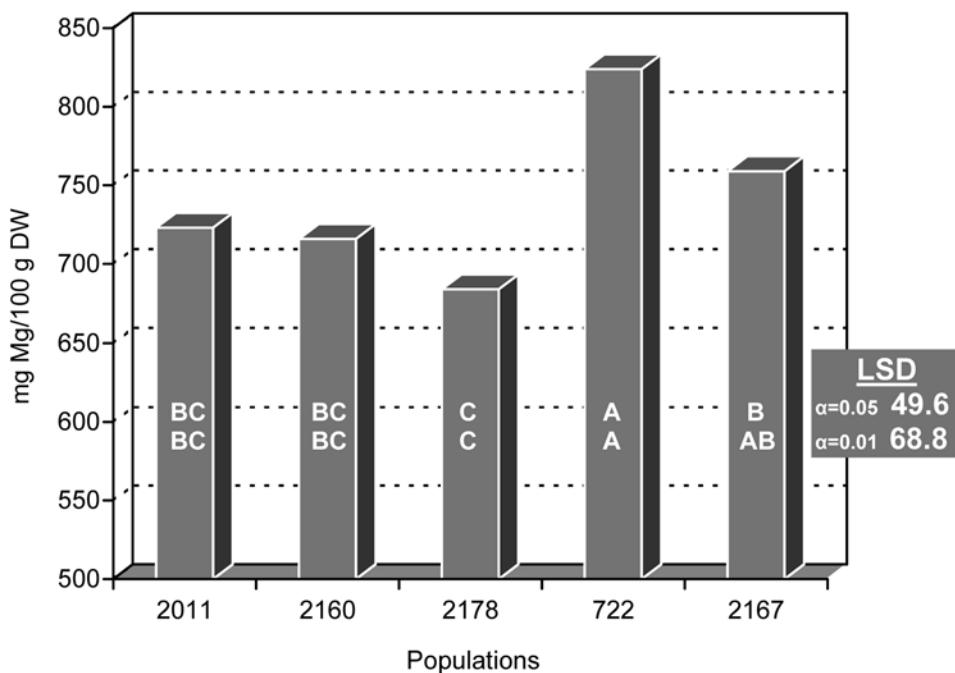


Fig. 2 — Magnesium concentration in different populations of *Helianthus petiolaris*

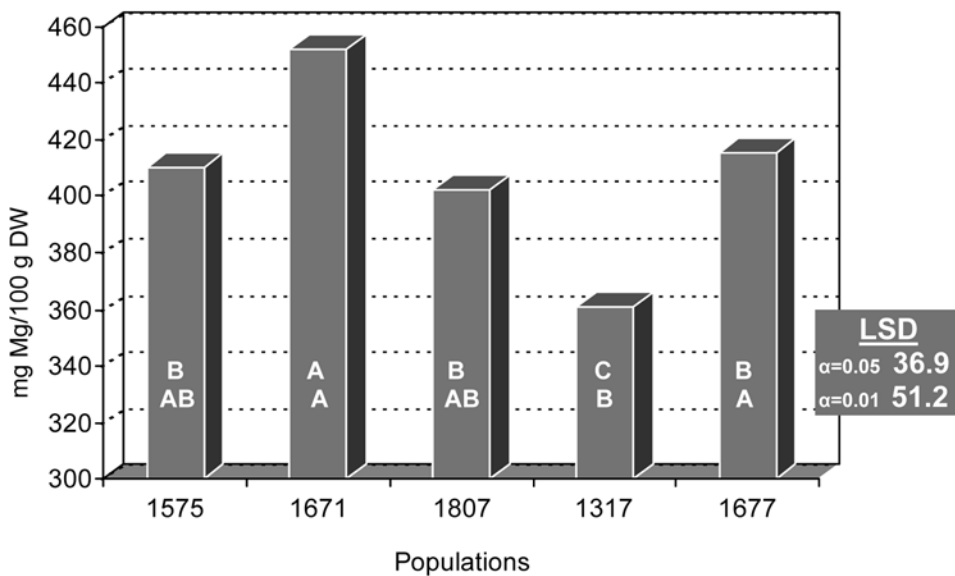


Fig. 3 — Magnesium concentration in different populations of *Helianthus agrophyllus*

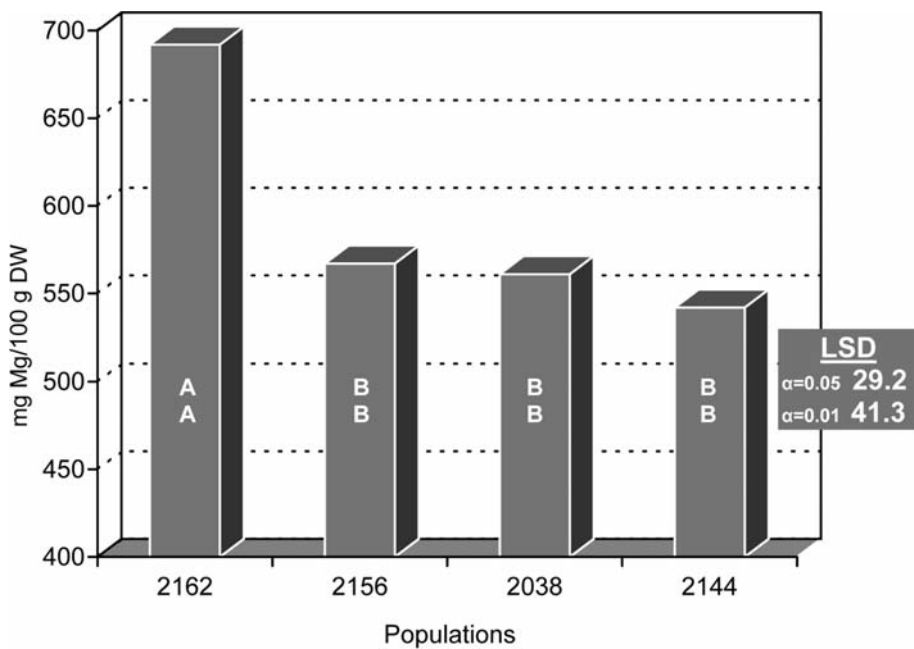


Fig. 4 — Magnesium concentration in different populations of *Helianthus annuus*

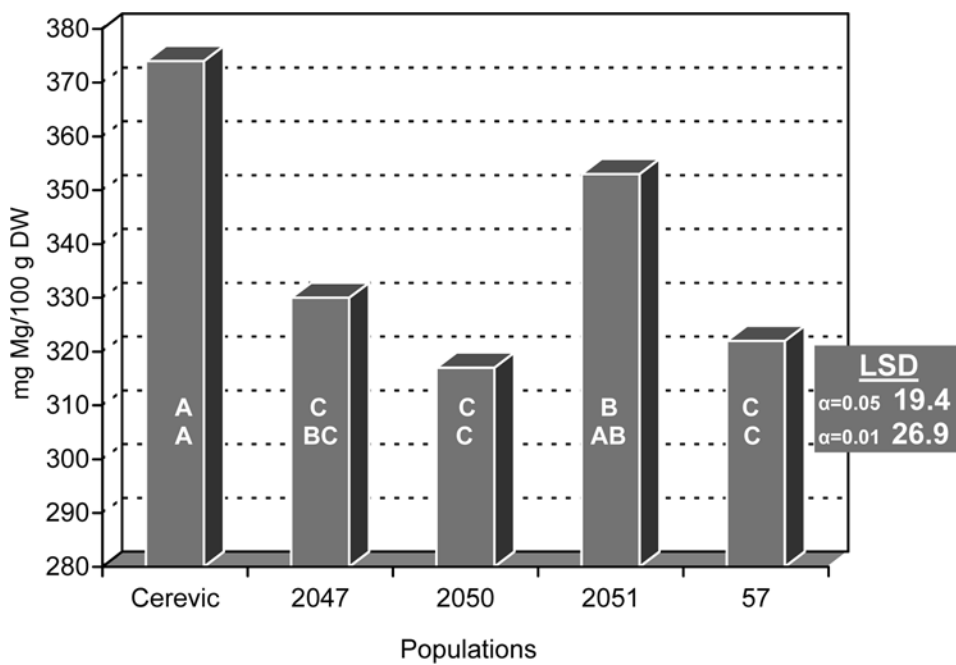


Fig. 5 — Magnesium concentration in different populations of *Helianthus tuberosus*

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ГЕНЕТСКА СПЕЦИФИЧНОСТ САДРЖАЈА МАГНЕЗИЈУМА У ДИВЉИМ ВРСТАМА И ХИБРИДИМА СУНЦОКРЕТА

Рудолф Р. Кастори^{1, 2}, Радован З. Маринковић², Петар Ђ. Секулић²,
Ивана В. Максимовић^{1, 2}, Мира М. Пуцаревић²

¹ Пољопривредни факултет, Нови Сад, Трг Доситеја Обрадовића 8,
Нови Сад, Србија

² Институт за ратарство и повртарство, Максима Горког 30, Нови Сад

Резиме

У циљу проучавања генетске специфичности исхране сунцокрета магнезијумом испитан је садржај магнезијума у пет најраспрострањенијих хибрида сунцокрета у Србији и у различитим популацијама дивљих врста сунцокрета пореклом из Северне Америке: *Helianthus neglectus* (3), *Helianthus petiolaris* (5), *Helianthus agrophylus* (5) *Helianthus tuberosum* (5) и *Helianthus annuus* (5).

Садржај магнезијума у испитиваним дивљим врстама сунцокрета кретао се у широким границама од 317 до 824 mg/100 g суве материје. Највећи је био садржај магнезијума код испитиваних популација *Helianthus petiolaris*, у просеку 741, а најмањи код *Helianthus tuberosum*, 330 mg/1000 g суве материје. У оквиру дивљих врста испитиване популације су се такође статистички значајно разликовале у садржају магнезијума. Највећи коефицијент варијације утврђен је код *Helianthus tuberosus* а најмањи код *Helianthus petiolaris*. Садржај магнезијума се и у испитиваним хибридимат сунцокрета значајно разликовао и за две године испитивања се у просеку кретао од 575 до 813 mg/100 g суве материје.

На основу добијених резултата може се закључити да је генетска варијабилност садржаја магнезијума у дивљим врстама и њиховим популацијама као и у гајеним хибридимат сунцокрета веома изражена, што треба имати у виду при њиховом обезбеђењу овим елементом, као и при коришћењу дивљих врста у оплењивачком процесу.

Ankica Đ. Kondić-Špika
Borislav Đ. Kobiljski
Nikola S. Hristov

Institute of Field and Vegetable Crops
M. Gorkog 30, 21000 Novi Sad, Serbia

EFFICIENCY OF ANTHHER CULTURE TECHNIQUE IN THE PRODUCTION OF WHEAT DOUBLE HAPLOIDS

ABSTRACT: The objective of the study was to investigate efficiency of anther culture in the production of spontaneous double haploids from randomly selected heterozygous genotypes of wheat (*Triticum aestivum* L.). Anthers of 20 F₁ wheat combinations were grown *in vitro* on a modified Potato-2 medium.

All of the examined genotypes have shown the ability to produce pollen calluses as well as to regenerate green plants. On average for the whole experiment material, 47.2 calluses were produced per 100 cultured anthers. The green plant regeneration ranged from 0.8 to 13.4 green plants per spike, with an overall mean of 5.8. From the total of 582 regenerated green plants, 47.9% (279) were spontaneous double haploids. The final average yield from the study was 2.8 double haploids per spike.

KEY WORDS: androgenesis, double haploid, *in vitro*, *Triticum aestivum* L.

INTRODUCTION

Double haploid techniques provide plant breeders with pure lines in a single generation, which may save considerable time in the breeding of new cultivars. There are two major techniques for haploid production in cereals — anther/microspore culture and chromosome elimination using wide hybridizations. The former technique is usually considered as simpler, more efficient, and more cost-effective than the latter (Ljevnaić, 2007).

The most important advantage of the anther culture method is occurrence of spontaneous chromosome doubling which results in production of homozygous DHs. Those spontaneous DHs are fertile and cytologically stable, except for a small percentage of them, which exhibit chromosomal abnormalities (Ahmed et al., 1999). However, the frequency of spontaneous DH plants depends of several factors and it is usually low (Armstrong et al., 1987; Ahmed et al., 1999).

Wheat in particular is known as a recalcitrant species with regard to *in vitro* androgenesis techniques such as anther and microspore culture. Use of anther culture in wheat breeding programs is limited by strong genotype specificity, low frequency of haploids, and a high rate of albinism in regenerants (Kisana et al., 1993; Sadasivaiah et al., 1999). Despite these problems, the anther culture technique has been successfully applied in some wheat breeding programs, resulting in new cultivars (Pauk et al., 1995; Kertesz et al., 2001; Sadasivaiah et al., 2004).

It is known that anther culture response is highly genotype-specific (Orlov et al., 1993; Moieni and Sarafi, 1995; Kondić and Šesek, 1999) and typically, it would produce many individuals from only a few selected crosses. One suggestion has been to use the anther culture technique only for breeding combinations in which at least one parent line is highly responsive (Zhou and Konzak, 1992; Tuvešson et al., 2000). The goal of this study was to investigate if it is possible to produce a large number of DHs from randomly selected wheat breeding combinations using the anther culture method.

MATERIAL AND METHODS

In this study, 20 randomly selected F₁ wheat combinations were used for anthers isolation. The breeding material was produced at the experimental fields of the Small Grains Department of the Institute of Field and Vegetable Crops in Novi Sad.

Donor plants were grown under field conditions. Five spikes were taken from each combination at the mid- or late uninuclear stage of microsporogenesis. After a temperature pre-treatment, sterilization of the material was carried out as described in Šesek and Denčić (1996) and anthers were isolated under aseptic conditions.

The Potato-2 inductive nutrient medium (Chuang et al., 1978) was used for callus induction. During their culturing on the inductive medium, anthers were kept in the dark and at 28–30°C. Plant regeneration from formed embryogenic calluses was performed on the modified 190-2 medium (Zhuang and Jia, 1980). This medium contained 190-2 mineral salt solution as well as some other components, namely (in g l⁻¹) agar (5), sucrose (30), and (in mg l⁻¹) glycine (2), thiamine-HCl (1), pyridoxine-HCl (0.5), nicotinic acid (0.5), myo-inositol (100), NAA (0.5) and kinetin (0.5). When green shoots reached 5–10 mm after approximately three weeks, calluses with green shoots were transferred to the rooting medium. A semi-solid agar medium was used for the development of the root system. It also contained the 190-2 mineral solution. The only difference between this medium and the one used for plant regeneration was that in this one the concentration of NAA and kinetin was reduced from 0.5 to 0.1 mg l⁻¹. During the plant regeneration and root development period, the temperature in the growing chamber was maintained at 25–27°C. The intensity of white fluorescent illumination was 2500–3000 lx, with a photoperiod of 14 hours of light.

Plants that had a well-developed root system were transplanted into containers with the sterilized substrate. Prior to transplanting, five to six root tips were taken from each plant and checked for chromosome number by the standard acetocarmine squash method. After acclimatization and vernalization periods, further plant growth and development until full maturity took place under field conditions. The plants of the H₁ generation were harvested in early July.

During the study, the following traits were analyzed:

- callus yield (CY — no. of calluses per 100 anthers)
- green plants yield (GP — no. of green plants per spike)
- DH plants yield (DH — no. of DH plants per spike)

RESULTS AND DISCUSSION

All genotypes had the ability to form callus tissue by growing anthers in the *in vitro* culture. The highest callus yield (found in the combination NS 111-95/Ana) exceeds 100% (119%), since each androgenic anther produced more than one callus. The lowest callus yield (21%) was found in NS 111-95/Tiha (Tab. 1).

Tab. 1 — Callus yield and numbers of green and DH plants per spike of 20 randomly selected F₁ wheat combinations

Genotype	CY (%)	GP (No.)	GP/spike	DH (No.)	DH/spike
Ana/NS 0-691	35.9	27	5.4	19	3.8
Balkan/Košuta	34.3	15	3.0	8	1.6
CHI 6/Tiha	27.7	7	1.4	1	0.2
CHI 6/Sremica	30.0	6	1.2	2	0.4
Kutječanka/Slavija	26.7	17	3.4	11	2.2
Mex.3/Tiha	69.7	39	7.8	16	3.2
Mex.3/NS 55-25	67.0	43	8.6	21	4.2
Mex.3/MV 18	85.1	67	13.4	43	8.6
NS 33-90/Fawwon-138	29.0	26	5.2	14	2.8
NS 38-93/Rusija	35.0	48	9.6	15	3.0
NS 38-93/Košuta	82.7	39	7.8	12	2.4
NS 92-205/Tiha	32.0	25	5.0	9	1.8
NS 95-95/Tiha	22.3	15	3.0	6	1.2
NS 95-95/NSP 11	92.7	7	1.4	2	0.4
NS 111-95/Tiha	21.0	36	7.2	14	2.8
NS 111-95/Renesansa	25.0	19	3.8	8	1.6
NS 111-95/Ana	119.0	56	11.2	26	5.2
NS 111-95/Sremica	54.0	64	12.8	39	7.8
NSP 41/NS 0—649	21.3	22	4.4	13	2.6
30-Sc.Smoc.88—89/Hays2	33.7	4	0.8	—	—
F ₁ (mean)	47.2	29.1	5.8	13.9	2.8
LSD	0.05	3.31	0.89		0.66
	0.01	4.43	1.19		0.89

The average callus yield in the experiment on the whole was 47.2%, which is close to the results obtained by Barnabas et al. (1991) and Marciniak et al. (2003) — 41% and 45%, respectively. Ekiz and Konzak (1994) as well as Bruins and Snijders (1995) reported significantly higher average values (70.9 and 77.8%, respectively), but they did not use randomly selected genotypes in their studies.

Regeneration ability was found to exist in all of the genotypes. A total of 582 green plants originating from 6000 isolated F₁ microspores were produced during the study. The number of green plants produced per spike varied from 0.8 (30-Sc.Smoc.88—89/Hays2) to 13.4 (Mex.3/MV 18). The average response per spike was 5.8 green plants (Tab. 1).

Studies with a large number of tested wheat genotypes have shown that such a large number of green plants is obtainable in general, which makes the anther culture technique applicable in wheat breeding. Tuvešson et al. (2000) in their screening study with 91 F₁ wheat combinations obtained very promising results: 4.7 green plants per spike.

From the total of 582 regenerated green plants, 279 (47.9%) were spontaneous DHs. The final average yield for this study was 2.8 DH plants per spike. The range was from 0 (30-Sc.Smoc.88—89/Hays2) to 8.6 (Mex.3/MV 18) DH plants per spike (Tab. 1).

Similar results were obtained by Šešek (1989) — 1.8 DH plants per spike, Snape et al. (1986) — 2.2 DH plants per spike and Tuvešson et al. (2000) — 2.1 DH plants per spike.

The results showed that significant genotypic differences have been found for callus yield, regeneration of green plants and DH production. It is in agreement with results of other authors (Tuvešson et al., 2000; Tersic et al., 2006; Ljevnaić, 2007). Among the large number of cultivated genotypes, the number of cultivars exhibiting a good level of androgenic response is usually small (Zamani et al., 2003). According to the results of Tuvešson et al. (2000), the ability to respond in anther culture is present in more modern cultivars compared with older material. The year of testing and the land of origin seem not to be important factors in determining the degree of response. Therefore, the results are of general value to wheat breeders. They also suggest that expensive tissue culture programmes should be concentrated on responsive breeding combinations, while unresponsive material should be improved via crossing.

CONCLUSION

Since the average production in this study was 5.8 green plants per spike and 2.8 spontaneous DH plants per spike, the efficiency of anther culture in DH production could be improved by inducing chromosome doubling using colchicine treatment. It will improve this technique enough for its effective use as an additional method in wheat breeding programs.

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ЕФИКАСНОСТ ТЕХНИКЕ КУЛТУРЕ АНТЕРА У ПРОИЗВОДЊИ ДВОСТРУКИХ ХАПЛОИДА ПШЕНИЦЕ

Анкица Ђ. Кондић-Шпика, Борислав Ђ. Кобиљски, Никола С. Христов

Институт за ратарство и повртарство, М. Горког 30,
21000 Нови Сад, Србија

Резиме

Циљ рада био је да се испита ефикасност културе антера у производњи спонтаних двоструких хаплоида из случајно одабраних хетерозиготних генотипова пшенице (*Triticum aestivum* L.). Антере из 20 F₁ комбинација пшенице гајене су *in vitro* на модификованој Потато-2 подлози.

Сви испитивани генотипови показали су способност да произведу калусе, као и да регенеришу зелене биљке. У просеку за цео експериментални материјал, произведена су 47.2 калуса на 100 изолованих антера. Регенерација зелених биљака кретала се од 0.8 до 13.4 зелене биљке по класу, са укупним просеком од 5.8. Од укупно 582 регенерисане зелене биљке, 47.9% (279) биле су спонтани двоструки хаплоиди. У просеку, у овом истраживању, произведено је 2.8 ДН биљака по класу.

*Mirjana M. Kresović, Vlado Đ. Ličina,
Svetlana B. Antić-Mladenović*

University of Belgrade, Faculty of Agriculture, Nemanjina 6, Zemun, Serbia
e-mail: ktomi@eunet.yu

EVALUATION OF OPTIMAL TIME AND PARAMETERS FOR MEASURING POTENTIALY MINERALIZED NITROGEN IN SOIL

ABSTRACT: Our research was done on brown forest soil with long-term experiments and with a system of fertilizing which is in use for 40 years. Experiment variants with an increasing dose of nitrogen fertilizer were chosen for this research. Two experiments have been performed: experiment in pots supplied with ammonium nitrate labeled with a stable isotope ^{15}N (11.8%) and experiment in the field. The aim of the research was to establish which plant and soil parameters group (obtained in the controlled conditions and/or in the field) could be considered as reliable for evaluation of aerobic and anaerobic incubation and of the best time for estimation of potentially mineralized nitrogen in soil. According to the determined correlative dependence, it could be concluded that reliability of aerobic incubation should be estimated in October by plant and soil parameters from field, anaerobic incubation should be estimated in early spring (March) by plant and soil parameters, from controlled conditions (pots) and from field.

KEY WORDS: parameters, available nitrogen, aerobic, anaerobic, incubation

INTRODUCTION

All methods used today to determine the availability of soil nitrogen could be classified in three groups, namely: methods for determination of potentially mineralized nitrogen in soil, methods for determination of residual nitrogen, and the method of “A”-values (Goh and Haynes, 1986; Dhanke and Johnson, 1990; Bundy and Meisinger, 1994; Brown, 1996, Kresović, 1999, Bogdanović et. al., 2005).

Two groups of methods are used to estimate potential mineralized nitrogen in soil, namely: biological and chemical (biological and chemical indexes of nitrogen availability (Goh and Haynes, 1986; Brown, 1996).

Reliability of biological methods was established most often by using the plant parameters from controlled conditions when the parameters used were gained yield and adopted nitrogen (Keeney and Bremner, 1996; Ozuş

and Hanway, 1966; Gasser and Kalembasa, 1976; Stevanovic, 1978). Reliability of before mentioned methods was rarely determined by using the field parameters (Robinson, 1968; Power, 1980; Hussian et al., 1994).

The purpose of these researches was to establish which of the used parameters of plants and soils (controlled conditions and/or field) could be considered reliable for estimation of aerobic and anaerobic incubation values. We also wanted to determine the most favorable time for estimation of potentially mineralized nitrogen (October, March, July).

MATERIAL AND METHODS

Researches were performed on a stationary experiment, on brown forest soil type.

The samples of soils were taken in October, March and July (0—30 cm depth) with experiment variance where the increasing doze of nitrogen fertilizer was applied.

a) Methods for determination of soil chemical properties

General chemical properties of investigated brown forest soil were determined in the soil samples taken in March, by using the following methods:

- Reaction of soil (pH-H₂O and 1 MKCl)
- Organic carbon and humus (Tjurin, modification Simakova, 1957)
- Total nitrogen (Semimikro Kjeldahl, Bremner, 1996)
- Available phosphorus and potassium (Al-method, Egner-Riehem, 1960).

b) Experiment in pots

Plastic pots containing 2 kg of soil were used to make the experiment in controlled conditions (greenhouse). The soil was taken three times in spring (in March) from the depth of 0—30 cm, from selected experiment variances in the field.

Before sowing, this soil was mixed with the following fertilizers: NH₄NO₃, KH₂PO₄ and KCl. Used ammonium nitrate was marked with a stable isotope ¹⁵N (11.8%).

Vegetation experiment was made with two variants, PK and NPK. For PK variance, 50 mg of P₂O₅ and K₂O per kg of soil was used. For NPK variance, 50 mg of N, P₂O₅ and K₂O per kg was used. The experiment was applied with oats plants, with 10 plants in each pot, grown to the stage of jointing.

c) Experiment in field

The variances chosen for the experiment in field were those with increasing nitrogen fertilizer dose, as follows: control (Ø), N₁ P₂ K₂ (N₁ — 60, P₂ —

120, K₂ — 120 kg/ha); N₂ P₂ K₂ (N₂ — 90 kg/ha); N₃ P₂ K₂ (N₃ — 120 kg/ha); N₄P₂ K₂ (N₄ — 150 kg/ha). Each of mentioned experiment variances in the field was applied three times.

Wheat was raised in stationary experiment in these researches.

d) Methods for estimation of potentially mineralized nitrogen
— aerobic and anaerobic incubation

Methods were applied to the soil samples taken from before mentioned experiment field variances in October, March and July.

Aerobic incubation was performed in accordance with proposed procedure (Bremner, 1965), while the anaerobic incubation was made with the procedure of Waring and Bremner, 1964, modification by Keeny (1982).

e) Method of isotopic labeled nitrogen (¹⁵N)

During the application of ¹⁵N isotope, the determination of percentage content of nitrogen isotope was made (¹⁵N/¹⁴N) by using the analyses of mass spectrometer. Isotope analysis was determined in accordance to Bremner procedure (1965).

f) Analysis of plant material

Nitrogen content in plants (oats and wheat) was determined by distillation method (Munsinger and McKinney, 1982).

g) Method of statistical analysis

A simple linear correlation analysis was applied for parameters of plants and soils in controlled conditions and in field, i.e. the methods for estimation of potentially mineralized soil nitrogen in all three times of taking samples.

This statistical analysis enabled us to determinate the value of correlation coefficient (Pirson) referring to the similarity degree, i.e. dependences of this compared value.

Based on the values of correlation coefficients, the percentage of relative dependences for $r = 0.50-0.99$ was calculated, with significance at the probability level of 0.05—0.01 and for $r = 0.70$, with significance at the probability level of 0.01.

This calculation was made in order to use these data for the purpose of defining which group of applied parameters of plants and soils (pots and/or field) could be considered as the most appropriate for estimation on reliability of applied methods, as well as to establish the most reliable time to make those estimations (October, March or July).

RESULTS AND DISCUSSION

Chemical properties of investigated brown forest soil are shown in Table 1.

Tab. 1 — Chemical properties of investigated soil

Field variants	pH		Humus (%)	Total nitrogen (%)	C/N	Available	
	H ₂ O	nKCl				P ₂ O ₅ (mg/100 g)	K ₂ O (mg/100 g)
Control	5.40	4.60	1.43	0.098	8.5	6.4	16.2
N ₁ P ₂ K ₂	5.10	4.30	1.67	0.111	8.7	18.0	21.8
N ₂ P ₂ K ₂	5.00	4.20	1.81	0.113	9.3	14.4	21.8
N ₃ P ₂ K ₂	4.90	4.15	1.85	0.113	9.5	16.0	25.0
N ₄ P ₂ K ₂	5.00	4.05	1.88	0.116	9.4	12.5	21.8

To establish which parameters of plants and soil might be considered reliable to evaluate the applied methods being one of the goals of these calculations, we have established the plant and soil parameters in the pots (controlled conditions) and in the field. Their values are shown in Tables 2, 3, 4, 5 and 6. Results are presented as mean values of three repetitions.

Tab. 2 — Parameters of plants in pot (g/pot)

Field variants	Yield		Total uptake of nitrogen NPK	Uptake of soil nitrogen NPK	Uptake of fertilizer nitrogen NPK	Total uptake of nitrogen PK
	NPK	PK				
Control	10.26	2.25	90.0	58.1	31.9	18.5
N ₁ P ₂ K ₂	10.84	3.58	105.8	72.4	33.4	22.9
N ₂ P ₂ K ₂	10.86	4.18	118.0	83.8	34.2	30.2
N ₃ P ₂ K ₂	11.22	4.69	110.1	78.0	32.1	30.1
N ₄ P ₂ K ₂	11.10	6.36	115.1	84.4	30.7	48.5

Tab. 3 — Parameters of plants in field (t/ha)

Field variants	Yield			% N grain	% N straw
	grain	straw	Total		
Control	0.95	1.21	2.16	1.239	0.209
N ₁ P ₂ K ₂	3.90	6.08	9.99	1.463	0.261
N ₂ P ₂ K ₂	4.38	6.30	10.68	2.015	0.272
N ₃ P ₂ K ₂	4.97	6.21	11.17	2.172	0.424
N ₄ P ₂ K ₂	5.13	6.77	11.90	2.359	0.426

Tab. 4 — Parameters of soil in field (kg/ha)

Field variants	Uptake of nitrogen (grain)	Uptake of nitrogen (straw)	Total uptake of nitrogen
Control	11.82	2.53	14.35
N ₁ P ₂ K ₂	57.07	15.88	72.95
N ₂ P ₂ K ₂	88.22	17.15	105.37
N ₃ P ₂ K ₂	107.88	26.33	134.21
N ₄ P ₂ K ₂	121.04	28.85	149.80

Taking into consideration the fact of most commonly used parameters and recommendations by Sapoznikov (1973), evaluated parameters for applied methods were divided in two groups: parameters from plants and parameters from field that are further grouped in plant and soil parameters (Table 6).

Quantities of mineralized nitrogen by aerobic and anaerobic procedure are shown in Table 5 as mean values of three repetitions.

Tab. 5 — Quantities of mineralized nitrogen in aerobic procedure (ppm)

Field variants	Aerobic incubation			Anaerobic incubation		
	October	March	July	October	March	July
Control	15.7	24.7	50.6	16.2	14.8	18.6
N ₁ P ₂ K ₂	21.5	24.2	48.1	17.7	18.0	19.0
N ₂ P ₂ K ₂	21.8	32.2	62.8	14.6	17.7	19.4
N ₃ P ₂ K ₂	28.3	30.6	103.4	21.6	19.7	20.4
N ₄ P ₂ K ₂	26.0	27.8	54.3	17.0	20.1	18.7

Data on quantities of mineralized nitrogen obtained by aerobic and anaerobic procedure were compared with plant and soil parameters (pots and field) and the values of established correlation coefficients are presented in Table 6.

Tab. 6 — Correlation coefficient values between plant and soil parameters and nitrogen mineralized in aerobic and anaerobic procedure in brown forest soil

Parameters of plats of soil (pot)	Aerobic incubation			Anaerobic incubation		
	October	March	July	October	March	July
Yield (NPK)	0.75**	NS	NS	NS	0.70**	NS
Yield (PK)	0.74**	NS	NS	NS	0.90**	NS
% N in plants (NPK)	NS	0.56*	NS	NS	NS	NS
% N in plants (PK)	NS	NS	NS	NS	-0.54*	NS
Total uptake of nitrogen (NPK)	NS	0.54*	NS	NS	0.67**	NS
Uptake of soil nitrogen (NPK)	0.58*	0.57*	NS	NS	0.75**	NS
Uptake of fertilizer nitrogen (NPK)	NS	NS	NS	NS	NS	NS
Uptake of soil nitrogen/fertilizer	0.73**	NS	NS	NS	0.88**	NS
Soil nitrogen uptake (PK)	0.61**	NS	NS	NS	0.77**	NS
Parameters of plants and soil (field)						
Yield of grain	0.75**	NS	NS	NS	0.81**	NS
Yield of straw	0.70**	NS	0.53*	NS	0.76**	NS
Total yield	0.72**	NS	0.51*	NS	0.79**	NS
% N in grain	0.73**	NS	NS	NS	NS	NS
% N in straw	0.70**	NS	NS	NS	0.93**	NS
Uptake of nitrogen-grain	NS	NS	NS	NS	0.79**	NS
Uptake of nitrogen-straw	NS	NS	NS	NS	0.82**	NS
Total uptake of nitrogen	0.69**	NS	NS	NS	0.80**	NS

** — significant of probability level 0.01

* — significant of probability levels 0.05

NS — no statistic value

In March, the established correlation coefficients between the quantities of mineralized nitrogen obtained by aerobic procedure and the plant and soil

parameters from the experiment in pots were nearer to low than to average values. As for parameters of experiments in field, statistically significant correlation dependences were not found.

In July, there was no statistically significant correlation dependence for plant and soil parameters in pots while for parameters in field, the dependence was low.

In October, for plant and soil parameters from the pots, the statistically significant correlation dependence was not found for half of observed values, while for the remaining parameters it was high, with a high degree of statistical dependence.

Based on established values of correlation coefficients, a percentage of correlation dependences for given criteria was calculated (Table 7).

Tab. 7 — Correlation dependences (in percents) $r = 0.50—0.90$ (** and *) and for $r \geq 0.70$ ** between the plants and soil parameters and nitrogen mineralized in aerobic procedure

Parameters	Percentage of correlation dependence for $r = 0.50—0.90$ (**, *)	Percentage of correlation dependences for $r \geq 0.70$ **
October		
Parameters of plants and soil in pots	55.5	33.3
Parameters of plants and soil in field	75.0	62.5
March		
Parameters of plants and soil in pots	33.3	0.0
Parameters of plants and soil in field	0.0	0.0
July		
Parameters of plants and soil in pots	0.0	0.0
Parameters of plants and soil in field	25.0	0.0

From obtained percentages of correlation dependences, it might be noted that the highest percentage was established in October. Thus, we can recommend applying the evaluation of potential mineralized nitrogen referring to the reliability of aerobic method in autumn (October), before the wheat seeding.

Based on established percentages of correlation dependences in October, it is obvious that the highest percentages were established for parameters from fields in both criteria. This percentage for the parameters from the pots, in both cases, was lower (55.5 and 33%). Thus, one can conclude that the evaluation of aerobic incubation value should be made by using the parameters from the field, being in conformity with the results of Robinson (1968). This is important having in mind that parameters from the field can be established by a much simpler way while it is more difficult to establish the parameters from the pots since it requires more work and use of expensive procedures (use of marked ^{15}N).

In anaerobic incubation, the same statistic analysis used in aerobic incubation was applied. The values of established correlation coefficients are presented in Table 6.

In March, the established correlation dependences between mineralized nitrogen obtained by anaerobic procedure and plant and soil parameters in pots

were mostly high, with a high degree of statistical significance, except for total adopted nitrogen (NPK) (0.67**) and for percentage of nitrogen in oats plants, where it was very low. A high correlation dependence between mineralized nitrogen obtained by mentioned procedure and plant and soil parameters from the field. In one case, it was very high ($r = 0.93^{**}$ —%N — straw) with a high degree of statistical significance.

In July and October, no statistically significant correlation dependences between plant and soil parameters (pots and field) and mineralized nitrogen were found when anaerobic procedure was applied.

Tab. 8 — Correlation dependences (in percents) $r = 0.50$ — 0.90 (** and *) and for $r \geq 0.70^{**}$ between the plants and soil parameters and nitrogen mineralized in anaerobic procedure

Parameters	Percentage of correlation dependence for $r = 0.50$ — 0.90 (**, *)	Percentage of correlation dependences for $r \geq 0.70^{**}$
October		
Parameters of plants and soil in pots	0.0	0.0
Parameters of plants and soil in field	0.0	0.0
March		
Parameters of plants and soil in pots	77.78	55.5
Parameters of plants and soil in field	87.5	87.5
July		
Parameters of plants and soil in pots	0.0	0.0
Parameters of plants and soil in field	0.0	0.0

On the basis of the results shown in Table 8, it might be concluded that the highest percentages of correlation dependence for given criteria are found in March. Consequently, we can recommend making the evaluation of potential mineralized nitrogen by the anaerobic procedure in early spring (in March). In addition, from presented results in March, it is possible to note that the highest percentages of correlation dependences were established for parameters in the field for both given criteria. As for the parameters in pots, the percentages were lower. Taking into consideration the value of established percentages of correlation dependences, for a strict criterion ($\geq 0.70^{**}$), we recommend to make the evaluation of anaerobic incubation value by using the parameters either in field or in pots.

CONCLUSION

On the basis of presented results, it can be concluded as follows:

— The evaluation of aerobic incubation reliability being a method for establishing potential mineralized nitrogen with conditions in brown forest soil should be performed in October.

— The evaluation of aerobic incubation should be made by using plant and soil parameters in the field.

— Evaluation of anaerobic incubation for given conditions should be performed in early spring (March).

— From used plant and soil parameters, to evaluate the value of anaerobic incubation, use equally the parameters from the field and from the pots.

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УТВРЂИВАЊЕ ОПТИМАЛНОГ ВРЕМЕНА И ПАРАМЕТАРА ЗА ОЦЕНУ ПОТЕНЦИЈАЛНО МИНЕРАЛИЗУЈУЋЕГ АЗОТА ЗЕМЉИШТА

Мирјана М. Кресовић, Владо Ђ. Личина, Светлана Б. Антић-Младеновић

Универзитет у Београду, Пољопривредни факултет,
Немањина 6, Земун, Србија

Резиме

Истраживања су обављена на земљишту типа гajњача (околина Младеновца) на којем је постављен стационарни оглед са одређеним системом ђубрења. За ова истраживања одабране су варијанте огледа из поља где је примењена растућа доза азота ђубрива. Циљ истраживања је био да се утврди који се параметри биљака и земљишта (контролисани услови и/или поље) могу сматрати поузданима за оцену вредности аеробне и анаеробне инкубације, као и да се утврди најповољније време за ту оцену. Да би се остварио постављени циљ истраживања, изведена су два огледа: оглед у судовима (контролисани услови) где је примењен амонијум нитрат који је био обележен стабилним изотопом ^{15}N (11,8%) и оглед у пољу. Да би се утврдило оптимално време за оцену поузданости аеробне и анаеробне инкубације, са стационарног огледа су узорци земље узимани у октобру, марту и јулу. На основу утврђених корелативних зависности, за услове који су владали у испитиваној гajњачи, оцену поузданости аеробне методе треба доносити у октобру коришћењем параметара биљака и земљишта из поља. Оцену поузданости анаеробне методе треба утврђивати у рано пролеће (март), при чему се за ту оцену могу равноправно користити како параметри биљака и земљишта из поља, тако и из судова (контролисани услови).

Marina Putnik-Delić

Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia

RESISTANCE OF SOME WHEAT GENOTYPES TO *Puccinia triticina*

ABSTRACT: Thirty-four wheat genotypes were tested for resistance characteristics to *Puccinia triticina* at different growth stages. At seedling stage in a greenhouse, latency period, infection frequency and reaction type were determined. In the field, time of spike appearance, and infection intensity were followed. Weak ($r = 0.322$) to strong ($r = 0.660$) correlation was found between resistance characteristics at seedling stage (LPxIFxRT, in the greenhouse) and in adult plants (infection intensity and AUDPC, in the field). The values of AUDPC (area under the disease progress curve) were also strongly related to resistance characteristics of seedlings ($r = 0.598$). As pseudoresistant in the field (especially equal to adult plant resistance) according to other stress reactions were considered genotypes which were the most sensitive at seedling stage (20°C) and at adult stage they did not express maximal infection intensity, particular Evropa 90 and Suvača. Adult plant resistance as the sum of specific and pseudoresistance was observed on genotypes Tiha, Zlatka and Eva.

KEYWORDS: resistance, *Puccinia triticina*, wheat genotypes, seedling stage, adult stage

INTRODUCTION

Since the seventeenth century frequent damages caused by *Puccinia triticina* were described (Chester, 1946). Consequences of attack were reduction of photosynthesis and collapse of metabolism. Infection in the beginning of plant development reduces resistance to low temperatures or reduces stool and seed number. The most often consequence is bad quality of seed (Jerković, 1995). Susceptibility of commercial varieties is the result of growing the same varieties for long time, on very large area in one region, and because wheat breeding does not always follow the dynamics of pathogen changes.

Many authors were studying a problem of expressing resistance as hypersensitive reaction (Jerković, 1992), low infection efficiency and prolonged latent period (Parlevliet, 1986). The most exploited method to evaluate resistance of wheat genotypes to *Puccinia triticina* at seedling stage is according to reaction type. This feature is dependent on temperature, and at seedling stage, optimum is 15—20°C. Even then, some results do not correlate with in-

fection efficiency and reaction type in the field. For discovering and using all genes responsible for expressing any kind of resistance, it is usual to test latent period and infection efficiency. Infection efficiency was the most used criteria in our region. Low infection efficiency and prolonged latent period are sufficient to reduce development of pathogen and make non-economic application of the other protection measurements (Jerko vić, 1992). Low infection efficiency is the most discussible parameter whose better explanation is necessary to fully understand the process of overcoming genes for resistance (Niks et al., 2000). Prolonged latent period is the last characteristic before resistance overcoming. In the field, reliable criteria of incomplete resistance are time and intensity of the beginning of infection, and AUDPC value (Putnik and Jerko vić, 2002). The first infection, according to modified Cobb scale (from five and higher, Peterson et al., 1948), in our experimental conditions appear almost in the third (wheat in phase > 70), and sometimes in the second decade of May in time of bloom (phase 60—69 according to Zadoks).

Parasite has the possibility of continuous and fast adaptation, so that it can multiply on each variety after shorter or longer period. Process of changes in pathogen population infecting particular genotype is concomitant with the breeding process (Parlevliet, 1986).

The aim of the study was to differentiate varieties according to adult plant resistance depends on specific resistance genes to *Puccinia triticina*. These adult plant level of resistance depends only on environmental conditions, could be activated the other stress reactive genes, and could be called pseudo resistant.

MATERIAL AND METHODS

Thirty-four wheat genotypes were tested on three resistance characteristics as latency period (LP), reaction type (RT) and infection efficiency (IF) at seedlings in greenhouse. In the field, times of spikes apperance and leaf senescence were marked, and infection intensity was evaluated also, in three times.

The resistance characteristics values of seedlings were achieved at approximate 20°C in greenhouse during the winter period (temperature rising below 24°C increased the leaf rust development). The seven days old seedlings were inoculated by trashing spores (Stakman, 1954). Incubation in high moisture was for 24 hours. The RT estimated after ten days from incubation was transformed as before present (0 = 6, 1 = 7, 2 = 8, 3 = 9, 4 = 10) (Jerko vic and Putnik-Delic, 2004). The latency period (LP), was observed visually six and seven days after incubation and marked adequately (1 = pustule appeared first day, 0.8 = some second day, 0.7 = all after second day), and infection frequency (IF) after 10 days. Infection frequency (IF) was the maximal number of pustules on the observed area (6 = less than 20 pustules in the middle of the first leaf, 7 = 20—30, 8 = 31—40, 9 = 41—50 and 10 = more than 50). The formula $RT \times IF \times LP$ results were correlated with maximal infection intensities in field, and AUDPC (area under the disease progress curve) (Bjarko and Line, 1988) too, on all varieties. The results of seedlings test in greenhouse and adult stage in field during 2004 were presented in the paper. The

infection intensities in field were estimated according to modified Cobb scale together with the leaf condition in June during the same year. Experiment was done at experimental fields of the Institute of Field and Vegetable Crops, Novi Sad. The correlation coefficients between estimated parameters were calculated using MstatC program.

RESULTS

In this paper, we showed the evaluation of resistance characteristics under controlled conditions (LP, IF, RT) and in the field (max infection intensity, AUDPC); and time of spikes appearance and leaf senescence according to number of estimation (Table 1). Together they were reliable criteria to distinguish between specific resistance and adult plant resistance of tested genotypes.

The seventeen genotypes (No. 1—17, Table. 1) showed no signal of resistance according to tests in greenhouse, at seedling stage, and they were evaluated as susceptible. Some early-headed varieties as Suvača, Evropa 90, and Kremna were relatively less attacked in field. The 12 genotypes were with prolonged LP and for six of them the latency period was the only single expressed characteristics of resistance, while lower IF was recorded for 3 genotypes (Pobeda, Sonja, Jarebica). Only 4 genotypes showed relatively lower values of all three parameters (Nova Banatka, Sava, Selektta, Eva). Another four genotypes (Pesma, Bajka, Slava and Sreća) were designated as completely resistant both according to results obtained in a greenhouse, at seedling stage, and under field conditions, as adult plants. Pesma, Bajka and Eva showed the late flag leaf senescence as Košuta, Suvača, and Mina (5 or after 5 estimation) related with other susceptible genotypes in the greenhouse (Table 2). Mina was specific according to erective leafs. The date of heading was correlated with leaf area duration in all cases, except Košuta, Suvača, Tiha, Jarebica, and Bajka, which were early, headed varieties with prolonged leaf area duration.

Coefficients between results of infection intensity in field, AUDPC, LPxIFxRT and LP show high correlation. Maximal value was obtained between infection intensity at last estimation in field (16th June) and LPxIFxRT (0.660), so AUDPC and LPxIFxRT (0.598). Correlation coefficient between LPxIFxRT and infection intensity in field was high, too ($r = 0.982$) (Jerko vi c et al., 2004)

Tab. 1 — The estimate values of resistance characteristics on all tested genotypes

Number	Genotype	LP	IF	RT	Max infection intensity in field	Time of spike appearance	Leaf senescence in 2004 (number of estimation)	AUDPC	LPxIFxRT
1	NSR 2	1	10	10	80	10 th May	4	670	100
2	PZ	1	10	10	70	11 th May	4	743	100
3	Košuta	1	10	10	70	11 th May	5	667	100
4	Milica	1	10	10	70	11 th May	4	487	100
5	Rodna	1	10	10	60	11 th May	4	812	100
6	Novosadska crvena	1	10	10	60	10 th May	4	622	100
7	Delta	1	10	10	60	10 th May	4	582	100
8	Dična	1	10	10	60	10 th May	4	582	100
9	Silna	1	10	10	60	10 th May	4	552	100
10	Rusija	1	10	10	60	11 th May	4	507	100
11	Renesansa	1	10	10	60	11 th May	4	358	100
12	Tera	1	10	10	50	10 th May	4	466	100
13	NSR 5	1	10	10	50	10 th May	4	447	100
14	Suvača	1	10	10	40	10 th May	5	552	100
15	EV 90	1	10	10	40	10 th May	4	342	100
16	Kremna	1	10	10	40	10 th May	4	172	100
17	Mina	1	10	10	30	16 th May	after 5	98	100
18	Sofia	0.8	10	10	50	17 th May	5	635	80
19	Prva	0.8	10	10	40	10 th May	4	477	80
20	Nizija	0.8	10	10	40	11 th May	4	380	80
21	Prima	0.8	10	10	40	8 th May	4	218	80
22	Tiha	0.8	10	10	20	10 th May	5	140	80
23	Zlatka	0.8	10	10	10	10 th May	4	70	80
24	Sonja	0.8	9	10	70	8 th May	4	382	72
25	Pobeda	0.8	9	10	50	15 th May	4	412	72
26	Jarebica	0.8	8	10	60	11 th May	5	822	64
27	Nova Banatka	0.8	9	9	60	16 th May	5	588	64.8
28	Sava	0.8	6	8	20	13 th May	5	134	38.4
29	Selekta	0.8	6	7	30	17 th May	5	81	38.4
30	Eva	0.8	6	7	10	18 th May	after 5	70	38.4
31	Pesma	0.7	6	8	40	17 th May	5	280	33.6
32	Bajka	0.7	6	7	20	10 th May	after 5	177	29.4
33	Sreća	0.7	6	7	15	16 th May	4	67	29.4
34	Slava	0.7	6	7	5	11 th May	4	18	33.6

Tab. 2 — Correlation value (r) based on data from field and greenhouse, for testing wheat varieties

Correlation	Infection intensity in field				AUDPC 2004.
	2004				
	28 th May	2 nd June	9 th June	16 th June	
2 nd June 2004	0.494				
9 th June 2004	0.451	0.826			
16 th June 2004	0.449	0.543	0.781		
AUDPC/04.	0.412	0.647	0.882	0.873	
LPxIFxRT	0.322	0.550	0.634	0.660	0.598
LP	0.359	0.572	0.574	0.580	0.543

DISCUSSION

Only 25% of tested varieties with maximal values of resistance characteristics at seedling stage (1 10, 10) showed infection intensity in field 70 and up. Although genotypes in this experiment should have been adapted for climate condition in this region, some of them did not show correlation among resistance expression at different stage. Short LAD (leaf area duration) and none reconciled mature result with relatively lower yields and lead to be favorable for other facultative parasites. Many factors, as the extremely unfavorable temperatures, dry conditions, or poor agricultural practice have the influence on wheat and leaf rust development in field, but on all varieties suitable for the region (Putnik and Jerković, 2002). In this experiment, it was not the case, climatic conditions and other factors were approximate ideal for plants and parasite, too. Resistance to *Puccinia triticina* was unconditionally proved.

In the period of 1970—1973, varieties Novosadska crvena, Sava, Partizanka, Nova Banatka were registered. Until today, the first one did not show any kind of resistance to *Puccinia triticina*, although Sava expressed low values of tested characteristics. From 1974—1981: Novosadska rana 2 (NSR 2) and Nizija whose resistance is still present and because of that Nizija could be potential source of genes for durable resistance.

Genotypes as Pasma, Eva, Jarebica, Pobeda, Tiha, Prima, Renesansa, Evropa 90, Tera, Rusija, Dična, Košuta were registered between 1988 and 1996 (Đokić, 2000). They are listed here according to their level of resistance, starting with the most resistant. The last six of them have no resistance characteristic even though they were registered later than, for example, Sava and Nizija. Their latent period is also the shortest as the consequence of resistance overcoming after long period in production. Such occasional resistance in the field also has to be declared as pseudo (Putnik-Delić, 2006).

Results of interaction between Mina and *Puccinia triticina* were not expected. At seedling stage, it showed total susceptibility. In former investigations, it was hypersensitive on higher temperatures. Mina has so-called marble leaf in adult stage, which was not in correlation with hypersensitive reaction. This variety had poor technological quality of seed, and it matured late.

Zlatka and Tiha were only genotypes with prolonged latent period in greenhouse, and in the field condition, they showed the lowest infection intensity (10, 20). They had early appearance of spike as Prva and Nizija, but lower infection intensity. It is very important in production although LP values is the last characteristics before resistance overcoming. Other genotypes with lower values of parameters at seedling stage had higher maximal infection intensity in the field except Eva.

All analyzed varieties Đokić (2000) characterized as very resistant. It was not confirmed in these experiments because of resistance overcoming.

According to Broers (1997) LP, IF and RT are highly correlated with AUDPC data. This fact suggests that selection based on greenhouse results allows selection of genotypes with high level of quantitative resistance. Infection intensity after uniform inoculation in a greenhouse could be used for monocycle estimation, because it is the simplest procedure for evaluation.

Genotypes with different resistance genes, which are susceptible today, probably could be used again as source of resistance in future, after a period of exclusion.

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ОТПОРНОСТ НЕКИХ ГЕНОТИПОВА ПШЕНИЦЕ ПРЕМА *Puccinia triticina*

Марина Путник-Делић

Пољопривредни факултет, Департман за ратарство и повртарство,
Трг Доситеја Обрадовића 8, 21000 Нови Сад, Србија

Резиме

Тестирањем 34 генотипа пшенице утврђене су вредности и степени повезаности показатеља отпорности према *Puccinia triticina* у различитим фазама развоја пшенице. У стадијуму сејанаца у стакленику су одређени: латентни период, успешност инфекције и реакциони тип, док је у пољу евидентирано време класања и сушење лисне масе, као и интензитет заразе, вишестратним читавањем. Корелацијом оцењених података у стадијуму сејанаца и интензитета заразе у различитим фазама развоја генотипова пшенице у пољским условима, установљена је слаба ($r = 0,322$) до јака ($r = 0,660$) повезаност. Највиша вредност коефицијента корелације карактера отпорности у стакленику је била с последњим читавањем у пољским условима. Корелације интензитета заразе у каснијим фазама указује на прилагођеност сорти регионалним условима. Вредности AUDPC су, такође, биле повезане с карактерима отпорности у стадијуму сејанаца (0,598). Генотипови пшенице код којих није било значајнијих одступања по карактерима отпорности од најосетљивијих у стадијуму сејанаца, а нису испољили максималне интензитета заразе у пољу сматрано је да су псеудорезистентни, односно да поседују пољску отпорност. Ову врсту отпорности као резултат специфичне и лажне испољили су генотипови као што су Тиха, Златка и Ева.

*Peter Bokor*¹, *Ján Tancik*¹, *Miroslav Habán*¹,
*Branko J. Marinković*², *Milan Poláček*¹

¹ Slovak University of Agriculture in Nitra
Faculty of Agrobiolgy and Food Resources
Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic

² University of Novi Sad, Faculty of Agriculture
Dositeja Obradovića Sq 8, 21000 Novi Sad, Serbia

THE OCCURRENCE OF PESTS ON LEMON BALM (*MELLISA OFFICINALIS*) AND GARDEN SAGE (*SALVIA OFFICINALIS*)

ABSTRACT: Production of medicinal plants is accompanied by many problems, out of which some are overcome. Problems in medicinal plant production, including the production of sage and lemon balm that cannot be avoided are pathogens, the presence of insects and weeds.

During the summer of 2003 and 2004, the occurrence of animal pests and pathogens was investigated in the medicinal plants lemon balm (*Mellisa officinalis*) and garden sage (*Salvia officinalis*). The pathogens *Alternaria alternata* and *Fusarium moniliforme* and the insects *Eupterix atropunctata* and *Empoasca pteridis* (Homoptera, Cicadellidae) were identified as the cause of various damages to medicinal plants.

KEY WORDS: diseases, garden sage, lemon balm, pathogens

INTRODUCTION

Garden sage and lemon balm are species cultivated on large areas in the world. Such mass production is favorable for the occurrence of animal pests and pathogens. Although garden sage and lemon balm are not often infested with microorganisms and pests, in some cases their yield and quality can be reduced in this manner.

Septoria mellisae infests lemon balm and causes leaf spot. The leaf spots are gray, sometimes black, and are usually limited by leaf nerves. *Puccinia melissae* causes rust on lemon balm. Gamliel and Yarden (1998) indicated that, besides the fungus *P. mellisae*, rust can also be caused by the fungi *P. menthae* and *P. angustata*.

Oidium erysiphoides causes sage mildew, while different leaf and stalk spots are caused by the fungi *Ascochyta vicina* and *Phoma salviae*. Plenk

(2002) indicated *Peronospora lamii* as the pathogenic fungus causing sage rot. This fungus was found on sage by other researchers as well, Ellis, Kellermann (1887) and Muller (1999), who uses the old term, *P. swinglei*.

Another very important sage disease is wilt. Salli (1997) indicated the following pathogens isolated from wilt-affected plants: *Fusarium oxysporum*, *Alternaria alternata*, *F. Solani*, *F. Moniliforme*, *Rhizoctonia solani* and *Fusarium culmorum*. This data leads to the conclusion that the most important causal agents of wilt are the *Fusarium* species of fungi.

Lemon balm pests are *Eupteryx atrapunctata* and *Cassida viridis*. Lemon balm can be damaged by the flea beetle, different caterpillars and some insect species.

Stepanović (1998) indicated the armyworm *Phytometra chrysitis* as a sage pest, while Habán (1996) indicated the caterpillar *Zygaena punctum* as causing damage to sage leaves.

Many sage insect species were determined by Kostić et al. (1999). Sage flowers were infested with *Zygaena punctum*, *Adelphocoris lineolatus*, *Euridema ornata* and *Dolicoris baccarum*. The following species were found on the leaves: *Disaneura salviae*, *Thrips tabaci*, *Empoasca pteridis*, *Aceria salviae*, *Tetranychus lubeniu*, etc.

MATERIAL AND METHODS

The occurrence of lemon balm and sage pests and diseases was investigated in the Kolonjani area, 10 km to the northeast of the town of Nitra. Crops of these medicinal plants were controlled once every two weeks. Diseased plants were marked. The leaves and other infested plant parts were examined in laboratory conditions. Fungi were isolated from the diseased plants. Isolation was conducted on potato stock. After sieving, determination of pathogens was carried out.

Pest numbers and dispersion were determined by the method of random catch using the $\emptyset = 50$ cm catcher. Three replicates were made in the middle of the plot (3 x 50 catches). The insects were starved and determination was done following that.

RESULTS AND DISCUSSION

The following sage and lemon balm pathogens were determined: *Fusarium equiseti* and the species *Alternaria alternata*, syn. *A. tenuis*. The determined pest species were *Eupteryx atrapunctata* and *Empoasca pteridis* from the family *Cicadaelidae* and some more species from families *Miridae*, *Aphididae*, *Chloropidae* and *Chrysomelidae*.

The health condition of the medicinal plant crops was good and disease symptoms were detected on the leaves and stems on the inside and on the lower plant parts. The leaves were wilted and black in these places. Pathogen *Fusarium equiseti* was isolated from stalks and leaves garden sage plants. The

identification was carried out by using of manuals and monograph Gerlach, Nirenberg (1982), and Samson et al. (1995). On PDA *F. equiseti* produced aerial, yellowish brown mycelium. On mycelium only macroconidia formed, mostly 4—5 septae and 26—60 μ long and 3.5—5.2 μ wide. Chlamydospores were formed in hyphae, less frequently in conidia. Kostić et al. (1999) determined the presence of *Fusarium*, *F. equiseti* in sage seeds, roots, stalk and leaves. One more species of fungus was isolated from the seeds, stalk, leaves and roots — *F. moniliforme*, var. *subglutinans* sage Kostić et al. (1999).

Alternaria tenuis was isolated from sage seeds and leaves. The pathogen caused round or shapeless black spots on the leaves. The spots often merged together and caused necrosis. The symptoms were observed on the older leaves first, Kostić et al. (1999). We observed similar symptoms on the older leaves, from which the fungus *A. alternata* was isolated. *A. alternata* on PDA produces aerial mycelium. The first mycelium was white and later it became dark grayish and black. Mostly 3—6 septae conidia formed on mycelium in chain. Measuring of conidia was 20—60 x 11—15 μ . The identification was carried out by using of manuals Ellis.

Hristov (1972) in Bulgaria determined the following pathogens: *Erysiphe salviae*, *E. cichoracearum*, *Peronospora swinglei*, *P. lamii*, *Ovularia ovata*, *Cercospora salvicola*, *Ramularia salviae*, *Septoria salviae*, *Pythium debaryanum*, *Rhizoctonia solani* and *Fusarium* spp. Pironethe (1996) in America found sage rust and rusts caused by the fungi *Puccinia salvicola*, *P. caulicola* a *P. farinacea*.

The occurrence of pests belonged to family *Cicadaelidae*, *Aphididae*, *Cassidinae* and *Chrysomelida* subfamily *Halticinae* (Tab. 1 and 2) was determinate on lemon balm (*Mellisa officinalis*) and garden sage (*Salvia officinalis*). Especially, the insects *Eupterix atropunctata* and *Empoasca pteridis* (*Homoptera*, *Cicadelidae*) were identified.

Tab. 1 — Occurrence of pests on lemon balm in 2003 and 2004

Pest	2003			2004		
	2.6.	5.6.	17.6.	20.5.	9.6.	17.6.
<i>Aphididae</i>	13	8	4	13	53	7
<i>Phyllotreta atra</i> and <i>P. undulata</i>	5	4	4	20	45	5
<i>Casida viridis</i>	0	0	0	0	1	0
<i>Eupterix atropunctata</i>	283	280	274	656	89	63

Tab. 2 — Occurrence of pests on garden sage in 2003 and 2004

Pest	2003			2004		
	2.6.	5.6.	17.6.	20.5.	9.6.	17.6.
<i>Aphididae</i>	10	7	2	68	48	18
<i>Phyllotreta atra</i> and <i>P. undulata</i>	3	3	3	3	1	16
<i>Casida viridis</i>	0	0	0	0	0	0
<i>Eupterix atropunctata</i>	63	17	6	132	101	28

The most numerous pests on lemon balm were *Eupterix atropunctata*. *Eupterix atropunctata* were most abundant pests on lemon balm; about 280 individuals per one day were recorded in 2003. The same species was the most abundant also on sage. The second rank belongs to *Empoasca pteridis*. There were recorded 3 individuals on sage and 12 ones on lemon balm. Tanasijević and Simova-Tošić (1987) and Simova-Tošić et al. (1997) found the following species from the family *Cicadaelidae* on sage in Serbia: *Eupterix atropunctata*, *Eupterix urticae* and *Eupterix* spp. They damaged the leaves, causing leaf spots, leaf curling, and different leaf deformations. *E. atropunctata* causes chlorophyll degradation, resulting in the appearance of round pale or gray spots, which can merge with one another in some cases, Neubauer et al. (1980).

We recorded same species from families *Chrysomelidae*, *subfamilia Halticinae* on lemon balm than on sage. In both years, species *Phyllotreta atra* and *P. undulata* was recorded and in 2004 *Longitarsus lycopi* on lemon balm was recorded, too. Dominated species were found on lemon balm in 2004, when the greatest number of individuals was recorded. Typical harmful caused by flea beetle — holes on leaves were recorded.

During 2004 more aphids were recorded on sage and lemon balm in comparison with 2003. *Aphis gossypii* Glover was dominated. This polyphagous species was recorded also on sage and many other crops in Serbia. In Serbia, as well as in other countries, on sage and other species belonging to *Salvia* spp. was recorded aphids *Aphis salviae* Walker, *Aphis passeriniana* del Guericco.

On lemon balm was recorded only one specimen *Cassida viridae* from family *Cassidinae*. This is polyphagous species attacking crops belonging to *Cemopodiaceae*, *Carduaceae* and *Malvaceae*.

CONCLUSIONS

Production of medicinal plants, such as lemon balm and garden sage, is accompanied by many problems, like weed, disease and pest occurrence.

During the summer of 2003 and 2004, the occurrence of pests and pathogens was investigated in lemon balm (*Mellisa officinalis*) and garden sage (*Salvia officinalis*). The pathogens *Alternaria alternata* and *Fusarium* spp. and the insects *Eupterix atropunctata* and *Empoasca pteridis* (*Hemiptera*, *Cicadaelidae*) were identified. All these pests can decrease the quantity and quality of drugs.

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ПОЈАВА (ПРИСУСТВО) ШТЕТОЧИНА И БОЛЕСТИ У УСЕВУ МЕЛИСЕ
(*MELLISA OFFICINALIS*) И ЖАЛФИЈЕ (*SALVIA OFFICINALIS*)

Петер Бокор¹, Јан Танцик¹, Мирослав Хабан¹,
Бранко Ј. Маринковић², Милан Полачек¹

¹ Словачки пољопривредни универзитет, Пољопривредни факултет,
Тр. А. Хлинку 2, 949 76 Њитра, Република Словачка

² Универзитет у Новом Саду, Пољопривредни факултет,
Трг Доситеја Обрадовића 8, 21000 Нови Сад, Србија

Резиме

При узгоју лековитог биља срећемо се са различитим проблемима, међу њима и са појавом корова, болести и штеточина. С овим штетним факторима срећемо се и при узгоју мелесе и жалфије.

Појава болести и штеточина у усеву мелисе (*Mellisa officinalis*) и жалфије (*Salvia officinalis*) праћена је током лета 2003. и лета 2004. године. Установили смо појаву следећих патогена: *Alternaria alternata* и *Fusarium* spp. и следеће врсте инсеката: *Eupterix atropunctata* и *Empoasca pteridis* (Homoptera, Cicadellidae), које могу оштећивати лековито биље и проузроковати снижење како квантитета тако и квалитета дроге.

Anita S. Klaus¹, Damir V. Beatović²,
Miomir P. Nikšić¹, Slavica Ć. Jelačić²,
Viktor K. Nedović¹, Tanja S. Petrović¹

¹ Dept. of Food Technology and Biochemistry

² Dept. of Crop Science, Faculty of Agriculture, University of Belgrade
Nemanjina 6, 11081 Belgrade, Serbia
e-mail: aklaus@agrifaculty.bg.ac.yu

INFLUENCE OF ETHEREAL OILS EXTRACTED FROM LAMIACEAE FAMILY PLANTS ON SOME PATHOGEN MICROORGANISMS

ABSTRACT: As pathogen microorganisms can be found in different kinds of food, using of natural antimicrobial compounds, like ethereal oils, could be important in the preservation of different groceries. To evaluate antimicrobial activity of ethereal oils extracted from *Lamiaceae* family plants — *Rosmarinus officinalis* L., *Thymus vulgaris* L., *Majorana hortensis* Moench, and *Salvia officinalis* L screening of their effects against food borne bacteria *Staphylococcus aureus*, *Enterococcus faecalis*, *Proteus mirabilis*, *Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Escherichia coli* O157:H7, *Listeria monocytogenes* and yeasts *Candida albicans* and *Saccharomyces cerevisiae* were applied. All investigated concentrations and pure *Majorana hortensis* and *Thymus vulgaris* ethereal oils showed microbicidal effect on majority of tested microorganisms.

KEY WORDS: ethereal oils, marjoram, rosemary, sage, thyme, antimicrobial effect

INTRODUCTION

We investigated antimicrobial activity of ethereal oils on some strains of gram-negative bacteria: *Proteus mirabilis*, *Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Escherichia coli* O157:H7; gram-positive bacteria: *Enterococcus faecalis*, *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Listeria monocytogene*; yeasts *Saccharomyces cerevisiae* and *Candida albicans*.

Medicinal, aromatic and herb spices from family *Lamiaceae* such as sage (*Salvia officinalis* L.), rosemary (*Rosmarinus officinalis* L.), marjoram (*Majorana hortensis* Moench.), and thyme (*Thymus vulgaris* L.) are widely distributed in Serbia growing as wild or cultivated species (Sarić, 1989). These

plants are used as stomachic, spasmolytic, carminative, and expectorant agents in folk medicine and in official medicine. Ethereal oils extracted from *Lamiaceae* family plants can contribute the quality of food with better odor and flavor what is consider as very important quality parameter in food manufacturing (K o v a č e v i ć, 2001). Other benefits could be application of ethereal oils in therapeutics purposes due to their antimicrobial (bactericidal and fungicidal) effects on some pathogen microorganisms (S t e f a n i n i et al., 2001, K l a u s et al., 2007).

The folium of sage (*Salvia officinalis* L.) contains 1% to 2.8% of ethereal oil. Monoterpenes are the major ingredients of this oil: tujon (30%—60%) as predominant constituent, cineol (15%) and camphor. The ethereal oils of sage have spasmolytic, astringent and carminative effects (B a r a t t a et al., 1998).

The folium of rosemary (*Rosmarinus officinalis* L.) contains up to 2% of ethereal oil. The major ingredients of ethereal oil of *Rosmarini aetheroleum* are cineol (35%), pinene, camphor and borneol. It is used in the food processing industry as a spice and aroma enhancer (A n g i o n i et al., 2004).

The herb of thyme (*Thymus vulgaris* L.) contains 1%—2% of ethereal oil. Monoterpenes are the major ingredients of this oil with thymol (40%) as predominant constituent and carvacrol, which are well known like antimicrobial substances. The ethereal oils of thyme have spasmolytic and expectorant effects (M a r i n o at al., 1999).

The herb of marjoram (*Majorana hortensis* Moench.) contains 1—2% of ethereal oil (terpinen-4-ol, borneol). It is also used in folk medicine as stomachic and as a spice and aroma enhancer in food processing industry (B i o n d i at al., 1993).

MATERIALS AND METHODS

Antimicrobial effects of ethereal oils extracted from *Lamiaceae* family plants on certain strains of microorganisms were investigated.

Test organisms

Antimicrobial activity was tested on gram-negative bacterial strains *Proteus mirabilis* ATCC 17576, *Salmonella enteritidis* ATCC 31806, *Pseudomonas aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *E. coli* O157:H7 ATCC 35150, *E. coli* O157:H7 ATCC 12900; on gram-positive bacterial strains *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* ATCC 11788, *Bacillus subtilis* BS-30B-0403001, *Listeria monocytogenes* ATCC 19115, *Listeria monocytogenes* ATCC 19112; and on yeasts *Saccharomyces cerevisiae* ATCC 9763, *Candida albicans* ATCC 24433 and *Candida albicans* ATCC 10259.

ATCC cultures were taken from American Type Culture Collection, Rockville, Maryland. *B. subtilis* were taken from Dept. of Food Technology and Biochemistry, Faculty of Agriculture, University of Belgrade. The cultures of

bacteria were maintained in Mueller-Hinton agar and for *Listeria monocytogenes* TSA-YE slants at 4°C throughout the study and used as stock cultures.

Preparing of ethereal oils

Ethereal oils were obtained from sage (*Salvia officinalis* L.), rosemary (*Rosmarinus officinalis* L.), marjoram (*Majorana hortensis* Moench.), and thyme (*Thymus vulgaris* L.). The folium of sage and rosemary and herbs of marjoram and thyme represent a domestic raw material from Serbia. The folia of sage (variety Primorska), rosemary (domestic variety), thyme (variety N-19), and marjoram (variety Holand olfactory) were used in this experiment (Anonymous, 2005). Medicinal herbs were provided by the Institute for Medicinal Plant Research "Dr Josif Pančić" from Belgrade. Materials were determined at the Faculty of Agriculture in Belgrade. Ethereal oils were extracted from dry folia and herbs of sage, rosemary, thyme and marjoram by distillation and vaporization with Clevenger according to the methods of Pharmacopeia Yugoslavica V (Ph. Jug. V, 2000).

Screening of ethereal oils for antimicrobial activity was done by the disk diffusion method with three different concentrations (pure oil, 1:1, and 2:1) (Klaus et al., 2007). Ethereal oils were dissolved in 96% C₂H₅OH in ratio 2:1 (2 ml ethereal oil: 1 ml 96% C₂H₅OH) and in ratio 1:1 (1 ml ethereal oil: 1 ml 96% C₂H₅OH). In cases of ethereal oils obtained from *Majorana hortensis* and *Thymus vulgaris* ethereal oils were additionally dissolved in 96% C₂H₅OH in ratio 1:2 (1 ml ethereal oil:2 ml 96% C₂H₅OH) and 1:3 (1 ml ethereal oil:3 ml 96% C₂H₅OH).

Preparing of microorganisms

Bacterial strains were inoculated in Mueller-Hinton broth except *Listeria monocytogenes* which were inoculated in TSB-YE and incubated at 37°C for 24 h, to reach concentration 10⁶ cells/ml. Yeasts were inoculated in malt broth and incubated at 30°C for 48 h, to reach concentration 10⁶ cells/ml. The inocula of microorganisms were prepared from 24 h and 48 h-old culture and suspensions were adjusted to 0.5 McFarland standard turbidity (~ 10⁶ CFU/ml).

Influence of ethereal oils on microorganisms

Screening of essential oils for antimicrobial activity was done by the disk diffusion method. The cultures were adjusted to approximately 10⁶CFU/ml with sterile saline solution. Petri dishes (100 mm in diameter) were inoculated with 0.2 ml suspension of certain strains of microorganisms and overlaid with 20 ml of the medium. For most of bacteria Mueller Hinton agar was used, for *Listeria monocytogenes* TSA-YE were used and for yeasts malt agar were used. Three filter disks (Sigma-Aldrich's Whatman® Schleicher & Schuell, 6 mm in diameter) were placed on every agar and diffusion method was per-

formed by adding 10ml of appropriate suspension on every disk. Every test was done in triplicate. Blind probe contained only 96% C₂H₅OH, without any ethereal oils. Bacteria were incubated at 37°C for 24h and yeasts were incubated at 30°C for 48h. After the incubation period, the zone of inhibition was measured. Small sectors from the zone of inhibition were taken and inoculated in nutrient broth for cultivating bacteria, TSB-YE for cultivating *Listeria monocytogenes* and in malt broth for yeasts. Sectors in nutrient broth and TSB-YE were incubated at 37°C for 24h and sectors in malt broth were incubated at 30°C for 48h to see if the effect of ethereal oils were microbicide or microbistatic (Klaus et al., 2007).

RESULTS AND DISCUSSION

After 24-hour incubation at 37°C for bacterial strains and 48-hour incubation at 30°C for yeasts the zone of inhibition around the filter disks were measured and results are presented in Table 1. If nutrient, TSB-YE or malt broth with sectors taken from the zone of inhibition showed turbidity after incubation, influence of applied ethereal oils were regarded as microbistatic. In the cases where nutrient, TSB-YE or malt broth with sectors taken from the zone of inhibition stayed clear after incubation, influence of applied ethereal oil were microbicide. Blind probe showed that 96% C₂H₅OH had no antimicrobial influence on investigated microorganisms.

Pure ethereal oil obtained from *Rosmarinus officinalis* showed microbistatic effect on all investigated bacteria, but had no influence on yeasts *Candida albicans* ATCC 24433, *Candida albicans* ATCC 10259 and *Saccharomyces cerevisiae* ATCC 9763. The most intensive microbistatic influence of pure ethereal oil were in cases of *Bacillus cereus* ATCC 11788 (inhibitory zone 14.67 mm) and *Proteus mirabilis* ATCC 17576 (inhibitory zone 11.67 mm). Suspensions of ethereal oils in 96% C₂H₅OH in ratio 2:1 showed microbistatic effect on all investigated microorganisms, with best results in contact with yeasts *Candida albicans* ATCC 10259 (inhibitory zone 22 mm), *Candida albicans* ATCC 24433 (inhibitory zone 21 mm) and *Saccharomyces cerevisiae* ATCC 9763 (inhibitory zone 20 mm) and bacteria *E. coli* O157:H7 ATCC 35150 (inhibitory zone 10.33 mm). Suspensions of ethereal oils in 96% C₂H₅OH in ratio 1:1 had microbistatic effect on all investigated microorganisms, too, but with best results in contact with *E. coli* O157:H7 ATCC 35150 (inhibitory zone 11 mm) and *Bacillus subtilis* ATCC BS-30B-0403001 (inhibitory zone 9.67 mm).

All concentrations and pure ethereal oils obtained from *Thymus vulgaris* showed microbicide effect on yeasts *Candida albicans* ATCC 24433 (inhibitory zone 29 mm with pure oil), *Candida albicans* ATCC 10259 (microbicide with pure oil and in suspensions 2:1 and 1:1) and *Saccharomyces cerevisiae* ATCC 9763 (microbicide with pure oil-inhibitory zone 30.67 mm, and in suspensions 2:1 and 1:1). Similar results were obtained at bacteria *Staphylococcus aureus* ATCC 25923 (microbicide effect with pure oil-inhibitory zone 25.33 mm and in suspension 2:1-inhibitory zone 25.67 mm), *Listeria monocytogenes*

Tab. 1 — The zone of inhibition procured by presence of pure ethereal oils and in concentrations 2:1 (2 ml ethereal oil:1 ml 96% C₂H₅OH) and 1:1 (1 ml ethereal oil:1 ml 96% C₂H₅OH).

microorganism	Average size of the zone of inhibition in diameter (mm)											
	<i>Rosmarinus officinalis</i>			<i>Thymus vulgaris</i>			<i>Majorana hortensis</i>			<i>Salvia officinalis</i>		
	pure oil	2:1	1:1	pure oil	2:1	1:1	pure oil	2:1	1:1	pure oil	2:1	1:1
<i>Staphylococcus aureus</i>	6.33	6.67	7	25.33	25.67	—	18	23.33	27	7	6.67	8.33
<i>Enterococcus faecalis</i>	8.67	7.67	8.67	69	58	39.67	49.33	32	20.33	3	11	8
<i>Proteus mirabilis</i>	11.67	7.33	7	39.33	25	41.67	37.67	47	52	14	11	7
<i>Salmonella enteritidis</i>	4	9	9	30	31.33	25.33	22	46.33	38	3	9	4
<i>Pseudomonas aeruginosa</i>	5	9	6	57	53.33	45	30.33	32.33	43.33	25	15.66	9.33
<i>E. coli</i>	9	7.67	9.33	54.33	+	36.33	33	47	60	5.67	9.67	8.67
<i>E. coli</i> (O157:H7) 35150	5	10.33	11	15.67	36.33	50	29	24.33	29	3	8.33	4
<i>E. coli</i> (O157:H7) 12900	3	8.33	8.33	29.67	+	+	22	22	31	2.67	7.67	9
<i>Bacillus cereus</i>	14.67	9.33	9	+	+	59	20.67	32	30	15.33	10.33	7.67
<i>Bacillus subtilis</i>	8	7	9.67	47.33	+	27.67	37	50	45	9	8.67	8.33
<i>Listeria monocytogenes</i> 19115	8.67	4.67	5.67	20.67	16	—	38	57	51.67	6.67	4.67	4.67
<i>Listeria monocytogenes</i> 19112	9	4	4	18.67	22.67	—	31	32	36.33	9.67	5.67	5.67
<i>Saccharomyces cerevisiae</i>	—	20	8	30.67	23.67	23	24.67	23	21	56	3.33	5
<i>Candida albicans</i> 24433	—	21	3.67	29	19.33	16	40	43.67	42.33	59	3.33	4
<i>Candida albicans</i> 10259	—	22	4	+	+	+	+	+	71	+	4	5

— With no influence on growth
+ With no growth

ATCC 19115 (inhibitory zone 20.67 mm with pure oil), *Listeria monocytogenes* ATCC 19112 (inhibitory zone 22.67 mm with suspension 2:1) and *E. coli* O157:H7 ATCC 35150 (microbicide in all cases, in suspension 1:1 inhibitory zone was 50 mm). The best microbistatic influence of suspension 2:1 noticed in the cases of *E. coli* ATCC 25922 with no any growth, *Enterococcus faecalis* ATCC 29212 when inhibitory zone was 58 mm (Fig.1) and *Pseudomonas aeruginosa* ATCC 27853 with inhibitory zone of 53.33 mm. In the presence of suspension 1:1, the best microbistatic effect appeared at *E. coli* O157:H7 12900 without growth, at *Bacillus cereus* ATCC 11788 with inhibitory zone 59 mm and at *Proteus mirabilis* ATCC 17576 (inhibitory zone 41.67 mm).

All concentrations and pure ethereal oils obtained from *Majorana hortensis* showed microbicide effect on *Staphylococcus aureus* ATCC 25923 (inhibitory zone 27 mm in suspension 1:1), *Salmonella enteritidis* ATCC 31806 (inhibitory zone 46.33 mm in suspension 2:1 and 38 mm in suspension 1:1), *Saccharomyces cerevisiae* ATCC 9763 (inhibitory zone 24.67 mm in the presence of pure oil), *Candida albicans* ATCC 24433 (inhibitory zone 43.67 mm in suspension 2:1), *Candida albicans* ATCC 10259 (with no growth in the presence of pure oil and suspension 1:1 and with inhibitory zone of 71 mm in the presence of suspension 1:1), *Listeria monocytogenes* ATCC 19115 (inhibitory zone 57 mm in suspension 2:1 and 51.67 mm in suspension 1:1, Fig.2), *Listeria monocytogenes* ATCC 19112 (inhibitory zone 36.33 mm in suspension 1:1), *E. coli* O157:H7 ATCC 35150 (inhibitory zone 29 mm in the presence of pure oil and in suspension 1:1), and *E. coli* O157:H7 ATCC 12900 (inhibitory zone of 31 mm in the presence of suspension 1:1). The most intensive microbistatic influence of suspensions of ethereal oils in 96% C₂H₅OH were in case of *Bacillus subtilis* ATCC BS-30B-0403001 (inhibitory zone 50 mm in suspension 2:1 and 45 mm in suspension 1:1), *E. coli* ATCC 25922 (inhibitory zone 47 mm in suspension 2:1 and 60 mm in suspension 1:1) and *Proteus mirabilis* ATCC 17576 (inhibitory zone 47 mm in suspension 2:1 and 52 mm in suspension 1:1).

All concentrations and pure ethereal oils obtained from *Salvia officinalis* showed microbicide effect on yeasts *Saccharomyces cerevisiae* ATCC 97639 (inhibitory zone 56 mm in the presence of pure oil) and *Candida albicans* ATCC 24433 (inhibitory zone 59 mm in the presence of pure oil). In the contact with other investigated microorganisms, pure oil and suspensions of ethereal oil in 96% C₂H₅OH in ratio 2:1 and 1:1 showed microbistatic effect. The best microbistatic influence of pure oil noticed in the cases of *Pseudomonas aeruginosa* ATCC 27853 (inhibitory zone 25 mm) and *Bacillus cereus* ATCC 11788 (inhibitory zone 15.33 mm). Suspension 2:1 showed the best results in the contact with *Pseudomonas aeruginosa* ATCC 27853 (inhibitory zone 15.66 mm), *Enterococcus faecalis* ATCC 29212 (inhibitory zone 11 mm) and *Proteus mirabilis* ATCC 17576 (inhibitory zone 11 mm). Suspension 1:1 showed best results in the contact with *Pseudomonas aeruginosa* ATCC 27853 (inhibitory zone 9.33 mm) and *E. coli* ATCC 25922 (inhibitory zone 9.33 mm).

In cases of ethereal oils obtained from *Majorana hortensis* and *Thymus vulgaris* ethereal oils were additionally dissolved in 96% C₂H₅OH in ratio 1:2

(1 ml ethereal oil:2 ml 96% C₂H₅OH) and 1:3 (1 ml ethereal oil:3 ml 96% C₂H₅OH), because previous investigations with dissolving of ethereal oils in 96% C₂H₅OH in ratio 2:1 (2 ml ethereal oil:1 ml 96% C₂H₅OH) and in ratio 1:1 (1 ml ethereal oil:1 ml 96% C₂H₅OH) showed very good results. Results are presented in Table 2.

Tab. 2 — The zone of inhibition procured by presence of ethereal oils in concentrations 1:2 (1 ml ethereal oil:2 ml 96% C₂H₅OH) and 1:3 (1 ml ethereal oil:3 ml 96% C₂H₅OH)

microorganism	Average size of the zone of inhibition in diameter (mm)			
	<i>Thymus vulgaris</i>		<i>Majorana hortensis</i>	
	s u s p e n s i o n			
	1:2	1:3	1:2	1:3
<i>Staphylococcus aureus</i>			11.33	11.67
<i>Salmonella enteritidis</i>	10	9	10	11.67
<i>E. coli</i> (O157:H7) 35150	9.67	9.67	11	12.67
<i>E. coli</i> (O157:H7) 12900			10	11.33
<i>Bacillus cereus</i>			19.33	31.67
<i>Listeria monocytogenes</i> 19115	31	37	36.33	36.33
<i>Listeria monocytogenes</i> 19112	38	34	34	42.33
<i>Saccharomyces cerevisiae</i>	27	30.67	21.67	23.33
<i>Candida albicans</i> 24433	27	22	21	26
<i>Candida albicans</i> 10259	84	75	60	45

Suspensions of *Thymus vulgaris* ethereal oil in 96% C₂H₅OH in ratio 1:2 and 1:3 showed microbicide effect in contact with all investigated microorganisms except *E. coli* O157:H7 ATCC 35150 when it was microbistatic with inhibitory zone 9.67 mm in both concentrations. The best microbicide effect of these two suspensions appeared when applied on *Listeria monocytogenes* ATCC 19112 (inhibitory zone 38 mm in suspension 1:2 and 34 mm in suspension 1:3) and *Listeria monocytogenes* ATCC 19115 (inhibitory zone 31 mm in suspension 1:2 and 37 mm in suspension 1:3).

Suspensions of *Majorana hortensis* ethereal oil in 96% C₂H₅OH in ratio 1:2 and 1:3 showed microbicide effect in contact with *Staphylococcus aureus* ATCC 25923 (inhibitory zone 11.67 mm in suspension 1:3), *Salmonella enteritidis* ATCC 31806 (inhibitory zone 11.67 mm in suspension 1:3), *Saccharomyces cerevisiae* ATCC 97639 (inhibitory zone 23.33 mm in suspension 1:3), *Candida albicans* ATCC 24433 (inhibitory zone 26 mm in suspension 1:3), *Candida albicans* ATCC 10259 (inhibitory zone 60 mm in suspension 1:2), *Listeria monocytogenes* ATCC 19115 (inhibitory zone 36.33 mm in suspensions 1:2 and 1:3) and *Listeria monocytogenes* ATCC 19112 (inhibitory zone 42.33 mm in suspension 1:3). These suspensions performed microbistatically when applied on *Bacillus cereus* ATCC 11788 (inhibitory zone 31.67 mm in suspension 1:3), *E. coli* O157:H7 ATCC 35150 (inhibitory zone 12.67 mm in suspension 1:3), and *E. coli* O157:H7 ATCC 12900 (inhibitory zone 11.33 mm in suspension 1:3).



Fig. 1 — Inhibitory effect of *Thymus vulgaris* etheral oil (pure oil) on the growth of *Enterococcus faecalis* ATCC 29212



Fig. 2 — Inhibitory effect of *Majorana hortensis* etheral oil (ratio 1:1) on the growth of *Listeria monocytogenes* ATCC 19115

CONCLUSIONS

There are many demands regarding the food safety in modern food production. Microorganisms can contaminate the food on different points, from raw to final product, so producers have to protect the food. Easy, promising and not harmful way to protect the food could be adding the specific herbs spices or their etheral oils, which could be harmonized with flavor, taste and odor of the particular groceries. Besides, historically it is confirmed that some etheral oils possess antimicrobial activities. This could be very important regarding the fact that microorganisms become resistant on numerous antibiotics. By adding some herbs or their etheral oils in food, it is possible to upgrade the quality of food and to protect the groceries from unwanted microorganisms.

In this work it was shown that medicinal, aromatic and herbs spice from *Lamiaceae* family-sage (*Salvia officinalis* L.), rosemary (*Rosmarinus officinalis* L.), marjoram (*Majorana hortensis* Moench.) and thyme (*Thymus vulgaris* L.) could be used for microbial control of some kinds of food by using pure oils or suspension in alcohol. As some of tested pathogen microorganisms could be presented in large numbers, addition of etheral oils is possibly the good way to inhibit their growth and they can be used as antimicrobial supplement for development of new therapeutic agents. Additional *in vivo* studies and clinical trials would be needed to justify and further evaluate the potential of etheral oils as an antimicrobial agent.

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УТИЦАЈ ЕТАРСКИХ УЉА ЕКСТРАКОВАНИХ ИЗ БИЉАКА ФАМИЛИЈЕ *LAMIACEAE* НА НЕКЕ ПАТОГЕНЕ МИКРООРГАНИЗМЕ

Анита С. Клаус¹, Дамир В. Беатовић², Миомир К. Никшић¹,
Славица Ђ. Јелачић², Виктор К. Недовић¹, Тања С. Петровић¹

¹ Институт за прехранбenu технологију и биохемију

² Институт за ратарство, Универзитет у Београду — Пољопривредни
факултет, Немањина 6, 11081 Београд, Србија
e-mail: aklaus@agrifaculty.bg.ac.yu

Резиме

Како патогени микроорганизми могу да се нађу у различитим прехранбеним производима, коришћење природних антимикуробних компонената, као што су етарска уља, може да буде важно за њихово чување. За процењивање антими-

кробне активности етарских уља екстракованих из биљака фамилије *Lamiaceae*-*Rosmarinus officinalis* L., *Thymus vulgaris* L., *Majorana hortensis* Моенсн. и *Salvia officinalis* L. посматран је њихов утицај на микроорганизме контаминенте хране, бактерије *Staphylococcus aureus*, *Enterococcus faecalis*, *Proteus mirabilis*, *Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Escherichia coli* O157:H7, *Listeria monocytogenes* и квасце *Candida albicans* и *Saccharomyces cerevisiae*. Све испитиване концентрације као и чиста етарска уља *Majorana hortensis* и *Thymus vulgaris* показала су микробицидни ефекат на већину тестираних микроорганизама.

Predrag L. Pap
Miroslav P. Marković

Institute of Lowland Forestry and Environment
Antona Čehova 13, Novi Sad, Serbia
e-mail: predragpap@ptt.yu

EFFECTS OF SOME ECOLOGICAL FACTORS ON *DOTHICHIZA POPULEA* SACC. ET BR. GROWTH

ABSTRACT: The aim of the present work was to evaluate the mycelial growth and fruiting vigor of *Dothichiza populea* Sacc. et Br. under various temperatures, pH values, and light regimes. Effect of temperature on the fungus growth was examined by growing isolates in polythermostat at 5°C to 30°C. The best mycelial growth occurred at 20°C, while at 30°C it was inhibited. Fruiting of the fungus was not observed at 5°C, 25°C, and 30°C. However, the best fruiting of the isolates appeared at 20°C. The influence of different pH of the cultivation medium (3,5—10) on the fungus isolates growth was also evaluated. Optimal pH for the fungus growth ranged between 6 and 8, while formation of reproductive organs occurred at all pH values. The influence of two light regimes (light/dark regime and continuous dark) on the fungus growth was also studied. Obtained results showed that mycelial growth and fruiting of the fungus were considerably better under the light/dark regime.

KEY WORDS: *Dothichiza populea*, ecological factors — temperature, pH, light regime

INTRODUCTION

During the second half of the 20th century fungus *Dothichiza populea* Sacc. et Br. has attracted considerable attention of scientists in our country and abroad (Butin, 1957, Taris, 1957, Donaubaue, 1957, Hubbes, 1959, Magnani, 1959, Marinković, 1965, Gojković, N., 1981, Avramović, 1988). A remarkable portion of these authors' investigations was directed to elucidate effect of different external and internal factors on its life cycle. It is known that the fungus actively responds to various external influences by producing or "filtering" of varieties compatible with those conditions. It is supposed that the fungus population comprises subpopulations or varieties (isolates) differing in their response to environmental influences. Responses of isolates to various temperatures, pH, and light regimes were evaluated *in vitro*, in order to study their ecological characteristics.

Considering that these factors also affect the host organism of the parasite fungi, it is not convenient for these investigations to be carried out in the environment. Hence, these investigations were performed at *in vitro* laboratory. Of course, effects of the environmental factors could not be completely realistic evaluated on the base of obtained results, but information about the importance of a certain factor for the fungus growth *in vitro* is valuable. Besides, these results do not have to be in accordance with the situation in nature, where factors other than mentioned also influence the fungus and its host plant, positively or negatively.

MATERIAL AND METHODS

The *Dothichiza populea* isolates were provided from the infected seedlings, originating from Experimental Estate of the Institute for Lowland Forestry and Environment — Novi Sad and from different localities in Vojvodina. Studies of the fungus ecological characteristics were performed using six isolates. Five of them (isolates 229, 1004, 447, 103/92, and 1—5) originate from the Institute's Experimental Estate, while isolate 214 Bč originates from Bečej.

All laboratory experiments started with sowing of the mycelial fragments (size 5 mm) on the carrot medium. Experiments were performed in four replicates, with five Petri dishes in each of them. In order to estimate the mycelial growth, the diameter of the colonies was measured at certain time intervals. The growth of *D. populea* was measured until the colony in whichever Petri dish covered the complete area of the medium. The presence of aerial and substrate mycelia, as well as form and appearance of the colonies, were evaluated by visual observation, during the period of the growth measurements.

Effects of temperature on the fungus growth were examined by growing isolates on the carrot culture medium in polythermostat at 5°C, 10°C, 15°C, 20°C, 25°C, and 30°C. This method for evaluation of different temperatures impact on the pathogen growth under laboratory conditions was used by many authors (Magnani, 1959, Marinković, 1965, Marković, 1970, Arsenijević and Veselić, 1997, Keča, 2001). The diameter of the colonies was measured 5, 12, and 16 days after the experiment beginning.

The pH values of the carrot culture medium were 3, 5, 5, 6, 7, 8, 9, and 10. Prior to sterilization, pH was adjusted using the pH meter, by adding 1N NaOH or HCl. Isolates were cultivated at 20°C, and the diameter of their colonies was measured after 5, 12 and 16 days, as suggested by other authors: Arsenijević (1963), Marković (1970), Borić (1985), Vučinić (1991), Arsenijević and Veselić (1997).

The influence of two lighting conditions (light/dark regime and continuous dark) on the fungus growth was also studied. The light/dark regime was performed in a 12-hour photoperiod. In both light regimes, the fungus was cultivated on the carrot medium at 20°C, while relative humidity was 65%—70%. After sowing, isolate 447 was cultivated in the climate chamber under light/dark regime, while mycelium exposed to total darkness was grown in the incu-

bator. Observations and measurements of the colony diameter were done after 7, 11, and 14 days, according to the method of Vučinić (1991).

RESULTS

Influence of different temperatures on the fungus growth

Appearance of the colonies

Colonies of all isolates formed thin, white, circular shaped mycelia, at 5°C. At higher temperatures (10° and 15°C), colonies formed denser and more compact mycelia, having a typical look of colonies formed on the carrot culture medium at 20°C. Significant differences in appearance of the colonies at 20°C and 25°C were not found. Dirty-white plates of the aerial mycelium occurred around the primary inoculum at 30°C.

Fruiting of the isolates

With exception of the isolate 1—5, all others formed stromata with pycnidia. The fruiting bodies were formed at 10°C, 15°C, and 20°C after 45 days (Tab. 1). The isolates showed the greatest fruiting at 20°C. At this temperature, fructification of isolates 214Bč, 1004 and 447 was intensive, of isolate 229 moderate, while of isolate 103/92 mild. The fruiting intensity was weaker at lower temperatures, with exception of the isolate 103/92 which showed an uniform poor fruiting at temperatures ranging from 10°C to 20°C.

Tab. 1 — Influence of different temperatures on the fruiting intensity of *D. populea* isolates after 45 days of cultivation

Temperature	229	214Bč	1004	447	103/92	1—5
Isolates						
5°C	—	—	—	—	—	—
10°C	+	+	+	++	+	—
15°C	++	++	++	+++	+	—
20°C	++	+++	+++	+++	+	—
25°C	—	—	—	—	—	—
30°C	—	—	—	—	—	—

In this experiment, temperature conditions significantly affected growth and characteristics of the studied isolates in all time intervals.

Growth of the colonies

According to results regarding the diameter of the colonies after 5 days of cultivation (Tab. 2), it could be seen that applied temperatures markedly influenced the rate of the colonies growth. The greatest growth isolates exhibited at 20°C; lower or higher temperatures decreased their growth. A negligible

growth of mycelium was observed at 5°C, 10°C, and 30°C. The isolate 447 was characterized by the highest values of the average diameter of the colony at almost all temperatures (Fig. 1). Lower values were recorded for isolates 103/92, 1004 and 229, whose diameters were similar. Isolates 214Bč and 1—5 had the weakest mycelial growth. After 5 days at 30°C, growth of the colonies was thoroughly stopped.

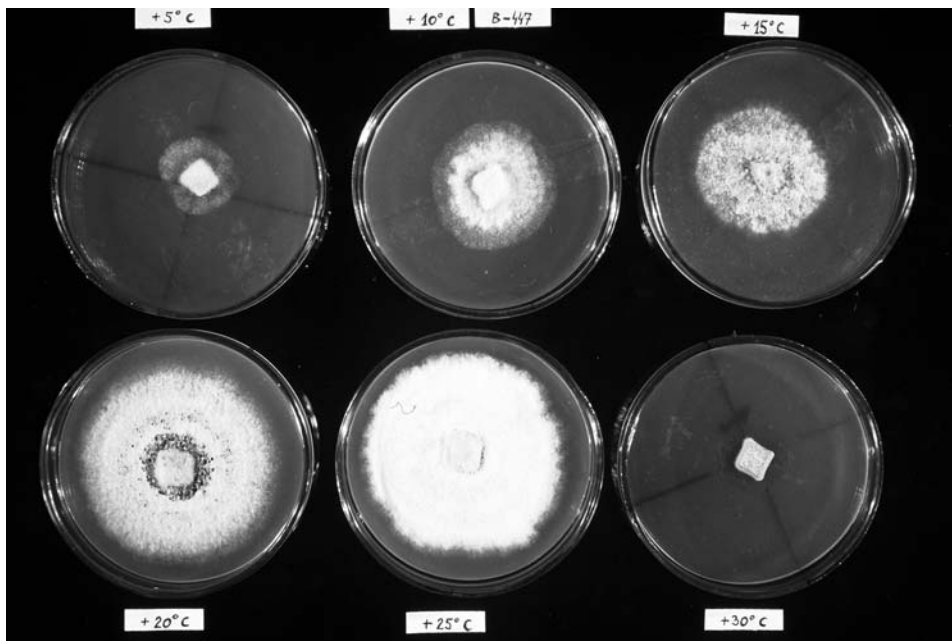


Fig. 1 — Growth of isolate 447 at different temperatures after 17 days of cultivation

Influence of various temperatures on the average diameter of the colonies cultivated for 5—12 days was pronounced. The colony growth was the greatest at 20°C, while at 25°C and 15°C it was significantly weaker. The lowest values were measured at 10°C and 5°C. The order of the isolates was changed after this period. The fast growth of isolate 214Bč caused this situation, because values of its average diameter were equal or higher than of isolates 103/92, 1004 and 229. The lowest values were recorded for isolate 1—5.

The last measurements were performed on the 16th day. The influence and order of the average values obtained for temperature intervals were not changed. Differences in growth between isolates were evident. Isolates 103/92 and 214Bč showed better growth and differentiation than 1004 and 229.

Tab. 2 — Influence of different temperatures on the mycelial growth of *D. populea* cultivated on the carrot nutrient medium

Isolates	Temperatures																	
	5°C			10°C			15°C			20°C			25°C			30°C		
	5	12	16	5	12	16	5	12	16	5	12	16	5	12	16	5	12	16
	Average diameter of the colonies after 5, 12 and 16 days of cultivation (mm)																	
447	13.2	29.2	34.7	16.2	39.2	49.0	27.3	50.0	59.0	38.6	72.2	78.0	40.5	71.7	76.5	13.5	13.5	13.5
103/92	11.0	24.2	30.8	13.7	38.0	47.0	23.8	49.2	57.2	39.3	79.7	89.7	31.2	57.3	68.0	14.7	14.7	14.7
214Bč	10.8	22.2	27.2	12.2	37.3	44.5	20.8	49.3	58.3	34.8	67.2	79.5	28.2	67.8	76.2	11.5	11.5	11.5
1004	12.3	20.8	24.0	16.0	34.5	39.3	24.0	49.3	55.7	41.0	64.2	69.2	31.8	54.5	60.8	15.5	15.5	15.5
229	12.3	21.2	23.3	16.0	34.3	38.3	24.0	42.5	46.8	35.5	61.2	67.2	35.3	44.8	49.7	14.8	14.8	14.8
1—5	11.2	16.7	22.2	14.0	27.0	31.7	18.2	29.5	33.8	25.0	50.0	59.5	28.0	47.3	56.0	14.0	14.0	14.0

*Influence of different pH values of the cultivation medium
on the fungus growth*

In this experiment, changes of the colony appearance, color of the aerial and substrate mycelia and fruiting intensity, caused by different pH values of the cultivation medium were described.

Appearance of the colonies

A grayish-brown pigmentation of the colony center occurred in isolate 229, following 11 days of cultivation at pH 3.5 and 5 (Fig. 2). At the same time, pigmentation of the substrate mycelium was more pronounced at lower pH.

Colonies of the isolate 1004 formed a discrete rings of the aerial mycelium at pH 3.5, 5 and 10 (Fig. 2). At pH between 6 and 9, colonies were identical in their morphology (uniform density on the entire area).

Observation of the colonies formed by isolate 447 revealed irregular, ambiguous, ring-shaped increase of the aerial mycelium at pH 3.5 and 5 (Fig. 2). All colonies had the same morphological characteristics at pH 6–10.

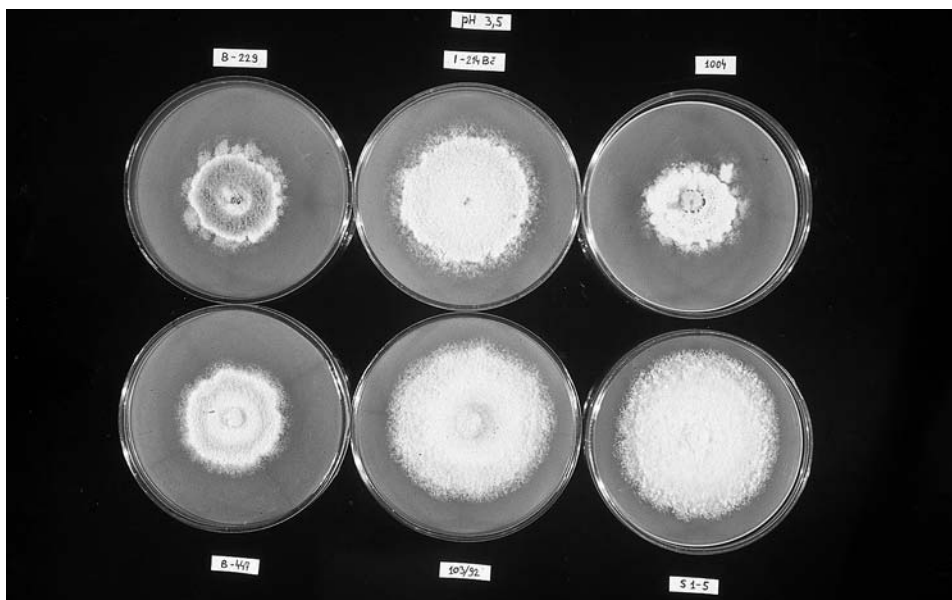


Fig. 2 — Appearance of the *D. populea* colonies at pH 3.5 after 16 days of cultivation (original)

Isolates 214Bč, 103/92, and 1—5 have retained their primary appearance at all pH values (3.5–10).

Fruiting of the isolates

Isolates formed exclusively stromatic aggregations with pycnidia. Isolate 229 produced fruiting bodies under all pH conditions (Tab. 3). After 30 days, a water drops occurred on the colonies. Pillow-shaped, blackish-brown agglomerations of hyphae, i.e. stromatic aggregations with pycnidia were formed under these drops. This isolate showed a vigorous fructification at pH 5 where stromata were formed. In addition, fructification was intensive at all pH values. Isolate 214 Bč produced a corpulent stromatic bulk following 35 days of cultivation, under all pH conditions. Fruiting was very intensive at pH 3.5–8, and weaker at pH 9 and 10 (Fig. 3).

Tab. 3 — Influence of the cultivation medium pH on fruiting intensity of *D. populea* isolates

pH	229	214Bč	1004	447	103/92	1—5
Isolates						
pH 3.5	+++	+++	+	++++	—	—
pH 5	++++	++++	++	++++	—	—
pH 6	+++	+++	+++	+++	—	—
pH 7	+++	+++	+++	+++	—	—
pH 8	+++	+++	++	+++	—	—
pH 9	+++	++	++	+++	—	—
pH 10	+++	++	+	++	—	—

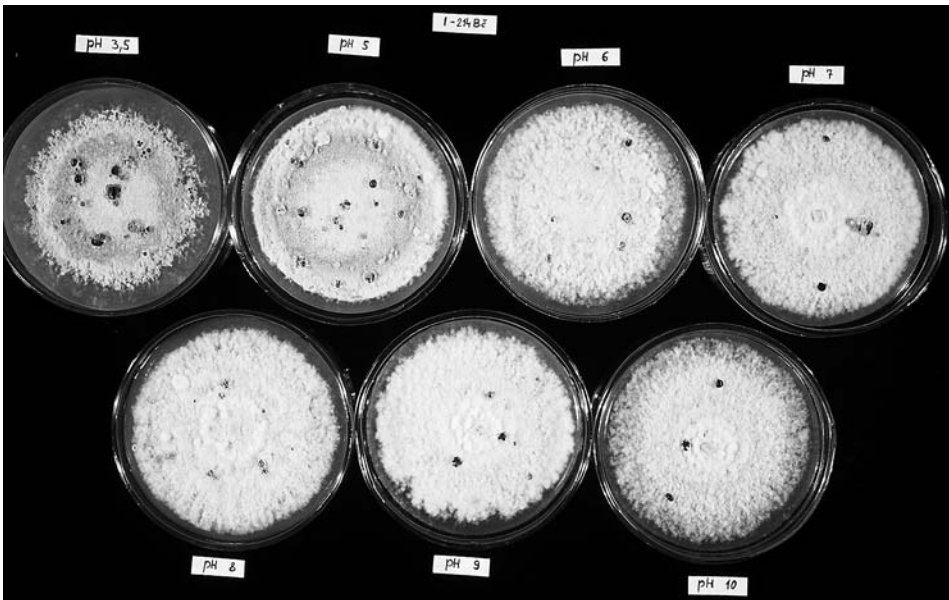


Fig. 3 — Fruiting of the isolate 214 Bč at different pH after 45 days of cultivation

The best fruiting of isolate 1004 was found at pH 6 and 7. With increase or decrease of pH, intensity of fructification evenly decreased (Tab. 3). Fruiting bodies were formed following 4 weeks of cultivation.

After 20 days, isolate 447 produced stromata at all pH values. Stromata were situated on the entire area of the colonies. Fructification was very intensive at pH 3.5 and 5, and numerous, irregularly shaped stromata provided a very specific appearance of the colonies. At pH 6—9, colonies had a similar fruiting intensity, characterized by strong fructification. Fruiting was evaluated as moderate only at pH 10. Fruiting bodies have not been formed at colonies of isolates 103/92 and 1—5.

Occurrence of the fruiting bodies at all pH values was observed in four isolates. However, two isolates failed to form reproductive organs. Isolates 229, 214Bč and 447 exhibited medium to very intensive fructification under all pH conditions. Cultivation media with acid reaction (pH 3.5 and 5) have stimulated fruiting of the isolates mentioned above. The best fruiting of isolate 1004 was found on neutral and slightly acid cultivation medium, and it decreased along with changes of pH values (increase or decrease).

Overall, colonies of *D. populea* isolates grew at a wide range of pH values.

Growth of the colonies

The mycelial growth after 5 days of cultivation was recorded under all pH conditions (Tab. 4). In this period, significant differences between average diameters of colonies at pH 3.5—8 were not found. Cultivation media with pH 9 and 10 markedly slowed down growth of the colonies. Isolates 103/92, 44, and 214Bč were grouped on the basis of great colony diameter, in comparison to isolates 1004, 22, and 1—5 (Tab. 4).

The results obtained following 12 days of the isolates cultivation elucidated high divergence of the average diameter of colonies at different pH.

Cultivation medium with pH 7 had the most beneficial effect on the colony growth. A very good growth was observed at media with a slightly alkaline (pH 8) and a slightly acid (pH 6) reactions. Average diameters of the colonies at pH 5 and 9 were smaller than those obtained at pH 6, 7, and 8. The slowest colony growth occurred on extremely acid medium (pH 3.5) and on a very alkaline medium (pH 10). At this period, colonies of isolates 103/92 and 447 showed the best growth under all pH conditions, when compared to others. Isolate 214Bč grew considerably slower than above mentioned ones (103/92 and 447). Isolates 1004, 229 and 1—5 constituted a group, characterized by slow growth of their colonies. Colonies of isolate 1—5 thrived well on a slightly acid media (pH 3.5 and 5), while on other media, their growth was the weakest (Tab. 4).

Results obtained on the 16th day, related to the growth characteristics at various pHs, have not revealed changes of relationship between their values, in comparison to the previous period. Alterations of the isolates order and of relations between the average colony diameters were not evident.

Tab. 4 — Influence of different pH on the mycelial growth of *D. populea* isolates on the carrot cultivation medium

Isolates	pH values																				
	pH 3.5			pH 5			pH 6			pH 7			pH 8			pH 9			pH 10		
	5	12	16	5	12	16	5	12	16	5	12	16	5	12	16	5	12	16	5	12	16
	Average diameter of the colonies after 5, 12 and 16 days of cultivation																				
103/92	33.2	58.0	68.3	32.2	67.7	80.3	34.7	67.7	82.8	33.2	69.3	82.7	30.3	68.3	82.5	27.8	62.7	80.0	21.0	57.8	72.7
447	30.7	59.5	70.8	29.3	63.3	75.0	33.7	72.0	85.3	34.0	72.8	89.5	31.3	71.2	89.7	28.2	62.2	87.0	21.7	59.3	75.0
214Bč	29.2	43.2	51.3	30.2	55.8	70.7	28.8	58.7	75.2	30.0	64.8	73.8	30.0	65.3	80.3	27.2	59.8	72.0	22.0	53.5	67.0
1004	24.7	39.7	46.2	28.3	41.2	49.2	27.2	54.5	73.7	30.2	61.3	73.5	29.3	62.3	75.2	26.2	59.5	70.7	19.7	51.5	64.7
229	26.8	40.5	50.5	27.5	47.2	54.5	27.7	61.7	72.7	26.7	60.0	72.7	27.5	61.8	74.7	24.2	57.5	69.3	21.3	51.3	64.3
1—5	27.0	56.3	68.0	26.8	61.0	74.8	26.8	50.8	66.0	25.7	50.7	60.7	26.2	52.3	64.5	19.2	46.7	58.8	9.8	30.8	43.0

Influence of light regime on the fungus growth

Appearance and fruiting of the colonies

Following 10 days of the light/dark regime, detached drops of water occurred on the colonies. They were arranged in a ring-shape manner, in area around the primary inoculum. After 15 days, numerous drops occupied the whole area around the fragment, which was 3—4 cm in diameter. Black concentrically arranged stromatic aggregations were formed below them. Fruiting was characterized by strong fructification. In this zone, aerial mycelium was grayish, while color of the substrate mycelium varied from pale yellow to brown. Out of this zone, both aerial and substrate mycelia were white. Concomitantly, pigmentation and water drops were not observed on the colonies cultivated in total darkness. However, the first drops were formed on these colonies after 20 days of cultivation, while stromatic aggregations after four weeks. The stromata were mainly uniformly spaced on the colony area, while fructification was intensive (Fig. 4). Therefore, the fruiting bodies appeared earlier and were more abundant on the colonies cultivated under the light/dark regime than in total darkness.

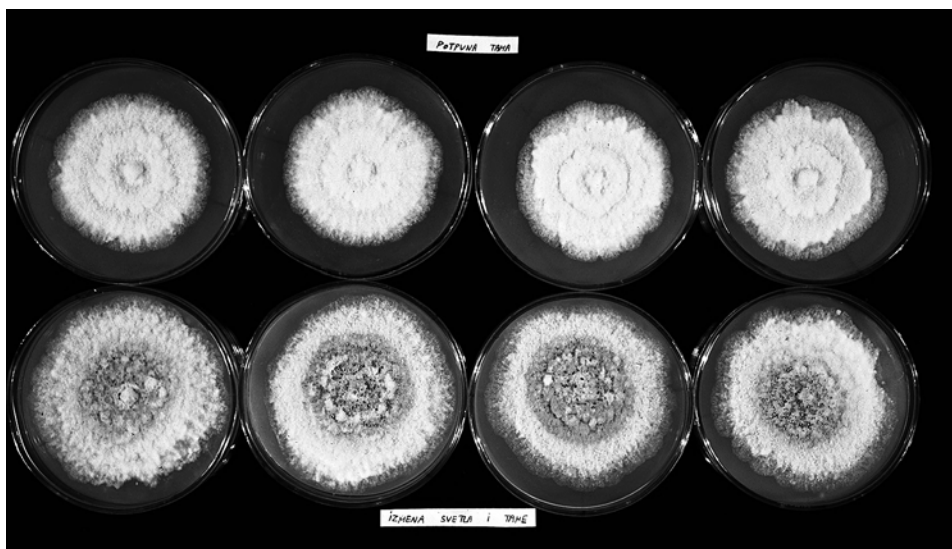


Fig. 4 — Appearance of colonies of isolate 447 exposed to various light regimes after 14 days of cultivation (orig.)

Growth of the colonies

Colonies exposed to the light/dark regime exhibited slower mycelial growth and markedly lower average values of their diameter for the first 7 days of cultivation, than those exposed to total darkness (Tab. 5).

Average values of the colony diameter, obtained after the second measuring period (i.e. after 11 days), indicated changed mycelial growth, and relationships between the varieties. In this period of cultivation, the light/dark regime induced a rapid increment of the mycelial growth, and differences between these colonies and those cultivated in total darkness became evident (Tab. 5).

Tab. 5 — Impact of light regimes on the mycelial growth of *D. populea* on the carrot cultivation medium

Isolate	Mycelial growth					
	Light/dark cycle			Total darkness		
	Average diameters of the colonies after 7,11 and 14 days of cultivation (mm)					
	7	11	14	7	11	14
447	31.9	63.6	81.3	38.7	58.8	71.5

Growth of the colonies under the light/dark regime remained very fast and dynamic in the last measuring period. According to average values of their diameters, variability between varieties became more evident.

DISCUSSION

Impact of temperatures, pH, and light regime, i.e. important ecological factors, on the fungus growth was studied in this work.

Effect of temperature on the fungus growth and development spontaneous and artificial infection of plants under both natural and *in vitro* conditions were studied by numerous authors. These investigations mainly referred to determination of temperatures optimal for the fastest mycelial growth and the best fruiting, in both the culture and plant tissue. Some authors have investigated impact of temperature on the fungus, using a wide range of values.

Donaubauer (1957) reported that optimal temperatures for the fungus growth in the culture were 20—22°C. Investigations were performed in Austria with isolates obtained from poplar plantations established on alluvium of the Danube. According to our results, the minimum limit of temperature reported by this author was optimal for the fungus growth in the culture.

Giving the retrospective view of the massive infection of poplars by *D. populea*, during 1956 in Croatia, Böhm (1957) found that optimal temperature was 18°C. He connected occurrence of epidemic spread of fungus with high mean daily temperatures during spring. These climate conditions were beneficial for the parasite growth, but unfavorable for physiological processes of plants, and massive infection occurred. Temperature reported by this author was lower than optimal values for the fungus growth obtained in our work.

In his doctoral thesis, Marinković (1965) studied characteristics of the fungus at temperatures ranging from 11 to 28°C, when mycelial physiological activity was high. Taking into consideration mean daily increment of the mycelia, the author concluded that optimal temperature was 23°C. This value was higher than that from our investigation.

Decrease of the moisture content in bark of poplar trees by 10% made them susceptible to *D. populea* attack at 20°C (Butin, 1957). Such results could be explained by high aggressiveness of the fungus at temperatures optimal for growth of its vegetative organs.

The mycelial growth at different temperatures (0°C—35°C) has been studied by Magnani (1959). The fastest growth of the colonies occurred at 25°C, and they reached the greatest dimensions after 15 days of cultivation. The temperature was higher for several degrees than that obtained in our work.

Keča (2001) investigated mycelial growth on MEA cultivation medium (malt extract agar) using temperatures from -1 to 35°C. The author found that temperatures between 22 and 26°C were optimal for the fungus growth. These values are higher than those obtained in our experiment, as well as in investigations of other authors.

According to average values of the colony diameter for all intervals (Tab. 2), the greatest values were reached at 20°C. Hence, we have supposed that this temperature and values close to it were optimal for the mycelial growth.

A very good growth of *D. populea* isolates at 5°C, 10°C, and 15°C was not a surprise, because the best growth in cortical tissues of plants in nature occurs in early spring, when temperatures range between 18°C and 22°C. Cambial activity does not occur at -1°C, but growth of the fungal mycelium and hyphae begins. Minimal reactions of cambium are possible at +5°C, when activity of the fungus is already high. This enables the fungus growth, which causes cell and tissue necrosis even at very low temperatures. According to Hubbes (1959), reactions of plants become more intensive than the activity of the fungus at 15°C. Studying artificial infections in laboratory, this author found particularly great number of infections at temperatures below 12°C, suggesting a good adaptation to these temperatures. Zycha (1955) have described a good mycelial development and growth in the cambium at +4°C, causing death of the cells.

There is a disagreement about the maximum temperatures compatible with the fungus growth between our and other authors' results. Following several days of minimal growth at 30°C, the mycelial growth was completely retained (Tab. 2). However, the mycelium was still alive after 16 days at this temperature. Several days after the transfer of Petri dishes into incubator at 20°C, it continued the normal growth, meaning that previous period was the stage of hypobiosis, i.e. of stagnancy. The same results were reported by Keča (2001), who have studied the influence of different temperatures on the mycelial growth. In contrast to our results, Donaubaer (1957) and Keča (2001) observed week, but monotonous and continuous growth during the entire period of cultivation at 30°C. The upper temperature limit, where the mycelial growth stops, amounts 32°C (Donaubaer, 1957) i.e. 33°C (Keča, 2001). Similar to these authors, Magnani (1959) described a good mycelial growth at 30°C for 15 days, while the mycelium failed to grow at 35°C.

The literature data related to effects of pH on the fungus growth have elucidated the basic characteristics of the fungus at detached pH values of the nutrient medium (Hubbes 1959, Magnani 1964, Brendel 1965, Goković 1981).

H u b b e s (1959) noticed that growth of *D. populea* in the Czapek-Dox nutrient solution was slow at pH 3.6, while the adjustment of pH at 5.2 has significantly increased the dry mass of the mycelium. In our experiment, the fungus grew well at pH 3.5, but we used the nutrient medium for its cultivation, in contrast to this author, who used the nutrient solution.

The mycelial growth was intensive at solid medium containing a poplar bark extract at pH 5.2—5.6 (B r e n d e l, 1965), while M a g n a n i (1964) and G o j k o v i ć (1981a) observed the best mycelial growth at the carrot cultivation medium at pH 6. Results of these authors suggest the beneficial effect of a slightly acid media on the mycelial growth. Results at Tab. 4 confirmed that pH 6 was the optimal in our experiment, while good growth of the colonies occurred also at pH 7 and 8.

V e l d e m a n and W e l v a e r t (1956) and T a r i s (1957) explored physiological activity of the fungus at different pH, and their investigations were focused on the germination of spores and growth of the initial hyphae. Spores were germinating even at pH below 3.5, while at 8.5 germination was stopped. These authors found that optimal pH for germination of spores was 4—6.4.

Effect of different pH values (1.9—9) on the spore germination and growth of the initial hyphae was studied by T a r i s (1957), who reported that germination occurs at pH 4—7, while the optimum was at pH 6.1. In addition, he has noticed a good growth of the initial hyphae at this pH. Taking into consideration the above-mentioned facts, it could be concluded that growth of the initial hyphae and spore germination occur at several pH values, while the range of optimal values for the mycelial growth obtained in our work was wider. This disagreement resulted from differences in analyzing of parameters between experiments. The above-mentioned authors studied physiological activity of reproductive organs (spores) and growth of the initial hyphae, unlike our experiment where the mycelial growth in the culture have been determined under different conditions.

The average colony diameter of the isolates (Tab. 4) was high not only on media with a very acid reaction (pH 3,5), but also with high concentration of H ions (pH 9 and pH 10).

Of many authors who have studied ecological characteristics of the fungus, only T a r i s (1957) has been interested in the impact of light on its development. Experiments of this author were related to spore germination and growth of the initial hyphae. Spores exposed to total darkness for three days showed higher percentage of germination and manifold longer initial hyphae than variants exposed to continuous light or a light/dark regime. Besides, the hyphae formed in total darkness were thin, thread-like, with rare branching, in contrast to a dense, branching hyphae formed at continuous light and a normal alteration of day and night. The author considered that the winter period with reduced light contributed to the success of infection. Results of our experiment showed that the mycelial growth and fruiting were considerably better at light/dark regime than in total darkness. These results could partly be compared with those of T a r i s (1957) because of the differences in analyzing of parameters. However, there are some agreements between experiments. The

mycelial growth in total darkness was better than at light/dark regime during the first days of experiment, similar with results obtained by T a r i s (1957), who measured growth of the initial hyphae.

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УТИЦАЈ НЕКИХ ЕКОЛОШКИХ ФАКТОРА НА РАЗВОЈ
DOTHICHIZA POPULEA SACC. ET BR.

Предраг Л. Пап, Мирослав П. Марковић

Институт за низијско шумарство и животну средину
Антонa Чехова 13, Нови Сад, Србија
e-mail: predragpap@ptt.yu

Резиме

Проучаван је утицај различитих температура, рН вредности и режима светла на развој мицелије и снагу плодношења *Dothichiza populea* Sacc. et Br. Утицај температуре на развој гљиве испитиван је гајењем изолата у политермостату на температурама од 5 до 30°C. Најинтензивнији пораст мицелије је био на 20°C, док је на 30°C развој био прекинут. Највећи интензитет плодношења изолата су испољили на 20°C, а гљива није плодносила на 5, 25 и 30°C. Проучаван је и утицај различитих рН вредности у подлози на развој изолата гљиве у распону од 3,5 до 10 рН. Оптимална рН вредност подлоге за пораст колонија гљиве кретала се у интервалу од 6 до 8. Репродуктивни органи су се образовали при свим рН вредностима. Утицај светлости на развој гљиве је испитан у условима смене светла и таме и условима потпуне таме. Резултати су показали да су пораст мицелије и плодношење гљиве били значајно већи при смењивању светла и таме него у условима таме.

Siniša M. Mirković
Ljubiša D. Džambas
Srećko Đ. Selaković

Faculty of Medicine Novi Sad, Clinic of Dentistry of Vojvodina
Hajduk Veljkova 12, Novi Sad, Serbia

INFLUENCE OF DIFFERENT TYPES OF SURGICAL SUTURE MATERIAL ON THE INTENSITY OF TISSUE REACTION IN ORAL CAVITY

ABSTRACT: Throughout the history the most diverse suture material have been used for closing and suturing surgical wounds. The four basic features of suture material are described: knot safety, stretch capacity, tissue reactivity and wound safety. Tissue reaction, even the minimum one, which develops during the first to seven days after applying the suture in the tissue.

The aim of this study was to investigate influence of a monofilament suture material (nylon) on the intensity of local tissue reaction in experimental conditions, and to compare it with the multifilament suture used in the routine practice of oral surgery (silk).

This investigation is a prospective experimental study carried out on Wistar rats. The experiment included 30 animals, in which Black Silk (thickness 4-1) and Nylon (thickness 4-0) were applied in the upper and lower jaw, respectively.

To monitor tissue reaction on different suture materials the following parameters were used: coagulum formation, presence of polymorphonuclear leukocytes, presence of macrophages and granuloma, formation of epithelial bridge and connective tissue, collagen synthesis, granulomatous tissue formation and presence of fibrous tissue.

After comparing parameters for the intensity of tissue reaction to the investigate suture materials by suturing the oral mucosa, certain advantage could be given to the monofilament suture materials.

KEY WORDS: suture material, tissue reaction, oral cavity

INTRODUCTION

Surgery is as old as the mankind, and use of sutures dates back to the times of earliest human knowledge in the field of surgery (6, 7, 8).

Throughout the history the most diverse suture material have been used for closing and suturing surgical wounds. The most ancient suture materials include animal tendons and hairs, as well as herbal fibers such as linen, hemp, and different grass species. Some Egyptian papers, dating back some 5000

years ago, display scenes of suturing wounds with linen fibers. A famous Roman *medicus*, Galen, introduced sutures made of animal intestines into the surgery (6, 7, 8).

Since Renaissance times until 1940 there were substantial changes with respect to the kind of suture materials used, which involved catgut, cotton and surgical silk. The first synthetic suture materials were nylon and polyester, produced in the 1940's. Soon afterwards polyethylene and polypropylene were developed. In 1970 the polyglycolic acid was "put on stage," which is known under the licensed name DEXON (Davies+Geck inc., Great Britain). During 197, the polyglactine 910, which is an essential component of the VICRYL-a (Ethicon Inc., Great Britain), was put on market (6, 7, 8).

The four basic features of suture material are knot safety, stretch capacity, tissue reactivity and wound safety. Knot safety implies capacity of suture material to retain the strength and firmness of the knot, without slackening in the unit of time. It is reverse-proportional to a suture thickness, and directly proportional to its quality and functional output. Stretch capacity of the suture material is defined as a stretch power capacity per unit of area. The wound safety is directly related with suture capacity that is required for safe healing of the wound. Tissue reaction is reflected through an inflammatory reaction, even the minimum one, which develops during the first two to seven days after stitch application into the tissue (6, 7, 8).

The wound healing process encompasses three basic stages — the inflammatory, fibroblastic and remodeling ones. The inflammatory phase begins at the moment of injury, and lasts 3—5 days provided that there is no prolonged inflammation. The main characteristic of the wound in this phase is formation of fibrins of decreased firmness. During the stadium of fibroblastic phase, the fibrin bands permeate the wound building a frame, on which fibroblasts accumulate the ground substance and tropocollagen. Despite poor collagen synthesis, the firmness of the wound increases rapidly during the fibroblastic stadium, lasting 2—3 weeks. At the end of this phase, the wound becomes tight — because of excess collagen, erythematous — because of high degree of vascularization and capable to withstand 70%—80% of the tension of a healthy tissue. During the remodeling phase, substantial amounts of randomly deposited collagen fibers are eliminated and replaced by new fibrils, arranged in a pattern that enables higher firmness of the wound. In this stadium the firmness of the wound increases, yet not exceeding the rate of 80—85% of the firmness of a healthy tissue. (6, 7, 8).

THE AIM

The aim of this study was to investigate influence of a monofilament suture material (nylon) on the intensity of local tissue reaction in experimental conditions, and to compare it with the multifilament suture used in the routine practices of oral surgery (silk).

MATERIAL AND METHODS

The investigation was performed on 30 Wistar rats, aged 3—6 months and with body mass ranging 250—350 g. All experimental animals were distributed into the 3 groups of 10 animals each, according to the time of sampling during the postoperative period (day 2, day 5, and day 7).

On a day of surgery, each animal was anesthetized with 10% urethane (1 ml per 100 gr. body mass). The research design implied making two mucoperiosteal flaps in the anterior segment, i.e. on the right side in the upper jaw and on the left side in the lower jaw. A mucoperiosteal flap was produced by horizontal incision in the attached gingiva, and two vertical relaxation incisions. Horizontal incision was placed 5 mm from the gingival margin. Flap mobilization was performed using Freer *raspatorium*. Each animal undergone cortex trepanation in the line with the tooth root, interradiarily. Cortex trepanation was performed using round steel borer, 3 mm in diameter, with abundant douching of the drill and working area with sterile physiological saline. Each flap was ecartered for 15 minutes, and then returned to its original location and sutured.

The following suture materials were used: (1) monofilament synthetic material (Nylon®), thickness 4/0, needle with thread, round bodied, curvature 3/8 circle "DR 25"; (2) polyfilament natural silk material (Black Silk®), thickness 4/0, round bodied needle with thread, curvature 3/8 circle "DR 25" (non-traumatic).

In each animal both types of sutures were used, i.e. monofilament in one jaw and polyfilament in another jaw. Each mucoperiosteal flap was sutured with four stitches (with fourfold knot), two in the horizontal, and one in each vertical incision. After suturing, a gauze tampon was placed onto the wound, followed by a digital compression in order to induce hemostasis and to prevent development of hematoma.

The second procedure (Days 2, 5 or 7 upon first surgery, depending on the experimental group), performed in general anesthesia with 10% urethane, encompassed excision of mucoperiosteal portion from the surgery region along with the suture material. The obtained preparations were fixed in 70% alcohol and subjected to histopathological analysis.

To monitor tissue reaction to suture materials the following parameters were used: coagulum formation, presence of polymorphonuclear leukocytes, presence of macrophages and granuloma, formation of epithelial bridge and connective tissue, and presence of fibrous tissue.

Statistical analysis of the obtained results was performed by scaling non-parametric data using Lancaster contingency tables, multivariate and univariate analysis, (MANOVA and ANOVA), and Student's *t*-test in proportions.

RESULTS

On a day 2 post-surgery presence of coagulum, i.e. fibrous clot, was observed in all animals from the experimental and the control groups (Table 1).

Identical results were recorded in both groups with respect to the presence of polymorphonuclear leukocytes (in all suture types), presence of granuloma (no evidence of granuloma in neither of the investigated groups), as well as epithelial bridge formation, collagen synthesis and presence of fibrous tissue (observed in neither of the investigated groups). As regard to the presence of macrophages in the wound, they were not observed only in two animals from the experimental group, whereas they were detected in all remaining animals on a day 2 post-surgery.

Tab. 1 — Prevalence of tissue reaction parameters on day 2 post-surgery

Group	Tissue reaction rate*	Tissue reaction parameters**								
		Koag	Pnm	Mfg	Gran	Epit	Vzvn	Sink	Grnt	Fibt
Exp.	0.	0	0	2	10	10	2	10	0	10
	1.	10	10	8	0	0	8	0	10	0
Control.	0.	0	0	0	10	10	10	10	10	10
	1.	10	10	10	0	0	0	0	0	0

* — 0. — Absent
1. — Present

** — Koag — coagulum formation
Pnm — presence of polymorph leukocytes
Mfg — presence of macrophages
Gran — presence of granuloma
Epit — epithelial bridge formation
Vzvn — connective tissue formation
Sink — collagen synthesis
Grnt — presence of granulomatous tissue
Fibt — presence of fibrous tissue

Granulomatous tissue and fibroblasts, however, were not detected in neither animal from the control group, whereas present in all, or almost all, animals in the experimental group (Table 1). Statistical analysis of the obtained data revealed high statistically significant difference between the experimental and the control group with respect to presence of fibroblasts and granulomatous tissue in the area surrounding the suture material. As regards the presence of macrophages, differences between investigated groups were not statistically significant (Table 2).

Tab. 2 — Significance of the difference in tissue reaction to different suture materials on day 2 post-surgery

	R	R2	x	F	p	koef. disk.
MFG	.333	.111	.316	2.034	.171	317.56
VZVN	.816	.667	.632	34.421	.000	350.21
GrnT	1.000	1.000	.707	2630.919	.000	328.29
Cr = .203						

Tab. 3 — Prevalence of tissue reaction parameters on day 5 post-surgery

Group	Tissue reaction rate*	Tissue reaction parameters**								
		Koag	Pnm	Mfg	Gran	Epit	Vzvn	Sink	Grnt	Fibt
Exp.	0.	0	0	0	10	0	0	0	0	0
	1.	10	10	10	0	10	10	10	10	10
Control	0.	0	0	0	3	1	1	1	0	0
	1.	10	10	10	7	9	9	9	10	10

* — 0. — Absent
1. — Present

** — Koag — coagulum formation
Pnm — presence of polymorph leukocytes
Mfg — presence of macrophages
Gran — presence of granuloma
Epit — epithelial bridge formation
Vzvn — connective tissue formation
Sink — collagen synthesis
Grnt — presence of granulomatous tissue
Fibt — presence of fibrous tissue

On day 5 post-surgery in all 10 animals from the control and experimental groups the presence of coagulum was observed, i.e. formation of a fibrous clot, as well as presence of polymorphonuclear leukocytes, macrophages, granulomatous and fibrous tissue. Certain differences in tissue reactions were, though, observed with respect to the presence of granuloma in the wound, formation of epithelial bands (epithelial bridge) and connective tissue, as well as collagen synthesis (Table 3). However, statistical analysis of the obtained results revealed statistically significant difference only for the presence of granuloma in the wound (Table 4).

Tab. 4 — Significance of the difference in tissue reaction to different suture materials on day 5 post-surgery

	R	R2	CHI	F	p	
GRAN	.734	.538	.592	21.000	.000	.95
EPIT	.229	.053	.224	1.000	.331	.00
VZVN	.229	.053	.224	1.000	.331	.00
SinK	.229	.053	.224	1.000	.331	—
Cr = .216						

Tab. 5 — Prevalence of tissue reaction parameters on day 7 post-surgery

Group	Tissue reaction rate*	Tissue reaction parameters**								
		Koag	Pnm	Mfg	Gran	Epit	Vzvn	Sink	Grnt	Fibt
Exp.	0.	0	0	0	10	0	0	0	0	0
	1.	10	10	10	0	10	10	10	10	10
Control	0.	0	0	0	9	0	0	0	0	0
	1.	10	10	10	1	10	10	10	10	10

* — 0. — Absent
1. — Present

** — Koag — coagulum formation
Pnm — presence of polymorph leukocytes
Mfg — presence of macrophages
Gran — presence of granuloma
Epit — epithelial bridge formation
Vzvn — connective tissue formation
Sink — collagen synthesis
Grnt — presence of granulomatous tissue
Fibt — presence of fibrous tissue

On day 7 post-surgery eight investigated parameters were recorded in all ten experimental animals from both investigated groups (Table 5). Minor divergence between the groups was observed only about the presence of granuloma in the wound (noticed only in one case in the control group), yet this difference was not statistically significant (Table 6).

Tab. 6 — Significance of the difference in tissue reaction to different suture materials on day 7 post-surgery

	R	R2	CHI	F	p
GRAN	.229	.053	.224	1.000	.331
Cr = .183					

Comprehensive statistical analysis revealed that on days 5 and 7 both experimental groups exhibited identical characteristics (Table 7), whereas certain variations were observed in the control group (Table 8).

Tab. 7 — Distance in the experimental group between all three days post-surgery

	Day 2	Day 5	Day 7
Day 2	.000	22.836	22.836
Day 5	22.836	.000	.000
Day 7	22.836	.000	.000

Tab. 8 — Distance in the control group between all three days post-surgery

	Day 2	Day 5	Day 7
Day 2	.000	10.292	10.831
Day 5	10.292	.000	1.703
Day 7	10.831	1.703	.000

DISCUSSION

Analysis of the obtained results suggests that suturing with monofilament synthetic material (nylon) induces more rapid initiation of reparatory and regenerative processes in the body in comparison with the polyfilament natural material (silk). The aforementioned premise is strongly supported by the facts on the presence of connective tissue, i.e. fibroblasts and granulomatous tissue, in the wound sutured with monofilament material. Similar results after parallel testing of several suture materials, carried out on rats in experimental settings, were reported by Okamoto et al.^{7, 8} and by other authors.^{3, 9, 11, 12}

However, the available data do not confirm effects of the applied suture materials on the persistence of fibrous clot in the wound five days post-surgery, or later. Nevertheless, the obtained results strongly indicate the continual progress of reparatory processes involving all cell elements necessary for an undisturbed wound healing in the group, in which monofilament suture material was used.

In the control group, in which polyfilament natural suture material was used, a delayed activation of reparatory processes is observed. Though such processes were not apparent on day 2 post-surgery, the tissue reaction on day 5 was almost completely equal to the reparatory processes observed after application of monofilament nylon suture. Such a delayed, yet intensified tissue reaction, was described by numerous authors.^{2, 3, 7, 8, 10, 12} Formation of granuloma, as an inflammatory reaction associated with the occurrence of giant cells surrounding the foreign body on day 5 post-surgery, can be explained because of multifilament silk material that may enable "wicking" of bacterial organisms in the interstices of the braided structure, as well as marked capillary action associated with liquid absorption and volume changes.⁴⁻⁶

Comparative postoperative analysis of experimentally created wounds sutured with monofilament or polyfilament suture material confirmed more rapid reestablishment of reparative processes when using monofilament material, reaching the peak value on day 5 and proceeding continually until the end of the investigation period. Application of polyfilament suture material resulted in milder tissue reaction on day 2 post-surgery, reaching the peak value on day 5 and remaining at similar level on day 7 post-surgery, and characterized by slight differences with respect to existence of granuloma. Such observation suggest that polyfilament suture material, after applied into the tissue, induces prolonged and intensive tissue reaction that reaches its peak on day 5 post-surgery. As regards the natural silk material, the tissue reaction is most distinctly reflected through formation of granuloma reach in giant cells, as a reaction to foreign body. This phenomenon is not observed when applying synthetic monofilament suture material.

CONCLUSION

After analyzing the results obtained in experimental model the following conclusions can be made:

1. After placing sutures inside the oral cavity the tissue reaction is manifested as an inflammatory process (even minimal one) occurring from Day 2 to Day 7 post-surgery.

2. After placed into the tissue, the BLACK SILK that belongs to the group of natural polyfilament suture materials produced a prolonged intensive tissue reaction, reaching its peak on Day 5 post-surgery.

3. The most distinct parameter of tissue reaction by using the BLACK SILK was formation of granuloma reach in giant cells associated with the presence of foreign body. Such phenomenon was not observed in monofilament NYLON suture.

4. After placed into the tissue the NYLON, which belongs to the group of synthetic monofilament suture materials, induces short-lasting moderate tissue reaction and rapid reestablishment of reparatory and regenerative processes.

5. After comparing parameters of the intensity or tissue reaction to the investigated suture materials in suturing oral mucosa, we may give certain advantage to the monofilament suture materials.

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УТИЦАЈ РАЗЛИЧИТИХ ВРСТА МАТЕРИЈАЛА ЗА ШИВЕЊЕ НА ИНТЕНЗИТЕТ ТКИВНЕ РЕАКЦИЈЕ УСНЕ ШУПЉИНЕ

Синиша М. Мирковић, Љубиша Д. Џамбас, Срећко Ђ. Селаковић

Медицински факултет Нови Сад, Клиника за стоматологију Војводине,
Хајдук Вељкова 12, Србија

Резиме

Историјски гледано, за затварање и зашивање хируршких рана коришћени су најразличитији шавни материјали. У стручној медицинској литератури описане су четири основне карактеристике шавног материјала: сигурност чворова, затезна снага, ткивна реакција и сигурност ране. Ткивна реакција приказана је инфламаторном реакцијом која се, макар и минимална, јавља током првих два до седам дана након апликације шавова у ткиво.

Циљ истраживања је био да се испита утицај монофиламентног материјала за шивење (Nylon) на интензитет локалне ткивне реакције у експерименталним условима и упореди с истоветним утицајем упређеног материјала који се рутински користи у оралној хирургији (свила).

Испитивање представља проспективну експерименталну студију спроведену на експерименталним пацовима соја Wistar. Обухваћено је 30 животиња код којих се у горњој вилици као шавни материјал користио Black Sylk дебљине 4-0, а у доњој вилици Nylon дебљине 4-0. За праћење реакције ткива на шавни материјал коришћени су следећи параметри: формирање коагулума, полиморфонуклеарни леукоцити, макрофаги, гранулом, формирање епителног моста, формирање везивног ткива, синтеза колагена, гранулационо ткиво, фиброзно ткиво.

Компарирајући параметре интензитета ткивне реакције испитиваних материјала, након сутурирања слузокоже усне шупљине, могла би се дати извесна предност синтетским монофиламентним шавним материјалима.

*Dubravka M. Polić¹, Ružica S. Igić¹,
Slobodanka J. Stojanović²,
Dejana M. Lazić²*

¹ Faculty of Sciences, Department of Biology and Ecology
Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia

² Faculty of Agriculture, Trg Dositeja Obradovića 8
21000 Novi Sad, Serbia

THE PLANT COMMUNITIES OF CLASSES *HYDROCHARI-LEMNETEA* OBERD. 1967 AND *POTAMETEA* TX. ET PRSG. 1942 OF THE LABUDOVO OKNO LOCALITY (SERBIA)

ABSTRACT: Labudovo okno locality (50 m—84 m elevation) is situated in the south-eastern part of the edge of the Pannonian Plains, resting along the left bank of the Danube between 1982 km and 1078 km. The investigated locality is the result of rise of the Danube level after dam building of the hydroelectric power station Đerdap I. The vegetation comprises aquatic associations of the classes *Hydrochari-Lemnetea* Oberd. 1967 and *Potametea* Tx. et Prsg. 1942. The class *Hydrochari-Lemnetea* Oberd. 1967 includes the following phytocoenoses: *Lemno-Spirodeletum* W. Koch 1954, *Salvinio-Spirodeletum polyrrhizae* Slavnić 1956, *Lemno minoris-Azolletum filiculoides* Br.-Bl. 1952, *Ceratophylletum demersi* (Soó 27) Hild 1956. The class *Potametea* Tx. et Prsg. 1942 includes the associations *Myriophyllo-Potametum* Soó 1934, *Nympaetum albo-luteae* Nowinski 1928, *Trapetum natantis* Müller et Görs 1960.

KEY WORDS: the Danube, aquatic vegetation, floating vegetation, submerged vegetation

INTRODUCTION

The Danube is the second largest river of Europe. Its length is 2850 km covering ten countries. The Danube length through Serbia is 588 km.

The Labudovo okno locality (50 m—84 m elevation) is situated in the south-eastern part of the edge of the Pannonian Plains, resting along the left bank of the Danube between 1982 km and 1078 km, in the vicinity of southern boundaries of the Deliblato Sands. The investigated locality is the result of rise of the Danube level after dam building of the hydroelectric power station Đerdap I. The system altered the hydrological conditions of the Danube such as increased water depth and width, decreased water velocity, increased sedimen-

tation, and great oscillations of water level, resulting in new river branches and pipes. An example is the littoral zone of the Labudovo okno locality where rare aquatic macrophytes have benefited from these changes. Frequent oscillations of water level and water velocity have substantial implications upon stability of plant habitats therefore altering the composition of plant communities in the accumulation and the occurrence of aquatic vegetation at different successional stages (Stevanović, 2001).

In the past 10 years, the investigations of this particular locality were undertaken within the investigations of aquatic macrophytes of the accumulation (Stevanović, 2001). Also, an expansion of adventive species, *Echinochloa oryzoides* and *Paspalum paspalodes*, invading great area at the Labudovo okno locality was reported (Stevanović et al., 2004).

The obtained results that are a part of complex two-year investigations of the Labudovo okno locality include aquatic phytocoenoses of class *Hydrochari-Lemnetea* Oberd. 1967 and *Potametea* Tx. Et Prsg. 1942 (Polić, 2005).

MATERIAL AND METHODS

In the vegetation period 2003—2004, the phytocoenological investigations were performed employing the Swiss-French standards (Braun-Blanquet, 1964). Syntaxonomic analyses of vegetation were done after Soó (1964—1980) while plant species were determined using standard keys (Jávorka, 1925; Hayek, 1927—1933; Komarov, 1934; Soó, 1964—1980; Tutin et al., 1964; Tutin et al., 1968—1980; Josifović, 1970—1986; Felföldy, 1990; Preston, 1995).

RESULTS AND DISCUSSION

Syntaxonomy of communities of classes *Hydrochari-Lemnetea* and *Potametea*:

Class *HYDROCHARI-LEMNETEA* Oberd. 1967

Order *Hydrocharietalia* Rubel 1933

Alliance *Lemnion minoris* W. Koch et Tx. Ex Oberd. 1957

1. Ass. *Lemno-Spirodeletum* W. Koch 1954

2. Ass. *Salvinio-Spirodeletum polyrrhizae* Slavnić 1956

3. Ass. *Lemno minoris-Azolletum filiculoides* Br.-Bl. 1952

Alliance *Ceratophyllion* Den Hartog et Segal 1964

4. Ass. *Ceratophylletum demersi* (Soó 27) Hild 1956

Class *POTAMETEAE* Tx. Et Prsg. 1942

Order *Potametalia* W. Koch 1926

Alliance *Potamion* W. Koch. 1926

5. Ass. *Myriophyllo-Potametum* Soó 1934

Alliance *Nymphaeion* Oberd. 1956

6. Ass. *Nympaeetum albo-luteae* Nowinski 1928

a) subass. *nupharetosum* Soó 1973

7. Ass. *Trapetum natantis* Müller et Görs 1960

1. Ass. *Lemno-Spyrodeletum* W. Koch 1954
(Tab. 1, column 1)

Stands of the phytocoenosis *Lemno-Spyrodeletum* observed at the Labudovo okno locality either occurred in shallower water in sunny habitats next to the shoreline or accompanied with reed grass where water flow slows and becomes laminar. The stands were absent only in the northeastern part of the locality. The investigated community was structured of not only characteristic species of the association *Lemna minor* and *Spirodela polyrrhiza*, but *Azolla filiculoides*, *Salvinia natans*, and *Ceratophyllum demersum* were also frequent.

2. Ass. *Salvinio-Spyrodeletum polyrrhizae* Slavnić 1956
(Tab. 1, column 2)

Stands of the association *Salvinio-Spyrodeletum polyrrhizae* belong to floating, unattached type of pond vegetation where *Salvinia natans* and *Spirodela polyrrhiza* are ones that build them. They primarily occur in shallow water in warm and sunny spots. When the development of *Salvinia* (*Salvinia natans*) reached its maximum, stands of the association were dominant, in northern part of the Labudovo okno in particular. Consequently, this phytocoenosis was one of the dominant communities of the investigated locality. Ecologically, a transitional area where there was a change between the association *Lemno minoris-Azolletum filiculoides* and the stands investigated occurred. In addition to the species that build these stands, *Lemna minor* and *Ceratophyllum demersum* were also frequent.

3. Ass. *Lemno minoris-Azolletum filiculoides* Br.-Bl. 1952
(Tab. 1, column 3)

Stands of the community *Lemno minoris-Azolletum filiculoides* in which *Azolla filiculoides* and *Lemna minor* were dominant occurred in smaller area than the phytocoenosis *Salvinio-Spyrodeletum polyrrhizae*. Stands of the phytocoenosis were recorded in northern and northeastern part of the Labudovo okno locality, whereas dense stands were found in central part of the locality. Their optimum development requires the same ecological conditions of the environment as association *Lemno-Spyrodeletum* and *Salvinio-Spyrodeletum polyrrhizae*. With respect to floristic structure, *Salvinia natans* and *Spirodela polyrrhiza* were as frequent as ones that build them.

4. Ass. *Ceratophylletum demersi* (Soó 1927) Hild 1956
(Tab. 1, column 4)

Stands of the community *Ceratophylletum demersi* occur along the shore in shallow water (distance 2 m—5 m). They are fully developed in summer.

With respect to their density, the highest values were obtained in northern part of the locality. Besides characteristic species of the association *Ceratophyllum demersum* that form very dense populations in some spots and build inaccessible submersed meadows, floating species *Lemna minor* and *Spirodela polyrrhiza* were also frequent.

Tab. 1 — Associations review of the alliance *Lemnion minoris* and *Ceratophyllion* of Labudovo okno locality

Species	Associations			
	1	2	3	4
Characteristic species ass. of the alliance <i>Lemnion minoris</i> , <i>Ceratophyllion</i>				
<i>Lemna minor</i> L.	V ₊₄	IV ₊₁	V ₊₃	V ₊₂
<i>Spirodela polyrrhiza</i> (L.) Schleid.	V ₊₅	V ₊₅	IV ₊₁	IV ₊
<i>Salvinia natans</i> (L.) Allioni	IV ₊₁	V ₁₋₅	IV ₊₁	II ₊
<i>Azolla filiculoides</i> Lam.	IV ₊₁	III ₊₁	V ₃₋₅	III ₊
<i>Ceratophyllum demersum</i> L.	IV ₊₁	IV ₊₃	IV ₊₃	V ₃₋₅
Characteristic species of the alliance <i>Lemnion minoris</i> and <i>Ceratophyllion</i> , the order <i>Hydrocharietalia</i> and the class <i>Hydrochari-Lemnetea</i>				
<i>Vallisneria spiralis</i> L.		II ₊₁		I ₁
Accessory species				
<i>Myriophyllum spicatum</i> L.	II ₊₂	II ₊₃	II ₊₁	I ₊
<i>Najas marina</i> L.	I ₊₁	II ₊	II ₊	II ₊₁
<i>Trapa natans</i> L.	II ₊₁	III ₊	II ₊	III ₊₁
<i>Elodea canadensis</i> Rich		II ₊		I ₊
<i>Potamogeton x fluitans</i> Roth	II ₊₁	II ₊	II ₊	II ₊
<i>Potamogeton gramineus</i> L.	I ₊	I ₊		I ₊
<i>Potamogeton pectinatus</i> L.	II ₊₁		II ₊	III ₊
<i>Potamogeton perfoliatus</i> L.	I ₊			III ₊₁
<i>Potamogeton lucens</i> L.			II ₊₁	
<i>Sagittaria sagittifolia</i> L.	I ₊			
<i>Nymphoides peltata</i> (S. G. Gmelin) O. Kuncze		II ₊₁		
<i>Potamogeton crispus</i> L.			II ₊	I ₊
<i>Nuphar lutea</i> (L.) Sibith. & Sm.			I ₊	

1 — *Lemno Spirodeletum*, 2 — *Salvinio-Spirodeletum polyrrhizae*, 3 — *Lemno minoris-Azolletum filiculoides*, 4 — *Ceratophylletum demersi*

5. Ass. *Myriophyllo-Potametum* Soó 1943 (Tab. 2, column 1)

Stands of the association *Myriophyllo-Potametum* occur in still water while also enduring water currents in the eastern part of the locality, where contact between water of Labudovo okno and the Danube is evident and where the influence of the Danube progresses. Their occurrence however, was also observed in the central part, in the center of body of water, where a rather great area was invaded, and where a transition from stands of the phytocoenosis *Ceratophylletum demersi* to the investigated stands was evident. Composition and structure of the association *Myriophyllo-Potametum* vary largely, re-

flecting the ecology of the environment and therefore occurring in a number of different facies (Slavnić, 1956). Since high values of both abundance and covering of species *Potamogeton pectinatus* and *Potamogeton perfoliatus* were recorded, we may discuss about the two facies where a facies with a dominant species *Potamogeton pectinatus* prevails. High percentages were obtained only with *Myriophyllum spicatum* and *Ceratophyllum demersum*.

6. Ass. *Nymphaeetum albo-luteae* Nowinski 1928 subass. *nupharetosum*
Soó 1973 (Tab. 2, column 2)

Since no white water lilies (*Nymphaea alba*) was observed at the locality, the occurrence of Spatterdock (*Nuphar luteum*) promotes the stands of the subass. *nupharetosum*. These stands occurred in fragments along the right pond shore (the Danube, downstream). This fragmentation was caused by the ecological and hydrological shift of the Danube. Not only high percentages of Spatterdock were recorded, but, also, the percentage of *Ceratophyllum demersum* was high.

Tab. 2 — Associations review of the alliance *Lemnion minoris* and *Ceratophyllion* of Labudovo okno locality

Species	Associations		
	1	2	3
Characteristic species ass. and subass. of the alliance <i>Potamion</i> and <i>Nymphaeion</i>			
<i>Myriophyllum spicatum</i> L.	V ₁₋₄		II ₊₃
<i>Potamogeton perfoliatus</i> L.	III ₊₄		
<i>Potamogeton pectinatus</i> L.	V ₊₅	II ₊₁	II ₊
<i>Nuphar lutea</i> (L.) Sibth. & Sm.		V ₃₋₅	
<i>Trapa natans</i> L.	III ₊	III ₊	V ₃₋₅
Characteristic species of the alliance <i>Potamion</i> and <i>Nymphaeion</i> , the order <i>Potametalia</i> and the class <i>Potametea</i>			
<i>Ceratophyllum demersum</i> L.	V ₊	IV ₊₃	IV ₊₂
<i>Potamogeton fluitans</i> Roth.	II ₊	II ₊	II ₊₁
<i>Najas marina</i> L.			II ₊
<i>Potamogeton gramineus</i> L.			I ₊
<i>Salvinia natans</i> (L.) Allioni		III ₊₂	II ₊₂
<i>Elodea canadensis</i> Rich			I ₊
<i>Potamogeton crispus</i> L.			I ₊₁
Accessory species			
<i>Lemna minor</i> L.	III ₊	IV ₊₁	III ₊
<i>Spirodela polyrrhiza</i> (L.) Schleid.	III ₊	IV ₊	III ₊
<i>Azolla filiculoides</i> Lam.	II ₊	III ₊	II ₊₂
<i>Vallisneria spiralis</i> L.			I ₊

1 — *Myriophyllo-Potametum* 2 — *Nymphaeetum albo-luteae* subass. *nupharetosum*; 3 — *Trapa natantis*

7. Ass. *Trapetum natantis* Müller et Görs 1960
(Tab. 2, column 3)

Trapetum natantis was a dominant community in floating belt of plant cover of the Labudovo okno locality. The stands of the phytocoenosis occurred in shallow and still waters. Highly dense *Trapa natans* populations of 3 m in width and exceeding 100 m in length were developed scattered (central part of the locality) at maximum developmental stage. Of characteristic species of the alliance, order, and class, the species *Ceratophyllum demersum* showing higher percentages was distinguished.

CONCLUSION

The structure of plant communities of the Labudovo okno locality is the result of processes running in the past 20 years. In other words, widening of river bed, elevation of water level, slower stream, and formation of branches and pipes characterized by altered ecological conditions has contributed to widening of a zone of aquatic and semiaquatic vegetation altering the original appearance of the region.

Even four associations of the class *Hydrochari-Lemnetea* and three of the class *Potametea* Tx. Et Prsg. 1942 were recorded. Taking into account the invasive character of the species *Trapa natans*, a particular attention should be paid at stands of the community *Trapetum natantis*. Ecological, morphological, and other traits make this species a serious threat to other phytocoenoses of the investigated locality. All these results demonstrate the importance of the control of this species in attempt to protect inundate zones of the Danube in Serbia.

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ВЕГЕТАЦИЈА КЛАСА *HYDROCHARI-LEMNETEA* OBERD. 1967 И *POTAMETEA* TX. ET PRSG. 1942 У ЛАБУДОВОМ ОКНУ (СРБИЈА)

Дубравка М. Полић¹, Ружица С. Игић¹,
Слободанка Ј. Стојановић², Дејана М. Лазић²

¹ Природно-математички факултет, Департман за биологију и екологију,
Трг Доситеја Обрадовића 2, 21000 Нови Сад, Србија

² Пољопривредни факултет, Трг Доситеја Обрадовића 8,
21000 Нови Сад, Србија

Резиме

Локалитет Лабудово окно (50—84 m надморске висине) налази се у југоисточном делу обода Панонске низије, дуж леве обале Дунава између речног километра 1082 и 1078. Истраживани локалитет настао је подизањем нивоа Дунава након изградње бране хидроенергетског система „Ђердап I”. Константовану вегетацијску разноликост чине акватичне заједнице класа *Hydrochari-Lemnetea* Oberd. 1967 и *Potametea* Tx. et Prsg. 1942. Из класе *Hydrochari-Lemnetea* Oberd. 1967. констатоване су фитоценозе: *Lemno-Spirodeletum* W. Koch 1954, *Salvinio-Spirodeletum polyrrhizae* Slavnić 1956, *Lemno minoris-Azolletum filiculoides* Br.-Bl. 1952 и *Ceratophylletum demersi* (Soó 27) Hild 1956. Из класе *Potametea* Tx. et Prsg. 1942 присутне су асоцијације: *Myriophyllo-Potameteum* Soó 1934, *Nymphaetum albo-luteae* Nowinski 1928 и *Trapaetum natantis* Müller et Görs 1960.

Vladimir M. Stojanović
Dragoslav J. Pavić
Minučer M. Mesaroš

Department of Geography, Tourism and Hotel Management
Faculty of Natural Sciences and Mathematics
Trg Dositeja Obradovića 3, Novi Sad, Serbia

THE USE OF NATURAL ASSETS OF REEDS MARSHLAND IN VOJVODINA IN VIEW OF SUSTAINABLE DEVELOPMENT

ABSTRACT: The idea of the application of sustainable development in the use of the natural resources of reeds area in Vojvodina has not been seriously considered so far, which has resulted in unwise management in this region. Similar situation was dominant in the past, especially during the twentieth century, which significantly influenced the degradation of the original characteristics of reeds marshland. A survey of economic activity that has been present in the area and their surroundings has emphasized the necessity of harmonized development so that the authentic natural entities would be preserved, for many reasons, for future generations. One of the ways for preserving original characteristics could be nature preservation. They have characteristics of wetlands, which have lately been given significant attention through the search for the models of wise use. An important prerequisite for familiarizing with and investigating reeds marshland is the establishment of the distinctive criteria, which has not been thoroughly investigated so far.

KEY WORDS: natural resources, nature preservation, reeds marshland, sustainable development

INTRODUCTION

Reeds marshland represents one of the most important elements of the entire landscape diversity in Vojvodina, whose importance has been confirmed throughout history more than once. Unfortunately, the most diverse functions of this area — from ecological to cultural and historical were not enough to raise more interest in the assessment and analysis of the more efficient contemporary use of this area. The results are reflected in the degradation of the reeds marshland, which has peaked in the second half of the twentieth century. Even the more precise scientific establishment of the borderline of these natural entities has not been the subject of elaborate discussions so far.

According to its basic characteristics reeds marshland are grouped as wetlands, which have key ecological function, as the regulators of the water regime and significant habitats of flora and fauna. In accordance with their endangered existence and increased loss, in a number of countries in the world there have been many actions aimed at protection and preservation. One of the main protected natural assets of Vojvodina is actually reeds marshland, thus making the activities of nature protection one of the important potentials for its more wise use.

DISCUSSION

Vojvodina along with its vast, low-lying, flat areas, rich in water flows and shallow underground waters, possesses favorable prerequisites for the formation of the flood areas with distinctive vegetation and pedological features. The traditional word among local inhabitants for such areas especially the ones near large rivers is *rit* — reeds marshland.

If observed in this way, the determination of the term *rit* and its borderline represents a relatively simple assignment. However, considering the results of the research conducted so far, it can be observed that there are disagreements about the understanding of the term *rit*.¹

Thus B. B u k u r o v (1975) when determining reeds marshland in Vojvodina, starts with geomorphologic, and then hydrological and biogeographical criteria. In fact, this author considers reeds marshland to be alluvial plateaus, i.e. low-lying flat areas along rivers with altitudes of 85 to 68 meters above sea level. This relief entity has mostly been spread along the rivers Danube, Tisza, and Sava, as well as along the shorter water flows such as Tamis, Begej, Karas, Nera, Galacka, Zlatica, and Moris.²

According to contemporary concepts, reeds marshland represents mainly a type of wetland, which is on the borderline area between land ecosystems and water systems, making it a distinctive form, but still greatly dependent on the other two (M i t s c h, G o s s e l i n k, 1986). From this point of view, we can conclude that, in addition to geomorphologic preconditions (low, coastal strip near the water), it is actually a hydrological criterion, i.e. occasional surface and underground watering, which is necessary for the formation of the specific wetland of reeds marshland type. Because of the mentioned facts, the reeds marshland has been developing specific soil, as well as a specific flora of the vegetable cover. What contributes to this view of reeds marshland is a well-developed methodology of mapping wetland, on whose basis the establishment of its borderlines is connected to the criteria of the distribution of certain plant species (indicators), the presence of hydromorphic types of soil and specific hydrological conditions (periodic or often floods). Depending on the available data, all three indicators are used or just some of them. Vegetation is

¹ From German Ried = reeds, cattail, pond.

² Although it does not flow through Vojvodina, its alluvial plateau partly goes into the territory of the Province (B u k u r o v, 1975).

usually considered the most reliable indicator because it is a result of the dominant natural conditions of the wetland (M a r s h, 2005).

On the territory of Vojvodina, where many marshland areas have been dried out through melioration, or converted into agricultural land, anthropogenic indicators have to be taken into account when determining the borderline areas of the contemporary reeds marshland. Thus, geomorphologically speaking, completely or partially dried reeds marshland still represents a unique relief entity, more precisely alluvial plateaus, but due to the changed hydrological and vegetation conditions, these areas, no longer can be considered wetlands, or reeds marshlands. In this way, on the territory of Vojvodina, the areas of reeds marshland have been greatly reduced by melioration of the alluvial plateaus. Still, in some cases, the areas where natural characteristics have been changed, have still kept some original features of the reeds marshland. Above all, hydrological and vegetation characteristics should be taken into consideration when analyzing their usage.

The importance of these areas can partly be seen in the interest certain economic activities have shown for their natural resources. Field research has shown that in the reeds marshland area and its surroundings, the following activities have been dominant: forestry, fishing, reeds processing, and tourism.

Alluvial terrain is beneficial for the development of forests. It is supposed that in the past these areas had the characteristics of ancient woodland. Autochthonous species have been significantly reduced through irrational management of these resources. Plantations have been widely spread, although these habitats do not have the characteristics of forests but of agricultural areas. The largest areas covered with wood are in the Apatinski, Monoštorski and Bukinski Rit, in the vicinity of Obedska Bara, and then in the remaining parts of the alluvial plateaus of the rivers Danube, Tisza and Sava. According to P u z o v i ć (2002), the way these areas were forested in the last few decades was not optimal for the totality of the environment. Wetlands of reeds marshland type were treated as areas of low quality and inadequately used terrain, so the forestation with cloned poplars and willows has been treated as a noble act. The changes of the basic conditions of the habitats because of such activity have had a multiple impact on the natural characteristics of the reeds marshland. The analysis of the possibilities of transforming plantation areas into natural habitats of forest or open type has not yet been widely spread.

Fishing is an activity with a longer tradition on big rivers and reeds marshland. The amenities of natural conditions and plenty of resources were determining factors in the past for the entire family living, for example, in the reeds marshland of the Danube Basin, who made living based only on this activity (B u k v i ć, 1969). Although the conditions of the habitats have changed due to the human influence on natural potentials, fishing is still one of the most important economic activities of the reeds marshland. The evidence can be found in the management programs for fishing which regulates these activities, including the ban on certain fish species; fishing ban during spawning time, the protection of the spawn area, the ban on the economic fishing on all fish species during certain periods (Program unapređenja ribarstva na ribolovnom području „Dunav I” i „Monoštorski rit” 1996—2000).

The use of reeds has had a long tradition in the surrounding areas of big rivers in Vojvodina, as well as in the settlements built around them. Reed is one of the key characteristics of the marshland landscape in Vojvodina. In addition to this, reed was a very important building material in the past. New age has brought new building solutions, but reeds had a comeback, this time as ecologically friendly building material. At the same time, its processing can be a profitable business. In Holland, there are about 300 industrial companies involved in reeds processing. It is mainly grown in households, and the business is so developed that this country imports reeds from Hungary, Austria, Poland, Romania, and Turkey. The economic activity based on reeds as a raw material has not yet been spread in our country. Still, processing, as an incentive to economic development is present in reeds marshland. Among the rare reeds processing lines based on the raw materials of reeds marshland in Vojvodina are the villages of Bački Monoštor (Monoštorski Rit) and Belo Blato (Farkaždinski Rit, the vicinity of Carska bara).

The use of reeds can be important for the conservation of protected natural heritage in reeds marshland. In the reeds marshland around Obedska bara, reeds with their biomass and reproduction is endangering the strip within the horseshoe area of Obedska bara and is threatening to completely dam it and dry it out in the near future. This is why a cooperation has been suggested between Keepers of the Special Nature Reserve Obedska bara and some companies dealing with reeds processing (Informacija o stanju, zaštiti i mogućnostima održivog korišćenja zaštićenog prirodnog dobra SRP „Obedska bara”, 2000).

As a special potential for the development of these areas, tourism should be analyzed. Reeds marshlands have mainly been seen as important areas for hunting tourism so far, in accordance with a number of hunting areas earmarked for domestic and foreign tourists. Some of the important hunting areas are Kozara, Apatinski Rit, Kamarište, Plavna, Koviljski Rit, and Lower Danube Basin (D r a g i n, 2006). Still, the potentials of these areas are far greater and can be found in the possibilities for the development of ecotourism, which has been essentially eco-friendly, ecologically responsible, taking care of the status and the development of local population and finally supporting the actions for the environment preservation (F e n n e l, 1999). Some of the activities could include bird watching, educational hiking, boat rides, and volunteer camps.

What is extremely important for the vast reeds marshland in Vojvodina is the activity of nature preservation. It influences the regulation of the mentioned economic activities largely. If having observed the geographic map of the protected natural heritage sites, we can see that a large number of them are present in the alluvial plateaus of the rivers in Vojvodina. Some of the most important ones are: Special Nature Reserve Upper Danube Basin, Special Nature Reserve “Karadorđevo”, Nature Park “Tikvara”, Nature Park “Begečka jama”, Special Nature Reserve “Koviljsko-petrovaradinski rit”, Special Nature Reserve “Stari Begej — Carska bara”, Special Nature Reserve “Obedska bara”. Three spatial entities which are protected under the Ramsar Convention in Serbia, out of the total of six (The Ramsar List of Wetlands of International Im-

portance, www.ramsar.org/key_sitelist.htm), are situated in Vojvodina, which is a sufficient evidence of the significant interest of the initiators of the protection for these areas.

Because of the number of protected natural assets in the reeds marshland in Vojvodina, there is a question of usefulness of this activity for the general welfare of the environment in the reeds marshland. If we assess these problems, according to guidelines outlined in the legal acts and decisions about environment protection, a potential influence on the area is clear. Thus, the following activities are usually forbidden: the change of water regime, the release of unprocessed waste waters, the introduction of foreign flora and fauna, planting Euro-American poplars (depending on the degree of protection), collection and use of protected species, building establishments which are not in accordance with the protection, and any activity which changes the living conditions in the wetland. At the same time, the measures and activities that preserve and bring back original characteristics of the natural scenery are usually allowed.

The initiative and declaration about protected natural heritage is one of the basic means of applying the idea of sustainable development in an area. As landscapes dedicated to protection and preservation of biodiversity, natural and cultural assets connected to nature, through legal and other means (Prato, Fagre, 2005), they (the protected natural assets) have a multiple impact on the environment conservation. On the one hand, the introduction of sustainable development would mean the respect of three criteria: the principles (justice, ethics, and protection promotion), planning (long term, proactive and integral), and management (interdisciplinary character). On the other hand, the protective measures are sometimes not completely carried out which also represents a danger for the autochthonous nature of the reeds marshland. If we take into consideration that these areas have already undergone numerous changes and strong influence during the past, such as building levees, the changes in water regime and drying, regulation of river beds, we can see more clearly the challenge standing in front of the activities for the protection and preservation of reeds marshland, as an original natural scenery in the valleys of the Pannonian rivers. The changes have been especially prominent in the region of Apatinski and Monoštorski Rit (Bukurov, 1975; Dedić, 1998), wider landscape of Bukinski Rit (Dedić, Božić, 1998; Milošev, 1998), the area around Carska bara, in the alluvial terrace of Tisza (Ham, 1975). Some of the ongoing problems in the protection are active cutting down of forests, forestation with inadequate species — mainly Euro-American poplars, intensive agricultural activity on arable land connected to reeds marshland through melioration canals and the influence of other economic activities.

The concept of sustainable development comprises detailed planning of the protection measures and wise use of the assets. In all planning phases, it is inevitable to map the protection area, so this is why the mapping of wetlands has become one of the most important activities into the planning of the environment protection (Marsh, 2005). The borderlines of the protected regions are most often determined based on the mentioned criteria of the vegetation

structure, flora structure, pedological structure and hydrolocal indicators depending on the characteristics of the area and available sources of spatial data.

CONCLUSION

When denoting the term *rit* (*reeds marshland*), in addition to the usual geomorphological, hydrological and biogeographical criteria, we need to include the anthropogenic factor as well. In some cases building of dams and some other regulation measures, for example in Monoštorski and Apatinski Rit, have changed the structure of reeds marshland in the area that was dammed. Still, some of the original natural features have been preserved, and natural resources influence the use of the area, which was not dammed (forestry, fishing, hunting). In any case, the concept of the nature protection (mainly special nature reserves), which is present in some reeds marshlands in Vojvodina, including the dammed areas, confirms their general importance.

Economic activities in the area of reeds marshland and its surroundings, based on their natural resources, should be brought together with a strategy of sustainable developments. In the cases of the protected natural assets, which cover the area of reeds marshland, measures for their future preservation are conducted or at least earmarked. Many vast reeds marshlands are not encompassed in the region of protected natural assets and a solution for their preservation should be sought.

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КОРИШЋЕЊЕ ПРИРОДНИХ ПОТЕНЦИЈАЛА РИТОВА У ВОЈВОДИНИ И ОДНОС ПРЕМА ИДЕЈИ ОДРЖИВОГ РАЗВОЈА

Владимир М. Стојановић, Драгослав Ј. Павић, Минучер М. Месарош

Департман за географију, туризам и хотелијерство,
Природно-математички факултет, Трг Доситеја Обрадовића 3, Нови Сад

Резиме

Ритови представљају специфичан вид влажних станишта карактеристичан за околину великих река у Војводини. Резултати досадашњих истраживања потврђују неусаглашена схватања у вези са дефинисањем појма рит. Неки од индикатора за дефинисање и одређивање граница ритова су геоморфолошког, хидролошког и биоегеографског карактера. Веома је важно принципу за разграничење и дефинисање појма рит додати и антропогени критеријум. Мелиорацијским радовима знатне површине ритских подручја су исушене, а неке су претворене и у оранице. Неке друге су успеле да задрже одлике које су претежно карактеристичне за овај тип влажних станишта. Неусаглашеност схватања о одређивању појма рит има утицаја на коришћење ових простора. У стручној и научној литератури је уобичајено користити појам идеје мудрог коришћења оваквих простора, које је

у тесној вези са одрживим коришћењем и одрживим развојем. Најчешће активности од стране људи у војвођанским ритовима су шумарство, рибарство, прерада трске, а у последње време и туризам. Свака од наведених делатности се на специфичан начин одражава на природне ресурсе и екосистеме ритова. Преовлађује мишљење да ове активности нису довољно међусобно усклађене и усаглашене са ресурсима ритова. У будућности би, сходно идеји очувања ових простора, требало много очекивати од активности заштите природе. Многбројне целине ритова у Војводини већ сада уживају неки статус заштите (Моношторски, Апатински, Ковиљско-петроварадински рит). Пошто је један од стандарда одрживог развоја неког простора везан и за проглашавање заштићених природних добара, очекује се да би овакав тренд могао знатно допринети квалитетнијем коришћењу ритова, па тако и очувању природе и њених изворних одлика.