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DYNAMICS OF GENERATING TRANSIENTS OF DELAYED FLUORESCENCE INDUCTION SIGNAL AND PHOTOSYNTHETIC ANTENNAS: A POSSIBLE RELATIONSHIP. MATHEMATICAL MODELING APPROACH*

ABSTRACT. A mathematical model was developed for resolved temporal transients of experimentally recorded delayed fluorescence (DF) induction signal. During an intermittent light regime, antennas of the photosynthetic apparatus were treated as targets, repeatedly hit by potentially absorbable photons within a series of consecutive light flashes. Formulas were derived for the number of antennas, cumulatively hit by a specific number of photons, as function of the flash serial number (time). Model parameters included: number of absorbable photons in one flash, antenna sizes and numbers. A series of induction curves were analyzed, obtained from a *Zea mays* L. leaf segment and differing in the previous dark period (t_d). Each curve, consisting of the two most prominent DF transients (*C* and *D*), was fitted with several model types, differing in the number of absorbed photons. For both transients, the best fitting result was achieved when DF induction was linked to the second absorbed photon. As expected, model parameters related to antenna sizes showed weaker dependence on t_d than those referring to antenna numbers. With restrictions applied in this model, the two DF induction transients may be related to two classes of photosynthetic antennas. Their different sizes may have a predominant influence on the efficiency of photon absorption, and possibly time-dependent appearance of DF transients.

KEY WORDS: delayed fluorescence, photosynthetic antennas, induction transients, mathematical modeling, *Zea mays* L.

* Authors dedicate this paper to the memory of a countenance and deeds of Dragan Fidler's.

INTRODUCTION

Delayed fluorescence (DF) phenomenon can be described as lighting of green plants, algae and photosynthetic bacteria in red range of the visible spectrum, immediately upon their illumination. In the final step, DF is created by the same $S_1 \rightarrow S_0$ transition as prompt fluorescence (Lang et al., 1991; Krause et al.,1991). But very different lifetimes, 1.5 ns or less for prompt fluorescence (Govindjee et al., 1990; Schmuck et al., 1992) compared to nanoseconds (Sonneveld et al., 1981; Mimuro et al., 1999), over microseconds (Haveman et al., 1975; Holzapfel et al., 1974) and milliseconds (Hipkins et al., 1974; Barber et al., 1974) to seconds' range (Rutherford et al., 1984) for DF, clearly indicate two very distinct mechanisms by which photoactive S_1 state of chlorophyll (Chl) is created. In case of prompt fluorescence, the S_1 state is created in a 10^{-12}—10^{-14} s period by internal conversion, following light absorption. In case of DF, the S_1 state is created through a recombination of products formed in the primary photochemical act (Govindjee et al., 1971; Jursinic, 1986). Therefore, unlike prompt fluorescence, which does not need more than one single Chl molecule to be emitted, the entire entity of the photosynthetic apparatus is necessary for DF emission, *i.e.* DF has been used as a criterion for its integrity (Zaharieva et al., 1999).

Delayed fluorescence induction trace reflects processes and phenomena occurring when a photosynthetic object is being kept in darkness for a while, and then illuminated, *i.e.* in a transition period from dark to light regime. Most DF induction traces were recorded under the millisecond working regime of a rotating disc, with intermittent illumination, consisting of a few milliseconds of light period, and consecutive few milliseconds of darkness in which DF is being recorded (Vučinić, 1983; Marković et al., 1987). The overall shape of a DF induction trace is highly dependent on the length of the dark period preceding illumination (Dzhibladze et al., 1988; Bukhov et al., 1989). If the preceding dark period (t_d) is longer than 30 and shorter than 300 s, DF induction trace is split into at least three transients (Radenović et al., 1985, 1994; Radenović, 1994, 1997; Radenović et al., 2003). Clearly distinct appearance time of their maxima (t_{maxA} = 31 ± 6 ms; t_{maxB} = = 5 ± 0.5 s; t_{maxC} = 15 ± 5 s and t_{maxD} = 300 ± 60 s; t_{maxE} = 670 ± 35 s) suggests that their origins are in various processes occurring during the dark/light transition period. Veselovsky and Veselova (1990) made a step forward in explaining the DF induction trace transients, by putting a DF induction trace on the same time scale with temporal variation of prompt fluorescence during continuous illumination of the photosynthetic apparatus (Kautzky effect), and with oxygen evolution changes. The Kautzky effect has been thoroughly investigated and it is reasonably well understood (Govindjee et al., 1971; Govindjee 1975; Lichtenthaler et al. 1988; 1992). The comparison revealed correlation of the *B* and *C* transients with electrochemical gradient (ECG), formed across thylakoid membranes upon illumination (Veselovskii et al., 1990; Radenović et al., 1981, 1985, 1994; Radenović 1994, 1997).

Another mathematical model of these transients, contained in our last report, was based on the chosen kinetic model for consecutive first order reactions (M a r k o v i ć et al., 2001). In the present work we approach the problem of modeling DF induction signal transients from another angle. If a leaf segment is subject to an intermittent light regime, consisting of a series of flashes, a mathematical procedure could be developed to track the most probable number of targets (antennas), hit by a particular number of projectiles (photons), as a function of time (light flash number). Parameters of such a model include antenna sizes and their relative numbers within the analyzed leaf segment, for each of the recorded transient within the DF induction signal. Since it was shown that DF induction transients depend on the previous leaf dark period t_d (R a d e n o v i ć et al., 1981, 1985, 1994; R a d e n o v i ć, 1994, 1997), a basic test of the model would be to fit a number of induction curves, differing in t_d, with model equations. As a result, one should expect that fitted parameter values concerning antenna sizes should not depend on t_d (at least not in a trend-like manner), while those related to relative antenna numbers (*i.e.* numbers of PSII responsible for DF emission) may exhibit such a behavior, depending on the complex processes during the leaf dark period.

MATERIALS AND METHODS

Objects of Studies

Inbred lines:

— ZP R70ž — developed by the ear to row method from the Rumski Golden Dent variety is the inbred of FAO maturity group 300, dent kernel type, with white cob; the inbred is a good combiner, non-resistant to lodging and it is a property of the Maize Research Institute, Zemun Polje.

— Oh43 — developed by the ear to row method from the F_2 population of a narrow genetic base that was derived by self-pollination of F_1 hybrid Oh40B x W-8 is the inbred of FAO maturity group 500, dent kernel type, with white cob; the inbred is a good combiner, tolerant to drought, has lower yielding per se, and it is of the USA origin.

The hybrid ZPSC 46A — derived by crosses of the inbred lined ZP R70ž to the inbred line Oh43, is the hybrid of FAO maturity group 400, dent kernel type, with white cob; the hybrid has a high yielding potential, is tolerant to drought and is very adaptive to the growth under different agroecological cultivation conditions.

Experimental Procedure

Maize (*Zea mays* L.) inbred lines ZP R70ž, Oh43 and hybrid ZPSC 46A leaf segments (2 cm^2) were cut under water and placed on a temperature controlled plate inside a phosphoroscope. They were adapted to plate temperature (23°C), and the delayed fluorescence emission was recorded. The DF intensity

was measured in the dark interval of intermittently illuminated leaves, using a Becquerel phosphoroscope and a 150 W quartz-halogen lamp. One cycle consisted of 2 ms of light and 10 ms of darkness. Delayed fluorescence was recorded from the 3rd to 7th ms of the dark interval, using a cooled photomultiplier. Signal from the multiplier was registered on a storage oscilloscope for the fastest processes, while slower variations of DF were recorded on a chart. Few minutes of recording produced a DF induction trace, with faster transients in the first two minutes, and slower changes afterwards. A schematic presentation of the experimental set up of the equipment for DF chlorophyll recording is given in Figure 1. Details of the experimental setup can be found elsewhere (Vučinić et al., 1983; Radenović et al., 1994; Radenović 1994, 1997).

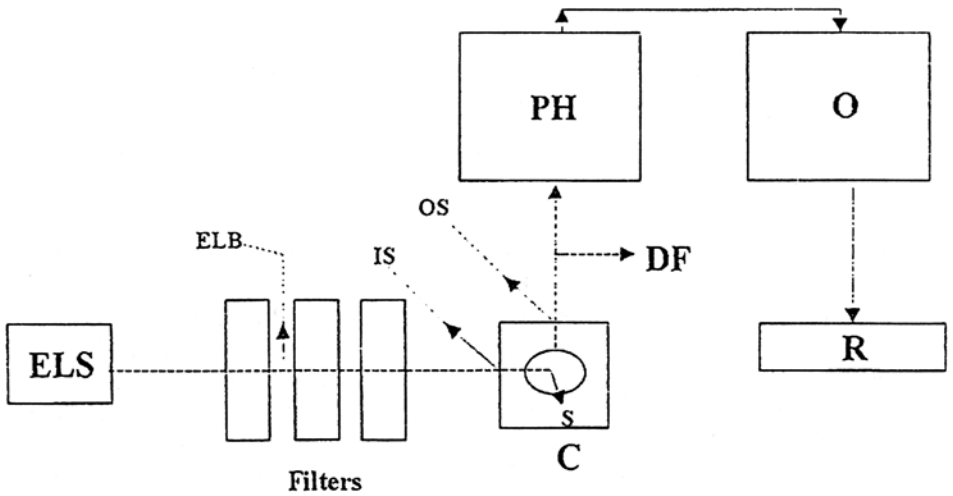


Fig. 1 — Experimental setup of the method and measuring equipment for delayed chlorophyll fluorescence: C — dark chamber with a sample stand; s — sample (leaf segment), filters, ELS — excitation light source, PH — photo-multiplier; O — oscilloscope, R — printer, ELB — excitation light beam, DF — luminescent light, IS — input chamber slot, OS — output chamber slot

A RETROSPECTIVE VIEW TO EXPERIMENTAL RESULTS WITH DISCUSSION

1. *Conditions for generation of transients of delayed chlorophyll fluorescence induction process*

Depending on the duration of the dark period (t) — the time of previous keeping of intact leaf segments of maize inbred lines and the hybrid in the dark — two induction curves of DF chlorophyll can be registered (Figure 2a, p, q).

The registered curves of DF chlorophyll induction processes have a reference connotation, hence they are used in preceding and initial measurements

of DF chlorophyll in the unknown objects of studies (R a d e n o v i ć et al., 1981, 1985, 2003; R a d e n o v i ć, 1994, 1997). The DF chlorophyll induction curve marked with p, Figure 2a, is always obtained when the maize intact leaf segment is kept in the dark for a longer period of time (t 15 minutes) prior to its intermittent illumination in the phosphoroscope.

The DF chlorophyll resolve induction curve, marked with q, Figure 2a, is obtained when the maize intact leaf segment is kept in the dark for significantly short period of time (500 s t 30 s) with a time rate of t = 30 or 60 seconds prior to its intermittent illumination in the phosphoroscope. It is shown that the DF chlorophyll induction processes resolved in 5 transients conditionally designated with A, B, C, D, E, Figure 2a (R a d e n o v i ć et. al., 1985; M a r k o v i ć et. al., 2001; R a d e n o v i ć, 1994, 1997).

2. *Resolution of delayed chlorophyll fluorescence induction processes into transients*

In the experimental resolution of DF chlorophyll induction processes, transients B, C, D and E were initially revealed by the application of standard measurements of DF chlorophyll (R a d e n o v i ć et. al., 1985, 1994; R a d e n o v i ć et. al., 1994, 1997). Much latter, the transient A was revealed (R a d e n o v i ć, 1997).

The revealed transients of DF chlorophyll (Figure 2b and 2c) are characterised with the time of their generation (tA, tB, tC, tD, and tE) amounting to:

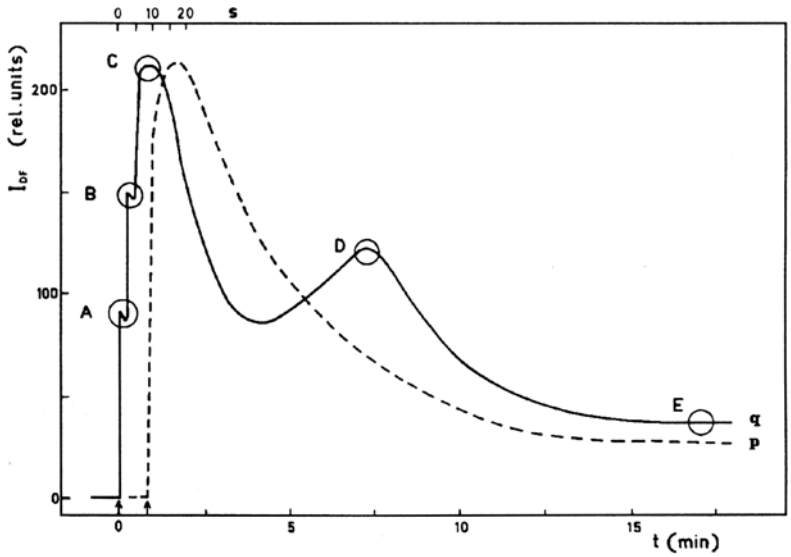


Fig. 2a — Schematic illustration of DF induction process. The curve **p** — DF induction processes registered from an intact leaf segment previously kept in the dark longer than 15 minutes (t15 min). The curve **q** — DF induction processes registered from an intact leaf segment previously kept in the dark for a significantly shorter period (t varies from 30 to 500s, with a rate of 30s) and than it is resolved transients A, B, C, D and E

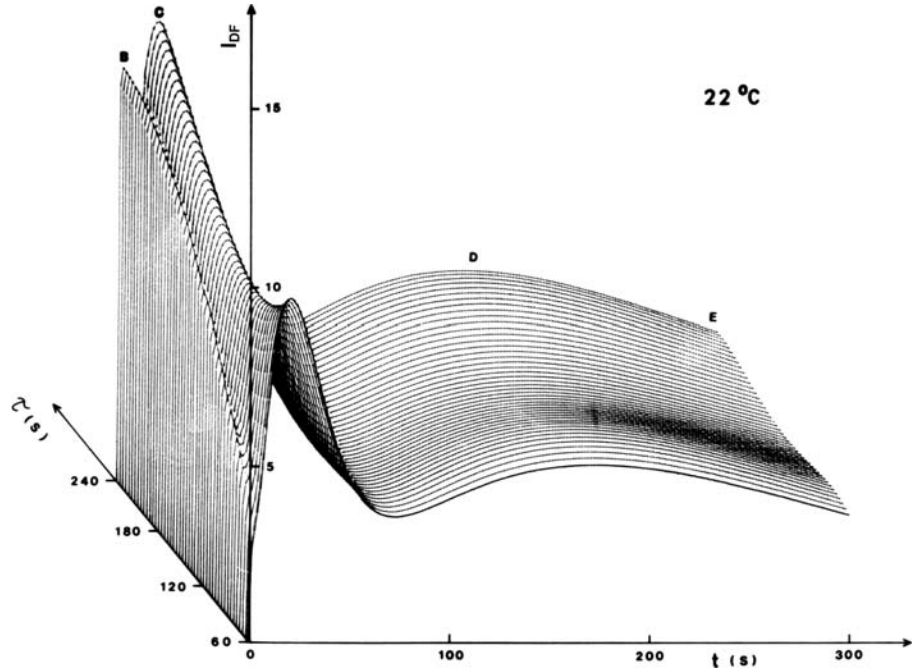


Fig. 2b — Three-dimensional plot of delayed fluorescence (DF) induction curve resolution into transients B, C, D and E at the temperature of 22°C

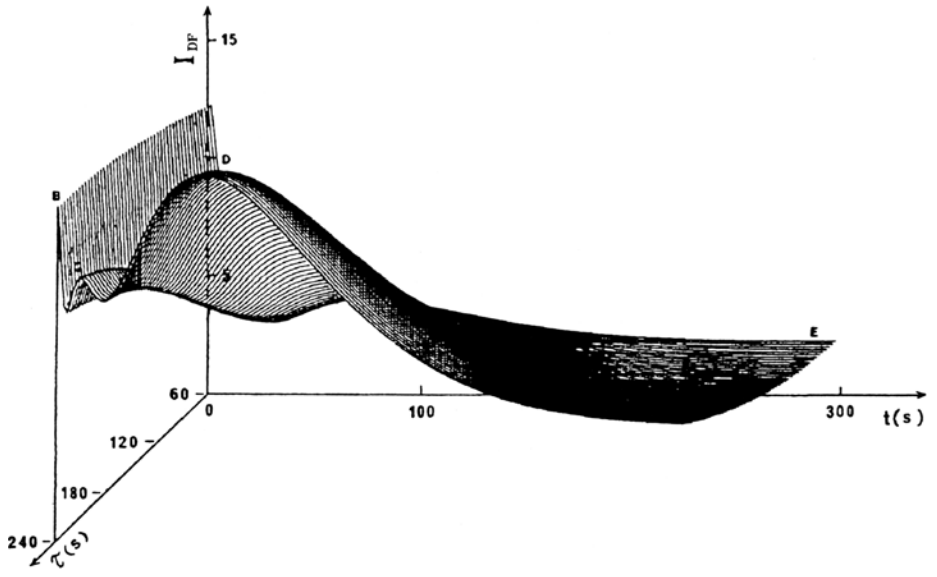


Fig. 2c — Three-dimensional plot of delayed fluorescence (DF) induction curve resolution, recorded from an intact maize leaf segment, at 32°C, following various preceding darkness periods (t). Peaks of the resolved transients are marked *B, C, D* and *E*

31.0 ± 6 ms (A), 5 ± 0.5 s (B), 15 ± 5 s (C), 300 ± 60 s (D) and 670 ± 35 s (E), continual change of transients intensity: IA, IB, IC, ID and IE, as well as, mechanisms of their generation (Radenović, 1994, 1997; Marković et al., 1999, 2001), which provide a possibility of their mathematical modelling (Kalauzi, 2006; Marković et al., 2001; Radenović et al., 2003).

THE MATHEMATICAL MODEL

Suppose that a group of identical targets, uniformly distributed over a particular surface, is being hit intermittently by flashes consisting of identical projectiles of infinitesimally small dimensions. Although relations resulting from this model may be applied on other objects, in this particular case targets represent antennas of the photosynthetic apparatus, and projectiles — potentially absorbing photons. Suppose, further, that each target may be associated with a number of discrete states, depending on the cumulative number of photons absorbed from the beginning of the intermittent regime. The following presumptions will be respected: (a) each target is hit not more than once during one flash; (b) "target history" can not be neglected, *i.e.* "target state" created by absorption of a particular number of photons is maintained throughout the process. This restriction may be overcome by deriving new equations, taking into account reversibility of target states.

Let us observe m uniformly dispersed targets, each with an area σ, within a target field with surface area S. If only one projectile approaches the field, geometric probability of a hit is:

$$p_1^1 = m\sigma/S.$$

If two projectiles are being directed towards the field, the corresponding probabilities would be:

— for both projectiles to hit the targets

$$p_2^2 = (m\sigma/S)^2;$$

— for one projectile to hit, the other to miss

$$p_1^2 = (m\sigma/S)\ (1 - m\sigma/S) + (1 - m\sigma/S)\ (m\sigma/S) = 2\ (m\sigma/S)\ (1 - m\sigma/S);$$

— for both projectiles to miss

$$p_0^2 = (1 - m\sigma/S)^2.$$

Probability for a given number of successful hits obviously obeys the binomial distribution. Therefore, for a flash containing n projectiles, probability of exactly r successful hits would be:

$$p_r^n = \binom{n}{r} (m\sigma/S)^r \ (1 - m\sigma/S)^{(n-r)}.$$

Most probable number of hits could then be calculated from the condition $p_r^n = p_{r-1}^n$, ich yields

$$r_{pmax} = [(m\sigma/S) \ (n + 1)] \cong (m\sigma/S) \ n = m \ (n\sigma/S).$$

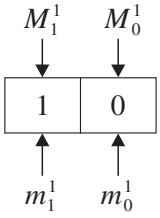
Let us observe a series of flashes, each flash consisting of n projectiles, and let us derive an expression for the most probable number of targets hit by j projectiles after k flashes. Schematically, the whole target field may be represented by a rectangle. After the k-th flash, it could be split into a series of $k + 1$ adjacent subfields (rectangles), each representing the group of targets cumulatively hit by a given number (0, 1, …, k) of projectiles. Although each subfield is drawn as a compact part of the whole field, it is presumed that subfield targets are uniformly dispersed within the field. In the centre of each rectangle, there is a numerical mark representing the cumulative number of hits received by each target in the subfield. After the k-th flash, there are two kinds of targets, cumulatively hit j times:

1) Targets, cumulatively hit j –1 times by projectiles before the k-th flash and receiving the j-th hit during the k-th flash ("newly hit targets"). Their number will be denoted with m_j^k.

2) Targets, cumulatively hit j times before the k-th flash, but missed by projectiles of the k-th flash.

Sum of the number of targets described by 1) and 2) will be denoted with M_j^k. Bearing in mind the presumption (b), concerning the conservation of target states, M_j^k represents the total number of targets cumulatively hit j times after k flashes. Schematically, it corresponds to the union of all subfields marked with number j in their centers.

According to the above defined labels, number of targets before the first flash is m_0^0. After the first flash, the following scheme appears:

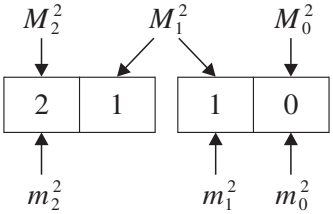


Following the presumption (a), there are only two kinds of targets — missed ones, and those hit by one projectile. Their numbers can be calculated from the following expressions:

$$m_1^1 = m_0^0 \ (n\sigma/S) \qquad M_1^1 = m_1^1$$

$$m_0^1 = m_0^0 \ (1 - n\sigma/S) \qquad M_0^1 = m_0^1.$$

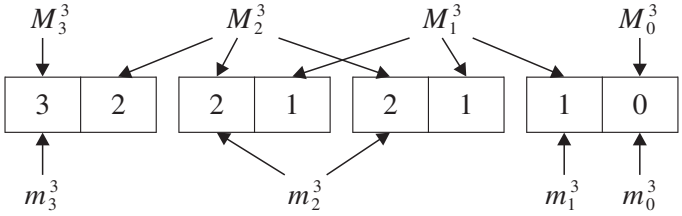
After the second flash, the same fraction of the number of targets that had previously been hit by one projectile (left subfield in the scheme above), is hit by the second projectile. Similarly, the same fraction of the number of targets previously missed (right subfield in the scheme above), is now hit by their first projectile. Therefore, each subfield from the previous scheme must be split into two new subfields, increasing the total number of subfields to four. In the new scheme (below), first subfield from the left denotes targets cumulatively hit by two projectiles, two subfields in the middle refer to targets hit by one projectile (one to targets hit during the first, the other during the second flash), while the last subfield stands for targets missed during both flashes:



The corresponding expressions are:

$$m_2^2 = M_1^1\ (n\sigma/S) \qquad M_2^2 = m_2^2$$
$$m_1^2 = M_0^1\ (n\sigma/S) \qquad M_1^2 = m_1^2 + M_1^1\ (1 - n\sigma/S)$$
$$m_0^2 = M_0^1\ (1 - n\sigma/S) \qquad M_0^2 = m_0^2.$$

After the third flash, the following situation arises:



resulting in the following relations:

$$m_3^3 = M_2^2\ (n\sigma/S) \qquad M_3^3 = m_3^3$$
$$m_2^3 = M_1^2\ (n\sigma/S) \qquad M_2^3 = m_2^3 + M_2^2\ (1 - n\sigma/S)$$
$$m_1^3 = M_0^2\ (n\sigma/S) \qquad M_1^3 = m_1^3 + M_1^2\ (1 - n\sigma/S)$$
$$m_0^3 = M_0^2\ (1 - n\sigma/S) \qquad M_0^3 = m_0^3.$$

From these few steps, general recurrent formulas can be derived:

$$(1)\quad m_j^k = M_{j-1}^{k-1}\ (n\sigma/S),\ \ j = 1, \ldots, k;$$

$$m_0^k = M_0^{k-1}\ (1 - n\sigma/S);$$

$$M_k^k = m_k^k;$$

$$(2)\quad M_j^k = m_j^k + M_j^{k-1}\ (1 - n\sigma/S),\ \ j = 1, \ldots, k-1;$$

$$M_0^k = m_0^k.$$

By sequential substitution of the corresponding quantities, one can derive explicit expressions for k = 1, 2 and 3:

$$m_1^1 = m_0^0\ (n\sigma/S) \qquad M_1^1 = m_0^0\ (n\sigma/S)$$

$$m_0^1 = m_0^0\ (1 - n\sigma/S) \qquad M_0^1 = m_0^0\ (1 - n\sigma/S)$$

$$m_2^2 = m_0^0\ (n\sigma/S)^2 \qquad M_2^2 = m_0^0\ (n\sigma/S)^2$$

$$m_1^2 = m_0^0\ (1 - n\sigma/S)\ (n\sigma/S) \qquad M_1^2 = 2m_0^0\ (1 - n\sigma/S)\ (n\sigma/S)$$

$$m_0^2 = m_0^0\ (1 - n\sigma/S)^2 \qquad M_0^2 = m_0^0\ (1 - n\sigma/S)^2$$

$$m_3^3 = m_0^0\ (n\sigma/S)^3 \qquad M_3^3 = m_0^0\ (n\sigma/S)^3$$

$$m_2^3 = 2m_0^0\ (1 - n\sigma/S)\ (n\sigma/S)^2 \qquad M_2^3 = 3m_0^0\ (1 - n\sigma/S)\ (n\sigma/S)^2$$

$$m_1^3 = m_0^0\ (1 - n\sigma/S)^2\ (n\sigma/S) \qquad M_1^3 = 3m_0^0\ (1 - n\sigma/S)^2\ (n\sigma/S)$$

$$m_0^3 = m_0^0\ (1 - n\sigma/S)^3 \qquad M_0^3 = m_0^0\ (1 - n\sigma/S)^3$$

If one observes the right column relations, an induction hypothesis can be established:

$$(3)\quad M_j^k = \binom{k}{j} m_0^0\ (n\sigma/S)^j\ (1 - n\sigma/S)^{(k-j)}.$$

Using the induction method, it is easy to prove that formula (3) is valid for every integer value of k.

Reversibility of Target States

Although the binomial distribution (3) could be derived more directly (starting from the fact that target states are independent and by calculating probabilities that a particular target received j hits from k flashes), the step-by--step tracking of target states, described above, turned out to be more suitable for model modifications. Specifically, the relations derived in that manner may be easily modified to account for reversibility of target states. The simplest

model modification would be by introducing only spontaneous transitions from a "more" to the "nearest less accumulated" state. Although these transitions probably occur during both light and dark intermittent intervals, for reasons of simplicity let us take into account only the dark transitions. Let us further denote with $(M_j^k)'$ number of targets hit by j photons during k flashes at the end of the k-th dark intermittent interval (after the reversible transitions had been completed). If the corresponding target state is denoted with (j, k), two opposite transitions occur during the dark interval:

1) From the first "higher" to the present state: $(j + 1, k) \rightarrow (j, k)$, increasing M_j^k, and

2) From the present to the first "lower" state $(j, k) \rightarrow (j - 1, k)$, decreasing M_j^k.

However, two exceptions exist: the number of targets in the "highest" state, M_k^k, may only decrease, while number of targets in the "lowest" state, M_0^k, may only increase. If the transition dynamics is exponential, so that at the end of the dark intermittent interval (T_{id} seconds long), from M_j^k targets in state (j, k) only $M_j^k \exp(-c_j T_{id})$ remain, two modified sets of recurrent equations could be written as:

$$
\begin{aligned}
m_j^k &= (M_{j-1}^{k-1})' \ (n\sigma/S), \ j = 1, \ldots, k; \\
m_0^k &= (M_0^{k-1})' \ (1 - n\sigma/S); \\
M_k^k &= m_k^k; \\
M_j^k &= m_j^k + (M_j^{k-1})' \ (1 - n\sigma/S), \ j = 1, \ldots, k-1; \\
M_0^k &= m_0^k.
\end{aligned}
$$

valid for the light and

$$
\begin{aligned}
(M_k^k)' &= M_k^k \exp(-c_k T_{id}); \\
(M_j^k)' &= M_j^k \exp(-c_j T_{id}) + M_{j+1}^k (1 - \exp(-c_{j+1} T_{id}), \ j = 1, \ldots, k-1; \\
(M_0^k)' &= M_0^k + M_1^k (1 - \exp(-c_1 T_{id}));
\end{aligned}
$$

describing the processes during the dark intermittent interval. However, unlike the initial model, complexity of the corresponding explicit relations increases considerably with k, even in case of equal transition rates: $c_k = c_k - 1 = \ldots = c_1 = c$. Therefore, in this work, we fitted the experimental data supposing that $c = 0$, leaving the derivation of explicit set of equations for $c \neq 0$ for our future work.

Model Summary

Observing formula (3), an expression relating the fraction of targets cumulatively hit by j photons during k flashes can be established:

$$(4)\quad h(k)=\frac{M_j^k}{m_0^0}=\binom{k}{j}\,(n\sigma/S)^j\,(1-n\sigma/S)^{(k-j)}.$$

Equation (4) defines a two-parameter (j, p), $p = n\sigma/S$, family of curves, presented on Figs. 3 and 4.

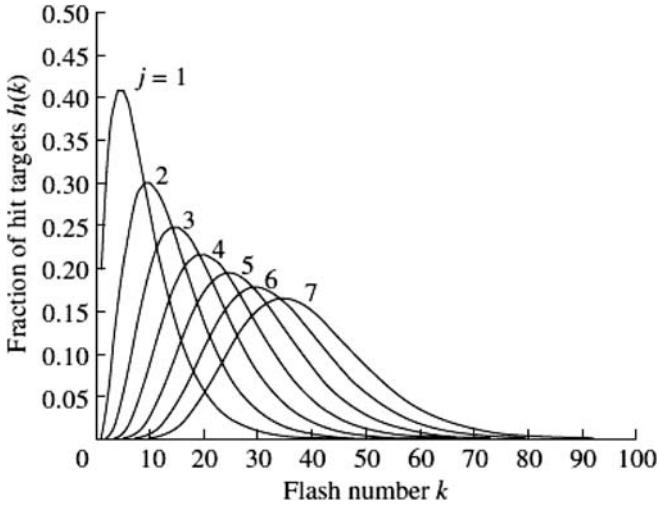


Fig. 3. — Family of curves, calculated by formula (4), showing fraction of the number of targets, $h(k)$, cumulatively hit by j = 1, …, 7 projectiles, as a function of time (*i. e.* number of flashes, k). Parameter $p = n\sigma/S$ (product of the number of projectiles in each flash, n, and relative target area, σ/S) was set to 0.2

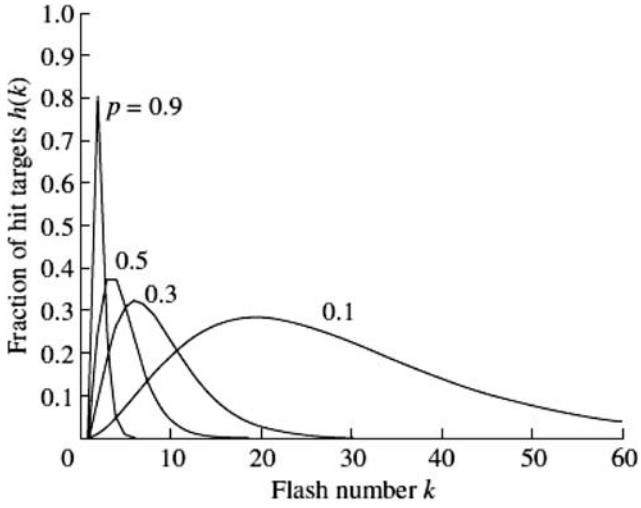


Fig. 4. — Family of curves, calculated by formula (4), showing fraction of the number of targets, $h(k)$, cumulatively hit by two projectiles (j = 2) as a function of time (*i. e.* number of flashes, k). Parameter $p = n\sigma/S$ was varied (0.1—0.9)

Abscissa variable is k, proportional to t, the illumination time of the intermittent regime in experiments with rotating disc (described in *Experimental Procedure*). These two quantities are related by the following equation: $t = (k-1)/f = (k-1)T$ [s], where f (83.3 Hz) denotes the frequency of disc opening appearances, T (12 ms) the corresponding light-dark cycle period. Ordinate variable is the fraction of targets hit by j projectiles during k flashes, $h(k) = M_j^k / m_0^0 < 1$. The curves presented in Figs 3 and 4 are products of polynomial and exponential functions. For the first few values of j, expression (4) reduces to the following functions:

$$j=1;\ \frac{M_1^k}{m_0^0} = k\,[(n\sigma/S)/(1 - n\sigma/S)]\ (1 - n\sigma/S)^k$$

$$j=2;\ \frac{M_2^k}{m_0^0} = \frac{k(k-1)}{2}\,[(n\sigma/S)/(1 - n\sigma/S)]^2\ (1 - n\sigma/S)^k$$

$$j=3;\ \frac{M_3^k}{m_0^0} = \frac{k(k-1)(k-2)}{6}\,[(n\sigma/S)/(1 - n\sigma/S)]^3\ (1 - n\sigma/S)^k$$

Since in this paper DF induction transients (DFIT) are modeled with functions defined by (3), each recorded DF induction curve, containing DFIT as components, should be related to (3) in the following manner:

$$I(k) = Sc\sum_{it=1}^{nt}(M_j^k)_{it} = Sc\sum_{it=1}^{nt}(m_0^0)_{it}\binom{k}{j_{it}}[(n\sigma/S)_{it}]^{(j_{it})}[1-(n\sigma/S)_{it}]^{(k-j_{it})},$$

where: Sc is the scaling factor, relating the number of targets hit and the ordinate value (in [mm]) of the experimentally recorded DF induction curve (depends on the experimental setup); it — index assigned to each induction transient; nt — number of induction transients. In addition, a steady state of DF induction trace, achieved after sufficient intermittent illumination time, was modeled with an exponential function: $I_s(k) = C_s(1 - \exp(-\tau_s\, k))$, where C_s represents the DF steady state level, τ_s — time constant defining the steady state dynamics. Final form of the fitting function is therefore:

$$(5)\ I(k) = Sc\sum_{it=1}^{nt}(M_j^k)_{it} = Sc\sum_{it=1}^{nt} m_{it}\binom{k}{j_{it}}[p_{it}]^{(j_{it})}[1-p_{it}]^{(k-j_{it})} + C_s(1-\exp(-\tau_s k)).$$

In (5), in case of it-th induction transient, m_{it} stands for its initial number of targets (m_0^0 of expression (3)), and p_{it} for $n\sigma/S$ of expression (3). These notations will be used throughout the **RESULTS** section. Additionally, in the same section, subscripts $it = 1$ and $it = 2$ will be substituted with conventional transient notations: C and D. Therefore, m_C, m_D, p_C, p_D, j_C and j_D will be used, rather than m_1, m_2, p_1, p_2, j_1 and j_2, as in (5).

Fitting Procedure

Each DF induction curve, within one series, was recorded from the same leaf segment after a previous dark period (t_d = 45, 60, 90, 120, 150, 180 and 240 [s]). The fitting procedure was performed applying the Nelder-Mead simplex algorithm, supplied with MATLAB for windows, Version 4.2c. A more detailed description of the procedure can be found in our previous paper (Marković et al., 2001).

RESULTS

As an example, four of the seven analyzed DF induction curves are presented on Fig. 5. After fitting the whole series of seven curves with three model types ($j_C = j_D = 1$; $j_C = j_D = 2$; $j_C = j_D = 3$), the resulting values of m_C and m_D parameters are presented on Fig. 6. The main difference between parameters m_C and m_D, as functions of the previous dark period t_d, for all three model types, was that m_C showed a tendency to increase with t_d, while m_D did not. The two parameters had similar values for short dark periods, while for t_d 200 s, m_C values were 2—3 times higher. Analogous analysis of p_C and p_D yielded

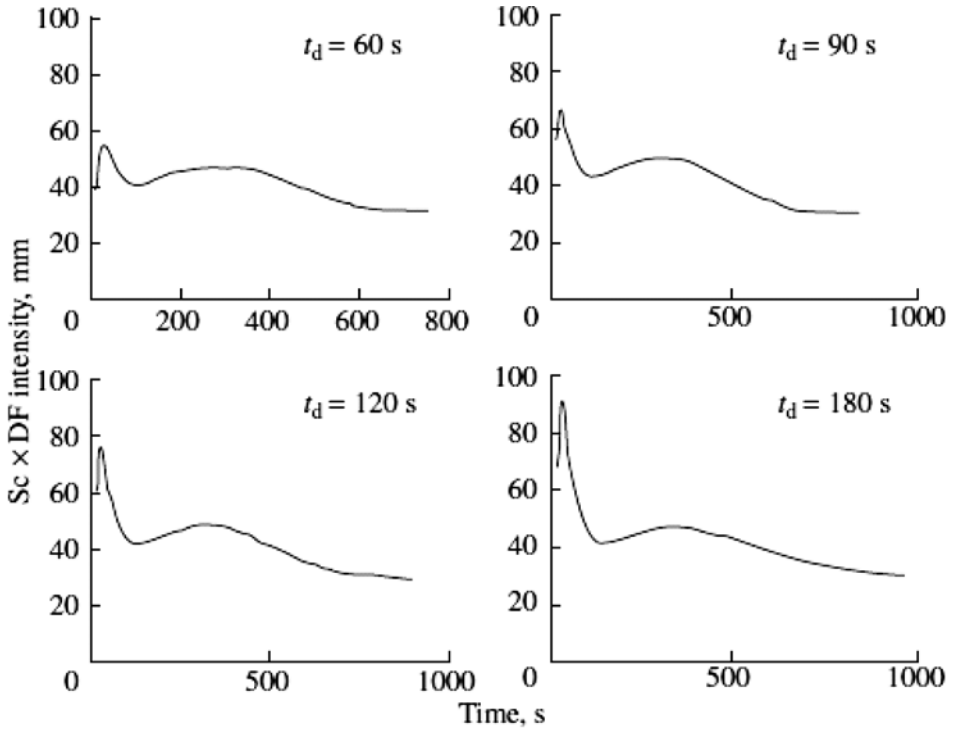


Fig. 5. — Four of the seven analyzed DF induction curves, obtained by intermittent illumination of a *Zea mays* L leaf segment (2 cm²), using a Becquerel phosphoroscope. As indicated, previous leaf dark periods were t_d = 60, 90, 120 and 180 s

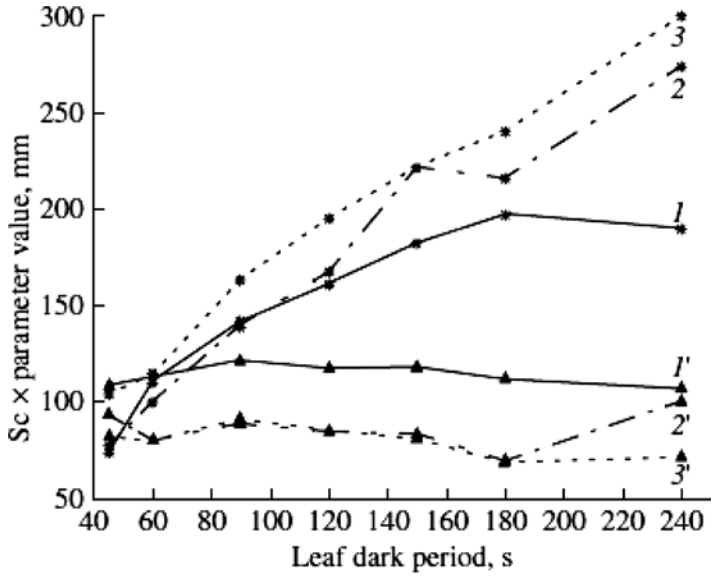


Fig. 6. — Dependence of the fitted model parameters, m_C and m_D, on the previous leaf dark period, t_d, obtained by fitting a series of DF induction curves with three model types: $j_C = j_D = 1$ (solid); $j_C = j_D = 2$ (long-short dashed); $j_C = j_D = 3$ (short dashed); point marks: (*) — m_C; (?) — m_D

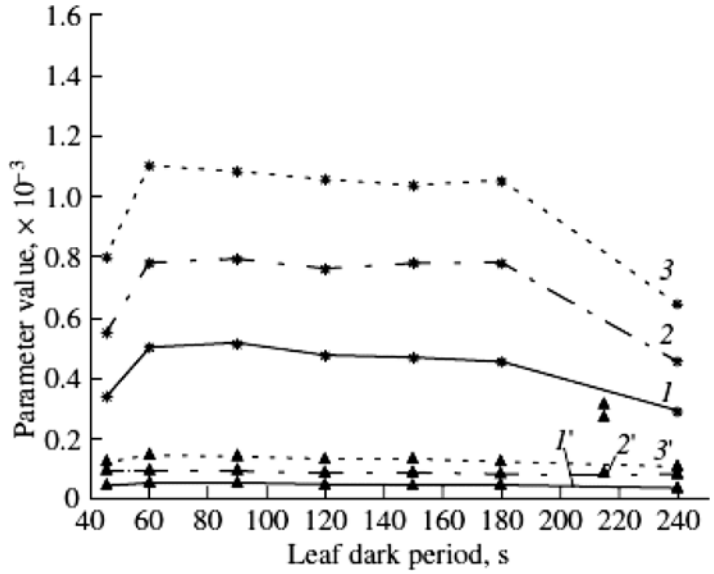


Fig. 7. — Dependence of the fitted model parameters, p_C and p_D, on the previous leaf dark period, t_d. The results were obtained by fitting the same series of DF induction curves, with the same model types, as in Fig. 4. Line style same as in Fig. 4; point marks: (*) — p_C; (?) — p_D

results shown on Fig. 7. These results show a dependence of p_C and p_D on t_d, different from m_C and m_D. Parameter p_C had no obvious linear trend with t_d as m_C had (Fig. 6), but rather an inverse "U" shape with a broad plateau, while p_D was close to a constant.

In order to obtain an answer to the question which model type is most appropriate, we compared their mean square errors:

$$E^2/N = \frac{1}{N}\sum_{ip=1}^{N}\left((DFIC)_{ip} - I(ip)\right)^2,$$

where: N is the number of experimental points for a particular DF induction curve (DFIC); $(DFIC)_{ip}$ — value of the experimental curve in point ip; $I(ip)$ — value of the fitted model line, according to (5), in the same point.

Six model types: j_C, j_D = 1,1; 1,2; 1,3; 2,2; 2,3; 3,3 were tested. Each of the seven experimental DF induction curves (t_d = 45, 60, 90, 120, 150, 180, 240) was fitted with every one of the six models. For each model type, all seven mean square fitting errors were averaged, and the result presented on Fig. 8. As shown, the smallest fitting error was obtained when both (*C* and *D*) DF transients had been modeled as targets cumulatively hit by two photons after *k* intermittent light flashes.

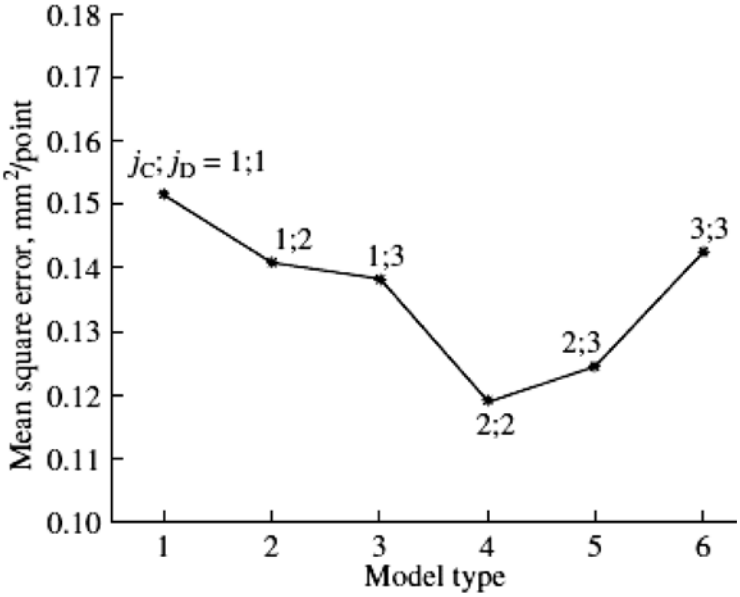


Fig. 8. — Comparison of fitting efficiency for different model types, according to their mean square errors. Each of the seven experimental DF induction curves was fitted with six model types and the fitting errors were averaged for each model type. The most accurate model was $j_C = j_D = 2$.

DISCUSSION

Induction kinetics of delayed fluorescence is dependent on the used photosynthetic object, as well as on the applied experimental method, and the same stands for very rare attempts of the transients modeling. For example, Goltsev and Yordanov (1997) recorded simultaneously prompt and delayed fluorescence emission from few photosynthetic objects, using a phosphoroscope disc, but with much shorter registration and dark periods. Their modeling was related to particular electron-transport steps involved in the mechanism of DF emission (primarily located in PSII), but antennas role was not considered. In a recently published paper, Goltsev et al. (2003) extended their research by analyzing prompt/delayed fluorescence relationship from barley-wild type and chlorophyll b-less mutant chlorina f2, but antennas role were interpreted in an indirect manner.

Although DF emission is a minor probability dissipation event, once the photons are absorbed inside antennas (0.03% of total absorbed energy (Jursinic et al., 1982)), delayed fluorescence is the only registered emission with the described experimental setup. In our case, a typical DF induction curve may be split into at least three components (Marković et al., 2001). In this work, similarity of shapes between theoretical (Figs. 3 and 4) and experimental DF induction curves (Fig. 5) was used as a starting point to relate two distinct DF transients (*C* and *D*) with subpopulations of targets, hit by a particular number of photons. This was achieved by means of theoretically introduced model parameters m_C, m_D, p_C and p_D (equations (4) and (5)). Since the shape of the DF induction signal is dependent on the preceding dark period t_d (Radenović et al., 1985, 1994; Radenović, 1994, 1997), and bearing in mind that DF induction transients are ECG controlled (Marković et al., 1999), investigation of a possible relationship between ECG induced structural changes and dependence of the model parameters m_C, m_D, p_C and p_D, shown on Figs. 6 and 7, appears as a challenge in future research. It was shown already that variation of t_d induces ECG controlled structural changes, before any temperature induced ones (Marković et al., 1999). Beside, in our previous report we showed that the two transients originate from different ECG controlled states (Marković et al., 2001). On the other hand, from Figs. 6 and 7 it is obvious that parameters m_C and p_C exhibit a clearly distinct behavior from parameters m_D and p_D. As stated in the INTRODUCTION, bearing in mind the physical interpretation of the *p* parameters ($p = n\sigma/S$; product of *n*, number of absorbable photons in one flash and the ratio of target area σ, over leaf area *S*), one should expect a smaller dependence of p_C and p_D on t_d, than m_C and m_D, which represent the number of targets (antennas of the photosynthetic apparatus). Really, p_D was very weakly dependent on t_d (Fig. 7), while p_C was characterized by a very broad plateau in the middle range of t_d values (60—180 s). The important result was that no obvious quasilinear trend was observed for p_C or p_D, as was for m_C on Fig. 6. However, the detected decrease of p_C for the smallest and biggest values of t_d, if confirmed, still remains to be explained. Since the leaf segment area *S* was an experimental constant, the only quantity responsible for any dependence of p_C or p_D on t_d, could

have been $n\sigma$ — product of the number of absorbable photons in a single flash, n, and the target area, σ. It is still an open question, though, whether based on these results solely, we are qualified to speak about two subpopulations of targets. Obviously, additional analyses are required in order to acquire reliable answers to these questions. But if this hypothesis would be confirmed, targets associated with transient *C* would have an order of magnitude greater value of the product $n\sigma$, than those associated with transient *D* (although it would still remain unclear whether this could be transferred to their areas: $p_C/p_D \rightarrow \sigma_C/\sigma_D$). As well, it would be interesting to check whether different dependences of their DF-emitting numbers on t_d (m_C showed a quasilinear increase while m_D did not, Fig. 6.), would also be confirmed.

Two types of photosynthetic antennas, dealing with DF, have already been described. They were related to the intensity of the incident light (I_L), depending on the light regime by which a photosynthetic object was illuminated. Two types of DF dependences on light intensity (I_L) have been found: square dependence (proportional to $(I_L)^2$), at lower I_L values, and linear dependence, at higher light intensities (McCauley et al., 1981). The $(I_L)^2$ dependence was obtained using a phosphoroscope (millisecond light/dark regime), while the linear dependence has been observed with microsecond and submicrosecond excitation flashes. Square dependence of DF intensity, for low I_L values, could also be associated with our model. Namely, since light intensity is proportional to n, number of absorbable photons contained in one light flash, let us transform expression (4) into the following form:

$$h(k) = \chi(n) = \frac{M_j^k}{m_0^0} = \binom{k}{j} [(n\sigma/S)/(1 - n\sigma/S)]^j \ (1 - n\sigma/S)^k.$$

For $j = 2$, and for sufficiently small values of n, since $n\sigma/S << 1$, this expression reduces to

$$\chi(n) = \frac{M_2^k}{m_0^0} \approx \binom{k}{2} (n\sigma/S)^2 = \text{Const} * (n^2),$$

which theoretically states that, in case of model type $j = 2$ (DF emission after two photon hits), intensity is directly proportional to the square of the number of photons in one light flash (light intensity). This expression additionally points to the absorption of second photon, as a possible event evoking DF, and is in accordance with the results of the error analysis, presented on Fig. 8.

Under present experimental conditions, large area antennas should have a higher hitting probability. However, since the existence of smaller antennas could not be neglected (McCauley et al., 1981), their participation and influence on the DF induction signal should not be excluded. Since bigger antennas absorb more efficiently, one could expect the transient *C* to appear earlier than transient *D*, associated, according to our model, with smaller antennas, which was experimentally verified.

Accuracy of any model is limited, among other factors, on the choice of its initial assumptions. In case of the presented model, whole procedure and consequent conclusions are based on somewhat restrictive presumptions (a) and (b). It is of interest, therefore, to suggest corresponding potential generalizations of this model. Namely, if one flash would consist of so many photons that the number of targets hit with two or more projectiles could not be neglected, then the flash itself should be treated as a series of successive, shorter sub-flashes, each of them respecting the condition (a). On the other hand, if the number of targets, with reversible history between two flashes, could not be neglected, present recurrent formulas should be modified to account for these "inter-flash state changes", by introducing expressions like $m_j^k = f(M_{j-1}^{k-1})$ (one simple case of this modification was described in the subsection *Reversibility of Target States*). Future introduction of these more sophisticated models will hopefully contribute to a better understanding of already known experimental facts.

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ДИНАМИКА НАСТАЈАЊА ТРАНЗИЈЕНАТА ИНДУКЦИОНИХ ПРОЦЕСА ЗАКАСНЕЛЕ ФЛОУРЕСЦЕНЦИЈЕ ХЛОРОФИЛА И ФОТОСИНТЕТИЧКИХ АНТЕНА: МОГУЋА ЗАВИСНОСТ. ПРИСТУП МАТЕМАТИЧКОГ МОДЕЛОВАЊА

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Резиме

Овај рад чине два међусобно повезана дела. У првом делу излажу се експериментални резултати сложених индукционих процеса закаснеле флуоресценције хлорофила и фотосинтетичких антена интактног сегмента листа инбред линије ZP R70ž и Oh43 и хибрида кукуруза ZPSC 46A. Проучавано је и откривено разлагање индукационих процеса на пет транзијената (кинетички облици промена) који су означени као A, B, C, D и E. Њих карактеришу времена настајања: t_A, t_B, t_C, t_D и t_E која износе: 31,0 ± 6 ms (A), 5 ± 0,5 s (B), 15 ± 5 s (C), 300 ± 60 s (D) и 670 ± 35 s (E), континуелне промене интензитета транзијената: I_A, I_B, I_C, I_D и I_E као и механизми њиховог настајања. Наведени резултати о карактеристикама транзијената индукционих процеса закаснеле флоуресценције хлорофила и фотосинтетичких антена била су добра основа за посебан приступ њиховог математичког моделовања.

У другом делу развијен је математички модел временског разлагања транзијената експериментално регистрованих индукционих сигнала закаснеле флуоресценције (ЗФ). За време интермитентног светлосног режима антене фотосинтетског апарата су третиране као мета и гађане понављајућим погоцима апсорбујућих фотона унутар серија узастопних светлосних снопова. Формула је изведена за број антена, кумулативно гађаних одређеним бројем фотона. Параметри модела укључују: број апсорбованих фотона у сваком снопу, величину и број антена. Анализиране су серије индукционих кривих, добијених из листа кукуруза, које су се претходно разликовале у тамној фази (t_D). Свака крива, која садржи два изабрана транзијента C и D индукционих процеса закаснеле флуоресценције, је оптимизована са неколико типова модела, који се разликују по броју апсорбованих фотона. За оба транзијента најоптималнији резултати су постигнути када су индукциони процеси закаснеле флоуресценције били повезани са другим апсорбованим фотоном. Као што се очекивало, параметри модела указују да величина антена показује слабију зависност t_D у односу на број антена. Уз ограничења овог модела, два транзијента индукционих процеса закаснеле флуоресценције могу се повезати са две класе фотосинтетских антена. Њихове разлике у величини могу да имају преовлађујући утицај на ефикасност апсорпције фотона и могућу временску зависност појаве транзијената индукционих процеса закаснеле флуоресценције.

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INHERITANCE OF PLANT HEIGHT, SPIKE LENGTH AND NUMBER OF SPIKELETS PER SPIKE IN *DURUM* WHEAT

ABSTRACT: Using the line x tester analysis we studied the combining ability and gene effects of plant height, spike length and number of spikelets per spike in *durum* wheat. The results of the study show that non-additive genes play more important role than additive genes in the inheritance of plant height, number of spikelets per spike in both years and in inheritance of spike length only in the first year of research. Variety Belfugito, the best general combiner for plant height and number of spikelets per spike, combined well in two best hybrids: Belfugito x Alifen and Belfugito x Yavaros 79, and these hybrids may be used in wheat breeding programs. In the majority of the cases, good specific combining ability (SCA) effects were associated with crosses of two genetically divergent parents having at least one parent as a good general combiner.

KEY WORDS: combining ability, gene effects, *durum* wheat, yield components

INTRODUCTION

The choice of parents is a very important task in a breeding program. Combining ability studies are used by plant breeders to select parents with maximum potential of transmitting desirable genes to the progenies. In autogamous crops like wheat, where the ultimate aim is to develop pure line varieties, the estimates of general combining ability (GCA) are very useful because the variance due to general combining ability is attributable to additive gene action and A x A interaction which can be fixed in further generations, while the variance due to specific combining ability is attributable to non-additive gene action. The gene effects and combining ability of yield components were already studied by a number of scientists using diallel analysis (Knežević and Kraljević-Balalić, 1993; Menon and Sharma, 1994; Menon and Sharma, 1995; Perović, 1995; Petrović et al., 1995; Joshi et al., 2002).

This study was therefore, undertaken to obtain information regarding the combining ability and gene effects of plant height, spike length and number of spikelets per spike in *durum* wheat using line x tester analysis.

MATERIALS AND METHODS

Five *durum* wheat (*Triticum turgidum durum*) genotypes: Mexicali 75 (MEX), Yantar odeskij (UKR), Belfugito (ITA), Monodur (FRA) and Kunduru (TUR) were crossed with each of the three testers: Durumko (SCG), Yavaros 79 (MEX) and Alifen (CHL). The parent varieties and their F_1 hybrids were examined in randomized block design, with three replications. All parents were selected on the basis of different phenotypic expression and geographic origin.

The experiment was conducted at the experiment field of the Institute of Field and Vegetable Crops, Novi Sad, during 2000—2002. Sowing was done in the beginning of the October, in 1.2 m^2 plot, with a 10—12 cm space inside the row, and a 20 cm space between rows. Three traits were studied at full maturity: plant height, spike length and number of spikelets per spike. All traits were determined in 5 plants per replication. The combining ability and gene effects were studied using GEN software package (Program for quantitative genetic analysis) — line x tester analysis, described by Sing and Choudhary (1979).

RESULTS

The analysis of variance for plant height, spike length and number of spikelets per spike showed highly significant differences amongst genotypes in both years. The genotype x environment interaction was also highly significant in both years of investigation.

The analysis of variance for line x tester for spike length indicated that significant differences existed between parents (both years), interaction parents vs. crosses (both years), crosses (second year), lines (second year), testers (second year) and interaction line x tester (first year). Analysis of variance for plant height showed that significant differences existed between parents (first year), parents vs. crosses (both years), crosses (first year), lines (both years), testers (first year) and interaction line x tester (both years). For number of spikelets per spike significant differences existed between parents (second year), parents vs. crosses (second year), crosses and lines (both years), testers (first year) and line x tester (both years) (Table 1).

The estimation of the genetic components of variation, as well as the ratio of GCA/SCA showed that the additive component was lower than the dominance component which suggests that, in both years of the investigation, plant height and number of spikelets per spike were predominantly controlled by non-additive gene action. Spike length was predominantly controlled by non-additive gene action in the first year, while in the second year spike length was controlled mostly by additive genes (Table 1).

Tab. 1. — ANOVA line x tester for yield components in *durum* wheat

Source of variation	DF	Mean squares					
		Plant height		Spike length		Number of spikelets	
		2001	2002	2001	2002	2001	2002
Replication	2	17.62	3.39	0.13	0.03	0.07	1.20
Treatments	22	877.94**	437.66**	0.81**	0.53**	8.63**	4.65**
Parents	7	1144.26**	459.25	1.47**	0.43**	7.01	6.49**
P vs. C	1	1360.21**	722.15**	2.23**	0.54**	–0.01	2.43*
Crosses	14	710.33**	406.55	0.38	0.57**	10.06**	3.88*
Lines	4	889.44**	727.14*	0.66	0.50**	22.44**	9.73**
Testers	2	2641.24**	440.23	0.05	0.67**	12.45*	0.15
L x T	8	138.05**	237.83**	0.33**	0.08	3.27**	1.89**
Error	44	7.86	4.77	0.10	0.07	0.38	0.58
Total	68						

Components of genetic variance						
	Plant height		Spike length		Number of spikelets	
	2001	2002	2001	2002	2001	2002
GSA	20.23	5,97	0.002	0.017	0.24	0.07
SCA	43.39	77.69	0.075	0.005	0.96	0.44
GCA/SCA	0.47	0.08	0.027	3.40	0.25	0.16

* $p < 0.05$; ** $p < 0.01$

The estimates of general combining ability pointed out that the best general combiner for plant height in the first year was Mexicali 75, while in the second year it was Belfugito (Figure 1). For spike length the best combiner in the first year was Yantar odeskij, while in the second year it was Mexicali 75 (Figure 2). For number of spikelets per spike the best combiners were Belfugito, in the first year, and Kunduru in the second year of research (Figure 3).

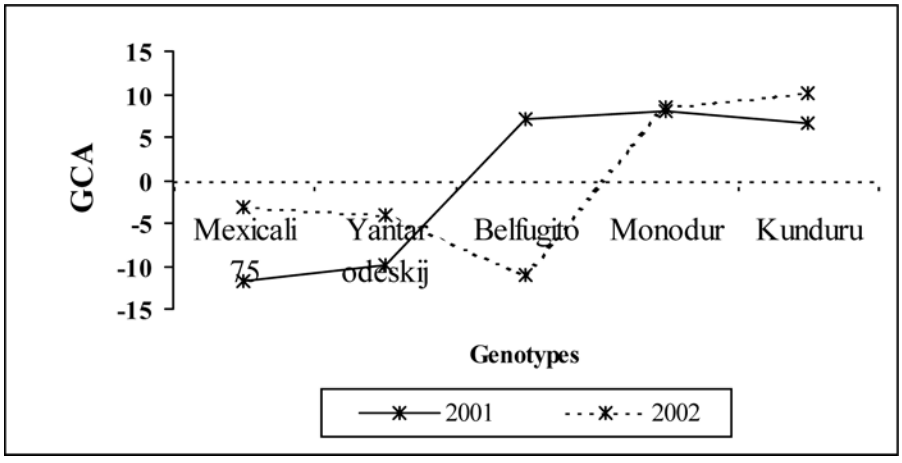

Fig. 1. — GCA for plant height in *durum* wheat

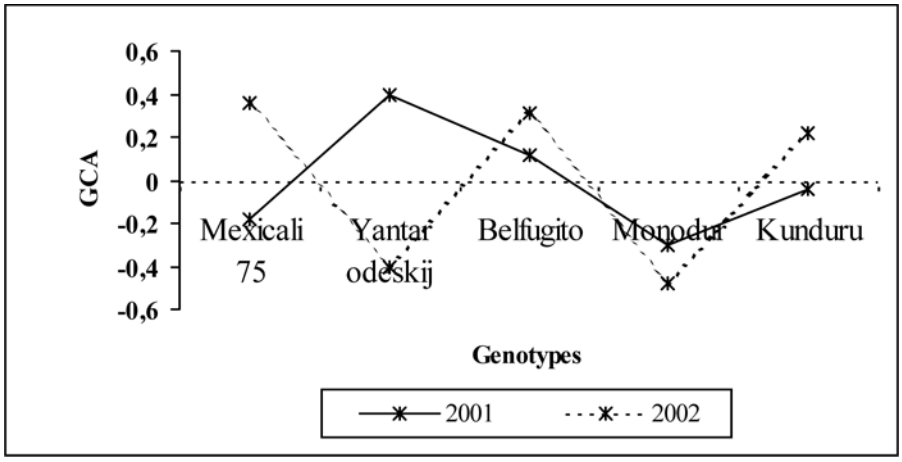

Fig. 2. — GCA for spike length in *durum* wheat

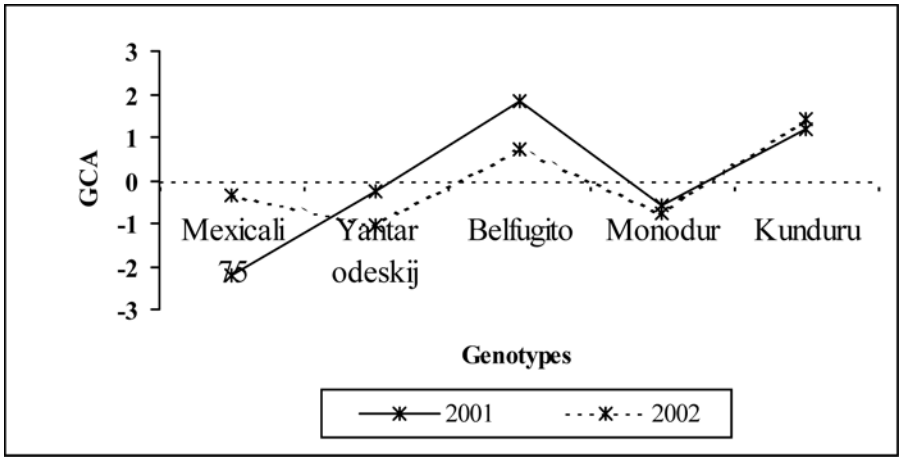

Fig. 3. — GCA for number of spikelets per spike in *durum* wheat

The hybrid which showed significant positive SCA for spike length in the first year was Belfugito x Yavaros 79, while in the second year there was no significant SCA. For plant height, the best specific combiner in the first year was Kunduru x Alifen, while in the second year it was Belfugito x Alifen. In case of number of spikelets per spike the best specific combiners were Mexicali 75 x Yavaros 79 in the first year, and Monodur x Alifen and Belfugito x Yavaros 79 in the second year of research (Table 2).

Tab. 2. — Specific combining ability for yield components in wheat

Hybrid	Plant height		Spike length		Number of spikelets	
	2001	2002	2001	2002	2001	2002
1. Mexicali 75 / Durumko	–5.04*	–8.59*	–0.07	0.12	–0.19	–0.49
2. Mexicali 75 / Yavaros 79	3.26*	4.97*	0.26	0.03	1.10*	0.48
3. Mexicali 75 / Alifen	1.78	3.62*	–0.19	–0.15	–0.91*	0.00
4. Yantar odeskij / Durumko	–5.03*	–2.94*	0.22	0.08	0.79*	0.75
5. Yantar odeskij / Yavaros 79	–0.33	–6.00*	–0.08	–0.13	0.01	–0.55
6. Yantar odeskij / Alifen	5.37*	8.94*	–0.13	0.05	–0.80*	–0.20
7. Belfugito / Durumko	–1.91	6.58*	–0.34	–0.14	–0.60	0.24
8. Belfugito / Yavaros 79	–2.43	8.42*	0.40*	0.19	0.86*	0.86
9. Belfugito / Alifen	4.35*	–15.00*	–0.06	–0.05	–0.26	–1.10*
10. Monodur / Durumko	2.01	2.82*	–0.12	0.12	–0.28	–0.12
11. Monodur / Yavaros 79	–2.16	–5.85*	–0.22	–0.18	–0.66	–0.75
12. Monodur / Alifen	0.14	3.03*	0.34	0.06	0.94*	0.87*
13. Kunduru / Durumko	9.97*	2.13	0.31	–0.18	0.28	–0.38
14. Kunduru / Yavaros 79	1.67	–1.54	–0.36	0.09	–1.30*	–0.05
15. Kunduru / Alifen	–11.63*	–0.59	0.05	0.09	1.03*	0.42
S.E. (PKS)	1.62	1.26	0.18	0.15	0.36	0.44

* $p < 0.05$

DISCUSSION

The estimation of genetic components of variation showed that non-additive gene effects were predominant in inheritance of plant height and number of spikelets per spike. Similar results were obtained by Sing et al. (1984), Menon and Sharma (1994), Petrović et al. (1995). However, some authors (Kraljević-Balalić and Dimitrijević, 1992; Knežević et al., 1995; Joshi et al., 2002; Sharma et al., 2002) reported that those traits were affected mainly by additive gene action. Spike length was predominantly controlled by non-additive gene actions in the first year, which is in agreement with studies of Srivastava et al. (1981) and Sharma et al. (2003). In the second year of research the spike length was predominantly controlled by additive gene action. Similar results were obtained by Mihaljev and Kraljević-Balalić (1981) and Joshi et al. (2002).

The best general combiners with maximum number of favorable alleles for traits under study are: Mexicali 75 and Belfugito (for plant height), Yantar odeskij and Mexicali 75 (for spike length) and Belfugito and Kunduru (for number of spikelets per spike). These genotypes may be exploited in the crossing programs in obtaining superior segregants.

Variety Belfugito, the best general combiner for plant height and number of spikelets per spike combined well in two best hybrids: Belfugito x Alifen and Belfugito x Yavaros. Therefore, suitable segregates may be expected from these cross combinations. In majority of the crosses positive SCA effect were associated with crosses of two genetically divergent parents having at least one parent as a good general combiner, which is in agreement with studies of

Kraljević-Balalić and Borojević (1985), or two poor general combiners. The crosses involving high x low and low x low combiners genetic interaction might be additive x dominance and dominance x dominance type in nature, respectively. Therefore, the heterosis observed in these crosses will be not-fixable and possibility of good segregants will be rare (Sing et al., 1980). The combinations of two good general combiners not showing positive SCA may be due to the fact that parents were not diverse, while in those crosses with high SCA involving high x high general combiners, the genetic interaction might be additive x additive, which is fixable in further generations and can be used in wheat breeding.

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НАСЛЕЂИВАЊЕ ВИСИНЕ СТАБЉИКЕ, ДУЖИНЕ КЛАСА И БРОЈА КЛАСИЋА ПО КЛАСУ КОД *DURUM* ПШЕНИЦЕ

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Резиме

У раду су помоћу линија х тестер анализе испитиване комбинационе способности и ефекти гена за висину стабљике, дужину класа и број класића по класу код *durum* пшенице, користећи пет линија, три тестера и њихове хибриде.

Резултати испитивања показују да су неадитивни гени имали већи значај у наслеђивању висине стабљике и броја класића по класу у обе године истраживања, док су у наслеђивању дужине класа имали већи значај само у првој години истраживања. Најбољи општи комбинатори за висину стабљике били су генотипови Mexicali 75 и Belfugito. За дужину класа најбоље опште комбинационе способности имали су Yantar odeskij и Mexicali 75, док су за број класића по класу најбољи општи комбинатори били Belfugito и Kunduru. Сорта Belfugito, најбољи општи комбинатор за висину стабљике и број класића по класу, дала је два хибрида са најбољим посебним комбинационим способностима (Belfugito x Alifen и Belfugito x Yavaros 79), који се као такви препоручују за даљи рад на оплемењивању пшенице. У већини случајева хибриди са добрим посебним комбинационим способностима настали су укрштањем два различита родитеља од којих је бар један био добар општи комбинатор.

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CHARACTERIZATION OF BEAN VARIETIES ON THE BASIS OF PROTEIN MARKERS

ABSTRACT: The biochemical marker phaseolin and isozymes were used in this work to display the variation of common bean germ plasm. Fifteen bean genotypes of different origin i. e. selections were studied. From 8 analyzed enzymic systems, enzymes MDH, SKDH, ME and IDH were polymorphic, while there were no differences in zymograms for enzymes PGM, PHI, PGD, and ADH. Analysis of phaseolin revealed two types: S and T. The S type of phaseolin was found in most of analyzed genotypes (9). Phaseolin type T was found in varieties of Novi Sad selection: Zlatko, Sremac and Aster, domestic population Žuto-zeleni Stepanovićevo and Jovandeka, Croatian variety Slavonski žuto-zeleni. Those varieties were developed from domestic populations from north-west region of Balkan, Slavonia, and Vojvodina.

KEY WORDS: common bean, germ plasm, phaseolin, isozymes

INTRODUCTION

Bean is an unavoidable food component in diets of people living in many Balkan countries, and elsewhere in the world. It is main source of protein and energy, and is gaining importance in human diet.

Origin of bean (*Phaseolus vulgaris*) is America. It was brought to Europe from Central America during second Columbus voyage. It was brought to Balkan from two directions: from Turkey — south, and From France and Italy — north. Crossing of main trade ways, soil and climatic conditions, and other differences led to great divergence of bean in our surroundings (Vasić, 2004). Domestic populations of tall, climbing beans, of short bean and introduced American varieties with shrubby straight stem, and small, white with round grains prevailed earlier. Today, mostly domesticated populations and modern, bred bean varieties are grown (Vasić et al., 2001).

It is thought that domestication of bean was done in two regions, one is Central America, and second is the area of Andes in South America (Gepts et al., 1986). It is still unclear if there was small centre in Columbia around that region, where transfer of genes from wild relatives to domestic varieties was done (Beebe et al., 1997).

Proofs supporting diversity of two centers of origin come from study of variability of grain size (Evans, 1973), phaseolin (Gepts et al., 1986), morphology (Singh et al., 1991), isozyme (Koenig and Gepts, 1989, Singh et al., 1991a), and DNK markers (Haley et al., 1993).

Storage protein phaseolin and isozymes

From total protein content in bean 50 and 75% are globulins (Alli et al., 1994). There are two types of protein inside this group, the dominating one — phaseolin, and lectin or phytohemagglutinin (Staswik et al., 1986). Phaseolin the main reserve bean protein is soluble in high salt concentration. It contains from 35 to 50% of total nitrogen in seed (Ma and Bliss, 1978, Lioi, 1989). Phaseolin is coded with loci complex from 6 to 9 genes. Alleles coding polypeptides of each phaseolin type are co-dominant. Reserve proteins are reliable markers in studies of domestication and dispersion of bean varieties, and in analysis of phytogene relationship between species inside *Phaseolus* genus. In comparison with *Phaseolus vulgaris* L., bean and string bean, other species of this genus have not been studied enough in the context of molecular characterization.

Bean as a self-pollinated plant species presents an excellent material for isoenzymic fingerprint. Low level of heterozygosity makes it possible for each species to be characterized with one or two isozymic profiles (Weeden, 1984).

The aim of this work was to evaluate 15 bean varieties, using phaseolin seed protein and isozymes analysis, the genetic variability as well as to relate their origin to the Mesoamerican and Andrean gene pools. The results may contribute to improvement of germ plasm bank management and may improve the efficiency of the breeding process.

MATERIAL AND METHODS

Fifteen bean genotypes of different origin i. e. selections were studied in this paper. Eight varieties of Department of vegetables, Research institute of field and vegetable crops (IFVC), Novi Sad: Zlatko, Sremac, Balkan, Belko, Dvadesetica, Levač, Maksa and Aster, domestic population: Greenish-yellow Stepanovićevo and Jovandeka, Bulgarian varieties Prelom and Ludogorje, variety Medijana from Smederevska Palanka, American variety C-20, and Slavonski žuto zeleni from Croatia.

Stem tissues of 5 days old seedling homogenized in 50mMTrisHCl, pH 6.8 in which 1% mercaptoethanol was added, was used for isozymic analysis.

Isozyme systems: malate dehydrogenase (MDH), malic enzyme (ME), phosphohexose isomerase (PHI), phosphogluconate dehydrogenase (PGD), phosphoglucomutase (PGM), shikimate dehydrogenase (SKDH), isocitrate dehydrogenase (IDH), alcohol dehydrogenase (ADH) were analyzed according to Stuber et al. (1988).

Preparation of samples and 1D-SDS PAGE electrophoresis of phaseolin were done according to Rodino et al. (2001). Four individual seeds were tested from each samples.

RESULTS

From 8 analyzed enzymic systems, enzymes MDH, SKDH, ME and IDH were polymorphic, while there were no differences in zymograms for enzymes PGM, PHI, PGD, and ADH (Fig 1). Genotypes Jovandeka and Aster had faster traveling variant of malic enzyme and malate dehydrogenase, while rest of them had slow traveling allelic variants (Fig. 2a and b). Three different allelic variants were found for enzyme shikimate dehydrogenase (Scheme 1) and two for locus *Idh1* isocitrate dehydrogenase.

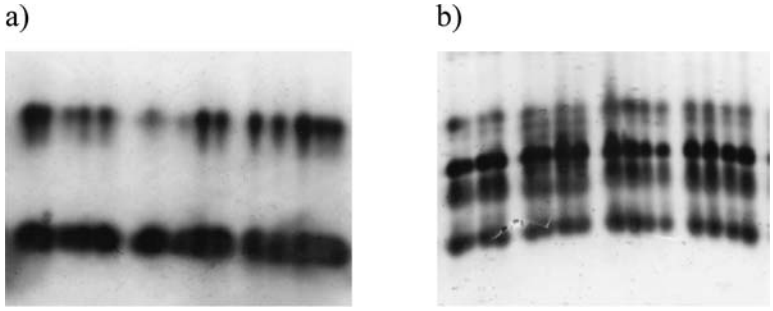


Fig. 1. — Zymogram pattern of PGM (a) and PHI (b) bean genotypes

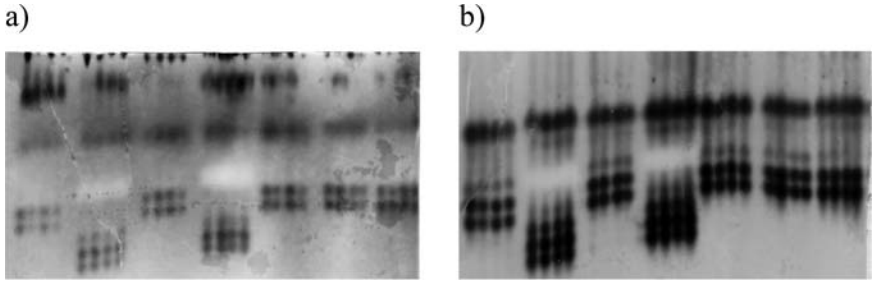
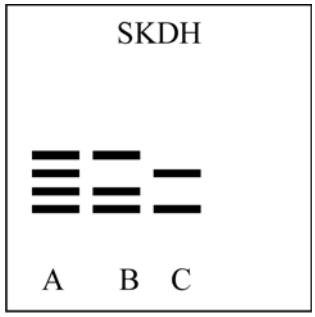


Fig. 2. ME (a) and MDH (b.) zymograms of bean genotypes from the left to the right: C-20, Aster, Ludogorje, Jovandeka, Prelom, Medijana, Greenish-yellow Stepanovićevo



Scheme 1. — Presentation of SKDH zymogram pattern of bean genotypes

Analysis of phaseolin revealed two types: S and T. S type of phaseolin was found in most of analyzed genotypes (9 from 14) (Tab. 1). Phaseolin type T is found in varieties of Novi Sad selection: Zlatko, Sremac and Aster, domestic population greenish-yellow Stepanovićevo and Jovandeka, Croatian variety Slavonski žuto-zeleni (Fig. 3).

Tab. 1. — Bean varieties, origin, type of phaseolin and isozymic variants

Bean variety	Origin	Type of phaseolin	MDH	ME	SKDH	IDH
1. Zlatko	IFVC	T	S	S	B	F
2. Sremac	IFVC	T	S	S	A	F
3. Balkan	IFVC	S	S	S	C	F
4. Belko	IFVC	S	S	S	C	F
5. Dvadesetica	IFVC	S	S	S	B	F
6. Levač	IFVC	S	S	S	C	F
7. Maksa	IFVC	S	S	S	C	F
8. Greenish-yellow Stepanovićevo	domestic population	T	S	S	A	S
9. Medijana	S. Palanka	S	S	S	C	F
10. Prelom	Bulgaria	S	S	S	C	F
11. Jovandeka	domestic population	T	F	F	B	S
12. Ludogorje	Bulgaria	S	S	S	C	F
13. Aster	IFVC	T	F	F	B	S
14. C-20	USA	S	S	S	C	F
15. Slavonski žuto-zeleni	Croatia	T	S	S	B	F

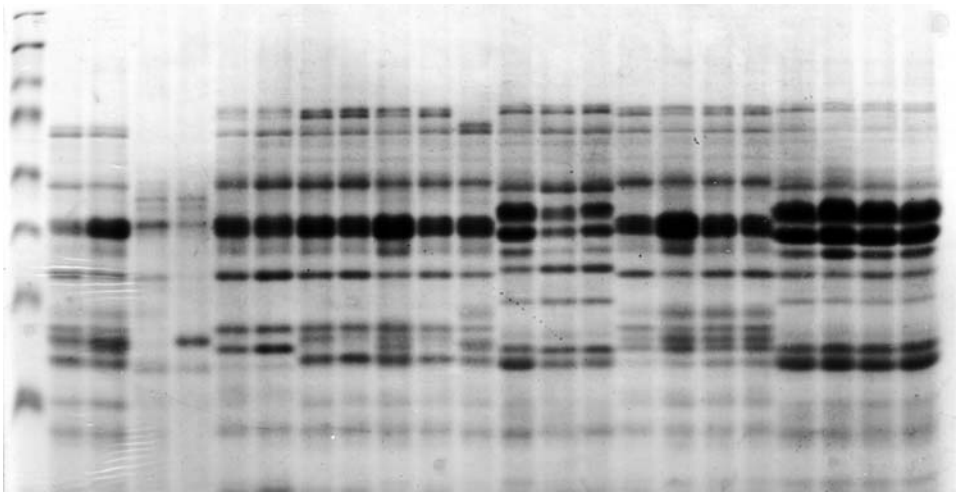


Fig. 3. — Different types of phaseolin obtained by SDS PAGE electrophoresis: 1. protein marker (170-11 kDa), 2,3 control S type phaseolin, 4,5 control C type, 6,7 control S, 8—11 C-20, 12—15 Aster, 16—19 Ludogorje, 20—23 Jovandeka

DISCCUSSION

The variability at the protein level has been well documented for *P. vulgaris* (Weeden 1984, Koening and Gepts, 1989). Isozyme analysis (Koening and Gepts, 1989) and the analysis of phaseolin seed storage protein pointed out to two different groups of *P. vulgaris*. It was found out that there was a relationship between geographic distribution and phaseolin type in wild and cultivated bean varieties. Samples from Central America had primarily S phaseolin type, with a few exceptions having M type. Samples from Andes had primarily T phaseolin type, and some had C, H, A, J, or I type. There are bean varieties with S and C/T phaseolin type, revealing that multiple events of gene recombination happened during domestication process (Brown et al., 1982, Gepts et al., 1986).

The origin of Serbian bean germ plasm is unclear. The biochemical marker phaseolin and isozymes were used in this work to display the variation of common bean germ plasm. The S type of phaseolin was found in most of analyzed genotypes (9 from 14) (Tab. 1), which revealed that in the process of development of new varieties under climatic conditions of our country and the region, germ plasm from Central America was used. According to Genčev et al. (2002) Bulgarian bean varieties with dominating S phaseolin were better adapted, to climatic conditions of high temperature, and irregular rain falls, in comparison to others.

Phaseolin type T was found in varieties of Novi Sad selection: Zlatko, Sremac and Aster, domestic population greenish-yellow Stepanovićevo and Jovandeka, Croatian variety Slavonski žuto-zeleni (Fig. 3). Those varieties were

developed from domestic populations from north-west region of Balkan, Slavonia, and Vojvodina.

Zeven et al. (1999) showed that T phaseolin type predominated in Holland gene bank. It was found in 132 genotypes from analyzed 157, which revealed Andes origin.

By combination of data for phaseolin and seed size one can conclude that at least three independent domestications took place. In Central America domestication led to varieties with small seed and S phaseolin type; in Columbia small seed and B phaseolin type, and in region of South Andes large seed and T phaseolin type. Low frequency of B phaseolin type pointed out that it was a minor center (Gepts et al., 1986). Origin of C, H and T phaseolin type has not been cleared yet. They have not been found in Central America. Brown et al. (1981) suggested that C phaseolin type could be created by translocation or uneven crossing over in hybrids between two lines having T and S type. This event could take place after introduction of varieties with S phaseolin type into Andes region. Results obtained in this work suggested that both gene pools were used in process of introduction and breeding of common bean in Serbia.

Data on isozymic variability in combination with data on phaseolin type give a fine picture on genetic diversity of bean varieties (Santalla et al., 2002). Analysis of specific region of genes for phaseolin, identification of variation in exon and intron, offers more precise data on genetic diversity (Kami et al., 1995).

CONCLUSION

It was confirmed by experiment that significant polymorphism of enzymic system was not expected since commercial bean varieties were studied. Different allelic variants were found for enzymes: MDH, ME, SKDH and IDH. Most of studied genotypes had S type of phaseolin, and T type was found in just a few. Germ plasm from Central and South America was used in the process of creation new varieties under climatic conditions of our country and the region. Analysis and characterization of varieties of Department of vegetables, Research institute of field and vegetable crops, Novi Sad at the level of protein was done for the first time. Obtained results present a solid starting base for further investigation of gene bank and application of molecular markers.

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КАРАКТЕРИЗАЦИЈА СОРТИ ПАСУЉА НА ОСНОВУ ПРОТЕИНСКИХ МАРКЕРА

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Резиме

У раду је проучено 15 сорти пасуља различитог порекла и селекција, из банке гена Завода за повртарство Научног института за ратарство и повртарство, Нови Сад. Анализирано је 8 ензимских система и резервни протеин фазеолин. Различите алелне варијанте нађене су за ензиме: MDH, ME, SKDH и IDH. Већина анализираних генотипова (9) има S тип фазеолина. Сорте новосадске селекције: Златко, Сремац и Астер, домаће популације Жуто зелени Степановићево и Јовандека, хрватска сорта Славонски жуто-зелени имају T тип фазеолина. Новосадске сорте су настале избором из домаћих популација из северозападног подручја Балкана, Славоније и Војводине.

На основу добијених резултата закључено је да се у процесу стварања нових сорти у климатским условима наше земље и региона користила гермплазме из Средње и из Јужне Америке. По први пут су извршене анализе и карактеризације сорти Завода за повртарство Научног института за ратарство и повртарство, Нови Сад, на протеинском нивоу. Резултати полиморфизма фазеолина и изоензима представљају добру полазну основу за даља истраживања банке гена пасуља и примену молекуларних маркера.

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EFFECT OF CYTOPLASM ON EXPRESSION OF GENES FOR RESISTANCE TO *PUCCINIA TRITICINA*

ABSTRACT: The F2 progenies from crosses of wheat varieties Pliska, Sreća, Vila, Holly, three BM lines as Lr 26, Lr 34 and Lr 38 near isogenic lines (NIL), were tested simultaneously in the greenhouse at seedling stage (20°C) and differentiated according to the reaction type to *Puccinia triticina* monopustule isolate collected from Pesma, the most resistant wide grown variety in Serbia. The presence of Lr 26 and Lr 26 + Lr 34 genes was found in incomplete but not in partialy resistant varieties Sreća and Vila. Pliska and three BM lines had a complete resistance controlled by other genes. Genes from Pliska acted complementary in authentic cytoplasm and were single effective in Lr 34 NIL (Thatcher background) one. Resistance of two BM lines was dependent of one gene. Lr 26 and Lr 34 had influence on resistance enhancement in the F2 of crosses with Sreća and Vila when NILs were donors of cytoplasm. They also increased frequency of completely resistant plants in progenies of Pliska. Resistance suppression controlled by gene linked with Lr 34 on 7D chromosome was evident.

KEY WORDS: *Puccinia triticina*, Lr 26, Lr 34

INTRODUCTION

According to gene-for-gene concept (Flor, 1971), the parasite population differentiation methods were developed (Johnson and Browder, 1966). Only few Lr genes in near isogenic lines (NIL, products of backcrossing with variety Thatcher), expressed the hypersensitive resistance to the virulence in the investigated European parasite populations (Mesterhazy et al., 2000). The logical consequence in applied breeding for the resistance was pyramiding of specific single genes effective to the population parts. There was attempt to use the term pyramiding for any case of genes for resistance relative to parent's efficient accumulation (Rolfs et al., 1992). The basis of the enhanced hypersensitive or partial resistance was the aim of many studies (Samborski and Dyck, 1982; Momčilović and Jerković, 1985). Inheritance of the trait was mostly explainable parallel by eliminating the sup-

pressor genes during the wheat breeding (K y o s a w a and N o m u r a, 1997). With aim to avoid mentioned parallelism, the method that involved the Lr near isogenic lines as fathers for gene identification was applied (J e r k o v i ć, 1992; M i ć a n o v i ć, 2002). The resistant genes identification across the molecular markers without observed interactions with pathogen has promoted the inventarisation of the genotype resistance potential. Benefit was in relatively quickly decreased number of involved genes. According to studies of F_2 generation defining necessary minimum of the genes for the efficient resistance in the particular wheat growing regions could be helpful for breeding to obligate parasites resistance purpose in future.

The aim of the study was to investigate the number and way of action in of the genes for hypersensitive seedling resistance to *Puccinia triticina* in Serbia in different cytoplasm as involvement of the very common Lr 26 and Lr 34 genes in the trait expression.

MATERIALS AND METHODS

The winter wheat genotypes with different expression of the resistance as partial: Lr 26 NIL (not Thatcher cytoplasm), Lr 34, Lr 38 NILs (Thatcher cytoplasm) (M c I n t o s h, 1988) and Holly; incomplete but hypersensitive: Sreća, Vila; complete: Pliska, BM 6, BM 26 and BM 242, to the *Puccinia triticina* isolate from the most resistant in the region nowadays grown winter wheat variety Pesma, by the virulence of race 2 (J o h n s t o n and B r o w d e r, 1966) were crossed. F_2 progenies were simultaneously tested in the greenhouse at air temperature around 20°C during the February 2005. The spores were trashed from susceptible variety Novosadska Rana 2. The estimation of the reaction types according to the scale 0—4 (RT) (S t a k m a n et al., 1962) was 12 days after infection. The plants with RTs 0—3 were counted as resistant (HR). The frequency of hypersensitive resistant plants (PR) was the result of divided resistant and total number of plants. The agreement probability of experimental and expected two or three hybrid segregation ratios (SR) beside resistant (R) and susceptible (S) plants were calculated according to the χ^2 test schedule.

RESULTS AND DISCUSSION

The partially recessive inherited suppressor of the resistance linked with Lr 34 was more efficient in cytoplasm of variety Pliska then in Thatcher one. Amount of at least three dominant genes (RlRlR2r2) controlled hypersensitive resistance of plants, were proved according to achieved SR near expected 5R: 11S in F2 Pliska x Lr 34 NIL (Table 1). Heterozygous suppressor on 7D chromosome and heterozygous two resistant genes produced susceptibility. Suppressor on mentioned chromosome for stem rust resistance genes was detected by D y c k (1987). Segregation ratio (SR) 9R:7S when Pliska was crossed with Lr 38 NIL was associated with the complementary effect of the resistant genes in the mentioned variety, as 45R:19S (FR = 0.70) did when Lr 26 was

added. The Lr 38 NIL was resistant to parasite population in Hungary while Lr 26 and Lr 34 NILs were susceptible according to RT (Manninger, 2002). In Serbia, Lr 38 NIL was susceptible. The high percent of the resistant plants in the progeny of Lr 34 NIL x Pliska, when mentioned NIL was the donor of cytoplasm leaded to conclusion that resistance genes inherited from variety had independent single effect. Expressed genes could be Lr 1 and some of the Lr 2 multiple alleles (Jerkovic et al., 2003). The presence of Lr 9, Lr 19 or Lr 24 in Pliska was not expectable according to previous study (Jerković, 1992). The hypersensitive resistance controlled by Lr 34 when air temperatures were lower (10°C) was also observed by Dyck (1987). Linked suppressor influence on resistance expression was dependable of the cytoplasm (Thatcher or Pliska). The similar SR as in progenies of crosses with Lr 34 NIL was achieved in one from another partial resistant variety Holly. The Lr 26 was effective throw complete resistance as complementary with resistance genes beside Lr 34. Results of progenies from Pliska and complete resistant BM lines, also indicated the presence of two dominant complementary resistance genes from Pliska.

Tab. 1 — Inheritance of the resistance to *Puccinia triticina* in F2 progenies from the crosses of incomplete and complete resistant wheat genotypes

Mother Father in combination	FR of complete R plants	FR of hypersensitive R plants	Conventional two, three hybrid SR	*P*	Total No. of tested plants
Pliska x Lr 38	0.1	0.56	9R:7S	0.99	160
Pliska x Lr 34	0.02	0.33	Suppressor on 7D in NIL 5R:11S	0,75	247
Pliska x Holly	0.04	0.28	Suppressor in Holly 5R:11S	0,10	243
Pliska x Sreća	0.04	0.40	27R:37S or suppressor in Sreća	0.75	166
Pliska x Vila	0.42	0.70	45R:19S	0.99	188
Pliska x BM 6	0.78	0.92	57R:7S	0.50	97
Pliska x BM 26	0.40	0.80	54R:10S or 4 genes	0.05	217
Pliska x BM 242	0.85	0.91	57R:7S	0.50	207
Lr 26 x Sreća	0.33	0.91	Lr 26 homozygous + 2 in Sreća effective throw complete resistance in NIL cytoplasm 60R:4S	0.01	300
Lr 26 x Vila	0.28	0.91	Lr 26 homozig. + 2 in Vila effective throw complete resistance in NIL cytoplasm 60R:4S	0.05	280
Lr 26 x Pliska	0.1	0.70	45R:19S	0.99	234
Lr 34 x Sreća	0.34	0.84	54R:10S	0.99	241
Lr 34 x Vila	0.34	0.90	Lr 34 homozygous + Lr 26 + one gene from Vila 60R:4S	0.05	225
Lr 34 x Pliska	0.34	0.93	2 single effective genes from Pliska in Thatcher cytoplasm 15R:1S	0.90	155

Such acted but different two genes were in BM 26. One single gene for resistance from BM 6 and BM 242 (according to same RT as in previous studies Lr 19) was detected. Last mentioned gene was located at the same chromosome as Lr 34. If these two genes were present in progenies, expected FR had to be 0.94. Lr 34 was in that case complementary to another gene from Pliska. The expected FR was near to achieve, as 0.89 also. So, the only prove of Lr 34 absence in Pliska was the result of progeny from the cross when Lr 34 NIL was not the cytoplasm donor. In the case of Lr 34 carrying chromosome presence in Pliska the linked suppressor effect had to be overcome by genes for resistance accumulation. Recessive inheritance of the resistance was excluded according to the results of F_2 Pliska x Lr 38 NIL. The SR of the F_2 Vila x Pliska was similar with those when Lr 26 from NIL was involved. The presence of this gene in Serbian varieties was logical according to pedigrees. Expression in resistance direction with other genes in used material (FR 0.70 in F_2 of Lr 26 NIL x Pliska, Pliska x Vila) was evident. The SR 3R:1S was achieved in F_2 Anastasia x Renesansa. Anastasia carried Lr 26. As seedlings, Renesansa was partially and Anastasia incomplete hypersensitive resistant (Jerković et al., 2003). In the cytoplasm of NILs the enhancement of the resistance from incomplete to complete was achieved by gene accumulation in the progenies from varieties Sreća and Vila. According to gene identification results Sreća possess the Lr 26 as Vila Lr 26 and 34. Three expressible genes in each of these varieties were present. Lr 26 was also complementary with resistance genes in Pliska in the NIL (not of Thatcher) cytoplasm. Sreća carried different suppressor than Lr 34 NIL. In the cytoplasm of Pliska the Lr 34 NIL was donor of suppressor linked with mentioned resistance gene. Lr 38 was not effective single or in combinations. The Lr 34 was also in resistance enhanced function together with resistance genes from Sreća and Vila in the Thatcher cytoplasm. The similar expressed complementary identified genes as two dominant in Pliska were Lr 3 in Selekta with another unknown, or Lr 14a and Lr 16 in the line NS 1923. In the investigation were involved Lr 1, Lr 2a, Lr 3, Lr 10, Lr 13, Lr 14a, Lr 16 and Lr 26. The mentioned NILs were used as fathers in the crosses. The relatively increased FR in comparison to other progenies was the signal of the particular of noticed known genes presence in the investigated resistant material (Mićanović, 2002). The one of conclusions from presented results was that Thatcher and NILs had to be used as fathers with purpose to prove complementary effect and linked resistance enhancement. The last trait was previously dependable to particular resistant genes amount in the progenies. Single effective dominantly inherited gene like Lr 9, Lr 19 or Lr 24 (sometimes called strong) was also recognized in two BM lines. They were more efficient throw lower RT without necrosis supported by other resistance genes. Such resistance of genotypes carried Lr 26, Lr 19 and Lr 24 accumulation was described by Wanishe and Milus (2004). The enhanced resistance and linked FR decrease was dependant to gene from B genome (Lr 26). Such was previously noticed by Kolmer (2003).

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УТИЦАЈ ЦИТОПЛАЗМЕ НА ИСПОЉАВАЊЕ ГЕНА ЗА ОТПОРНОСТ ПРЕМА ПРОУЗРОКОВАЧУ ЛИСНЕ РЂЕ ПШЕНИЦЕ

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Резиме

Потомства Ф2 генерације из укрштања сорти пшенице Плиска, Срећа, Вила, Холи, три БМ као и Лр 26, Лр 34 и Лр 38 изогене линије (НИЛ), су тестирана истовремено у стадијуму сејанаца у стаклари (20°C) и разликована по типу реакције према монопустулном изолату лисне рђе ц Песме, најотпорније проширене сорте у Србији. Присуство Лр 26 и Лр 26 + Лр 34 гена је установљено у некомплетно али не и парцијално отпорним сортама Срећа и Вила. Плиска и три БМ линије су испољиле потпуну отпорност на основу других гена. Гени из Плиске су деловали комплементарно у цитоплазми наведене сорте, а појединачно у оној од Лр 34 НИЛ. Отпорност две БМ линије је била наслеђена преко једног гена. Лр 26 и Лр 34 су утицали на повећање отпорности у Ф2 када су Срећа и Вила биле донори цитоплазме. Такође, повећали су фреквенцију потпуно отпорних биљака у Плискиним потомствима. Установљено је смањење отпорности контролисано геном везаним ц Лр 34 на 7Д хромозому.

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ANALYSIS OF ANATOMICAL AND MICROMORPHOLOGICAL CHARACTERISTICS OF *IVA XANTHIFOLIA* NUTT.

ABSTRACT: *Iva xanthifolia* is a North American weed species, which was introduced and naturalized in Europe. Anatomical and micromorphological characteristics of this species were investigated, in order to get better knowledge of its biology, which could help in development of strategies for prevention of its spreading. Detailed descriptions of lamina, petiole, stem and inflorescence axis anatomical structures were given, together with micromorphological characteristics of epidermis and indumentum of lamina, petiole, stem, inflorescence axis, involucre and fruit. All vegetative organs had mesomorphic structure, with some xeromorphic adaptations. Mechanical tissue was well developed, which gave those plants additional strength and resistance. Trichomes were the most numerous on lamina and in the region of inflorescence, while rare on petiole and stem epidermis and their distribution varied according to plant organ.

KEY WORDS: anatomy, *Iva xanthifolia*, indumentum, micromorphology

INTRODUCTION

In last few decades one more adventive species is spreading in the North Serbia (Vojvodina) — *Iva xanthifolia.* It is North American species, which was introduced and naturalized in European flora (Hansen, 1976). It belongs to Pontic-Pannonian (Sarmatian-sub-Atlantic) element of flora in Europe (Meusel and Jäger, 1992), with values of ecological indexes around optimal (Soó, 1970). In Vojvodina, it was first recorded by Šajinović and Koljadzinski (1966). Ecological conditions of the Pannonian plane proved to be very suitable for its fast and aggressive spreading. As the result, the area of distribution of *Iva xanthifolia* in our country expanded. This species was also recorded in floras of our neighboring countries, in the region of Pannonian plane — Romania (Ciocârlan, 2000), Croatia (Marković, 2000) and

Hungary (H o r v á t h et al., 1995). *Iva xanthifolia* is also plant that can cause different allergic reactions in humans (I g i ć et al., 2005). Considering its progressive spreading in this region, it could be supposed that this species, together with *Ambrosia artemisiifolia*, could cause significant problems for human's health in near future. In order to perceive biological characteristics of this species more completely, it is necessary to get better knowledge of the structure of its vegetative and reproductive organs. For that purpose, detailed analysis of anatomical structure of lamina, petiole, stem and inflorescence axis, as well as micromorphological analysis of epidermis and indumentum of lamina, petiole, stem, inflorescence axis, involucre and fruit were conducted.

MATERIAL AND METHODS

Dry plant material was taken from Herbarium of the Department of Biology and Ecology (BUNS), University of Novi Sad (voucher numbers 2-1969, 2-1970 and 2-1971). Plants that were used for analyses originated from different localities in North Serbia (Vojvodina)—saline in Orlovat, neglected habitat in Novi Sad and ruderal habitat in Apatin. Structure of the lamina, petiole, stem and inflorescence axis was determined using light microscopy. Cross sections were made using Leica CM 1850 cryostat, at temperature –18°C to –20°C, at cutting intervals of 25 μm. Sections were observed using Image Analyzing System Motic 2000. Indumentum characteristics of lamina, petiole, stem, inflorescence axis, involucre and fruit were observed using scanning electron microscopy (SEM). Small pieces of dry parts of the plants were sputter coated with gold for 180 seconds, 30 mA (BAL-TEC SCD 005) and viewed with JEOL JSM-6460LV electron microscope at an acceleration voltage of 20 kV.

RESULTS AND DISCUSSION

The results of lamina anatomical analysis showed that it had dorsiventral structure. Mesophyll was differentiated on palisade and spongy tissue, which were almost equal in thickness. Palisade cells were elongated, rectangular in shape, arranged in one layer. Spongy cells were round to oval in shape, arranged in several layers. Crystals in the form of crystal druses were observed in idioblasts, which were placed among the mesophyll cells. Collateral closed vascular bundles in mesophyll were arranged in a line. The main vein and the most of the lateral veins were prominent on the abaxial side. One vascular bundle, surrounded by parenchyma sheath, occurred in them. Collenchyma tissue was present in large veins, subepidermally. Epidermis was one-layered, covered with dense indumentum (Fig. 1). Nonglandular trichomes were multicellular, uniseriate, narrow or very wide. On adaxial epidermis they were short and erect, while on abaxial epidermis more numerous, longer and flattened. Two types of glandular trichomes were present — short and long ones (Fig. 2). Short glandular trichomes were multicellular and biseriate. A large subcuti-

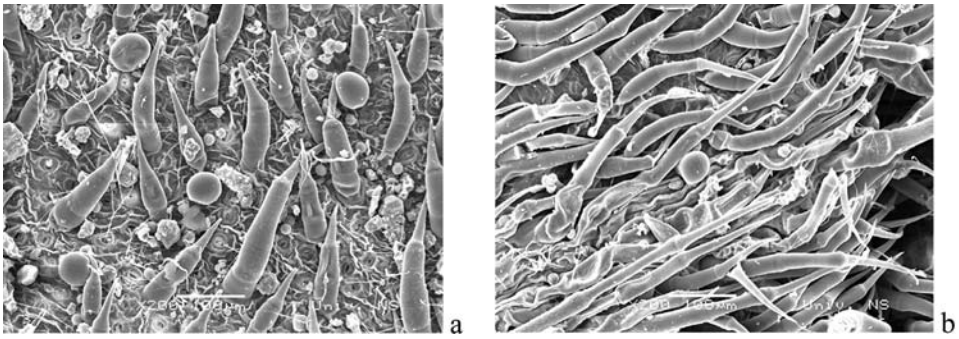

Fig. 1. — SEM micrographs of lamina epidermis; a. adaxial epidermis; b. abaxial epidermis

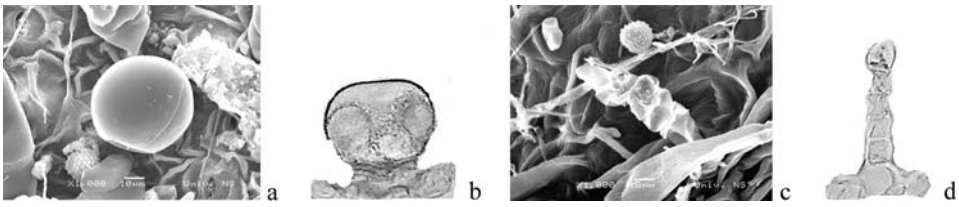

Fig. 2. — SEM micrographs of lamina epidermis. Glandular trichomes: a-b. short glandular trichomes; c-d. long glandular trichomes

cular chamber, filled with secretions, was formed by the elevation of joint cuticle of upper secretory cells. Those trichomes were placed at the level of epidermal cells, or slightly above the epidermis, and resembled peltate trichomes very much. Long glandular trichomes were multicellular, uniseriate, with apical secretory cell. They were less numerous compared to short trichomes. Both types of glandular trichomes were present on both epidermal sides, but were more numerous abaxially.

On cross section, petiole was round in shape, with two wing-like expansions and more or less prominent lateral ribs (Fig. 3a). Epidermal cells were small, with thick walls. Subepidermally, a few layers of collenchyma tissue occurred. Collenchyma was especially well developed in wing-like expansions and in the part of the cortex opposite to wings. Under collenchyma, multi-

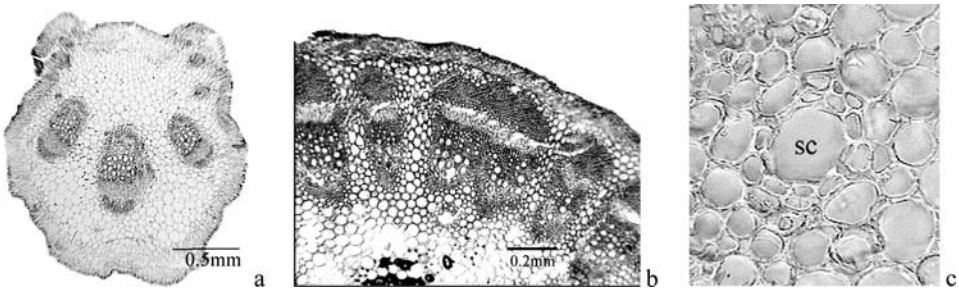

Fig. 3. — Cross-section: a. petiole; b. stem; c. secretory canals in stem medulla (sc)

layered assimilatory parenchyma occurred. Collateral closed vascular bundles, surrounded by parenchyma sheath, were arranged in simple arc. Usually, three large and several smaller vascular bundles were present. Groups of sclerenchyma tissue occurred next to phloem and xylem part of the vascular bundles. Large parenchyma cells were in the central part of petiole. Solitary crystals of different size, prismatic, wide rectangular or narrow-rectangular in shape, were recorded in them. Indumentum analysis using electron microscopy, revealed the presence of nonglandular and long glandular trichomes, while short glandular trichomes were not recorded. The number of trichomes was smaller compared to lamina.

Stem cross-section was round in shape (Fig. 3 b, c). Epidermis was made of small, thick-walled cells. Cortex was relatively thin, composed of few layers of collenchyma cells and assimilatory parenchyma. Endodermis was not prominent. More than twenty collateral closed vascular bundles were arranged in a circle. Larger groups of sclerenchyma cells occurred next to phloem, while smaller groups next to xylem. The cell walls of parenchyma cells between vascular bundles were thickened, and they formed sclerenhymatous parenchyma. Parenchyma cells in the central cylinder were large and contained numerous small solitary crystals of narrow-rectangular shape. Among the parenchyma cells in perimedullar zone, under vascular bundles, secretory canals occurred. They were lined with epithelium and filled with secretions. Secretory canals in family *Asteraceae*, where this species belongs to, could be present in stem cortex, in the region of the endodermis, in stem medulla, root, petiole and lamina (M e t c a l f e and C h a l k, 1957). The distribution of stem secretory canals was said to be valuable for the identification of genera. Observation of the stem epidermis under electron microscope showed that stem was mostly glabrous, or with rare nonglandular and long glandular trichomes, while short glandular trichomes were not recorded.

The anatomical structure of inflorescence axis was very similar to the stem structure. On cross-section it was round in shape, with small ribs above vascular bundles. Usually, it contained 15 to 17 vascular bundles. Number of crystals in parenchyma cells of the central cylinder was much lower, compared to the stem. Differences were also recorded in indumentum characteristics. On inflorescence axis it was denser. Besides nonglandular and long glandular trichomes, short glandular trichomes were also recorded.

Inflorescences of panicoid type consisted of large number of solitary capitula. Each capitulum was surrounded by involucre. Observation of involucre leaves under electron microscope revealed the presence of a very large number of trichomes (Fig. 4). Besides nonglandular trichomes, short glandular trichomes were very numerous, while the long ones were very rare. Short glandular trichomes were especially numerous in the region of flowers, on the inner side of involucre leaves.

The fruit was obovate in shape. Micromorphological analysis of pericarp surface showed the presence of parallel, shallow, longitudinal ridges (Fig. 5). They were more prominent on wider part of the fruits. Along the ridges, tuberculate structures occurred, which were also more prominent on the ridges of the wider part of the fruits.

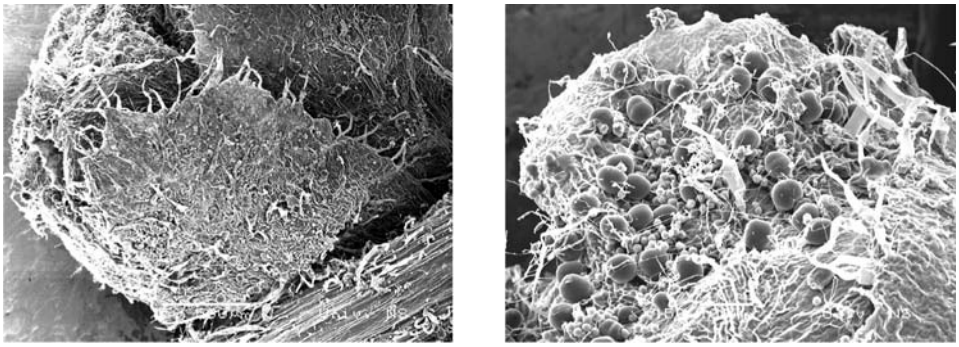

Fig. 4. — SEM micrographs of involucre leaves

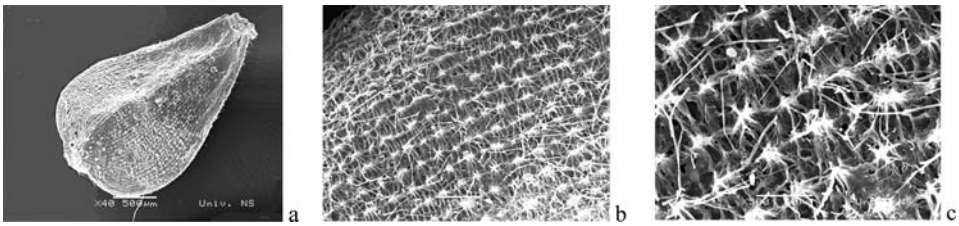

Fig. 5. — SEM micrographs of pericarp surface: a. fruit; b-c. detail of pericarp surface

Anatomical and micromorphological analyses of leaf and stem showed that they had mesomorphic structure, with some xeromorphic adaptations. Mechanical tissue was well-developed in all examined organs: collenchyma tissue subepidermally in larger lamina veins, petiole, stem and inflorescence axis; sclerenchyma tissue next to the phloem and xylem parts of vascular bundles; sclerenchymatous parenchyma between the vascular bundles of the stem and inflorescence axis. Epidermal cells and the cells of the nonglandular trichomes also had thick walls. This way of distribution of mechanical tissues gives those plants additional strength and resistance to different biotic and abiotic factors of the environment. Our analyses also showed that trichomes were the most numerous on lamina and in the region of inflorescence, while rare on petiole and stem epidermis. The distribution of glandular trichomes varied according to plant organ. Long glandular trichomes were present on all examined organs, but were not numerous. Short glandular trichomes were recorded only on lamina surface and in the region of inflorescence, where they were more numerous than the long trichomes. Because of such distribution of glandular trichomes, it would be interesting to conduct biochemical investigations of their secretions, in order to get better knowledge of their significance and role they had in ecology of this species.

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АНАЛИЗА АНАТОМСКИХ И МИКРОМОРФОЛОШКИХ КАРАКТЕРИСТИКА *IVA XANTHIFOLIA* NUTT.

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Резиме

Iva xanthifolia је северноамеричка коровска врста која је интродукована и натурализована на подручју Европе. Истраживане су анатомске и микроморфолошке карактеристике ове врсте, у циљу доприноса бољем разумевању њене биологије, што би могло допринети развијању стратегије за превенцију њеног ширења. У раду су дати детаљни описи грађе лиске, лисне дршке, стабла и осовине цвасти, као и микроморфолошке карактеристике епидермиса и индументума ла-

мине, лисне дршке, стабла, осовине цвасти, инволукрума и плода. Сви испитивани вегетативни органи имају мезоморфну грађу, са појединим ксероморфним адаптацијама. Механичко ткиво је добро развијено, што обезбеђује овим биљкама већу чврстоћу и отпорност. Трихоме су најмногобројније на епидермису лиске и у региону цвасти, док су ретке на епидермису лисне дршке и стабла. Њихова дистрибуција варира у зависности од биљног органа.

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THE IMPORTANCE OF EXTREMOPHILE CYANOBACTERIA IN THE PRODUCTION OF BIOLOGICALLY ACTIVE COMPOUNDS

ABSTRACT: Due to their ability to endure extreme conditions, terrestrial cyanobacteria belong to a group of organisms known as "extremophiles". Research so far has shown that these organisms posses a great capacity for producing biologically active compounds (BAC). The antibacterial and antifungal activities of methanol extracts of 21 cyanobacterial strains belonging to *Anabaena* and *Nostoc* genera, previously isolated from different soil types and water resources in Serbia, were evaluated. In general, larger number of cyanobacterial strains showed antifungal activity. In contrast to *Nostoc*, *Anabaena* strains showed greater diversity of antibacterial activity (mean value of percentages of sensitive targeted bacterial strains 3% and 25.9% respectively). Larger number of targeted fungi was sensitive to cultural liquid extract (CL), while crude cell extract (CE) affected more bacterial strains. According to this investigation, the higher biological activity of terrestrial strains as representatives of extremophiles may present them as significant BAC producers. This kind of investigation creates very general view of cyanobacterial possibility to produce biologically active compounds but it points out the necessity of exploring terrestrial cyanobacterial extremophiles as potentially excellent sources of these substances and reveals the most prospective strains for further investigations.

KEY WORDS: cyanobacteria, *Nostoc*, *Anabaena*, extremophiles, biologically active compounds

INTRODUCTION

At present, cyanobacteria generally remain as potential sources for further investigations as prospective and excellent sources of biologically active constituents produced during primary and especially secondary metabolism (Skulberg, 2000). Primary metabolites have been defined as low molecular weight compounds that are necessary for growth (Staley and Stanley, 1986). Thus, these compounds are produced by microorganisms during active growth, i. e., in the logarithmic growth phase. They include amino acids, nucleotides, coenzymes, organic acids and vitamins, as well, as intermediates in the bi-

osynthetic pathways of these compounds. Secondary metabolites have been defined as low molecular weight compounds that are not necessary for microbial growth in pure culture continuously grown. These compounds are usually produced when the cyanobacterial culture enters the maximum stationary growth phase.

Among microorganisms, cyanobacteria are rapidly proving to be an extremely important source of biologically active secondary metabolites with potential benefits against human disease. They have been shown to produce a variety of antibacterial, antifungal, antilarval, antiprotozoan, antialgal, antihelmintic and generally cytotoxic secondary metabolites (Meeting and Pyne, 1986; Glombitza and Koch, 1989), some of which have potential for use as therapeutic or agrochemical agents (Moore, 1996). Among the commercially significant secondary metabolites are antibiotics and other pharmacologically active compounds, toxins and pigments (Staley and Stanley, 1986). Since they were first encountered, the toxins were always considered a threat to the health of living organisms.

Research of microalgae and cyanobacteria has mostly been directed towards examining water cultures. During the recent years, the emphasis has been shifted to organisms that do not primarily depend on aquatic surroundings, for not only survival but also reproduction. A large number of microalgae and cyanobacteria belong to the terrestrial types, which inhabit the soil (soil microalgae), rocks, tree bark, roofs, caves, city walls and other surfaces that are in contact with the atmosphere (Cvijan and Blaženčić, 1996; Petrovska, 1997). Due to their ability to endure extreme conditions (for algae and cyanobacteria, water represents the optimal environment), these organisms have become known as "extremophiles". Extremophiles thrive on the edge of temperature, pH, pressure, hypersalinity, dryness and desiccation. Research so far has shown that these organisms posses a great capacity for producing biologically active compounds.

Significant biological activity *in vivo* and *in vitro* is found to happen in the cases of extracts of cyanobacterial strains isolated from freshwater and marine environments. It appears that extracts of terrestrial cyanobacteria have been found to possess even greater biological activity (Patterson et. al., 1994; Reisser, 2000). Because of their special growing conditions, terrestrial cyanobacteria posses survival and adaptation mechanisms not found in aquatic species. Some scientists believe that the more harsh and extreme conditions lead to a wider amplitude of metabolic extremities and possibilities, which causes the production of a most diverse range of, more or less, specific substances, reveling and pointing towards brilliant candidates for biotechnological application (Svirčev, 2005).

Terrestrial collections have frequently provided too little material for isolation and identification of the active agents (Barch et. al., 1983). Among 976 cyanobacterial isolates, we managed to culture a great number of terrestrial cyanobacterial strains which display significant growth rate (higher than 2g/l). On this basis, we have been engaged in a search for biologically active constituents in cyanobacteria isolated from the soil samples. In this work, we

present the significance of cyanobacterial extremophiles in the production of biologically active compounds.

MATERIALS AND METHODS

Cyanobacteria and culture conditions

Cyanobacterial strains used for antibacterial and antifungal screening tests were cultivated in Novi Sad Cyanobacterial Culture Collection (NSCCC), Department of Biology, University of Novi Sad, Serbia. They were previously isolated from different soil types (S, SB, C, CB, LC, LCB, 3, 4, 14) and water resources (L, D, W, G) in Serbia. Isolates used to belong to genus *Anabaena*: *2S6B, 2S7, S8, C2, C3, C5, LC1B, LC2, 4, L, D*, and genus *Nostoc*: *S1, S2, 2S3B, S9B, 2S9, 2C1B, 3, 14, W, G.* All the strains were cultivated in eight Erlenmeyer flasks of 500ml volume, at the temperature of 26°C and fluorescent light intensity of 50 $\mu molm^{-2}s^{-1}$, for thirty-five days. They were grown in BG11 liquid nutritive medium (R i p p k a et al., 1979), without supplemented nitrogen.

Methanol extracts preparation (Modified method by Ø s t e n s v i k, 1998)

After incubation, microalgal biomass was separated from supernatant by centrifugation, at 3500 g/min and than freeze-dried. Final dry biomass was precisely measured. 15ml of solvent MeOH/EtOAc (ratio 10:1) was added to each biomass sample. The dissolving process was improved by using the ultrasonificator (10x10 sec). The mixtures were kept over night at 4°C, in the dark. After extraction, extracts were filtrated through sterile filter paper to eliminate the cells and left at 37°C, in the dark for 48 hours to evaporate to dry residue. In supernatant fluid, the same volume of MeOH/EtOAc was added as solvent and all the fluid was evaporated to dry residue. Evaporated cell-free extracts and dry supernatant residue were dissolved in 1% (v/v) MeOH/EtOAc in 0.9% NaCl and sterilized by filtration through ∅ 0.22 μm filter. Finally, the extracts of biomass and supernatants were administrated to obtain different dilution rates.

Antibacterial-antifungal screening in agar plates

Bacterial strains *Staph. oxfordii, Staph. aureus, Str. pyogenes, E. coli* and nine bacterial strains isolated from the river Danube water were used in the investigation. The following fungal isolates, as part of the Department of Biology, University of Novi Sad collection, were used in the antifungal screening: yeasts *Saccharomyces* sp., *Schizosaccharomyces* sp. and *Candida albicans* and molds *Trichoderma* sp., *Cladosporium* sp., *Penicillium* sp., *Aspergillus* sp. and *Rhizopus* sp. Bacterial or fungal suspensions were added in sterile plates with

MPA medium. Using the opposite end of sterile pipette, 4 mm wells were made. In two different experiments, 100 µl volume of methanol extracts of a) cell-free supernatant (CE) and b) supernatant residue (CL) of all 21 cyanobacterial strains were added in the wells. After 24h of incubation at 37°C, the inhibition zones were measured (distance in [mm] from the edge of the well to the point of normal colony size of the test bacteria).

RESULTS AND DISCUSSION

Among all cyanobacterial strains tested, some representatives of *Anabaena* and *Nostoc* genus showed no biological activity (19%), some strains detected less or more significant activity against different number of bacterial (19%) and fungal (29%) species, but the most of the strains with detected biological activity, influenced both group of targeted organisms, bacteria and fungi (Tab. 1, Fig. 1). *Anabaena* strains, however, showed greater diversity of antibacterial activity (mean value 25.9%), while *Nostoc* strains produced active compounds against less number of bacterial strains tested (mean value 3%) (Fig. 2). According to activity shown against the fungi, approximately the same number of fungal species was sensitive to *Nostoc* and *Anabaena* active compounds (mean values 15.3%: 16.9% respectively).

Tab. 1. — The percentage of bacterial and fungal strains affected by cyanobacterial extracts (CE — crude cell extract, CL — cultural liquid extract)

BIOASSAY	ANTIBACTERIAL		ANTIFUNGAL	
STRAINS	CE	CL	CE	CL
Anabaena				
2S6B	26.8	13.4	5.9	5.9
2S7	60.3	53.6	5.9	11.8
S8	26.8	13.4	0	0
C2	80.4	33.5	0	0
C3	6.7	6.7	0	0
C5	0	0	11.8	23.6
LC1B	53.6	33.5	23.6	29.5
LC2	46.9	33.5	47.2	64.9
4	53.6	26.8	64.9	64.9
L	0	0	5.9	5.9
D	0	0	0	0
Nostoc				
S1	0	0	0	0
S2	13.4	6.7	11.8	11.8
2S3B	13.4	6.7	29.5	29.5
S9B	0	0	11.8	41.3
2S9	0	0	53.1	41.3
2C1B	0	0	0	0
3	13.4	6.7	0	0
14	0	0	0	0
W	0	0	29.5	35.4
G	0	0	5.9	5.9

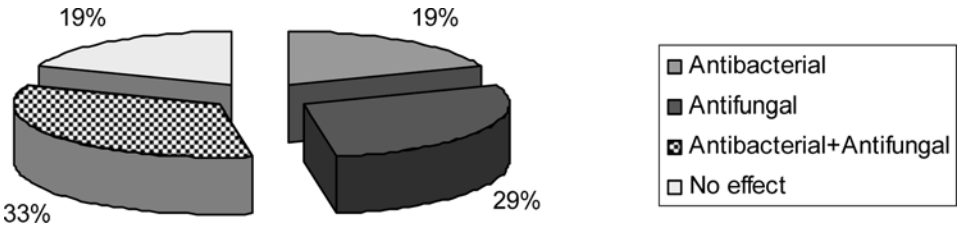

Fig. 1. — The percentage of cyanobacterial strains with only antibacterial, only antifungal, both antibacterial and antifungal effect, and without influence on the targeted organisms

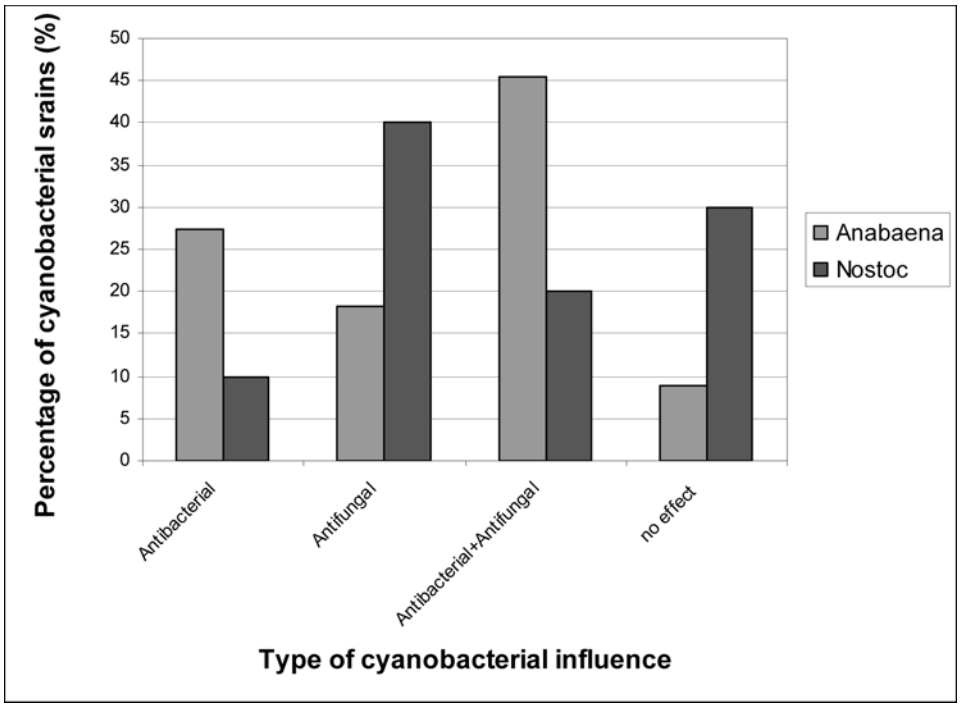

Fig. 2. — Mean values of percentages of targeted bacterial and fungal strains sensitive to extracts obtained from *Anabaena* or *Nostoc* strains

We can notice that tested *Anabaena* strains detected greater antibacterial then antifungal activity, but most strains were producing both antibacterial and antifungal substances (Fig. 2). It is opposite with *Nostoc* strains, which exhibit mostly fungicidal influence (only 3% of the investigated bacterial strains were sensitive to cyanobacterial extracts comparing to 15.3% of sensitive fungal strains). Investigations of 50 cyanobacterial isolates, belonging to *Nostoc* genus, showed that more than 80% of the strains had significant biological activity, mostly antifungal. Antibacterial activity was less frequent (only 8 of 50 strains) (P i c c a r d i et al., 2000). According to other research, less then 10% of 255 strains of microalgae showed fungicide or fungistatic activity (N e -

m e t h and Ö r d ö g, 2000). Different results of various investigations conducted as primary screening for production of biologically active compounds (S v i r č e v, 2005), could lead us to a conclusion that this capability is not genus dependent but vary from strain to strain, and the obtained differences are only the result of small number of tested strains. It is also obvious that biological activity can be directed towards one target, but can also refer to more then one secondary metabolite, which is also depending on the cyanobacterial strain (Tab. 1, Fig. 1). For example, C2 strain showed great antibacterial and no antifungal effect, while S2 strain had similar biological activity on both bacteria and fungi. At the same time, S1 strain showed neither antibacterial nor antifungal effect.

In general, larger number of cyanobacterial strains showed antifungal activity (Fig. 1). It was detected with 13 of 21 cyanobacterial strains. In all cases but one (2S9 strain), cultural liquid extract affected exact or larger number of targeted fungi then crude cell extract (Fig. 3). 12 of 21 investigated cyanobacterial strains showed antibacterial influence. All of those, except C3 strain, affected larger number of targeted bacteria using CE then when CL was used in experiment. This is most obvious with C2 strain, where CE: CL ratio is 80.4%: 33.5%. Similar results were obtained in some previous researches where terrestrial strains were included (S v i r č e v, 2005). Investigations of 50

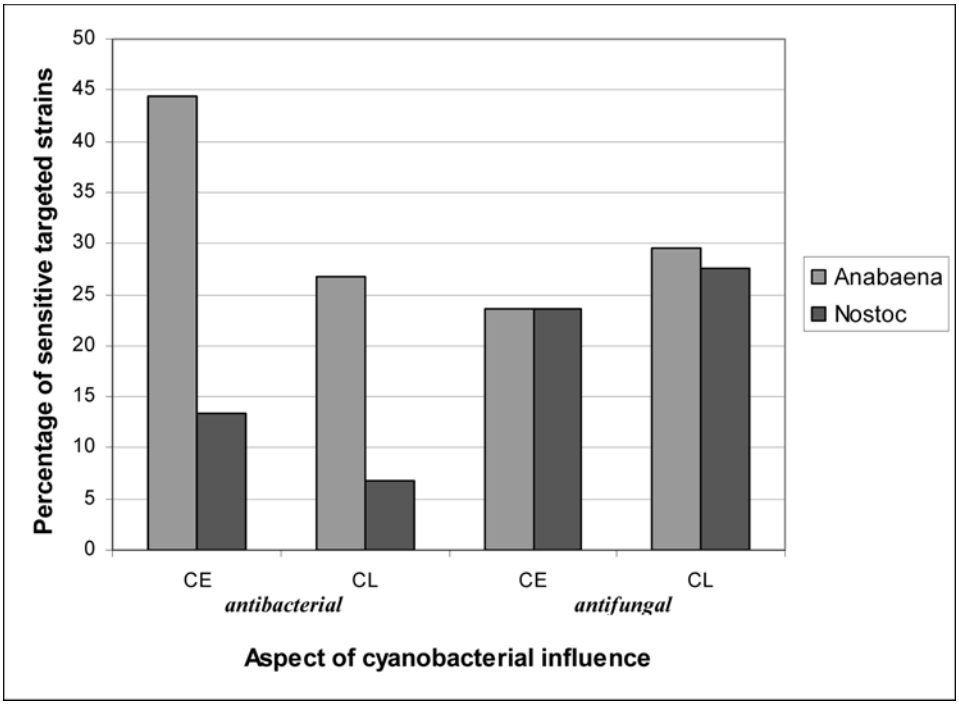

Fig. 3. — Mean values of percentages of targeted bacterial and fungal strains sensitive to different aspects of cyanobacterial extracts: crude cell extract (CE) and cultural liquid extract (CL); cyanobacterial strains with no detected influence excluded

Nostoc strains showed that the bioactivity is equally distributed between lipophilic and hydrophilic extracts of lyophilized cyanobacterial biomass. Methanolic extracts of *Nostoc* strain ATCC 53789, a known cryptophycin producer, obtained from both thawed biomass (BE) and thawing water (WE) were active against 9 of 12 fungi tested, but higher concentrations of WE were necessary to obtain activity of this extract (B i o n d i et al., 2004). Various investigations of different aspects of cyanobacterial influence pointed that antifungal substances are mostly not excreted out of the cell, but stay within the biomass of cyanobacteria observed. Nevertheless, screening of larger number of cyanobacterial strains for antifungal activities showed significant influence of excreted bioactive compounds (K u l i k, 1995). Various results from different investigations points that type of extract, as well as method of extraction, are an important factor in intensity of BAC activity.

Aquatic strains used in this experiment showed no antibacterial influence and less significant activity against fungal species then terrestrial strains (Fig. 4). According to these results, terrestrial strains, as representatives of extremophiles, should be taken into consideration as potent BAC producers more seriously. Some scientists believe that revealing new capabilities and possibilities of terrestrial strains presents a beginning of new era in biotechnology of microalgae (R e i s e r, 2000, S v i r č e v, 2005).

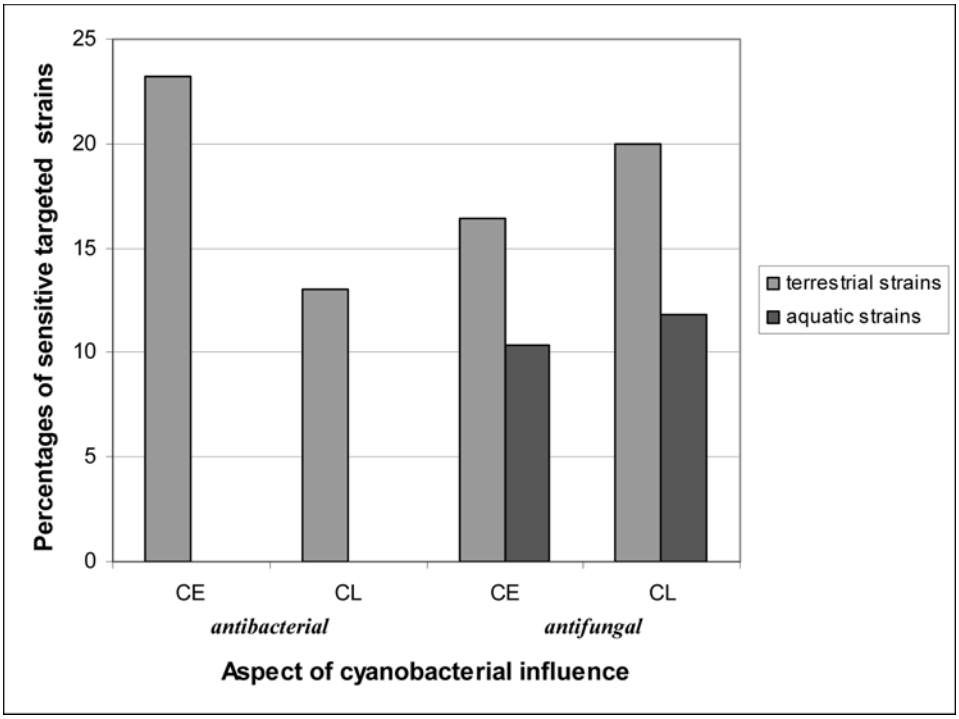

Fig. 4. — Mean values of percentages of targeted bacterial and fungal strains sensitive to extracts obtained from terrestrial or aquatic strains

This kind of investigation creates very general view of cyanobacterial possibility to produce biologically active compounds but it points out the necessity of exploring terrestrial cyanobacterial extremophiles as potentially excellent sources of these substances and reveals the most prospective strains for further investigations. The action spectrum and the potency of different cyanobacterial extracts is strain dependent. Therefore, the screening type of investigations, with as many cyanobacterial strains and targeted organisms as possible, are necessary to provide a large scale of information for each strain, which could help in further researches toward possible application in biotechnology.

CONCLUSION

In general, larger number of cyanobacterial strains showed antifungal activity. *Anabaena* strains, however, showed greater diversity of antibacterial activity (mean value 25.9%), while *Nostoc* strains produced active compounds against less number of bacterial strains tested (mean value 3%). According to activity shown against the fungi, approximately the same number of fungal species was sensitive to *Nostoc* and *Anabaena* active compounds (mean values 15.3%: 16.9% respectively). Tested *Anabaena* strains detected greater antibacterial then antifungal activity, but most strains were producing both antibacterial and antifungal substances. In contrast, *Nostoc* strains exhibit mostly fungicidal influence. Cultural liquid extract (CL) affected exact or larger number of targeted fungi then crude cell extract (CE), while more targeted bacterial strains were affected by CE then CL used in experiment. According to these investigation, the higher biological activity of terrestrial strains as representatives of extremophiles may present them as significant BAC producers.

This kind of investigation creates very general view of cyanobacterial possibility to produce biologically active compounds but it points out the necessity of exploring terrestrial cyanobacterial extremophiles as potentially excellent sources of these substances and reveals the most prospective strains for further investigations.

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ЗНАЧАЈ ЕКСТРЕМОФИЛНИХ ЦИЈАНОБАКТЕРИЈА У ПРОДУКЦИЈИ БИОЛОШКИ АКТИВНИХ МАТЕРИЈА

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Резиме

Земљишне цијанобактерије припадају групи организама под називом „екстремофили”, захваљујући способности да опстају у стаништима са екстремним условима средине. Досадашња истраживања су показала да ови организми имају велик капацитет продукције биолошки активних материја (БАМ). Посматране су антибактеријске и антифунгалне активности метанолских екстраката двадесет и

једног цијанобактеријског соја сврстаних у родове *Anabaena* или *Nostoc*, а претходно изолованих из различитих типова земљишта и водених станишта Србије. Већи број свих испитиваних цијанобактерија показао је антифунгалну активност. За разлику од *Nostoc*, сојеви из рода *Anabaena* показали су већи диверзитет антибактеријске активности (средња вредност процента бактеријских сојева на које је испољено дејство екстраката била је 3% и 25.9% редом). Екстракт фугата (CL) је испољио дејство на већи број испитиваних гљива него бактеријских сојева, док је код ћелијског екстракта (CE) био обрнут случај. Према овим истраживањима, већа биолошка активност земљишних сојева као представника екстремофила у односу на водене сојеве може их истаћи као значајне продуценте биолошки активних материја. Овакав вид истраживања даје уопштену слику могућности цијанобактерија да продукују БАМ, али такође истиче неопходност проучавања земљишних цијанобактеријских екстремофила као потенцијално веома важног извора у добијању ових материја, и указује на најперспективније сојеве за даља истраживања.

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IMPROVEMENT OF PRODUCTION OF HIGH-YIELD POPLAR VARIETIES SEEDLINGS BY MYCORRHIZA APPLICATION

ABSTRACT: Research related to the effects of treatment by mycorrhiza preparations Ectovit, Rhodovit (preparations Symbio-m Ltd., Czech Rep.) and their combination on growth of four high-yield poplar clones of *Populus deltoides* and one variety of *Populus x euramericana* are presented in this paper. In order to make more accurate assessment of mycorrhiza effect, soil characteristics such as morphology, texture and chemical composition were determined.

The study results indicate that mycorrhized cuttings had the same or the better survival in all the study clones compared to the control. The application of the preparation Ectovit and Rhodovit resulted averagely in the first class planting stock of all the study clones. The combination of the preparations Ectovit and Rhodovit produced averagely the first class planting stock only of the clone *Populus x euramericana*.

KEY WORDS: mycorrhiza, planting stock, poplar

INTRODUCTION

Production of black poplar nursery stock is a necessary precondition for the successful establishment of plantations. Nowadays, plantations are mostly established by one-year-old rooted cuttings, type 1/1. The production of nursery stock of the above age is derived from the black poplar property of vegetative reproduction (Marković et al., 1986). The production of black poplar nursery stock in the section *Aigeiros* is specific, because it depends on the physicochemical characteristics of the soil, climate and hydrological conditions, characteristics of poplar clone, selection of planting space, with the consequentially applied tending measures.

Poplar nurseries are located on the soils formed in the alluvial plains, characterised by a high variability of characteristics over small areas (Živ-

k o v i ć et al., 1972; Ž i v a n o v, 1979; Ž i v a n o v et al., 1986; I v a n i š e v i ć, 1991). Ž i v a n o v et al. (1986) report that the soils in the Danube alluvial plain are poor in readily available nutrients. The poor supply of the alluvial soil with readily available nutrients, after I v a n i š e v i ć (1991), becomes acute as the consequence of the absence of floods, under the application of the hydrological regime, or under intensive utilisation, such as in the nurseries.

The permanent improvement of the nursery stock production technology is necessary because of the constant generation of new genotypes, and also to reduce the production cost. The improvement should be realised by the production of the higher percentage of good-quality rooted cuttings and by the reduction of the costs of establishment and tending.

The aim of this paper is to contribute to the improvement of nursery stock production technology by the application of the preparations containing mycorrhizal inoculum. Mycorrrhiza is a symbiotic relationship between soil fungi and plant roots. Two major types of mycorrhiza occur in Nature: endomycorrhiza and ectomycorrhiza. Subtypes of endomycorrhiza are arborous, ericoid and orchidaceous mycorrhiza. Endomycorrhiza is common in about 90% of plant species whereas ectomycorrhiza is specific to conifers and some broadleaf species. A basic principle is similar for all mycorrhiza types. When new plant roots get in contact with the mycorrhizal inoculum the roots are colonised with fungal hyphae and after a few weeks the external mycelium network extends to the soil. Under normal condition fungus reproduces itself and persists in the roots or in the soils throughout the entire life of plant.

The uptake of water and solutes by woody species in forests of the Mediterranean, temperate and boreal zones largely depends on the mycorrhizal status of the roots. In forest ecosystems, ectomycorrhizas serve as a major organ for nutrient uptake by trees (M a r j a n o v i ć et al., 2005). Ectomycorrhizas are commonly assumed to enhance water uptake of their hosts (B o y l e et al., 1990; M e x a l et al., 1973). The fine roots of forest trees occupy the top 10 cm of the soil, where nutrient cycling is the most intense, and are dominated by ectomycorrhizal symbiosis (B r u n s, 1995; B a k k e r et al., 2000). Poplar cutting are treated with Ectovit and Rhodovit (preparations Symbio-m Ltd. Czech Republic). Ectovit based on ectomycorrhizal symbiotic fungi. It is a natural mycorrhizal bio-product improving growth of the majority of woody plant species. Rhodovit is a natural mycorrhizal bio-product, based on ericoid symbiotic fungi.

MATERIAL AND METHODS

The research was performed in the field polyclonal experiment established by planting the cuttings of four experimental clones of eastern cottonwood *Populus deltoides* B-229; *Populus deltoides* PE 19/66; *Populus deltoides* B-81 and *Populus deltoides* 182/81 and the clone of Euramerican black poplar *Populus x euramericana* M-1, planting space between rows 0.8 m, and within rows 0.3 m.

Physical characteristics of the soil were determined by the following methods (B o š n j a k et al., 1997):

— Particle size distribution (%) by international B-pipette method with the preparation in sodium pyrophosphate,

— Classification of particle sizes by Atterberg's classification.

Chemical characteristics were determined by the following methods (H a d ž i ć et al., 2004):

— $CaCO_3$ (%) volumetric method, by Scheibler unit calcimeter

— pH in H_2O electrometric method with combined electrode on Radiometer pH meter

— Nitrogen content (%) by the modified Kjeldahl method

— Readily available phosphorus and potassium (mg/100 g) by Al-method Egner-Riehm-Dom.

Humus (%) were determined by Turin's method, modification by Simakov (Š k o r i ć and S e r t i ć, 1966)

Root collar diameter and seedling height were measured at the end of the growth period. The plants were classified according to the following categories (I v a n i š e v i ć, 1991):

Extra class, seedling height above 3 m

I class, 2.51—3 m

II class, 2.01—2.5

III class, 1.51—2 m and

below 1.5 m.

Poplar cuttings are treated with Rhodovit and Ectovit (Symbio-m Ltd., Czech Republic). Rhodovit is ericoid mycorrhizal inoculum contains a dry component A, liquid component B and a powdered gel C.

A — perlit-peat carrier with bioadditives supporting the development of mycorrhizal symbions (seaweed extracts, natural humates, natural source of nitrogen and magnesium)

B — Liquid medium with sterile grown mycelium of several ericoid mycorrhizal fungi (*Hymenoscyphus* spp., *Oidiodendron* spp., and others)

C — powdered gel

Ectovit: ectomycorrhizal inoculum contains a dray component A, a liquid component B and a powdered gel C.

A — perlit-peat carrier with the spores of two ectomycorrhizal fungi *Sclerodema* spp., *Pisolithus* spp., and bioadditives supporting the development of mycorrhizal symbiosis (seaweed extracts, natural humates, natural sources of nitrogen and magnesium)

B — Liquid medium with sterile grown mycelium of another three ectomycorrhizal fungi (*Lactarius* spp., *Hebeloma* spp., *Laccaria* spp. and others)

C — powdered gel

Treatment with preparation Ectovit and Rhodovit was done by soaking cuttings in prepared solutions.

RESULTS AND DISCUSSION

In this experiment, the soil has the following morphological properties:

A_p (0—40 cm): brown sandy loam, spheroid structure, calcareous, humus, rich with roots, gradually transits into,

C (40—90 cm): gray-yellow sandy loam, structureless, calcareous, with ingrown roots, gradually transits into,

G_{so} (90—180 cm): marmorized loamy sand, affected by intense oxidation-reduction processes. Gray-blue gley continues under it and clearly transits into

G_r (deeper than 180 cm): gray blue gley

Based on the morphological description, it can be seen that the sample plot soil is humofluvisol on the level of the type. The soil has a clearly differentiated humus-accumulative horizon up to 40 cm thick, and a sandy loamy texture. Under it, the C horizon continues also with a sandy loamy texture alternating with the G horizon, in which the zone of oxidation-reduction processes and the zone of iron reduction are clearly differentiated. The stratigraphic composition of the soil is A_p — C — G_{so} — G_r.

The soil textural class varies between loamy sand and sandy loam. Content of the silt+clay fraction in this soil varies between 21.2 and 34.8%. In the distribution of particle sizes, fine sand content is the most important, and its share is from 64.4 to 78.2%. The fraction of coarse sand has the lowest content, from 0.5 to 0.8%. This soil contains from 15.2 to 23.2% silt, and from 6.0 to 11.6% colloid clay.

Tab. 1. — Physical characteristics of soils

Horizon	Depth	Granulometric composition %						Texture class
		> 0.2	0.2—0.02	0.02—0.002	< 0.002	Total > 0.02	Total < 0.02	
	cm	mm	mm	mm	mm	mm	mm	
A_p	0—40	0.5	65.9	23.2	10.4	66.4	33.6	Sandy loam
C	40—90	0.8	64.4	23.2	11.6	65.2	34.8	Sandy loam
G_{so}	90—180	0.6	78.2	15.2	6.0	78.8	21.2	Loamy sand
III G_{so}	72—110	1.8	40.5	37.8	19.9	42.3	57.7	Clay

The content of humus is highest in the humus-accumulative horizon and amounts of 2.19%, decreasing uniformly with depth (Table 2). The content of carbonates is between 11.3 and 14.25%, so it can be claimed that this soil is a very calcareous soil. Accordingly, this soil has a highly alkaline reaction of the soil solution. As for the content of readily available nutrients, this soil is in the category of poorly to moderately supplied soils. Due to the low content of organic matter, a marker effect of carbonates is evident, which is expressed in the form of increased alkalinity.

Tab. 2. — Chemical characteristics of soils

Horizon	Depth (cm)	pH in H_2O	Humus (%)	$CaCO_3$ (%)	Nitrogen (%)	P_2O_5 (mg/100 g soil)	K_2O (mg/100 g soil)
A_p	0—40	8.68	2.19	12.1	0.077	7.8	7.4
C	40—90	9.01	0.53	14.2	0.030	7.2	6.4
G_{so}	90—180	9.44	0.95	11.3	0.030	5.6	4.6

The survival percentage in the control amounted to 77—94% (Table 3). The percentage of rooted cutting survival compared to the control was higher only for the clone *Populus x euramericana* M-1. Other clones did not show a higher percentage of rooting under the treatment with mycorrhized cuttings.

Tab. 3. — Survival percentage (%), diameter (d_s-mm) and height (h_s-m) of rooted cuttings

Clone	Control			Rhodovit			Ectovit			Ectovit+Rhodovit		
	%	d_s	h_s	%	d_s	h_s	%	d_s	h_s	%	d_s	h_s
M-1	84	12.80	1.69	91	18.5	2.55	97	19.08	2.52	94	19.33	2.57
B-229	94	19.78	2.30	94	20.33	2.76	93	23.4	2.72	100	19.65	2.40
PE19/66	77	17.64	2.01	74	21.89	2.51	71	20.58	2.40	78	20.31	2.38
182/81	89	15.95	2.16	84	17.90	2.42	73	16.80	2.27	71	14.45	1.93
B-81	89	21.00	2.28	84	22.89	2.47	79	20.95	2.31	49	17.95	1.91

Mean height was higher for all study clones treated by Rhodovit and Ectovit (Table 3). Mean height in the control ranged between 1.69 and 2.30 m. After Rhodovit treatment, the height was from 2.42 to 2.76m, and after Ectovit, it was from 2.31 to 2.72 m. After the combined treatment of the clones *Populus deltoides* 182/81 and B-81 by the above two preparations, mean height was lower than in the control. The results have shown the positive influence of application of mycorrhizal inoculum on diameter.

The statistically significant difference in mean heights compared to the control was determined after the application of the preparations of microbiological fertilisers for the clones *Populus x euramericana* M-1 and *Populus deltoides* PE19/66 (Table 4). The statistically significant difference compared to the control was determined for the clone *Populus deltoides* B-229 for the preparation Ectovit, while for the clone *Populus deltoides* 182/81, the statistically significant difference compared to the control was determined for Rhodovit.

Tab. 4. — Mean heights, analysis of variance and LSD test of poplar rooted cutting

Populus x euramericana M-1		Ectovit+Rhodovit	2.57 a
		Rhodovit	2,55 a
		Ectovit	2,52 a
	Sign***	Control	1.69 b
Populus deltoides B-229		Ectovit	2.73 a
		Rhodovit	2.49 b
		Ectovit+Rhodovit	2.40 b
	Sign***	Control	2.30 b

Populus deltoides PE 19/66		Rhodovit	2.51 a
		Ectovit	2.40 a
		Ectovit+Rhodovit	2.38 a
	Sign ***	Control	2.01 b
Populus deltoides 182/81		Rhodovit	2.42 a
		Ectovit	2.27 ab
		Control	2.16 b
	Sign ***	Ectovit+Rhodovit	1.93 c
Populus deltoides B-81		Rhodovit	2.47 a
		Ectovit	2.31 a
		Control	2.28 a
	Sign***	Ectovit+Rhodovit	1.91 b

The application of microbiological preparations increased the percentage of plants of extra class and I class (Table 5). The highest effect of the application was determined for the clone *Populus x euramericana* M-1, and the lowest variation was determined for *Populus deltoides* B-229.

Tab. 5. — Percentage of rooted cuttings (first and extra class, height up 2.5 m)

Clone	Control	Rhodovit	Ectovit	Rhodovit + Ectovit
P. x euramereicana M-1	11.00	58.24	51.37	69.14
Populus deltoides B-229	40.55	51.72	60.09	52.00
P. deltoides PE 19/66	25.97	60.81	49.30	55.12
Populus deltoides 182/81	30.34	52.38	30.14	19.72
Populus deltoides B-81	47.19	47.61	51.89	28.97

The total number of produced plants of the first class and extra class (height above 2.5 m) in the control ranged from 4,583 to 19,662 (Table 6).

Tab. 6. — Amount of rooted cuttings for normal planting, per unit area

Clone	Control	Rhodovit	Ectovit	Rhodovit + Ectovit
P. x euramereicana M-1	4583	21467	21404	28808
Populus deltoides B-229	16896	21550	25037	21667
P. deltoides PE 19/66	10820	25337	20541	22997
Populus deltoides 182/81	12642	21825	12559	8213
Populus deltoides B-81	19662	19837	21621	12070

The highest effect of preparation application on the nursery stock production was found for the clone *P. x euramericana* M-1, and then for the clone *Populus deltoides* PE19/66. Compared to the control, the clone *Populus x euramericana* M-1 produced more rooted cuttings of the first class and extra classes, from 16,884 to 24,225 respectively. For the clone *Populus deltoides* PE 19/66 the difference was between 9,721 and 14,517 rooted cuttings. The differences for the other analysed clones were lower. The results have shown the positive influence of application of mycorrhizal inoculum on the amount of rooted cuttings for normal planting.

Results of application of mycorrhizal inoculum were compared with results in experiment with planting space 1,3 x 0,2 m (Andrašev et al.,

2002). Number of rooted cuttings of clone *Populus x euramericana* were in range 21404 to 28808 in treatments with mycorrhizal inoculum, and in planting space 1,30 x 0,2 m were 17094 rooted cuttings (Andrašev et al., 2002). It means that number of rooted cuttings of clone *Populus x euramericana* M-1 were higher for 4304 to 11714 (respectively) in treatments with mycorrhiza. Number of rooted cuttings of clone *Populus deltoides* PE 19/66 were 20086 (Andrašev et al., 2002). In comparison to our results (table 6.) number of rooted cuttings of clone *Populus deltoides* PE 19/66 were higher in range of 455 to 4951 in treatments with mycorrhiza. This results show possibilities of improvement in production of poplar rooted cuttings with mycorrhiza.

CONCLUSION

This paper analyses the effect of microbiological preparations Rhodovit and Ectovit on the production of poplar planting stock for normal planting.

The percentage of rooting after the application of the above preparations increased only for the clone *Populus x euramericana* M-1.

Mean seedling height was higher for all the study clones after the application of Rhodovit and Ectovit. The statistically significant difference compared to the control was determined after the application of the preparations of microbiological fertilisers for the clones *Populus x euramericana* M-1 and *Populus deltoides* PE19/66.

The highest effect of the preparations on the nursery stock production was determined for the clone *P. x euramericana* M-1, and then for the clone *Populus deltoides* PE19/66.

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ЕФЕКТИ ПРИМЕНЕ МИКОРИЗА НА ПРОИЗВОДЊУ САДНОГ МАТЕРИЈАЛА ВИСОКОПРОДУКТИВНИХ СОРТИ ТОПОЛА

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Резиме

На интензивну производњу квалитетног садног материјала високопродуктивних клонских сорти топола утиче обезбеђеност хранљивим материјама у земљишту. Ово се јавља као последица дугогодишњег коришћења земљишта. Различитим методама ђубрења и прихрањивања постиже се надомештање недостајућих хранљивих материја. Осим наведеног се у производњи садног материјала високопродуктивних клонских сорти топола истражује и могућност примене биолошких средстава у циљу производње адекватног садног материјала.

У раду су приказани ефекти микоризираних резница препаратима Ectovit, Rhodovit и комбинацијом Ectovit и Rhodovit (препарати Symbio-m Ltd., Чешка Република). Микоризиране су резнице четири високопродуктивне клонске сорте топола *Populus deltoides* и једне сорте *Populus x euramericana*.

Резултати истраживања упућују на чињеницу да су микоризиране резнице имале исти или бољи пријем код свих истраживаних клонова у односу на контролу. Применом препарата Ectovit и Rhodovit је у просеку добијен садни материјал прве класе код свих истраживаних клонова. Комбинацијом препарата Ectovit и Rhodovit у просеку је добијен садни материјал прве класе само код клона *Populus x euramericana*.

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PRELIMINARY INVESTIGATION ON THE EFFECTS OF BIOLOGICAL AND SYNTHETIC INSECTICIDES ON LARGE WHITE BUTTERFLY (*PIERIS BRASSICAE* L.) LARVAE

ABSTRACT: Control of cabbage pests is oriented towards the use of efficient but high-risk insecticides, some of them being endocrine disruptors. Biopesticides are more environment-friendly, operator- and consumers-safe, but they have low initial toxicity, low efficacy to advanced larval stages, and they require certain knowledge of pest and host biology. In our laboratory experiments we have investigated the effects of formulated synthetic pyrethroid cypermethrin (0.3 l/ha) and biological products — formulations based on *Bacillus thuringiensis* subsp. *kurstaki* (2 and 3/ha) and Spinosad (0.1 l/ha) — on large white butterfly (*Pieris brassicae* L.) larvae-instars 2, 3, 4 and 5. The effect of insecticides was inversely proportional to larval instars. Btk effect could be improved if tank-mixed with cypermethrin. The mixing of ready-made products allows a reduction 3 and 6 times compared with the recommended dose, still obtaining satisfactory results. Rate of leaf damage was reduced when tank mixtures were used. Use of two products in mixture would be of significance especially for control of advanced late instars late in season, when Btk action alone is insufficient. Spinosad was effective in inducing mortality and reducing leaf damage by all larval instars, therefore we assume that the dose could be reduced. Feeding rate and mortality are equally important parameters when assessing biopesticide efficacy. This strategy should also reduce the possibility of inducing resistance in pest population. It also tends to reduce the residues in commodities and is good solution in production of hygienic and health safe food.

KEY WORDS: *Pieris brassicae* L., biopesticides, *Bacillus thuringiensis* subsp. *kurstaki*, spinosad, cypermethrin, tank mix

INTRODUCTION

Cruciferae pests are a limiting factor in cabbage production. The monitoring of cabbage fields at Rimski Šančevi in 2005 showed high densities of large white butterfly (*Pieris brassicae* L.) populations, low densities of small

white butterfly (*P. rapae* L.) and diamond cabbage moth (*Plutella xylostella* L.), while cotton bollworm (*Helicoverpa armigera* Hbn.) and *Mamestra* and other noctuids were not registered. This situation was opposite to those registered in 2003 and 2004.

Bioinsecticides are distinguished for their favorable ecotoxicological traits and low initial toxicity. In organic insect management, formulations based on *Bacillus thuringiensis* and spinosad are officially permitted in Europe and USA (A n o n y m o u s, 2004 a; and 2004 b). Synthetic pyrethroids have high initial toxicity, but are less attractive from ecological point. Some pyrethroids have recently been suspected of being endocrine disruptors — ED.

The efficacy of biopesticides depends considerably on application timing. Aim of this investigation were (1) to assess the effect of insecticides from three different classes on mortality and leaf consumption rates of different caterpillar instars; (2) to determine which parameter is more important — mortality or leaf damage — when assessing the effectiveness of the three classes of insecticides.

MATERIAL AND METHODS

Pieris brassicae caterpillars were collected in a cabbage field at Rimski Šančevi. Laboratory experiments were conducted separately for larval instars 2 to 5. D-Stop, a *B. thuringiensis* var. *kurstaki* (Btk) — based insecticide, was applied at doses of 2 and 3 l/ha. Cipkord 20-EC, a cypermethrin formulation representing pyrethroids, was applied at the dose of 0.3 l/ha. The biological insecticide Laser KS (spinosad), formulated as SC with 24% of a.i. (spinosyns A and D), was used at the dose corresponding to 0.1 l/ha. Tank mixtures with reduced quantity of both formulations — D-Stop + Cipkord 20-EC — were tested at the rates 2+0.1, respectively, and 1+0.05 l/ha, respectively. Experimental solutions were based on water consumption of 400 l/ha.

Because of a heavy waxy cuticle of cabbage leaves, Sillwet L-77, a surfactant containing organosilicone trisiloxane, was added to each insecticide at the dose of 0.3 l/ha. Cabbage leaves were immersed in spray liquids and offered to larvae. The experiment was set up in four replications. Different numbers of caterpillars (12, 20, 15 and 5) depending on the instar (2, 3, 4 and 5, respectively) were used. During the experiment, temperature varied from 22 to 24°C, day/night light period was 16/8 h and relative air humidity was 80—85%. The larval mortality was determined after 24 and 48h, and leaf consumption after 48h.

RESULTS AND DISCUSSION

The results of mortality bioassay are presented in Figures 1 and 2, those of feeding bioassay in Figure 3.

Understanding caterpillars' field biology may provide some insight regarding the use of active ingredients in a cost-effective manner. The speed at which a particular active substance causes mortality through contact or inge-

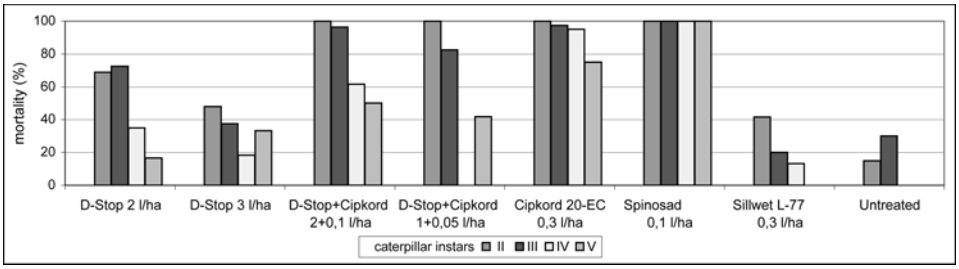

Fig. 1. — *Pieris brassicae* caterpillar mortality (%) after 24 h exposition

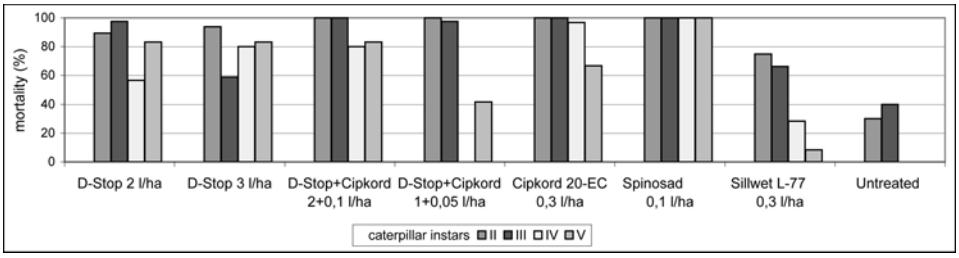

Fig. 2. — *Pieris brassicae* caterpillar mortality (%) after 48 h exposition

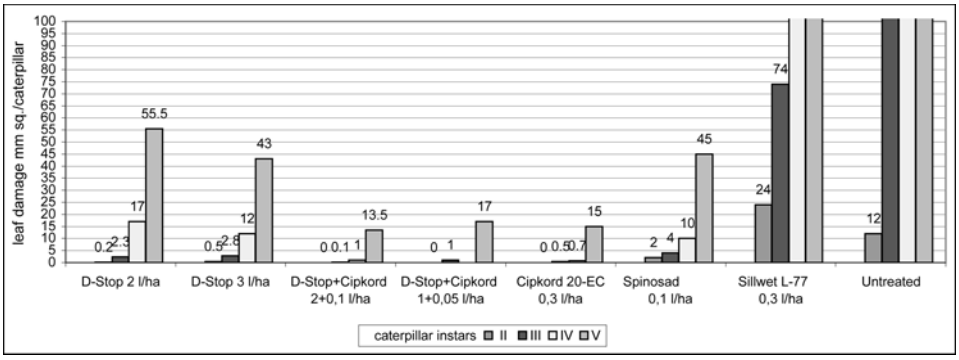

Fig. 3. — *Pieris brassicae* caterpillar cabbage leaf consumption [mm sq./caterpillar/48 h]

stion can be influenced by its mode of action and route of activity. The primary routes of activity differ among the products used in this study. Organophosphate (OP), carbamate and pyrethroids are primarily effective against 2nd larval instar due to numerous chemical and biological factors.

The mortality of caterpillars feeding on leaves treated with cypermethrin, *Btk.* and their mixtures depended on larval instar. Mortality in the experimental units with D-Stop exceeded 80% after 48h in contrast to cypermethrin, which exceeded 90% already after 24 h for the larvae in instars 2nd and 4th. In 5th instar, however, the mortality achieved with the two formulations hardly exceeded 60%. Even the reduced field recommended dose of cypermethrin is capable of providing satisfactory mortality when it was tank mixed with

D-Stop. As cypermethrin is an increased risk insecticide, suspected of being an ED, it is important that its dose in a mixture may be reduced without reducing its efficacy. In mixture with *Btk*, it was possible to reduce cypermethrin dose 3 and 6 times (100% mortality in 2nd instar and 80% mortality in 3rd instar, respectively). The effects on caterpillars in 4th and 5th instars varied from 60 to 80% and from 40 to 80%, respectively. Therefore, repeated treatments will be required for 4th and 5th instars for better control. Leaf damage (Figure 3) was reduced in all experimental units after application of tank mixed products (0—55.5 mm^2/caterpillar) compared with the untreated leaves (12—3100 mm^2/caterpillar). When used alone, Sillwet L-77 reduced the leaf consumption of instars 2, 3 and 5.

Opposite to the quick knock down effects of organophosphate and pyrethroids, *Bt* product first has to be ingested. This results in low initial toxicity. Because *Bt* is UV light and heat sensitive some growers find spraying at night will give the longest period of efficacy and best control and of cutworms (Anonymous, 2001).

When using *Bt*, one cannot expect fast, 100% control and must accept a certain level of crop damage. For enhancing the efficacy of *Bt* to lepidopterous larvae of older instars, some authors suggest adding pyrethroids. In our field experiments (Inđić et al., 2005) we proved that *Btk.* could be equally effective in larvae control as synthetic pyrethroids if used in two narrow-spaced application. The efficacy of tank mixture of this biopesticide and cypermethrin at reduced rate was lower, but still at the same significance level as when cypermethrin is used alone.

Products containing endotoxin *Btk.* should be used as alternative to chemical insecticides. This would reduce the danger of resistance appearance (Roush and Tingey, 1994). In our country, resistance towards this type of insecticides has not been registered. Their current use in agricultural production is small; therefore, we do not expect the appearance of resistance under field conditions soon. However, there are already numerous data in scientific literature on *H. armigera* sensitivity changes (Armes et al., 1994), as well as *Plutella xylotella* resistance to *Btk* since 1990 (Anonymous, 1999; Joia and Chawla, 1995).

Btk products are rather safe for operators and consumers. In our country, the *Btk* post-harvest interval (PHI) for cabbage for pickling is restricted to 21 days (Mitić, 2004). A similar period has been set in Croatia (Maceljski, 2002). In our country, the re-entry interval has not been determined. In Russia, PHI for cabbage is 5 days (Anonimus, 2004), in Hungary one day (Ocskó, 2006). In spite of environmental compatibility, *Btk* bioinsecticides are rarely used in cabbage production. Kandibin (cit. Glez et al., 2002) explains the low use of biopesticides in general with scant information of experts and agriculture producers about ecological advantages, poor marketing strategy and low production volume — insufficient to meet the needs of cabbage growers.

Volovik and Glez (cit. Glez and Čerkašin, 2002) proved the possibility of tank mixing the biological product *Bt* with synthetic pesticides. They controlled a high-density population of Colorado potato beetle with tank

mixtures of *Bt* and Fastac EC (alpha-cypermethrin) or *Bt* and esfenvalerate (97% mortality). *Pieris rapae* was more efficiently controlled when *Btk* was used tank mixed with lambda-cyhalothrin (Inđić et al., 2003). Biopesticide use in tank mix is restricted, due to the sensitivity of living organisms comprising *Bt* to active substances or solvent content in partner product. *Btk* is compatible with a number of insecticides, acaricides, fungicides and growth regulators. *Bt* + pyrethrin are formulated as ready mix (Tomlin, 1997). *Bt* products should not be mixed with alkali products, captan, folpet, oils (Mitić, 2004), or with copper-based products. Formulated product is not compatible with azinfos-ethyl, captafol, demeton-S-methyl, dimethoat, dinocap, isoprocarb, phenthoate, phosalon, propoxur, tetrachlorvinphos, alkali compounds (Bordeaux mixture), or under certain conditions with foliar fertilizers (Tomlin, 2000).

At the dose used, Spinosad induced high mortality of all larval instars already after 24 hrs and it probably could be used at a lower rate for the control of this pest. To enhance the efficacy on 5th instar, some authors suggest adding pyrethroids to Spinosad for late-season control. Adding cypermethrin to biological products is justified when older instars predominate in a treated population.

Spinosad acts as a neurotoxicant and its route of activity is translaminar. Some authors have estimated that it takes 1—2 days to achieve > 90% mortality of small larvae (< 5 mm) and 2 days for large larvae (> 10 mm) (Palumbo, 1999). In our experiment, the initial toxicity and mortality achieved were high probably due to the high rates used.

Spinosad's mode of action is invariably toxic for *P. brassicae* at the rates applied. Routes of activity involve both ingestion and contact activity (Anonymous, 2004). Moving into and across the leaf tissue, toxin reservoirs are formed on and within the treated foliage. This exposes the larvae to much greater amounts of the active ingredient and their chances for intoxication become much higher. The rate of foliage consumption is greater for old, large larvae, which potentially consume larger amounts of the active ingredient.

For effective control, the optimum timing of Spinosad treatment should be directed primarily at the newly hatched larvae, although large larvae are easily controlled too. The 3rd instar larvae consume large amounts of foliage (Anonymous, 1999). Delay in control increases the rate of plant injury proportionally. As far as enhancement of efficacy is concerned, some authors have suggested adding pyrethroids to Spinosad for late-season control and to *Bt* for midseason- and late-season control.

Wetting agents, spreaders and stickers are typically toxic to biocontrol agents. In our experiments, there was no reduction in mortality or adverse effect on feeding on leaves as a consequence to D-Stop mixing with Sillwet L-77 (0.3 l/ha). This commercially formulated trisiloxane affected 2nd instar larvae, inducing mortality after 24h and 48h (40—70%) and 3rd instar larvae (65%).

Lepidoptera ability to develop resistance to organochlorine, organophosphate, carbamate and pyrethroid classes of insecticides is known (Wolfensbarger cit. Gore & Adamczyk, 2004). Therefore, different strategies are applied to postponing the appearance of resistance.

To sustain product efficacy it is of importance for management of lepidopterous larvae during the growing season to avoid an overuse of a single product, rotate chemistries through the season, and not to apply than at the rates below the labeled ones. This strategy will optimize the control of the lepidopterous larval complex and maximize the longevity of new formulations.

CONCLUSION

Leafy and cole vegetables are endangered by a number of harmful insects. Due to frequent insecticide treatments, risk of residues is always present. As raw vegetables are part of regular diet, it is recommended to use bioinsecticides with low risk for consumers and operators.

The mortality induced by *Btk* is inversely proportional to larval instar.

Btk effect could be improved if tank mixed with cypermethrine.

Cypermethrin, potential endocrine disruptor, could be used at reduced rates if tank mixed with *Btk* and still produce satisfactory results.

Spinosad, effective on all instars could be used at rates below 0.1 l/ha, which remains to be proved experimentally.

Feeding rate and mortality are equally important when assessing efficacy of biopesticides.

Btk could resolve the problem of cabbage protection in integrated and organic production.

Organosilicone wetter Sillwett L-77 could be used on cabbage, as it did not affect adversely the effect of biological products.

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ПРЕЛИМИНАРНА ИСПИТИВАЊА ЕФЕКТА БИОЛОШКИХ И СИНТЕТИЧКИХ ИНСЕКТИЦИДА НА ЛАРВЕ ВЕЛИКОГ КУПУСАРА (*PIERIS BRASSICAE* L.)

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Резиме

Сузбијање штеточина купуса оријентисано је на примену ефикасних али високо ризичних инсектицида од којих су неки и ендокрини дисруптори. Биопестициди су мање опасни за животну средину, безбеднији за оператера и конзументе, али ниже иницијалне токсичности и ниске ефикасности на гусенице у касним узрастима и захтевају знање о биологији штеточине и домаћина.

У нашим лабораторијским испитивањима упоредили смо ефекат синтетског пиретроида циперметрина (0,3 l/ha) и биолошких препарата на бази *Bacillus thu-*

ringiensis subsp. *kurstaki* (*Btk*) (2 и 3 l/ha) и препарата на бази спиносада (0,1 l/ha) на гусенице великог купусара *Pieris brassicae* L. у 2, 3, 4. и 5. узрасту. Ефекат препарата је био обрнуто пропорционалан узрасту гусеница. Ефекат *Btk* препарата може бити побољшан мешањем с препаратом на бази циперметрина у смањеној количини 3 и 6 пута у односу на препоручену, а да се притом постигну задовољавајући резултати. Мешање синтетског пиретроида и биолошког препарата је нарочито важно у касним узрасним развојним фазама када је дејство *Btk* недовољно. Биолошки препарат на бази спинпсада је био врло ефикасан за ларве свих узраста, те је претпоставка да се количина примене може и смањити. Примена мешавине интензивира обуставу исхране. Интензитет исхране и морталитет су једнако важни параметри у процени ефиканости биопестицида. Оваква примена треба да спречи рану појаву резистентности штеточина у популацији, да доведе до смањења резидуа у намирницама и добро је решење у производњи здравствено безбедне хране.

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ETIOPATHOGENETIC CONSIDERATION AND DEFINISHON OF THE CLINICAL MANIFESTATION OF EROSIVE DENTAL DEFECTS

ABSTRACT: Dental defects of errosive nature are defined as irreversible losses of dental tissue, caused by long lasting and repeated action of acids that disolve top layer of hydroxyapatite and fluorideapatites cristal structure, under assumption that agressive factor is not of bacterial nature. Acids that cause changes on teeth according to their origin are gastric, dietetic, or they are of environmental origin. Current way of life, as well as nutritional habits create potentially dangerous conditions for the hard dental tissue, for prevention of mineralization process causes defects of oral system homeostasis. Defects occur on primary teeth, as well as on permanent teeth. However, this happens once and a half time more frequently on primary teeth due to the weaker primary maturation. In initial phases, changes are localized in enamel and by their development the bottom locates in dentine. Defects appear as smooth, shainy, round concavities on caries immune positions, or as cupping of occlusal surfaces. The depth of an eroded lesion consists of the depth of the crater plus the depth of tissue demineralisation at the base of the lesion. Early verification of the etiological factor, together with good knowledge of the manifested shape change has influence to the prevention of the crown of tooth loss, complete occlusion, mastication and speech.

KEY WORDS: dental erosions, diagnosis, etiology, pathogenesis

INTRODUCTION

Enamel, highly mineralized crystal structure, the densest and the hardest biological tissue of the human organism after teeth eruption remains exposed to the aggressive and biologically active environment of the oral ecosystem. Enamel minerals have crystal structure, but hydroxyapatite is not stoichimetric; it does not have fixed element proportion, for relation of calcium and phosphates is always lower than the assumed one in theoretic formula. Along with calcium, phosphorous, carbonate and fluoride enamel contains over forty other elements. Presence of carbonate and magnesium impurities in hydroxyapatites

petals increases its solubility in acid environment, while fluoride and strontium make enamel more stable.

Enamel content is not a homogenous category. Changes are evident toward referent surface, as well as toward observed depth. Enamel top layers contain significantly higher fluoride concentration than those on enamel and dental enamel junction, while remaining elements such as magnesium, carbonates, water and organic compounds are in an inverse proportion. Cervical enamel has significantly lower mineral density from one that is on occlusal surface of a tooth, or on incisal edge (Sturdevant et al., 1995, Vulović et al., 2002). Mineralization level of primary teeth is connected to the period of teeth eruption into oral cavity. During posteruptive maturation, the duration of which is one to two years, into enamel top layers about 10% more minerals are incorporated.

Enamel layer, tartar and saliva are specific and unique biosystem. In cases of neutral saliva pH values, there is equilibrium in mineral quantity that goes into and out of the enamel. If pH values lower under 5.5, or more, calcification is more intensive from decalcification, and whole activity is determined above all by saliva content, such as calcium, phosphates, fluoride, but also by saliva buffer capacity, protein quantity and quantity of stimulated and unstimulated saliva (Leach, 1986).

By local fluoride application we can influence to enamel "solubility". If its concentration is higher than 100 ppm, changes are manifested to the depth of 0.1—0.2 mm. Saliva free calcium react with fluoride ions, and calcium fluoride that deposits to unmineralised enamel is created on a teeth top layer. Fluoride can be considered anticariogenic, but tooth wear factor as well.

PATHOGENESIS OF THE EROSIVE CHANGES

Inadequate function of saliva puffer system, as well as eventual existence of xerostomia together with long lasting and frequently repeated incorporation or existence of different kinds of acids in oral microenvironment causes lowering of saliva pH values and occurence of deficient balans between demineralization and mineralization. Using interprismatic and intercrystal layers hydrogen ions penetrate enamel and cause solution of hydroxyapatite that cases reduction of the crystal size itself (Vulović et al., 2002). Ultra structural studies suggest that erosive lesions are seen in prismatic enamel as characteristic demineralization patterns where either the prism cores or interprismatic areas dissolve, leading to a honeycomb structure. In aprismatic enamel the pattern of dissolution is more irregular and areas with various degrees of mineral loss are seen side by side. In dentine, the first area to be affected is peritubular dentine. With lesion progression, the dentinal tubules become enlarged, but finally disruption is seen also in the intertubular area. According to current knowledge, there are no differences in lesion shape, size or depth, depending upon kind of acid that caused the defect occurence (Meurman, Ten Cate, 1996).

The depth of an eroded lesion consists of the depth of the crater plus the depth of tissue demineralization at the base of the lesion that is microradiographic detectable (A m a e c h i, H i g h a m, 2005).

Study of erosive changes occurence led to the conclusion that clinical manifestation of the erosive defect is not only result of erosive agents action, but also consequence of the combined action of demineralization of the tooth structure by erosive agents and abrasive action of the surrounding oral soft tissue, as well as by action of abrasive food during mastication and by use of abrasive foreign substances. Abrasive activity of the specified soft tissue structures determines defect location (A m a e c h i et al., 2003, I m f e l d, 1994).

Lesion expansion is a cause of cumulative process caused by:

— Frequency and time period of exposure to acids;
— Maintaining of oral hygiene basic principles;
— Individual sensitivity.

Dental top layer exposed to the action of acids undergoes process of demineralization making the enamel itself, as well as dentin more fragile to the abrasive factors, especially if the exposure to the abrasive force is performed directly after acid intake without previous leveling of pH values by salivary buffer capacity. Need for shortening of the time period from the acid intake to the moment of teeth brushing that lasts thirty minutes is imposed. Attrition of incisal edges and abfractions on cement enamel junction can increase defect caused by erosion, making by it diagnosis of the defect cause more difficult.

Dental erosions are also diagnosed in primary teeth. Attacked structures, enamel and dentin are of significantly less thickness, weaker mineralization (W i l s o n, B e y m a n, 1989), and enamel porous levels increase (F e j e r s k o v et al., 1987) than in permanent teeth. Weaker mineralisation and big pulp cavity cause fast "wearing out" of the dental tissue and formation of larger defects and earlier occurence of dental hypersensitivity, causing also opening of the pulp cavity accompanied by pulp development (S h a w, O'S a l l i v a n, 2000). Fast development of the process causes complete loss of the crown of a tooth and dental organ in whole, which can disturb bite, mastication and speech. Dental erosions in primary teeth can be predictors of the higher risk of erosive dental defects in permanent teeth. *In vitro* conditions they exhibit once and a half time higher susceptibility to erosion than permanent teeth.

ETIOLOGY AND CLASSIFICATION

Dental erosions are defined as irreversible loss of the hard dental tissue caused by long lasting and repeated action of acids that dissolve top layer of the hydroxyapatite and fluoroapatites crystal structure, even when aggressive cause is not cased by bacteria (P i n t b o r g, 1970, E c c l e s, 1974, K i d d et al., 2003, S u t a l o, N j e m i r o v s k i, 1981). Occurence of the saliva low pH value is cased by different kinds of acids, according to their chemical composition, chloral hydrogen acid, ascorbic acid, lemon acid: phosphorous acid, milk acid, but also gastric juices, remedies with the vitamin C, fruit, and car-

bonated beverages. Classification of dental erosions was performed according to the origin of acids that can cause defect occurence to the following ones:

— Endogenous;
— Exogenous;
— Idiopathic.

Endogenous erosions — develop as a consequence of penetration of gastric chlorine hydrogen acid into oral cavity in such a high quantity, often long lasting, that saliva buffer system proved unsuccessful (S c h e n t z e l, 1996). Sometimes gastric acid has pH serial value that starts from one, reaches oral cavity through gastroesophageal reflux or by chronic vomiting (Figure 1).

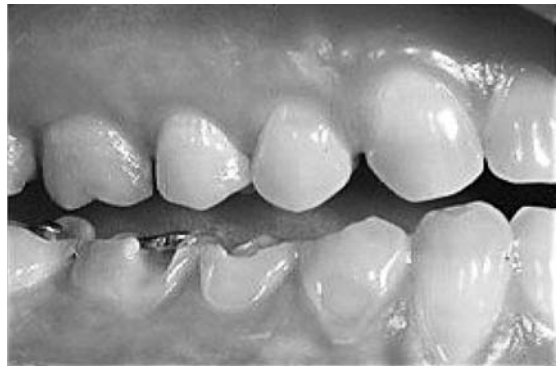

Fig. 1. — Endogenous erosions

The gastroesophageal reflux can occur in the aspect of a disease or it can be provoked. It occurs as a disease in a case of increased abdominal pressure, in a case of inadequate relaxation of lower esophageal sphincter, or in cases of increased production of gastric juices. Exaggerated consuming of a chocolate, coffee, peppermint, spices, as well as fat food causes provoked regurgitation that, when frequently repeated, can be a cause of such dental defects.

Chronicle vomiting in cases of nutritional disorders, such as anorexia, bulimia neurosis, rumination, chemotherapy, alcoholism, and even gravity, peptic ulcers, as well as gastritis place chlorine hydrogen acid into oral cavity and enable its negative action to the surrounding tissue structures, i. e. negative effect of life habits and style is exhibited. Erosions emerged as a consequence of xerostomia make special category. According to the origin of the acids themselves, they could be classified into mixed changes, for the presence of endogenous acid, as well as many exogenous acids introduced with food often coexist. At the same time there exist reduced quantity of saliva and all its elements that make buffer capacity. Some medical diseases and conditions cause occurence of xerostomia:

— Endocrine diseases — diabetes, hyperthyroidism;
— Autoimmune diseases — HIV infections, Systemic lupus, Rheumatic arthritis, Sjögren's syndrome;

— Medicine intake — vitamin C, antipsychotics, antidepressants, appetite suppressants, diuretics, sedatives, hypnotics, antihistamines, and antihypertensives (A m a e c h i et al., 2005, M a r o n, 1996, H a y s et al., 1992).

Exogenous erosions — develop under the action of acids that reach oral cavity from environment by dietetic pathway, or in certain environments as air pollutants (Z e r o, 1996). Dietetic acid source can be food such as: fruit (lemon, apple, plum), tomato, mustard, ketchup, carbonated soft drinks (Coca Cola, carbonated water), squeezed juices (orange, grape, kiwi), alcohol drinks (vine, bear). Several studies have proved that consummation frequency is of the identical importance as the acid level (M i l l w a r d et al., 1994), as well as the method of intake for it is better to take drinks by straw, than to drink directly from glass, and the drink should be kept in mouth as shortly as possible. Time period of teeth brushing is also important, for after food intake teeth brushing should be postponed to thirty minutes from the last intake of acid drink in order to allow buffer capacity of saliva to increase pH value and reduce abrasive action of toothpaste and a brush. This all has significant role in determination of defect size (G a n g a r a et al., 1999).

Consummation of cocaine and ecstasy is related to intake of greater quantity of acid drinks due to the occurence of dehydration and hyposalivation that cause erosive dental changes (D u x b u r y, 1993).

Dental erosions can also be classified into category of professional diseases. Persons who test vine or carbonated beverages on regular basis, as well as professional swimmers can detect this type of the defect on their teeth. (M a n d e l, 2005). Evaporation of industrial acids from battery plants, sanitary cleaning solutions, crystal glass are also dental erosion causers (M i l o š e v i ć, 1998).

Idiopathic erosions — are erosive changes whose existence can not be explained by any of the currently known causer (S u t a l o, N j e m i r o v s k i, 1981).

Current way of life and nutritional habits create conditions that are dangerous for hard dental tissue, for they prevent mechanism of enamel mineralization and in this way disturb homeostasis of the oral system (S m i t h, R o b b, 1996). This is especially expressed in childhood. Therefore, due to inadequately large quantity of juices, carbonated beverages and fruit, this kind of disease is considered typical for the standard of living. In age of adulthood it represents part of the clinical picture of psychosomatic diseases and the cause of certain therapy procedures. In sporadic cases it can also be considered as a professional disease.

According to the clinical picture changes on dental top layers are classified to:

— Superfficial and profound
— Localized and generalized (Z e r o 1996, I m f e l d, 1996);
— Manifested and latent;
— Eccles's and Jenkin's scale for erosive changes in cavity depth 0—3,

And according to the attacked surface they can be localized to:

— Palatal and occlusal surface of the upper jaw teeth;
— Buccal and occlusal surface of the lower jaw teeth.

CLINICAL PICTURE

Due to the lack of any method or procedure that could be used for early detection and quantification of the change, diagnostic of dental erosions is problematic. In early stadium, by erosion changed surface is smooth, shine, without macroscopic defects (A m a e c h i, H i g h a m, 2005), but it can be dim, without expressed colored lines, or clear frontiers toward unchanged part of the dental tissue.

If the defect is localized at inicisal edge, the incisial groove at the dentin is formed (Figure 2). However, if endogenous source is an acid, defect occurs at palatinal surface of the incisors, that becomes smooth, shine and hard. Vestibular diameter is also reduced making inicisal edge thinner and transparent and gingival region existence of an enamel collar (Figure 3).

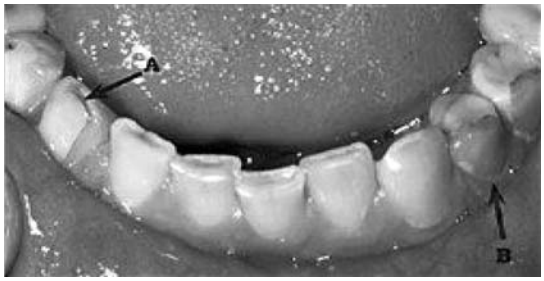

Fig. 2. — Incisal grooving and broad concavities

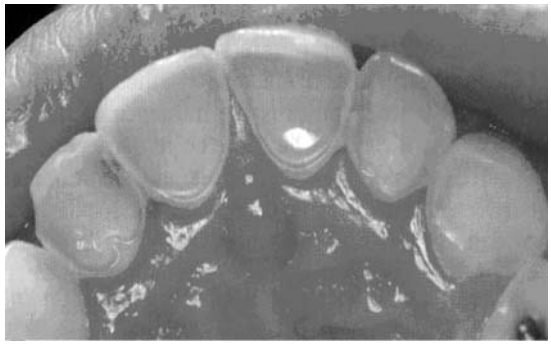

Fig. 3. — Endogenous erosion-enamel collar

At vestibular surface changes are manifested as broad concavities (Figure 4), and at occlusal surface as cup-like recesses (Figure 5). Amalgam fillings on such teeth appear as grown out, i. e. they occur above dental structure (Figure 6).

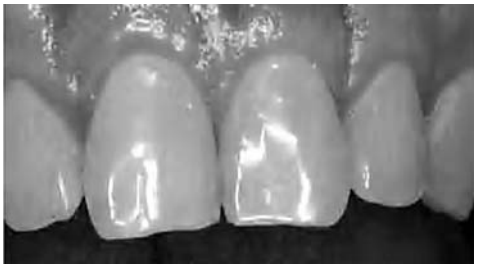

Fig. 4. — Broad concavities

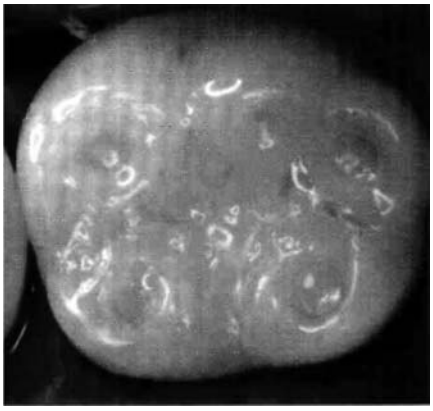

Fig. 5. — Capping of occlusal surfaces

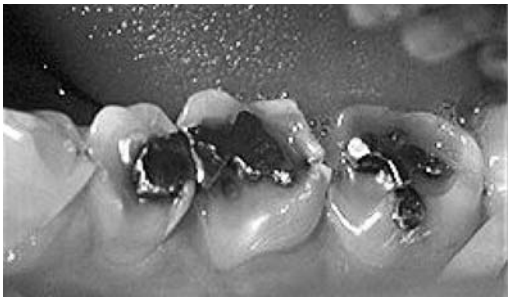

Fig. 6. — Raised amalgam restorations

Difficulties in diagnostics of erosive changes increase significantly at primary teeth (S h a w, O'S u l l i v a n, 2000). Enamel and dentin are thinner, less mineralized and porous, and acids aggressive activity appears even more, i. e. primary teeth are more susceptible to erosive changes. Initial phases are hard to detect. These changes on childrens' teeth are most frequently localized at occlusal surfaces of molars and inicisal surfaces that result in morphology loss (Figure 7), occurence of the dentine hypersensivity, as well as complete loss of the crown of the teeth, the pulpites, and premature extraction of the

primary teeth with all resulted consequences. Dental defects at primary teeth must always be observed from cumulative multi factor aspect. Attrition of incisal edges in primary dentition is frequent during exfoliation and it is than very hard to estimate cause of the change.

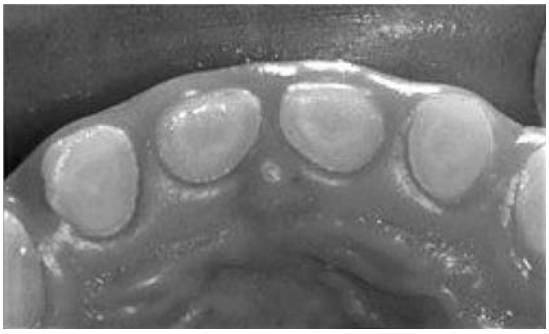

Fig. 7. — Loss of surface characteristics

Diagnostics of dental erosions requires serious, objective examination, analysis and evaluation. Well made questionnaire with precisely defined questions in regard to etiology is necessary. Saliva analysis related to determination of stimulated and unstimulated saliva quantities, extent of calcium and phosphates, buffer capacity, urea quantity is also of the highest importance.

After established diagnosis, progradation dynamic is necessary to be monitored. For that purpose silica index, dental erosion index and study models according to Wickens and taking a photograph are used (G a n d a r a et al., 1999).

PREVALENCE

At the beginning of the 19th century, the first data on existence of this disease were registered (M a h o n e y, K i l p a t r i c, 2003). It occurs in each life time, and it is distributed evenly between sexes. In child age it is considered a disease of living standard (B a r d s l a y et al., 2004), for it is a consequence of intake of great quantities of carbonated beverages, as well as juices and fruits.

Changes are more frequently located at upper jaw tooth, then lower one. The most common changes are on incisives, that are followed by changes on caninus and molar teeth. In lower jaw, defects are predominantly located on caninus and molars.

Regurgitation erosions appear at palatal surface of the upper front teeth, as well as at occlusal and buccal surface of lower lateral teeth. Dental erosions localized at vestibular surface of front teeth with hole-like defects are determined as professional diseases.

DIFFERENTIAL DIAGNOSIS

Erosions as causers of the health tooth tissue loss are a part of a detailed picture of dental defects to which attrition, abrasion and abfraction also belong.

Attrition — causes defects of dental tissue as well as of the established filling, and it is caused by teeth contact during mastification or parafunction. Occlusal surface are smooth, shiny, flat and hard, and at amalgam filling are observed shiny mark. Bottom of the defect can be located in enamel, as well as in dentin.

Abrasion — is caused by direct contact of teeth and foreign substance such as whitening toothpaste, antinicotin paste, sodiumbicarbonate. Changes localized in cervical region are always wider than deeper, and they are most frequently found on premolars and molars.

Abfraction — is characterized by dental tissue loss in cervical region caused by compression and pulling force that occurs during dental flexure. Changes are localized vestibular and they are wedge-shaped (G a n d a r a et al., 1999).

Based upon all stated, it can be concluded that dental erosions represent problem of human population. Many aspects require further research and more precise defining. This is especially important in relation to early diagnostics and quantification of changes for further longitudinal monitoring. Further studies are to be started by epidemiological studies for the purpose of more adequat prevalence, seriousness and spread of changes in different populations. Widening of knowledge from the aspect of pathophysiology is necessary, as well as detection of protective factors, preventive techniques and hemioterapeuticals. It is essential to develop preventive strategy, that should be followed by procedures for limitation of further damages. Protection of the remaining tissue with adequate reconstruction by contemporary dental materials is also of the highest importance. We are expected to fullfil a huge task.

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ЕТИОПАТОГЕНЕТСКО РАЗМАТРАЊЕ И ДЕФИНИСАЊЕ КЛИНИЧКЕ МАНИФЕСТАЦИЈЕ ЗУБНИХ ДЕФЕКАТА ЕРОЗИВНЕ ПРИРОДЕ

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Резиме

Зубни дефекти ерозивне природе дефинишу се као иреверзибилни губици зубних ткива, изазвани дуготрајним и понављаним дејством киселина које растварају површински слој кристалне структуре хидроксиапатита и флуороапатита а да агресивна нокса по свом пореклу није бактеријске природе. Киселине које изазивају промене на зубима по свом пореклу су гастричне, дијететске или потичу из животне средине. Данашњи стил живота као и нутрициона навике креирају стања која су потенцијално опасна за тврдо зубно ткиво јер се спречавањем механизма реминерализације ремети хомеостаза оралног система. Дефекти се јављају како у млечном тако и у сталном зубљу али један и по пут више у млечном због слабије примарне матурације. Промене су у иницијалним стадијумима локализоване у глеђи да би се прогредирањем дно лоцирало у дентин. Дефекти су у виду глатких, сјајних конкавитета овалног облика, на каријес имуним местима, или у виду шољастих удубљења на оклузалним површинама. Дубина дефекта настала денталном ерозијом одређена је дубином кратера којој је додата дубина ткивне деминерализације. Рана верификација етиолошког фактора уз ваљано познавање манифестног облика промене утиче на спречавање губитка комплетне крунице зуба што би довело до ремећења загризаја, мастикације и говора.

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THE INFLUENCE OF DIFFERENT LEVELS OF DIETARY SELENIUM ON ITS DISTRIBUTION IN THE ORGANS OF BROILER CHICKENS

ABSTRACT: The content of selenium in basic broiler diets was 50 ± 10, 100 ± 10, 150 ± 10 and 250 ± 10 μgkg^{-1}. At day 14, 28 and 42 tissue samples were collected. Dynamics and selenium deposits in blood, liver, muscle and heart were estimated in relation to the level of dietary selenium and age of broilers. When broilers were fed with diet containing 250 μgkg^{-1} Se, at day 42 the highest concentration of Se was in liver (570.8 ± 44.1 μgkg^{-1}), blood (430.3 ± 46.5 μgL^{-1}) and heart (237.5 ± 42.8 μgkg^{-1}). At day 14, increase of dietary selenium content from 50 μgkg^{-1} to 250 μgkg^{-1} was followed by decrease of selenium deposits in heart: when broilers were fed with basic diet containing 50 μgkg^{-1} Se measured content of Se in heart muscle was 49.8 ± 15.6 μgkg^{-1} (99.6%) while in broilers fed with basic diet containing 250 μgkg^{-1} Se measured content of Se in heart was 147.2 ± 33.4 μgkg^{-1} (58.9%), respectively. The point of saturation as well as maximal concentration of selenium in liver was reached in period between fourth (556.7 ± 40.6 μgkg^{-1}) and sixth (570.8 ± 44.1 μgkg^{-1}) week of age. Also, feeding with basic diet containing 250 μgkg^{-1} Se the selenium blood level reached 162.8 ± 28.9 μgL^{-1} already at day 14 that represent 65.1% of dietary selenium.

KEY WORDS: broilers, blood, heart muscle, liver, meat, selenium

INTRODUCTION

Selenium is one of the most significant essential microelement. It is present in all living systems and because of its high significance for the health of people and animals interest for selenium is recently rising. Many of the present knowledge is pointing out the significance of selenium such as: micro levels of selenium: in many tissues and organs which have high functional acti-

vity; selenium is showing simulative effect on development and growth, influences on reproductive ability and is a part of ante oxidative system (burst) of the organs of animals and people. Selenium also inhibits harmful effects of toxic elements (As, Cd, Hg, Pb).

Selenium is a cofactor in some enzyme's systems from which two are the most extensively studied: glutathione, where selenium is cofactor of glutathione peroxides acting as a antioxidant and destroying peroxide radicals which have harmful effect on cell membrane; and in the system of ioditreonin 5'-deiodinase which translates thyroxin in triiodtireonin and iodine is released (Jacques, 2001).

Research has shown that on the crops there are much more areas where ground does not contain enough selenium then there are the cases that it could be present at the toxic level (Mayland and James, 1989). Results of measurements of the level of selenium in the ground from the different localities in Serbia (Maksimovic et al., 1992) show that the level of whole or in water soluble selenium in the samples that were investigated is extremely low. This has led to the conclusion that the home substrate on which the crops are established is very poor in selenium. Results provided when selenium content was measured in different regions in Serbia (Mihailović et al., 1996) also show that plants in Serbia are poor in selenium.

Feed of plant origin and animal feed stuff in which there is low level of selenium are not suitable for successful agricultural production. Indirectly, over food chain, selenium reaches men so if there is insufficient supply of selenium that will reflects on plants or animals and also on humans.

Taking in to consideration the importance of selenium in the metabolism of animals and it its insufficiency as an important factor of risk in the majority of human population it is indispensable that appropriate intake of the optimal level of selenium for animals and humans is provided. To realize this goal there is a need of giving selenium in order to achieve appropriate concentration in the granule and vegetative mass of plants because amount of selenium in food from plant or animal origin depends of the concentration of the selenium in the crop of certain quality (Gissel et al., 1984).

Although laboratory investigations have been done the role of selenium in the biochemical process is still not understood well. Also, needs of certain domestic animals for selenium is not defined clearly and there is no general agreement about this issue. Adding adequate level of selenium in the animal feed will lead to the better production results, and over "functional" food of animal origin, mostly meet or eggs there will be possible to enhance ingestion of selenium in humans. This will prevent various diseases among which carcinogen or cardiovascular diseases are the most significant (Whanger, 2003).

When it was discovered that selenium is integral part of the enzyme glutathione peroxides Rotruck et al. (1973) proved its role in cell antioxidative metabolism. Assumption was that selenium is an integral part of the 30—50 proteins in the organism (selenoprotein) (Koerhle et al., 2000) that have a role in the function of triode hormones, immune system, in forming and viability of the sperm and the function of the prostate gland. As a consequence of the deficiency of selenium, in animals, there is a low immuno-

competence, high embryonic rate, lower fertility, and high rate of mortality in chickens in the first days of life (S u r a i, 2002).

Intensive growth of animals is often connected with various stresses. The major cause of the stress in animals could be divided in three major categories: nutritive stress (high amount of unsaturated acids, deficiency in vitamin E, selenium zinc or manganese, higher amount of irons, hypervitaminosis A or presence of various toxic elements); ambient (higher temperature or humidity, hyperoxia or irradiation) inside stress causes (bacteria and viral infections and allergic reaction-activity of macrophages). Laying eggs is stress for hens. Prolonged keeping in the hatchery cabinet, transportation from the hatchery to the farm and vaccination all presents stress and causes free radicals to evolve (S u r a i, 200).

Our goal was to investigate the influence of selenium the feed and its mass in tissue (whole blood, meat) and organs (liver and hurt) in broiler chickens.

MATERIAL AND METHODS

Experimental animals

Our experiments were performed *in vivo* on chickens of heavy hybrid Arbor Acres, which were treated from first to forty-two days of life. Feeding and water were given *ad libitum*. At the time when the experiments were starting chickens appear healthy and vital and they were raised according to the requirements for the hybrid.

Preparation of the feed for the chickens

In order to create low selenium intake through feed we tested the level of selenium in the plant feed from different regions (this included testing of corn, soybean meal and sunflower meal), (M i h a l j e v et al., 2003). During the mixing process we used only plant feed for which we discovered that selenium concentration was very low (5—10 μg Se/kg). As a source of selenium the specific amount of sodium selenite (Na_2SeO_3) was added. This way we achieved that feed mixture for the chickens have exactly the planed level of selenium, such as: < 10, 50 ± 10, 100 ± 10, 150 ± 10, 250 ± 10, μg Se/kg.

Experiment design

Five groups of chickens were formed in total. Each group consisted of 60 one-day old chickens of both sex (300 chickens). First group was fed with feed without additional selenium, second group was fed with the feed that was supplemented with 50 μg Se kg^{-1}, third group was fed with the feed containing 100 μg Se kg^{-1}, fourth group received feed with 150 μg Se kg^{-1} and fifth group was fed (given) with the feed containing 250 μg Se kg^{-1}. After day 14, 28, and 42, chickens were measured and blood samples were taken.

Preparing of samples and measurement of selenium content

Samples were prepared for the measurement by the method of wet digestion with automatic regulation of the temperature in the aluminum thermo block AC 300. The destruction of the collected samples of blood, meat and liver was done with HNO_3 and H_2O_2 at the temperature of 20°C per hour. Since only Se (IV) ion forms hydrides, reduction of Se (VI) was done with 8M HCl at t = 120°C (S t o e p p l e r, 1997 and A m p a r o, 1998). Hydrogen selenide is generated with sodium tetrahydroborate (0.6% in 0.5% NaOH) in the system VGA-76, and concentration of selenite is measured on atomic absorption spectrophotometer VARIAN SpectrAA-10. The conditions for the measurement were as follows (L u c i n d a B e a c h, 1992):

Hollow cathode lamp
λ = 196.0 nm
Lamp current = 10 mA
Slit = 1.0 nm
Delay time = 60 sec
Measurement time = 2.0 sec
Replicates = 3
Acetylene = 3.5 flow units/min
Air = 1.0 flow units/min
Inert gas = Nitrogen

Statistical analysis

For liver samples (n = 12), whole blood (n = 12), meat (n = 12), and heart muscles (n = 12) the results are given as a mean value ± standard deviation. The statistical significance between experimental groups was done by analysis of variance and by Student *t* test.

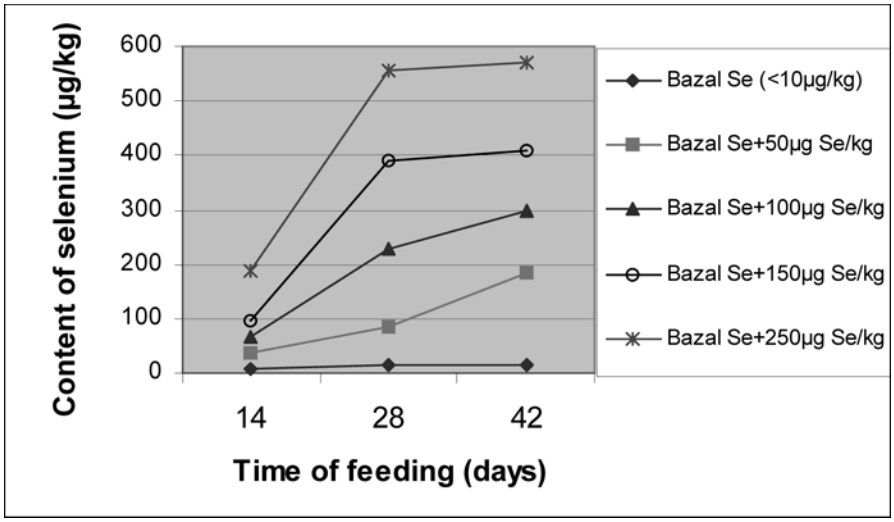
RESULTS

Results given in Table 1 lead to the conclusion that among granular feed the highest level of selenium in native sample was for soybean (42.7 μg/kg). The medium level of selenium in corn was 26.6 μg/Kg. In protein feed the averaged measured level of selenium in native samples of soybean meal was 92.7 μg/kg, and in sunflower meal 77.2 μg/kg.

Tab. 1. — Content of selenium in feed of plant origin

No.	Type of feed	Se [μg kg^{-1}]	Range [μg kg^{-1}]
1.	Corn (northern part — Vojvodina)	36.3 ± 5.4 n = 9	25.5—40.5
2.	Corn (central part)	25.3 ± 3.8 n = 7	20.8—31.1
3.	Corn (southern part)	18.1 ± 3.7 n = 6	12.5—22.2
4.	Soybean	42.7 ± 8.5 n = 6	30.3—50.1
5.	Soybean meal	92.7 ± 17.4 n = 7	75.8—122.4
6.	Sunflower meal	77.2 ± 15.1 n = 8	61.1—102.5

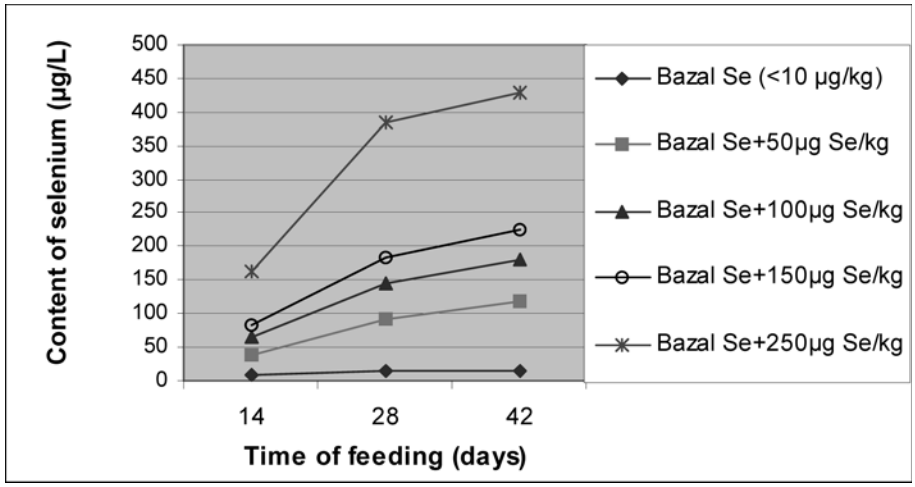
The values represent the mean ± SD



Dietary Se [µg/kg]	Content of selenium [µg kg^{-1}]		
	Time of feeding		
	14 day	**28 day**	**42 day**
Bazal (< 10)	9.0 ± 2.8	14.3 ± 3.4	13.1 ± 4.6
Bazal + 50	37.5 ± 16.9	85.9 ± 35.6	183.3 ± 22.7
Bazal + 100	67.8 ± 13.9	228.6 ± 42.1	297.6 ± 29.6
Bazal + 150	97.5 ± 24.1	388.5 ± 30.7	409.9 ± 36.5
Bazal + 250	187.9 ± 30.6	556.7 ± 40.6	570.8 ± 44.1

The values represent the mean ± SD

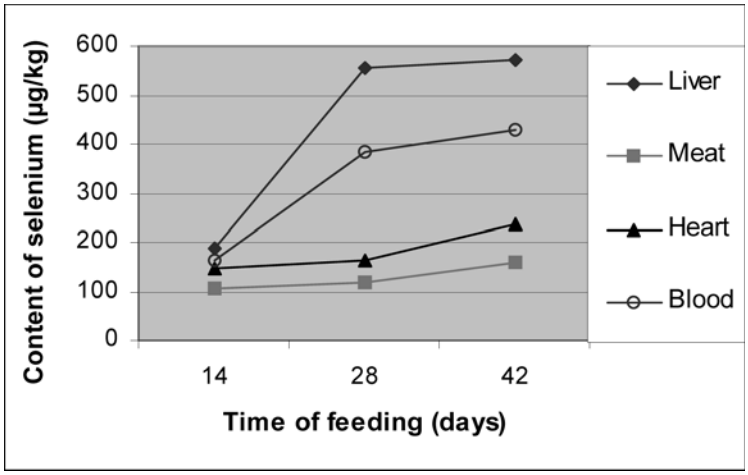
Fig. 1. — Measured amount of selenium in native samples of liver



Dietary Se [µg/kg]	Content of selenium [µg kg^{-1}]		
	Time of feeding		
	14 day	**28 day**	**42 day**
Bazal (< 10)	8.5 ± 2.6	15.3 ± 4.0	15.8 ± 4.5
Bazal + 50	38.5 ± 10.7	92.2 ± 18.0	117.0 ± 20.7
Bazal + 100	64.9 ± 22.8	145.9 ± 30.5	181.9 ± 29.4
Bazal + 150	81.9 ± 28.7	182.0 ± 31.5	224.8 ± 30.0
Bazal + 250	162.8 ± 28.9	385.1 ± 39.5	430.3 ± 46.5

The values represent the mean ± SD

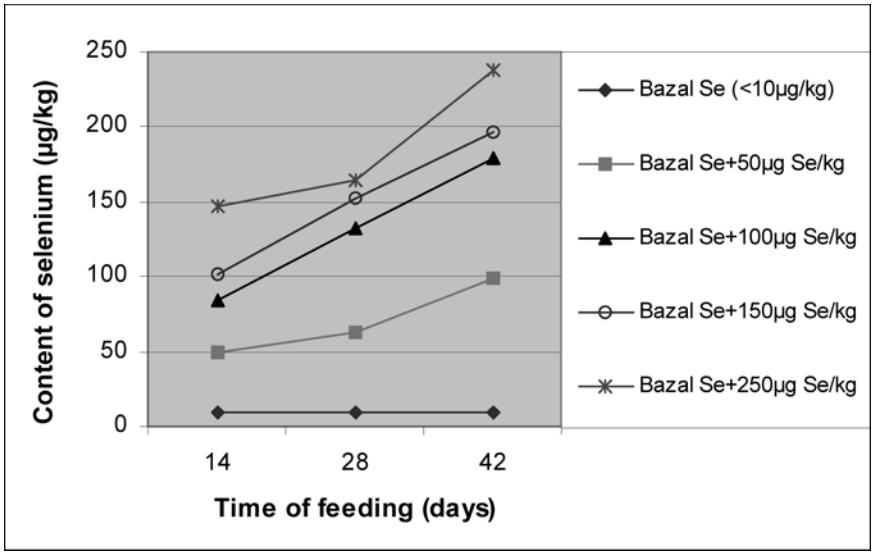
Fig. 2. — Measured amount of selenium in the whole blood



Organs	Content of selenium [µg kg^{-1}]		
	Time of feeding		
	14 day	**28 day**	**42 day**
Liver	187.9 ± 30.6	556.7 ± 40.6	570.8 ± 44.1
Meat	105.4 ± 27.4	118.4 ± 35.8	160.0 ± 35.8
Heart	147.2 ± 33.4	164.0 ± 30.1	237.5 ± 42.8
Blood	162.8 ± 28.9	385.1 ± 39.5	430.3 ± 46.5

The values represent the mean ± SD

Fig. 3. — Selenium deposition in organs and tissue of broilers fed with the feed with basal level of selenium + 250 µg Se/kg



Dietary Se [µg/kg]	Content of selenium [µg kg^{-1}]		
	Time of feeding		
	14 day	**28 day**	**42 day**
Bazal (< 10)	9.2 ± 2.6	9.3 ± 3.1	9.7 ± 3.0
Bazal + 50	49.8 ± 15.6	62.7 ± 17.6	99.4 ± 20.4
Bazal + 100	84.1 ± 19.2	132.2 ± 27.9	178.5 ± 32.9
Bazal + 150	101.9 ± 24.1	152.3 ± 28.9	196.9 ± 28.9
Bazal + 250	147.2 ± 33.4	164.0 ± 30.1	237.5 ± 42.8

The values represent the mean ± SD

Fig. 4. — Selenium level in native samples of heart muscle

DISCUSSION

Inorganic selenium (sodium selenite) is still the most frequently added form of selenium into animal diets. After resorption in gut, sodium selenite gets in the liver where is transformed into biologically usable form of selenide (Se^{-2}). In the presence of cystein, selenocystein is built through specific catalysed reactions. However the above mentioned mechanism becomes saturated when Se reached level of 300 mgkg^{-1} Se in liver (P e h r s o n, 1993). Our experimental results lead to similar conclusion (Fig. 1). In broilers fed with diet containing 250 µgkg^{-1} Se, at day 28 concentration of selenium in liver was 556.7 ± 40.6 µgkg^{-1} and at day 42 only 570.8 ± 44.1 µgkg^{-1}, respectively. Since there was no significant difference between these two values, it can be concluded that the point of saturation as well as maximal concentration of selenium in the liver reached the maximum levels in a period between fourth and sixth week.

The mechanism of biotransformation inorganic to organic form decelerates in time. Also, production of selenium deposits in different tissues is limited. A certain quantity of inorganic selenium that was not used in selenoprotein synthesis in liver is excreted in urine. In short time selenium is being incorporated in liver, the site of selenoprotein P synthesis, subsequently increasing the level of selenium in blood plasma that is good indicator of supply with this mineral (Žust et al., 1998). The effect is particularly prominent with content of dietary selenium of 250 μgkg^{-1} (Fig. 2). According to our experimental results, already at day 14 the blood level of selenium reached 162.8 ± 28.9 μgL^{-1} that represents 65.1% of dietary selenium.

The affinity for selenium as well as duration of its deposits varies among tissues. For example, feeding with diet containing 250 μgkg^{-1} Se at day 42 the highest concentration of selenium of 570.8 ± 44.1 μgkg^{-1} measured in liver (Fig. 3), than 430.3 ± 46.5 μgkg^{-1} in whole blood, 237.5 ± 42.8 μgkg^{-1} in heart muscle. The lowest concentration of selenium of 160.0 ± 35.8 μgkg^{-1} was measured in meat. Tissue deposits of selenium in such order in chickens coincide with findings of Echevarria (1988).

Certain tissues bind less selenium with increase of dietary selenium content. Our results (Fig. 4) show that at day 14 after the increase of dietary selenium from 50 μgkg^{-1} to 250 μgkg^{-1} concentration of selenium in heart muscle subsequently decreased: when broilers were fed with basic diet containing 50 μgkg^{-1}, 100 μgkg^{-1}, 150 μgkg^{-1} and 250 μgkg^{-1} Se measured content of Se in heart muscle was 49.8 ± 15.6 μgkg^{-1} (99,6%), 84.1 ± 19.2 μgkg^{-1} (84,1%), 101.9 ± 24.1 μgkg^{-1} (67,9%) and 147.2 ± 33.4 μgkg^{-1} (58,9%), respectively.

Knowledge of speed of forming and quantity of deposits of selenium in meat is important for production of so-called "functional food". There is constantly increase of number of food products with improved nutritive value by adding selenium, like food and drink for sportsmen and children, eggs, meat and milk. Studies suggest that deposits of inorganic selenium in meat are significantly lower than in other tissues and organs (Beale et al., 1990). In our investigation similar results were obtained. The level of selenium in meat was significantly lower than in other evaluated tissues and organs (Fig. 3). We also observed that considerably higher deposits of selenium were determined at day 42 when diet with 250 μgkg^{-1} Se was used.

The amount of selenium deposits in tissue and organs is in relation to quantity and form of selenium, age and animal species. Increase of intake of selenium increases its concentration in tissue nag organs, but in nonlinear regression line. After assessing a certain determined level of dietary selenium, concentration of selenium in tissue reaches and remains at maximum and is no longer dependent on concentration of dietary selenium.

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УТИЦАЈ РАЗЛИЧИТИХ НИВОА ДИЈЕТАРНОГ СЕЛЕНА НА ЊЕГОВУ ДИСТРИБУЦИЈУ У ОРГАНИЗМУ БРОЈЛЕРА

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Резиме

Потпуне смеше за исхрану бројлера садржавале су 50 ± 10, 100 ± 10, 150 ± 10 и 250 ± 10 μg Se kg^{-1}. Узорци су узимани 14-ог, 28-ог и 42-ог дана огледа. Праћене су динамика и количина накупљања селена у крви, јетри, месу и срчаном мишићу, у зависности од нивоа дијетарног селена и времена његовог уношења у организам. После 42 дана това смешом са 250 μg Se/kg највећи садржај селена измерен је у јетри (570.8 ± 44.1 μg/kg), затим у пуној крви (430.3 ± 46.5 μg/l) и срчаном мишићу (237.5 ± 42.8 μg/kg). Најнижи садржај селена измерен је у месу (160.0 ± 35.5 μg/kg). Повећање садржаја дијетарног селена у храни од 50 до 250 μg Se/kg, после 14. дана това праћено је смањењем количине депонованог селена у срчаном мишићу: за смешу са 50 μg Se/kg количина селена у срчаном мишићу износила је 49,8 ± 15.6 μg/kg (99,6%), а за смешу са 250 μg Se/kg измерена количина селена у срчаном мишићу износила је 147,2 ± 33.4 μg/kg (58,9%). Између четврте (556.7 ± 40.6 μg Se/kg) и шесте недеље (570.8 ± 44.1 μg Se/kg) огледа дошло је до засићења и постигнут је плато концентрације селена у јетри. Такође се може закључити да са храном од 250 μg Se/kg, већ после 14 дана, ниво селена у пуној крви достиже вредност од 162,8 ± 28.9 μg/l, што представља 65,1% од количине дијетарног селена.

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SEASONAL VARIATION IN NUTRITION OF CHUB (*LEUCISCUS CEPHALUS* L., *CYPRINIDAE, OSTEICHTHYES*) IN ONE RESERVOIR OF WEST SERBIA

ABSTRACT: *Leuciscus cephalus* L. is abundantly present fish species in the Balkan freshwaters, which indicates a good adaptation to the environmental conditions existing in the most of reservoirs. The fish species is abundant in the Međuvršje reservoir situated on the Zapadna Morava river (West Serbia, the Danube basin) as the only predator found here. In the period from 1996 to 2002, the intestinal content of 88 individuals of different age was analyzed. The zoophytophage character of diet was found to be largely shared with plant components all the year round. The differences in the trophic spectrum over the various seasons existed between the individuals of different age with high individual variations being found, too.

KEY WORDS: *Leuciscus cephalus*, the Međuvršje reservoir, nutrition, seasonal variation

INTRODUCTION

The Međuvršje reservoir is one of the oldest reservoirs in Serbia. It was erected in 1953 by the construction of a dam across the Zapadna Morava river (the Danube basin) 31 m high and 182 km long in the downstream river course (Fig. 1). In 1955, hydroelectric plant Međuvršje of the power 6.9 MW was built. The reservoir is 9,312 km long, 1.5 km^2 large, 292 m at widest point, and 12 m at deepest point immediately under the dam. Originally, the reservoir had a volume of 15.4 x 106 m^3. However, more than 70% of the original volume has been drifted. Its current annual flowing power is roughly 34 m^3s^{-1}. The reservoir's banks are steep and its bottom silty. The major river course is near the right bank causing its being pooled while the left bank is intensely being drifted and shallow. This has brought about a gradual protrudence of some small islands covered by hydrophilous macrophyte vegetation (S i m o v i ć

et al., 2000). Water transparency in summer has been found to range from 0.5—0.8 m and in winter 4.3 m.

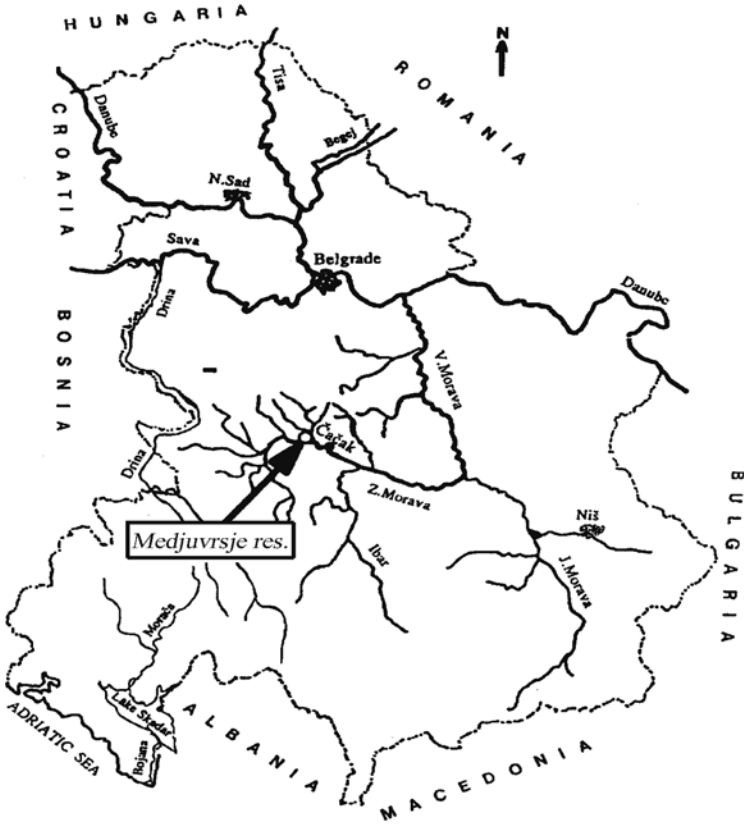

Fig. 1. — Location of the Međuvršje reservoir

The ichthyofauna of the Međuvršje reservoir was reported to contain 20 fish species from 7 families, with an expressed dominance of *Alburnus alburnus* (25.86%), subdominance of *Chondrostoma nasus* (13.85%), *Carassius auratus gibelio* (11.42%), abundance of *Rutilus rutilus* (9.43%), *Leuciscus cephalus* (8.30%), *Abramis brama* (7.27%) and *Pseudorasbora parva* (6.46%). The decrease in the economically significant fish species accounts for worsened ecological conditions in the reservoir. This is also due to an over catching over spawning and disobedience of the fishermen despite existing fish caretakers who are keeping an eye on them. Specifically, the ecosystem ichthyofauna has a huge abundance of *C. nasus* and *L. cephalus*, originally reophilous species. Chub (*L. cephalus*) is the only abundantly present predator within the ecosystem (M a r k o v i ć and V e l j o v i ć, 2005). The trophic spectrum of such a European species has been focused on.

MATERIALS AND METHOD

The ichthyological studies on the Zapadna Morava river sector lasted from 1996 to 2002. The fish were caught using the standard fishing gear — nets and hooks. The fish trophic spectrum determinations were based on analysing the intestinal content taken from the anterior one-third of the alimentary canal. Binoculars MBS-2 and MBDS-10 and the microscope "Studar" were used as the differing magnifying needs might have been. Semi-quantitative (per cent) presence of single components in the intestinal content was determined by using of different handbooks (G r a n d i, 1960; S t r e b l e and K r a u t e r, 1973; H i n d a k, 1978; J e z e k et al., 1980; A P H A, 1985; K e r o v e c 1986). After being analysed, the intestinal content could help show the seasonal diet spectrum.

RESULTS

The intestinal content analysed in 88 samples of the fish caught in the Međuvršje reservoir indicated that the animal (55.7%) dominated over the plant components (31.0%) and detritus (13.3%) (Tab.1).

Tab. 1. — The intestinal content of chub (%) in the Međuvršje reservoir

Group	Spring	Summer	Autumn	Total
Cyanobacteriophyta	< 0.1	0.5	1.5	0.5
Pyrrhophyta	< 0.1	—	< 0.1	< 0.1
Bacillariophyta	10.0	4.5	5.5	6.2
Chlorophyta	12.2	12.5	14.2	12.6
Macrophyte vegetation	5.5	15.7	10.7	11.6
Rotatoria	0.1	0.5	0.1	0.3
Nematoda	0.1	—	—	< 0.1
Mollusca	—	3.5	8.5	3.5
Oligochaeta	0.1	0.5	0.1	0.3
Cladocera	0.1	0.1	0.1	0.1
Copepoda	< 0.1	—	—	< 0.1
Odonata	25.5	18.5	5.5	17.2
Heteroptera	0.5	1.0	—	0.5
Coleoptera (aquatic)	10.5	5.2	2.5	6.1
Diptera — Chironomidae	0.5	0.1	0.1	0.2
Diptera — Tipulidae	0.5	4.5	2.5	2.6
Insecta (terrestrial)	4.0	3.0	7.5	4.2
Pisces	20.0	10.0	13.5	16.6
Amphibia	—	4.5	10.5	4.5
Detritus	10.1	15.4	17.1	13.3

Speaking about chub diet belt over the spring season the larvae *Odonata* of 25.5% seemed the most abundant (Tab. 1). Of the diverse fauna of this inherently insect order (*Lestes* sp. *Aeshna* sp. *Anax imperator*, *Gomphus vulgatissimus*), the finding on larvae *Brachytron pratense*, protected as the natural rarity of Serbia (H e r a l d RS 50/93), seemed interesting. Fish are an impor-

tant component of the spring diet (20.0%) of the larger chubs (age 6+ and older ones), found in the aquatic *Coleoptera* (primarily *Ilybius fuliginosis*) in abundant amounts in their intestines of up to 10.5%. Plant material accounted for 27.9% intestine content characterised by an abundance of filamentous *Chlorophyta* (12.2%) and *Bacillariophyta* (10.0%).

Over the summer period, the intestines had the larvae *Odonata* (18.5%) most, the fish share being decreased (10.0%). Among the animal components of diet, the presence of aquatic *Coleoptera* (5.2%), larvae *Diptera*, mainly *Tipulidae*, (4.6%) and amphibians (4.5%) was detected. The plant material occupied one third of the intestine content characterised by a frequently encountered macrophyte (15.7%) and filamentous *Chlorophyta* (12.5%).

Fish and amphibians were found to highly exist (13.5% and 10.5%, respectively) in the chub diet in the autumn. Their presence along with land insects (7.5%) was identified in the early fall, with an abundance of *Mollusca* (*Anodonta* sp.) with 8.5% and plant material full of filamentous *Chlorophyta* (12.6%) and macrophyte components (11.6%).

Annually, chub diet belt within the ecosystem under way denoted the dominance of macrozoobenthos (23.9%) (Fig. 2) with larvae *Odonata* being the most present (17.2%). The littoral of the reservoir covered in macrophyte paves the way for growing juveniles of this insect order representative so spreading the trophic belt of chub. Further, vertebrates, particularly those fish significantly sharing the diet of older chubs over a longer period of the year (from the end of March to the beginning of November) accounted for 16.6%. Also, an abundant presence of other animal groups in the intestines is mainly connected with some of the periods (*Coleoptera* in the spring, *Mollusca* in late autumn).

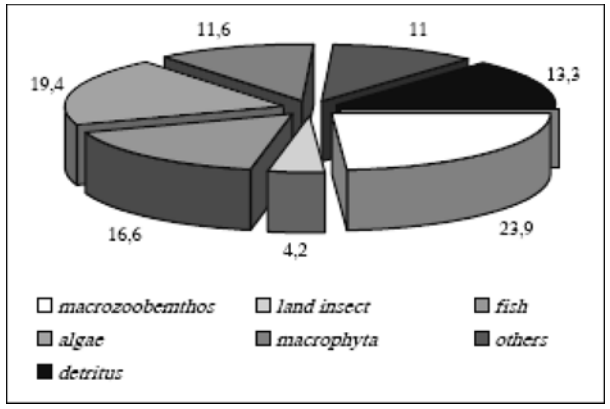

Fig. 2. The presence of different components in the chub diet (%)

The specificity of chub diet within this biotope is that the organisms of the lake bottom being edible within macrozoobenthos (*Oligochaeta* and *Chironomidae*) community are barely contained in the chub intestines. The presence of *Bacillariophyta* (6.2%) in all the samples was found to be the highest in colder intervals of the year (early spring and late autumn).

DISCUSSION

Numerous researchers have been discussing in favour of omnivorism of *L. cephalus* in the lake ecosystems of Europe simultaneously suggesting a high variability in such a presence when components are taken individually.

The characteristics of the fish diet in the Skadar Lake (Montenegro) showed an enormously wide trophic belt-classifying chub as a member of euryphage and zoophytophage sets (Janković and Trivunac, 1978). Basically, chubs eat insects (land ones, larvae, *Trichoptera, Hemiptera* and *Trichoptera*) then, crayfish, shells, snails and fish largely appearing in the autumn, winter and in the early spring. All year around, the algae (*Chlorophyta, Cyanobacteriophyta* and *Bacillariophyta*) were present in the chub intestinal content as well as the plant parts and detritus. A steady competition between *L. cephalus* and *Cyprinus carpio* was manifested in light of *Trichoptera*, snail, shell and detritus consuming.

Re-researches made on the same biotope corroborated a high diversity in diet of the species under consideration (Stanković-Trivunac, 1981). In the samples caught over the winter and early spring, there were *Trichoptera* and other insects and crayfish in large numbers. Over summer and autumn months, algae and detritus were dominating; Crustaceans and molluscs were frequently appearing in the late spring and autumn. The intestinal content of chub indicated that its diet went on throughout the whole year with no component being typical of the particular period, but it could have appeared for a longer while.

A wide euryphage features chub diet in the Dalesice reservoir on the Jihlava river (Adamek et al., 1987). Its peculiarity is a high presence of small rodents (*Rodentia*), falling into the water from the steep banks.

Chub diet in the Međuvršje reservoir went on from 1984—1985 indicating chub to have a longer trophic spectrum than *R. rutilus, Ch. nasus, A. brama* and *B. barbus* (Veljović et al., 1986). Fundamentally, chub diet consists of the larvae *Chironomidae* and other insects, with presence of the *Bacillaryphyta* (*Melosira granulata, M. varians* and *Synedra acus* representatives). Neither other organisms in chub diet nor the relationships among its single components have been stated in the literature so far except for certain species *Bacillaryophyta*. Hence, the results did not essentially coincide with analyses on dietary characters we have been concerned above. Zoophytophage character of the chub diet was also noticed with a significant share of the plant component all the year round, particularly in the intestinal content over the summer and autumn months.

The analysis of the juvenile individual intestinal content pointed to a relative uniformity of the diet. Thus, at the age of 0+ the highest portion of the intestinal content was consisted of *Bacillaryophyta*, with scarcely present *Chlorophyta* and individual zooplankton forms (plankto-phytophage diet). The intestinal contents of the older juveniles (age 1+), apart from silicate algae, were abundant in filamentous green algae (particularly *Cladophora* sp.), but also in other types of diet (macrophyte vegetation, larvae *Diptera*). Juvenile chubs of ages 2+ and 3+ were found to largely consume animal diet. In addition to the

insect larvae, land insects and other organism groups were also present. Characteristically, the diet of the sexually immature individuals was, in one word, plankto-phyto-zoo-phage. The high proportion of vegetation in the diet of juvenile chubs is a possible consequence of the habitat degradation caused by human activity (B a l e s t r i e r i et al., 2006).

When an adult individual intestinal content was analysed, a progressive increase in carnivorous was observed with aging of the samples caught. Younger adults (age 3+, 4+ and 5+) largely consumed insect larvae and land representatives *Odonata, Trichoptera, Diptera* and *Ephemeroptera*. Piscivorousness was expressed in the chubs of age 6+ and in the older ones, with the remaining diet components (*Mollusca, Amphibia* and others) equally present in the individuals of different ages. A huge heterogeneity in chub diet within the same biotype was noted. The samples of the same age and dimensions were caught at the same profile, with some of them having intestinal content entirely filled with filamentous green algae, whereas the others and larger in number consumed insects, fish or other animal component. The diet of adult individuals had an expressed zoophytophage character.

CONCLUSION

The analysis of the intestinal content of the specimens *L. cephalus* caught in the Međuvršje reservoir on the Zapadna Morava river (Western Serbia, the Danube basin) is in favour of the zoophytophage type of the diet. High variations in chub diet existed over different seasons with different ages, but also in the diet of the individuals of the same age. The results once more confirmed not only the euryphage characteristic of the species existing in the analysed ecosystem, but also its getting accustomed to certain diet resources.

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СЕЗОНСКА ВАРИРАЊА ИСХРАНЕ КЛЕНА (*LEUCISCUS CEPHALUS* L., *CYPRINIDAE, OSTEICHTHYES*) У ЈЕДНОЈ АКУМУЛАЦИЈИ ЗАПАДНЕ СРБИЈЕ

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Резиме

Клен (*Leuciscus cephalus* L.) представља масовно заступљену рибљу врсту у слатким водама Балкана која показује добру адаптираност на еколошке услове у већини акумулација. Врста је масовна у акумулацији Међувршје, лоцираној на реци Западна Морава (Западна Србија, Дунавски слив) и представља једину масовнију грабљивицу. Анализиран је цревни садржај 88 јединки клена различитог узраста уловљених у периоду 1996—2002. године. Констатован је зоо-фитофаг карактер исхране са великим уделом биљних компонената током целе године. Уочене су разлике у трофичком спектру врсте у различитим сезонама између јединки различитог узраста, као и велика индивидуална варирања.