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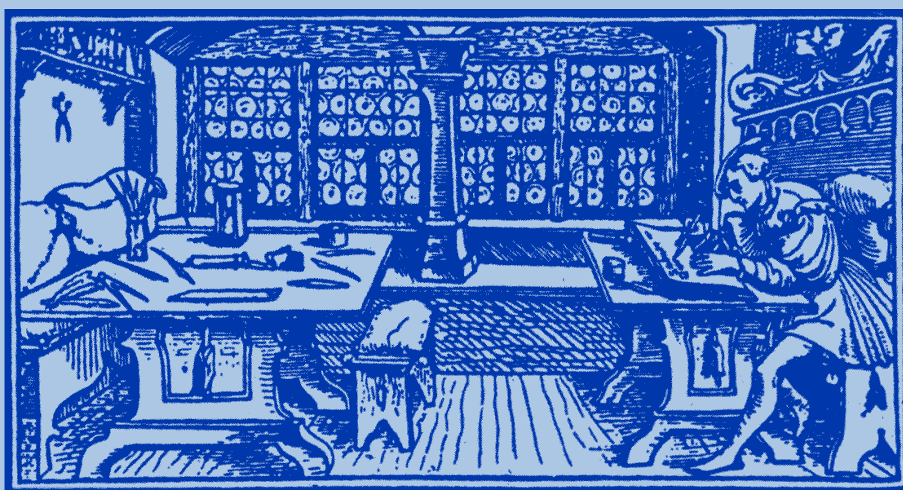

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ENZYMOLOGY OF SOILS INOCULATED WITH MICROORGANISMS

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SUMMARY.- This review article consists of two parts dealing with enzymology of soils inoculated with natural and genetically engineered microorganisms, respectively.

Part I comprises four chapters reviewing the literature on soil enzymological effects of the inoculation of different groups of natural microorganisms. Chapter 1 is devoted to inoculation of plant growth-promoting microorganisms: N_2-fixing bacteria (*Rhizobium, Azotobacter,* cyanobacteria); phospate- and potassium-solubilising bacteria; green algae; mycorrhizal plus saprophytic fungi. The topic of Chapter 2 is the inoculation of biocontrol microorganisms: *Trichoderma* sp. for controlling fungal diseases of plants caused by *Verticillium dahliae* and *Pythium ultimum; Bacillus thuringiensis* for controlling harmful insects of forests; *Pseudomonas fluorescens* F113Rif - a prospective biocontrol agent. Chapter 3 deals with inoculation of bioremediating microorganisms: hydrocarbon-oxidising microorganisms for remediation of oil-contaminated soils; bacteria for remediation of soils polluted by emissions from coking plants; bacteria for remediation of heavy metal-polluted soils; bacteria enhancing phytoremediation of contaminated soils. Chapter 4 describes other microorganisms (*Pseudomonas* sp. RCl rif^{100} and *Flavobacterium* sp. P25) inoculated into soil for fundamental studies.

In Part II, the soil enzymological effects of the inoculation of the following genetically engineered bacteria were reviewed: *Pseudomonas putida* PPO301(pRO103), *Escherichia coli* W3110(R702), *Streptomyces lividans* TK23.1, *Pseudomonas fluorescens* 10586s/FAC510, *Pseudomonas fluorescens* SBW 25 EeZY.

Introduction. Soils are inoculated with potentially beneficial microorganisms, with the aim to promote plant growth, to control phytopathogens and insect pests or to enhance bioremediation. Besides the potentially beneficial microorganisms, other microorganisms were also used for studying fundamental aspects of microbial inoculations such as survival (fate) of the inoculated microorganisms in soil and their effects on physical, chemical and biological soil properties. The inoculation of microorganisms is either direct or indirect. The direct inoculation means that the soils are treated with the microorganisms. The inoculation is indirect when the soils are sown with seeds previously treated with microorganisms.

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The investigations on microbial inoculation of soils have intensified in the last decades which is related to the extensive concern over the risks of accidental release and intentional use of genetically engineered animals, plants, microorganisms and viruses, including inoculation of soils with genetically engineered microorganisms (*e. g.* [3, 9, 17, 45, 63, 64, 66]). At the same time, the soil enzymological effects of the inoculation with natural (non-engineered) microorganisms continued to remain an important topic of the investigations.

The present review article consists of two parts. In Part I, enzymology of soils inoculated with natural microorganisms is dealt with, whereas Part II is devoted to enzymology of soils inoculated with genetically engineered microorganisms.

The enzymological investigations, in which natural or genetically engineered microorganisms were inoculated into sterile (autoclaved) soils (*e. g.* [12, 27, 39, 61, 65]), are not considered in this review.

PART I. ENZYMOLOGY OF SOILS INOCULATED WITH NATURAL MICROORGANISMS

The literature data reviewed will be grouped into 4 chapters, dealing with the soil enzymological effects of plant growth-promoting, biocontrol, bioremediating and other inoculants, respectively.

I. 1. Soil enzyme activities as affected by inoculation of plant growth-promoting microorganisms

The microbial inoculants promote plant growth by:

- improving the nutrient status of soil (increasing the available N, P, K contents);
- producing plant growth hormones and vitamins;
- enriching the soil in organic matter;
- improving the soil structure.

I. 1.1. Inoculation of N_2-fixing bacteria. I. 1.1.1. Inoculation of Rhizobium. The most frequently used name of the commercial *Rhizobium*-containing preparations for inoculation of legume seeds is Nitragin.

Orlyanskaya [48] carried out a field experiment on a soddy-podzolic soil, using broadbean (*Vicia faba* cv. Altai violet) as test plant. Before sowing, the experimental plots (20.5 m^2 each) were fertilised with superphosphate (300 kg/ha) and potash salt (150 kg/ha) and the seeds were soaked in water (control) or in suspension of Nitragin (*Rhizobium leguminosarum* strain 74). During the

vegetation period, soil samples were taken from the rhizosphere and analysed for determination of catalase, invertase, amylase and urease activities. Table 1 shows that each rhizosphere enzyme activity at each growth stage was higher in the Nitragin treatment than in the control. It should be added that the Nitragin treatment also resulted in higher contents of B-group vitamins (thiamine, pyridoxine, nicotinic acid, inositol, biotin and pantothenic acid) in both rhizosphere soil and roots and root nodules, and in increased crop yield.

Table 1

Enzyme activities in the rhizosphere of broadbean plants developed from non-inoculated and Nitragin-inoculated seeds [48]

Treatment	Enzyme activities*												
	Catalase				Invertase			Amylase			Urease		
	Growth stages**												
	A	B	C	D	A	B	C	A	B	C	B	C	D
Soaking of seeds in water	1.5	2.2	2.9	3.0	5.93	3.73	4.03	1.71	2.85	1.14	0.374	0.544	0.578
Soaking of seeds in Nitragin suspension	3.3	3.0	3.7	3.3	7.63	5.03	6.03	4.55	3.41	2.56	0.445	0.710	0.816

* Expression of enzyme activities: catalase in ml of O_2/g soil/minute; invertase in mg of glucose/5 g soil/24 hours; amylase in mg of maltose/5 g soil/24 hours and urease in mg of ammonia/g soil/24 hours.

** A - 5-6-leaf stage. B - Bud formation stage. C - Flowering stage. D - Maturation stage.

In two short reports, Redžepović *et al.* [52, 53] described field experiments, in which acid soils were limed at rates of 6, 10 and 14 t $CaCO_3$/ha, then sown with alfalfa (*Medicago sativa*) seeds, either non-inoculated or inoculated with a *Rhizobium meliloti* strain. Soil protease and cellulase activities were found to be higher in the inoculated variants. For example, C_x cellulase activity (expressed in mg of glucose/g soil) ranged from 0.655 to 0.695 in the inoculated variants and from 0.365 to 0.640 in the non-inoculated ones. The number of proteolytic microorganisms was lower, but the number of cellulolytic microorganisms was higher in the soil of the inoculated variants than in the soil of the non-inoculated ones.

In Strniša and co-workers' [60] experiments, the effect of inoculation and fungicide treatment of pea (*Pisum sativum*) seeds on the rhizosphere dehydrogenase activity was studied. For inoculation, *Rhizobium leguminosarum* bv. *viciae* was used. The pea varieties Karina and Pioneer AF were tested. The fungicide (tetramethylthiuram disulphide) was applied at a rate of 200 g/100 kg seeds. The experimental variants were: 1. not inoculated and not treated with fungicide (control); 2. inoculated; 3. fungicide-treated and 4. inoculated and fungicide-treated.

The rhizosphere dehydrogenase activity was assayed 21 and 45 days after germination. At day 21, dehydrogenase activity was higher in the inoculated and fungicide-treated variant and lower in the inoculated or fungicide-treated variants than in the control, in the rhizosphere of both pea varieties. At day 45 (flowering stage), essential differences were found in the rhizosphere dehydrogenase activity between the two pea varieties. In the case of the Karina variety, the highest dehydrogenase activity was registered in the rhizosphere of control plants, whereas, in the rhizosphere of the Pioneer AF variety, dehydrogenase activity, taken as 100% in the control, decreased in the following order: 144.44% (fungicide-treated) > 129.63% (inoculated) > 38.89% (inoculated and fungicide-treated). One can draw the conclusion that inoculation alone led to increased rhizosphere dehydrogenase activity only at the flowering stage of the Pioneer AF variety.

I. 1.1.2. Inoculation of Azotobacter. In the laboratory experiment performed by Kucharski and Niklewska [35], 400-g air-dried samples of a sandy soil were amended with wheat straw (at rates of 0.15 and 1.5 g/100 g soil) and inoculated with *Azotobacter* sp. ($5.4.10^4$ cells/g soil) or with other microorganisms: *Bacillus subtilis* (1.10^3 cells/g soil), *Streptomyces* sp. (6.10^5 cells/g soil) or *Penicillium* sp. (400 spores/g soil). The control soil samples were not inoculated; some controls were not amended with straw and not fertilised; other controls were amended but not fertilised; there were also controls which were amended and fertilised with urea (10 mg N/100 g soil). All samples were then incubated at 27^0C and at constant moisture content (60% of maximum water-holding capacity) for 150 days. At 30-day intervals, the soil samples were analysed for determination of their dehydrogenase, urease and acid and alkaline phosphatase activities.

Comparison of the results obtained in inoculated and non-inoculated soil samples has shown that inoculation of each microorganism led to diminution of dehydrogenase, urease and acid phosphatase activities in the samples amended with the smaller dose of straw (0.15 g/100 soil) and to increases in dehydrogenase activity and to little changes in urease and acid phosphatase activities in samples which received 1.5 g straw/100 g soil. Dehydrogenase activity, expressed in mm^3

H_2/100 g soil, presented the following order in the control and inoculated samples: 346 (N-fertilised control) > 285 (*Azobacter* sp.) > 220 (*Bacillus subtilis*) > 213 (*Streptomyces* sp. and *Penicillium* sp.) > 211 (unfertilised control). Consequently, of the four microorganisms inoculated, *Azotobacter* sp. was most efficient in increasing soil dehydrogenase activity. Finally, it should be mentioned that alkaline phosphatase activity was not affected by any of the inoculated microorganisms.

Jarak *et al.* [28] installed experimental plots on a hydromorphic black soil in Padinska Skela (Yugoslavia). The test plant was wheat (*Triticum vulgare* var. PKB-Mlinarka). The seeds, before being sown into the plots, were inoculated with one of seven *Azotobacter chroococcum* strains designated 3, 10, 20, 76, 84, 86 and 201. The control plot was sown with non-inoculated seeds. After harvest, the soil was analysed for determination of dehydrogenase activity. It was found that the activity increased only in the soil, into which seeds inoculated with strain 86 were sown. In the case of the other six strains, the soil exhibited lower dehydrogenase activity than the soil in the control plot. Contrarily, inoculation of seeds with the strains, excepting strain 76, brought about 9.3-13.6% increases in grain yield.

I. 1.1.3. Combined inoculation of Azotobacter *and* Rhizobium. The Indian researchers Narula and Gupta [44] used the ammonia-secreting *Azotobacter chroococcum* strain AC2 for inoculation of cereals and *A. chroococcum* AC2 alone or in association with the specific rhizobia for inoculation of legumes. Local varieties of wheat (*Triticum vulgare*), maize (*Zea mays*) and barley (*Hordeum vulgare*) as well as mungbean (*Vigna radiata* var. K-851) and chickpea (*Cicer arietinum* var. H-208) were studied. The seeds treated with *A. chroococcum* AC2 or with equal amounts of *A. chroococcum* AC2 and *Rhizobium* sp. were sown in vegetation pots containing a mixture of garden soil and sand (at 2:1 ratio) amended with 0.5% K_2HPO_4 and 0.1% $CaCO_3$. Non-inoculated plants served as controls.

The soil-sand mixtures were sampled after 35 days of plant growth for enzymological analyses. The enzyme activities determined are specified and the results obtained are reproduced in Table 2. It is evident from this table that soil invertase and amylase activities were negligibly low in all treatments. Inoculations led to significant decreases in soil phosphatase activity. Arylsulphatase activity was significantly higher in soil under inoculated plants than in the control soil, excepting the soil under chickpea inoculated with *Rhizobium* sp. only: in this soil arylsulphatase activity was significantly lower than in the control soil and in the soil under chickpea inoculated with both *A. chroococcum* AC2 and *Rhizobium* sp. Levels of significance were at 0.01.

Table 2

Effect of Azotobacter chroococcum AC2 alone and in association with Rhizobium sp. on enzyme activities in soil planted with wheat, maize, barley, mungbean and chickpea [44]

Plant	Treatment	Enzyme activities*			
		Invertase	Amylase	Phosphatase	Arylsulphatase
Wheat	Control	0.013	0.021	9.59	4.91
	A. chroococcum	0.023	0.040	5.00	5.54
Maize	Control	0.024	0.046	13.02	1.30
	A. chroococcum	0.049	0.050	6.70	1.36
Barley	Control	0.001	0.046	12.81	3.70
	A. chroococcum	ND	0.046	10.58	6.99
Mungbean	Control	0.020	0.034	14.25	4.46
	Rhizobium sp.	0.006	0.080	13.12	5.25
	Rhizobium sp. + A. chroococcum	0.024	0.040	7.97	5.29
Chickpea	Control	0.010	0.002	9.96	7.24
	Rhizobium sp.	0.010	0.040	6.09	7.06
	Rhizobium sp. + A. chroococcum	0.012	0.029	6.15	7.50

* Expression of enzyme activities: invertase in µmoles of glucose/g soil/3 hours; amylase in µmoles of glucose/g soil/24 hours; phosphatase and arylsulphatase in µmoles of *p*-nitrophenol/g soil/hour.

ND - Not determined.

I. 1.1.4. Inoculation of cyanobacteria (blue-green algae). For a field experiment, Dzhumaniyazov *et al.* [23] used wheat seeds soaked in suspension of a mixture of seven species of cyanobacteria (the species names are not given by the authors) or in a suspension of *Stratonostoc linckia* f. *muscorum*. The control seeds were soaked in water. The seeds were then sown into an NP-fertilised non-irrigated grey soil in Uzbekistan. At the inspication and maturation stages of wheat growth, peroxidase and polyphenol oxidase activities were determined in the 0-22- and 22-50-cm soil layers.

Peroxidase activity measured at the inspication stage was higher in both soil layers of both inoculated variants than in the control, but at the maturation stage this activity exceeded the control value only in the 0-22-cm layer of the variant, in which *Stratonostoc linckia* f. *muscorum* was used for inoculation of seeds.

At the inspication stage, polyphenol oxidase activity gave increased values in the upper soil layer in both inoculated variants and in the deeper soil layer only in the *Stratonostoc*-inoculated variant. At the maturation stage, polyphenol oxidase activity of both soil layers exhibited no evident differences between the inoculated and non-inoculated variants.

Taubaev *et al.* [62] also used *Stratonostoc linckia* f. *muscorum* for seed inoculation. The test plant was cotton. Its inoculated and non-inoculated (control) seeds (soaked in cyanobacterial suspension and water, respectively) were sown into an NPK-fertilised irrigated grey soil. The experiments were carried out in vegetation pots and in the field. Peroxidase and polyphenol oxidase activities in soil were assayed at bud formation, flowering and maturation stages of cotton growth.

In the pot experiment, both enzyme activities at all growth stages were higher in the inoculated variant than in the control, whereas in the field experiment only polyphenol oxidase activity measured at the bud formation stage was considerably higher in the inoculated variant than in the control.

In the field experiment described by Nafasov [43], rice seeds were, before sowing, inoculated with *Nostoc muscorum* (soaked in the cyanobacterial suspension) or left non-inoculated, *i. e.* soaked in water (control). Seasonal variation of peroxidase and polyphenol oxidase activities was determined in the 0-25- and 25-50-cm soil layers sampled in May, July and October.

In each month, both activities in both soil layers were higher in the inoculated variant than in the control. Exceptionally, the reverse was true for peroxidase activity in the 0-25-cm layer in October.

The upper soil layer was more enzyme-active than the deeper one. An exception was registered, again in peroxidase activity in October: in the inoculated variant the deeper layer was more active than the upper one.

Peroxidase activity was highest in July, and polyphenol oxidase activity showed increased values in May and October.

Rao and Burns [50,51] used a mixture of six cyanobacteria (*Calothrix* sp., *Nostoc* sp. 6719, *Nostoc muscorum* ARM-221, *Tolypothrix distorta* CCAP 1482/2, *Tolypothrix tenuis* ARM-76 and *Westiellopsis prolifica* ARM-365) for inoculation of a brown earth of silt-loam texture.

Soil, collected from the 0-20-cm layer, was placed in specially constructed multicompartment polybutylchloride columns (5, 10 and 15 cm in height and 15 cm in diameter) that allowed the collection of soil samples at different depths without disturbing the adjacent layers. The cyanobacterial mixture was surface-inoculated on wet soil. The inoculum was equivalent to 4.1 μg of chlorophyll *a*/cm^2. The columns with the inoculated soil were incubated at 25^{0}C in a growth chamber (18-hour light and 6-hour dark cycle). Dark and light controls served for comparison. In the dark control, the soil was inoculated, but the columns were

incubated with a PVC plate covering the surface. The light control was not inoculated, but exposed to light. The soil in all columns was maintained water-logged with 1 cm standing water, to simulate the soil conditions for rice growth.

After 5, 13 and 21 weeks of incubation, soil layers were removed from the comuns and analysed for a series of microbiological, enzymological, chemical and physical properties.

In the inoculated columns, although a mixture of six species was introduced, *Nostoc muscorum* ARM-221 became dominant. In the light control columns, the moss *Barbula recurvirostra* was dominant and restricted the growth of blue-green and other algae for the first 13 weeks. Later, there was partial disintegration of the moss mat and indigenous green and blue-green algae developed. By week 21, the total count of blue-green algae and the count of N_2-fixing blue-green algae in the surface soil layer (0-0.7 cm) in the light control were identical to the counts recorded in the inoculated colums (2.10^6 and $1.2.10^6$/g dry soil, respectively).

The enzyme activities (dehydrogenase, urease and neutral phosphatase) were determined in the surface (0-0.7-cm) layer and in four subsurface layers (0.7-1.7, 1.7-2.7, 2.7-3.7 and 3.7-4.7 cm). The results are presented in Table 3, from which one can see that dehydrogenase activity increased in both the inoculated and light control columns in the surface soil layer. The increase over the dark control was 3.1- and 1.7-fold after 5 weeks, 1.2- and 1.6-fold after 13 weeks and 2.1- and 2.7-fold after 21 weeks for the inoculated soil and light control, respectively. This means that by weeks 13 and 21 the surface soil was more dehydrogenase-active in the light control (non-inoculated) than in the inoculated columns. Dehydrogenase activity decreased with depth in all treatments at all times.

Urease activity in the surface soil layer of the inoculated columns was increased 2.5-fold over the dark control after 5 weeks, 2.4-fold after 13 weeks and 2.8-fold after 21 weeks, whereas the increase of this activity in the surface layer of the light control columns was 3.0- and 4.3-fold after 13 and 21 weeks, respectively. In other words, the surface layer of the light control columns exhibited higher urease activity after 13 and 21 weeks than the same layer of the inoculated columns. In all treatments, urease activity of the subsurface soil layers varied to a lesser extent.

Phosphatase activity increased with time in the surface soil layer of both inoculated and light control columns as compared to the dark control. The increase was 1.7- and 2.4-fold after 5 weeks, 3.1- and 5.5-fold after 13 weeks and 3.1- and 5.5-fold after 21 weeks in the inoculated and light control columns, respectively. Again, the surface soil layer proved to be more enzyme-active in the light control than in the inocualted columns. Phosphatase activity, like urease activity, exhibited smaller changes in the subsurface soil layers.

It should be mentioned that the aggregate stability index increased from 0.70 (dark control) to 0.75 (inoculated) and 0.76 (light control) after 13 weeks, and from 0.79 (dark control) to 0.82 (inoculated) and 0.81 (light control) after 21 weeks, *i. e.* soil aggregation in the inoculated and light control columns was similarly improved.

Table 3

Effect of the inoculation of soil with cyanobacteria on enzyme activities in surface (0-0.7 cm) and subsurface (0.7-4.7 cm) soil layers [51]

Enzyme activity*	Treatment	Soil layer	Incubation time		
			5 weeks	13 weeks	21 weeks
Dehydrogenase	Dark control	Surface	88.9±8.2	67.0±2.4	50.9±1.1
		Subsurface**	27.5±3.4	13.1±2.4	9.1±0.6
	Inoculated	Surface	276.4±10.5	81.2±1.2	107.5±3.0
		Subsurface**	28.5±1.3	9.0±0.7	9.5±0.1
	Light control	Surface	154.9±10.8	105.8±2.0	139.1±4.2
		Subsurface**	23.9±3.3	8.3±0.5	9.8±0.2
	LSD (P=0.05)	Surface	44.5	6.6	13.8
Urease	Dark control	Surface	0.77±0.1	0.87±0.1	1.31±0.2
		Subsurface**	1.06±0.3	1.37±0.3	1.46±0.2
	Inoculated	Surface	1.92±0.1	2.12±0.3	3.61±0.4
		Subsurface**	1.29±0.4	1.89±0.3	1.71±0.2
	Light control	Surface	ND	2.67±0.3	5.60±0.5
		Subsurface**	ND	1.40±0.1	1.52±0.1
	LSD (P=0.05)	Surface	0.43	0.78	1.31
Phosphatase	Dark control	Surface	0.49±0.07	0.84±0.05	0.44±0.05
		Subsurface**	0.73±0.09	0.37±0.04	0.63±0.09
	Inoculated	Surface	0.84±0.16	1.87±0.06	1.40±0.13
		Subsurface**	0.66±0.06	0.38±0.07	0.66±0.08
	Light control	Surface	1.18±0.18	2.74±0.07	2.47±0.17
		Subsurface**	ND	0.37±0.08	0.71±0.06
	LSD (P=0.05)	Surface	0.21	0.43	0.47

* Expression of enzyme activities: dehydrogenase in μg of triphenylformazan/g soil/24 hours; urease in μmoles of ammonia/g soil/hour and phosphatase in μmoles of *p*-nitrophenol/g soil/hour.

** Average of activities measured in the four subsurface layers. All differences in enzyme activities for subsurface layers within treatments are not significant at $P \leqq 0.05$.

LSD - Least significant difference.

ND - Not determined.

Based on this observation and on the finding that after 13 and 21 weeks all enzyme activities were higher in the light control than in the inoculated soil, one can draw the conclusion that, under appropiate conditions of light and moisture, the indigenous species may be as effective as inoculated species in bringing about favourable changes to soil.

I. 1.2. Inoculation of phosphate-solubilising bacteria. Burangulova and Khaziev [6-8, 29] studied the effect of the commercial preparation Phosphorobacterin (containing phosphate-solubilising bacteria, PSB) on phosphohydrolase (phosphomonoesterase and RNase) activities in typical, calcareous and leached chernozems. Field experiments were carried out in different areas of Bashkiria. Spring wheat was the test plant. They plots were: 1. not inoculated and not fertilised; 2. inoculated; 3. inoculated and fertilised with urea (45 kg N/ha) or superphosphate (60 kg P/ha) and 4. not inoculated but fertilised with urea (45 kg N/ha), ammoniated coal humate (2 t/ha), superphospahte (60 kg P/ha), NPK (45, 60 and 60 kg/ha, respectively) or farmyard-manured (20 t/ha). During the vegetation period, rhizosphere and non-rhizosphere soil samples were analysed monthly. The grain yields were also recorded.

It could be deduced from the results obtained that inoculation of Phosphorobacterin had increasing effect only on the rhizosphere enzyme activities. This effect was strongest when inoculation was combined with urea fertilisation. However, the activities, like grain yields, were highest in the non-inoculated but farmyard-manured plots.

Badr El-Din and Saber [2] used a local strain of PSB - *Bacillus megaterium* var. *phosphaticus* for inoculation of 400-g potted samples of an Egyptian calcareous soil. The soil samples, containing 0.2% total soluble salts (TSS), were supplied with sodium chloride to establish four levels of TTS: 0.2, 0.4, 0.8 and 1.2%, then fertilised with ammonium phosphate (0.13 g/kg soil) and sown with barley seeds. The soil surface was inoculated with 10 ml of suspension containing 10^8 viable PSB cells/ml or with 10 ml of water (non-inoculated, control). During the growth period (60 days), soil humidity was maintained at about 50% of field capacity. Numbers of PSB and dehydrogenase activity in the rhizosphere of barley plants were assayed at 10-day intervals, and dry weight and P content of the plants were determined at day 60.

The results showed that the numbers of viable PSB cells were persistently higher in the inoculated soil and reached their peak almost at day 40 after sowing, then declined. The results also indicated that the numbers of these cells decreased with increasing salinity. The rhizosphere dehydrogenase activity was also negatively affected by increasing salinity, but its values were only a little higher in the inoculated than in the non-inoculated soils. Inoculation also resulted in increased dry weight and P uptake by the barley plants growing at 0.2%-0.8% TSS; at 1.2% TSS, the seeds failed to germinate in both non-inoculated and inoculated soils.

The phosphate-solubilising bacterium *Enterobacter agglomerans*, the culture of which was used by Kim *et al.* [31] for soil inoculation, had been isolated from wheat rhizosphere. The effects of organic energy and carbon sources on the survival of *E. agglomerans* and on several soil parameters were studied.

The silt loam soil used was collected from the AP horizon (0-30 cm) at Bradford Agronomy Research Center, Columbia, MO. Samples (400 g) of air-dried soil were placed in 600-ml beakers and 4 g of hydroxyapatite (HA) were mixed to yield soil containing 1% HA. *E. agglomerans* was cultured in LB medium (peptone 10 g; yeast extract 5 g; NaCl 5 g; distilled water 1 l; pH 7.0). The treatments of the soil-HA mixtures included:

C1 (control 1): 120 ml LB medium; without *E. agglomerans* and additional energy source;

C2 (control 2): 120 ml culture suspension (3.10^9 cells/ml); without additional energy source;

GL: 120 ml culture suspension + 1% glucose;

PA: 120 ml culture suspension + 1% phytic acid dodecasodium salt;

GP: 120 ml culture suspension + 1% glycerol-2-phosphoric acid disodium salt; and

SS: 120 ml culture suspension + 1% soluble starch.

We remark based on this list of treatments that non-inoculated controls with addition of organic energy sources which could it make possible to evaluate the contribution of the indigenous microbiota to the effects observed in the GL, PA, GP and SS treatments, were not included.

The beakers were incubated at 28^0C for 55 days. During incubation, the 30% soil moisture content (on dry weight basis) was maintained. Number of *E. agglomerans* cells and some soil parameteres, including alkaline and acid phosphatase activities, were determined at days 2, 10, 22 and 55.

In the C1 treatment, in which, as mentioned above, no *E. agglomerans* had been added to the soil, no PSB were detected, *i.e.* the soil used was devoid of detectable native PSB. Consequently, in the other (=inoculated) treatments all effects attributable to PSB were caused by the inoculated *E. agglomerans.*

In the C2 treatment, *E. agglomerans* was not detectable after 22 days, but it survived for the 55 days in all organic energy treatments, in which, however, the *E. agglomerans* population declined significantly with time. The survival of *E. agglomerans* in the GL treatment was about 2 to 15 times greater compared to the other treatments at days 10, 22 and 55.

In the C1, C2, GL and SS treatments, alkaline phosphatase activity at day 2 was significantly higher than at days 10, 22 and 55 and showed a progressive decrease with time. This decrease correlated with the decrease in the *E. agglomerans* population. In the PA and GP treatments, the activity was almost constant, but low during the incubation excepting PA at day 55.

Acid phosphatase activity at day 2 was higher than at day 10. After 10 days, this activity continuously increased to day 55 in all treatments.

Both phosphatase activities were higher in the GL and SS treatments than in the PA and GP treatments, as well as in the C2 treatments at day 2 than in the C1 treatment.

E. agglomerans in C2 treatment increased P solubilisation compared to C1 at day 2; however, with time there was no significant difference in C2 and C1, as well as in GL and SS. High soil P concentrations were found in PA (at day 55) and in GP (at days 22 and 55), exceeding 1.5-2.0 times the P concentrations measured in the other treatments on the same day.

I. 1.3. Combined inoculation of phosphate-solubilising and associative N_2-fixing bacteria. A combined bacterial preparation consisting of a mixture of three active PSB strains isolated from a typical chernozem and Rhizoenterin (a commercial product, in which peat serves as carrier of the culture of N_2-fixing *Enterobacter aerogenes*) was used by Shatokhina and Khristenko [57] for inoculation of maize seeds before sowing them (for silage production) into a typical chernozem of heavy loam texture located in the forest-steppe zone of Ukraine.

The experiment was carried out on 12.6-m^2 plots and had five variants: 1. farmyard-manured (40 t/ha) and heavily NPK-fertilised (120 kg N, 120 kg P and 80 kg K/ha); 2. manured (40 t/ha) and NPK-fertilised at a lower rate (60 kg N, 60 kg P and 40 K/ha); 3. the same as variant 2, but sown with inoculated seeds; 4. manured (60 t/ha), amended with straw (2 t/ha) and N-fertilised (20 kg/ha); 5. the same as variant 4, but sown with inoculated seeds.

The experiment lasted 4 years (1992-1995). The soil (arable layer) under the maize monoculture was periodically analysed for determination of several parameters, including peroxidase, polyphenol oxidase and dehydrogenase activities. Mean values of the activities measured during the 4-year experimental period showed that inoculation of seeds led to increased peroxidase activity (it was higher in variant 3 than in variant 2, and in variant 5 than in variant 4), to no evident changes in polyphenol oxidase activity, and to decrease or increase in dehydrogenase activity (it was lower in variant 3 than in variant 2, but higher in variant 5 than in variant 4).

The 4-year average of silage yields in the five variants presented the order: variant 2 = variant 4 < variant 5 < variant 1 < variant 3. In other words, inoculation had a beneficial effect on crop yield and, thus, the rate of NPK fertilisers can be reduced by 50% without affecting crop yield.

I. 1.4. Combined inoculation of potassium-solubilising and N_2-fixing bacteria. In a short report, Ozhiganova *et al.* [49] summarise the results of an experiment carried out in the Botanical Garden of the State University in Kazan (Russian Federation). Seeds of purple amaranth were inoculated with cultures of local strains of alumo-silicate bacteria (solubilising K from silicates) and *Azotobacter*.

Under the influence of inoculation, the N, P and K contents in the over-ground biomass of budding amaranth plants increased, and the yield increases ranged from 42 to 80%. Considerable (68-241%) increases were also registered in soil enzyme activities. Oxidoreductases and hydrolases were studied, but their names are not specified in the report.

I. 1.5. Inoculation of green algae. Dzhumaniyazov and his associates [1, 18-23, 41, 42, 62] have carried out laboratory and field experiments for increasing crop production by inoculation of seeds and soil with green algae. Most attention was paid to the cotton plants as cotton production has a great importance in the economy of Uzbekistan. Inoculation of wheat was also dealt with. For inoculation, suspensions of *Chlorella vulgaris* or of mixtures of this alga with *Scenedesmus obliquus* were used.

The technology elaborated by these investigators for treatment of cotton is outlined below. The field is fertilised (N 250, P 175 and K 100 kg/ha). Twenty-four hours before sowing, the seeds are soaked in a *Chlorella* suspension containing 30.10^6-40.10^6 cells/ml. The volume of algal suspension for soaking seeds for 1 ha of field is 80-100 l in the case of downy seeds and 40-50 l in the case of denuded seeds.

During the vegetation period, the soil is sprayed with *Chlorella* suspension twice, namely at the 5-6-leaf and bud formation stages, using 3 and 2 t of suspension/ha, respectively, for sprayings. Each spraying is followed by application of irrigation water.

For comparison, two controls were used. In one control, the seeds were soaked in the *Chlorella* suspension, but during the vegetation period the soil was not sprayed with the algal suspension. In the other control, the seeds were soaked in water. The seeds in both controls were sown into $N_{250}P_{125}K_{100}$-fertilised soil which, during the vegetation period, was also irrigated.

For exemplification of the results obtained, we reproduce a table published by Dzhumaniyazov in 1979 (see Table 4).

Table 4 shows that the technology applied proved to be efficient: excepting soil respiration, the other soil parameters studied, including peroxidase and polyphenol oxidase activities as well as the yield of raw cotton increased. Diminution of soil respiration (evolution of CO_2) is attributed to intense assimilation of CO_2 by the algae. Another observation was that the *Chlorella* treatment exerted a protective effect against cotton wilt and other diseases or, at least, reduced their incidence.

Table 4

Effect of the Chlorella treatment of cotton seeds and soil on some parameters of the arable layer and on the crop yield in an irrigated grey soil [19]

Treatment	Humus content (%)	Enzyme activities (mg purpurogallin/g soil)		Number of microorganisms (thousands/g soil)		Total amount of free amino acids (mg/ 100 g soil)	Soil respiration (mg CO_2/ 100 g soil)	Yield of raw cotton (kg/ha)
		Peroxidase	Polyphenol oxidase	Ammonifiers	Algae			
NPK + soaking of seeds in water	0.93	3.95	11.10	3500	66.6	1.60	46.1	3933
NPK + soaking of seeds in *Chlorella* suspension	1.29	4.10	11.60	5400	72.7	1.76	40.6	4435
NPK + soaking of seeds in *Chlorella* suspension and spraying the soil with *Chlorella* suspension during the vegetation period	1.38	4.36	11.90	6200	84.5	1.92	36.6	4693

I. 1.6. Combined inoculation of mycorrhizal and saprophytic fungi. Based on literature data, according to which inoculation with arbuscular mycorrhizal and saprophytic soil fungi may cause additive or synergistic growth enhancement of the inoculated host plants, Camprubí *et al.* [10] performed a pot experiment, using a selected strain of the mycorrhizal fungus *Glomus intraradices* isolated from a citrus nursery (Tarragona, Spain) and a strain of *Trichoderma aureoviride* isolated from a compost. The test plant was *Citrus reshni*. Three mixtures placed in 1-l pot containers were used: 1. sphagnum peat and autoclaved sandy soil (1/1, volume/ volume); 2. sphagnum peat, quartz sand and perlite (1/1/1, volume/volume/volume) and 3. fertilised pine bark compost (a commercial product) prepared for container-grown plants in nurseries. The potting mixtures planted with *C. reshni* were submitted to the following treatments: no inoculation (control); inoculation with *G. intraradices* (G.i.); inoculation with *T. aureoviride* (T.a.) and combined inoculation with *G. intraradices* and *T. aureoviride* (G.i. + T.a.).

The *G. intraradices* inoculum consisted of 10 g of rhizosphere soil from 1-year-old leek (*Allium porrum*) pot cultures. The suspension of *T. aureoviride* conidia used for inoculation contained 9.10^7 colony-forming units (cfu)/ml.

After 6 months of growth, the plants were harvested. The shoot dry weight was recorded. Segments of cleared and stained roots were examined microscopically and the percentage of the mycorrhizal roots was established. The potting mixtures were analysed to estimate the number of viable microbial cells and to determine four enzyme activities, namely fluorescein diacetate (FDA) hydrolysis, trehalase, phosphatase and β-N-acetyl-D-glucosaminidase activities in rhizosphere samples.

The effects of the single and combined inoculations largely depended on the potting mixtures used. The growth of *C. reshni*, as indicated by shoot dry weight, was stimulated by the combined inoculation of *G. intraradices* and *T. aureoviride* in potting mixtures 2 and 3. Moreover, there was a synergistic effect of the combined inoculation in potting mixture 3. *T. aureoviride* inoculated alone did not promote plant growth; contrarily, it reduced plant growth, the reduction being insignificant in potting mixtures 1 and 2 and significant ($P<0.05$) in potting mixture 3. The single inoculation with *G. intraradices* was effective in promoting plant growth in potting mixture 3 only.

The percentage of roots colonised by *G. intraradices* was not affected by *T. aureoviride* in any potting mixture. Number of viable bacterial cells in potting mixture 3 was significantly higher than in mixtures 1 and 2 in each of the four treatments. *T. aureoviride* inhibited the development of the indigenous fungi in all potting mixtures, but the inhibition by *T. aureoviride* vs. control and by *T. aureoviride* plus *G. intraradices* vs. *G. intraradices* were both significant in the mixture 3 only.

Table 5 shows that each activity in each treatment was highest in potting mixture 3 and lowest in mixture 1. None of the four enzyme activities was significantly different in the control and inoculated potting mixtures 1 and 2, but the activities behaved differently in potting mixture 3. FDA hydrolysis, trehalase and phosphatase activities gave the highest value in the G.i. treatment, and β-N-acetyl-D-glucosaminidase activity in the control. Taking into account the significance of differences, one can see from Table 5 that FDA hydrolysis activity was significantly lower in the G.i.+T.a. treatment, whereas trehalase activity was significantly higher in the G.i. treatment than in the other inoculated and control mixtures 3; phosphatase activity did not exhibit significant treatment-dependent changes, while β-N-acetyl-D-glucosaminidase activity was significantly higher in the control than in the inoculated mixtures 3.

Table 5

Enzyme activities in the rhizosphere of *Citrus reshni* grown in potted mixtures non-inoculated (control) and inoculated with *Glomus intraradices* (G.i.), *Trichoderma aureoviride* (T.a.) or with both fungi (G.i.+T.A.) [10]

Potting mixture	Treatment	Enzyme activities*			
		FDA hydrolysis	Trehalase	Phosphatase	β-N-Acetyl-D-glucosaminidase
1 (sphagnum peat + sandy soil)	Control	2.23 abc	25.54 a	17.48 a	6.11 a
	G.i.	1.11 a	32.29 a	16.40 a	6.46 a
	T.a.	1.23 ab	23.47 a	14.03 a	5.64 a
	G.i.+T.a.	1.76 ab	27.21 a	18.64 a	5.69 a
2 (sphagnum peat + quartz sand + perlite)	Control	5.36 d	46.81 a	35.45 b	16.73 b
	G.i.	4.24 cd	22.37 a	46.95 b	15.69 b
	T.a.	3.33 bcd	41.21 a	47.91 b	17.38 b
	G.i.+T.a.	4.23 cd	39.59 a	49.06 b	19.12 b
3 (pine bark compost)	Control	20.08 f	440.04 b	92.11 c	100.46 d
	G.i.	20.12 f	585.30 c	101.62 c	80.54 c
	T.a.	18.35 ef	441.49 b	95.38 c	82.97 c
	G.i.+T.a.	17.73 e	366.78 b	91.49 c	81.76 c

* Expression of enzyme activities: FDA hydrolysis in µg of fluorescein/2 g soil/4 hours; trehalase in µg of glucose/2 g soil/3 hours and phosphatase and β-N-acetyl-D-glucosaminidase in µg of *p*-nitrophenol/2 g soil/4 hours.

Numbers followed by the same letter in a column are not significantly different at P=0.05.

I.2. Soil enzyme activities as affected by inoculation of biocontrol microorganisms

I.2.1. Inoculation of Trichoderma sp. for controlling fungal diseases of plants. I.2.1.1. Inoculation of Trichoderma sp. against Verticillium dahliae. Shadmanova *et al.* [55, 56] have described two experiments, in which they determined some enzyme activities in grey soils cultivated with Tashkent-1 cotton variety. In both experiments, *Trichoderma* strains antagonistic to the agent of cotton wilt, *Verticillium dahliae* were tested.

In the first experiment, a part of the plots *naturally* infected with *V. dahliae* were left untreated (controls) and the others were treated with *Trichoderma* sp. (120 kg/ha) or glauconite (675 kg/ha) or with both *Trichoderma* sp. (120 kg/ha) and glauconite (675 kg/ha). After 20 days, the plots were sown with cotton. During the vegetation period (at 4-5-leaf, bud formation and fructification stages), soil samples were collected from the 10-20-cm depth for determination of acid and alkaline phosphatase and cellulase activities. At all growth stages, acid phosphatase activity was highest in the soil treated with both *Trichoderma* sp. and glauconite, alkaline phosphatase activity - in the *Trichoderma*-treated soil and cellulase activity - in the glauconite-treated soil. As the cotton plants were affected by wilt to a lesser extent and the yield of raw cotton was highest in the *Trichoderma* + glauconite treatment, it could be stated that acid phosphatase activity may be considered as a sensitive indicator of the biocontrol efficiency.

In the other experiment, the grey soil in vegetation pots was, before sowing of cotton, *artificially* infected with *V. dahliae* in the following variants: 1. not infected; 2. not infected but treated with 60 kg of *Trichoderma* sp./ha; 3. not infected but treated with 120 kg of *Trichoderma* sp./ha; 4. mildly infected (8 g of infected oats for 200 kg soil); 5. severely infected (75.6 g of infected oats for 200 kg soil); 6. mildly infected and treated with 60 kg of *Trichoderma* sp./ha and 7. severely infected and treated with 120 kg of *Trichoderma* sp./ha. For enzymological analyses soil samples were taken from the 5-25-cm layer before applying the treatments specified above and during the growth period of cotton, namely at the bud formation and fructification stages. Acid phosphatase activity was found to be highest in the infected and *Trichoderma*-treated variants at the fructification stage, at which invertase activity gave the lowest values. Alkaline phosphatase activity was less effected by the treatments. Reduction of the incidence of cotton wilt was most pronounced in the variant in which acid phosphatase activity was highest at the fructification stage. Thus, this activity was found again to be a sensitive indicator of the biocontrol efficiency.

I. 2.1.2. Inoculation of Trichoderma sp. against Pythium ultimum. In order to make biocontrol more effective, Morishita and Chiba [40] have prepared capsules from spores of *Trichoderma* sp., C- and N-nutrients (cellulose and amino acids) and various granular agents. During storage of these capsules,

viability of spores is preserved for long time. When the capsules are introduced to soil, the spores and nutrients are released. Due to the nutrients, the germination of spores and production of antimicrobial substances by the mycelia are stimulated.

In a greenhouse experiment, cucumber seedlings were transplanted in a soil infected by *Pythium ultimum* and amended with the *Trichoderma*-containing capsules. The control soil was not amended. Cellulase activity, determined after 2-month growth of cucumber plants, was higher in the amended than in the control soil. The increase in cellulase activity can be attributed to the cellulose released from the capsules: cellulose induced the microbial synthesis of new cellulase molecules. It was also established that the amendment decreased the damage caused by the infection to the cucumber plants, which proves that *Trichoderma* sp. exerted a suppressive effect on *Pythium ultimum*.

I. 2.2. Inoculation of Bacillus thuringiensis for controlling harmful insects (lepidopterous larvae) of forests. Sarkisyan [54] has determined invertase, amylase, phosphatase and catalase activities in grey forest soils under Siberian spruce and Siberian larch situated on the territory of the Tuva Autonomous Republic. Plots treated with Insectin, a commercial preparation containing the insecticide bacterium *Bacillus thuringiensis (Bacillus insectus)* and untreated (control) plots were studied. The bacterial suspension used for the treatment was prepared by diluting Insectin (30 l/ha) up to a density of 10^9 cells/ml. Soil samples were taken monthly in the May-October periods of 1976 and 1977. The seasonal variation in each of the four soil enzyme activities determined showed the same trend in the treated and control plots. But comparison of soil enzyme activities in the treated and control plots revealed that invertase and amylase activities were two times higher in the treated than in the control plots, while in respect of phosphatase and catalase activities there were no significant differences between the treated and control plots.

I. 2.3. Inoculation of Pseudomonas fluorescens F113Rif - a prospective biocontrol agent. The soil enzymological effects of the inoculation of this strain were studied by Naseby *et al.* [47]. *Pseudomonas fluorescens* F113Rif (hereafter referred to as F113Rif) is a spontaneous, rifampicin-resistant derivative of the wild-type F113. F113Rif produces the antifungal compound 2,4-diacetylphloroglucinol and, like its wild-type parent, inhibits *Pythium ultimum* (as proved under *in vitro* conditions) and, therefore, it may be considered a prospective biocontol agent.

The experiment was carried out at a field site not infected by pathogens such as *Pythium* and *Aphanomyces*, in order to avoid the possibility that eventual negative effects of the inoculant on soil properties, including enzyme activities, could be masked by its disease-suppressing ability. The test plant was sugar beet (*Beta vulgaris* var. *altissima*). The seeds were inoculated with F113Rif at a rate of 10^6 cfu/seed, with the use of a commercial powder formulation. Non-inoculated seeds served as controls.

The field site was located near Bandon (Country Cork, Ireland) on a brown podozolic soil, the A_p horizon (0-22 cm) of which corresponded to a loam. The soil was prepared by ploughing in October 1993, and was limed at the equivalent rate of 5.5 t $CaCO_3$/ha, 25 kg P/ha and 25 kg N/ha in March 1994 (to a pH of 7.2 in water). A composite fertiliser was applied 3 days prior to sowing. The fertiliser supplied nutrients at the following rates (per ha): 160 kg N, 49 kg P, 173 kg K, 62 kg Na, 37 kg S and 4 kg B. The sugar beet was sown on 15 April 1994. A further addition of N (51 kg/ha) was made 45 days after sowing. The sugar beet was harvested on 13 October 1994 (*i. e.* 181 days after sowing).

The numbers of viable F113Rif cells were determined periodically during the growth period, and approximately 10^6 cfu/root system were found several months after inoculation, but the numbers were below the detection limit at harvest time.

Activities of eight enzymes participating in nutrient (C, N, P and S) cycles, pH and amounts of readily available nutrients were determined in the rhizosphere soil of 15 inoculated and of 15 non-inoculated sugar beet plants as well as in 5 bulk soil samples obtained from places half-way between rows where non-inoculated sugar beets were grown. All samplings were made from the surface horizon, at harvest time.

The enzyme activities are expressed in mg *p*-nitrophenol released/g dry soil/hour, excepting urease activity which is expressed in mg NH_3 released/g dry soil/hour.

Mean values of enzyme activities in the rhizosphere soil of inoculated and non-inoculated plants and in the bulk soil, respectively, were the following:

alkaline β-galactosidase: 0.29, 0.28 and 0.33;
acid β-galactosidase: 0.33, 0.27 and 0.18;
β-N-acetyl-D-glucosaminidase: 0.30, 0.29 and 0.23;
urease: 0.71, 0.79 and 0.80;
alkaline phosphatase: 1.88, 1.52 and 1.66;
acid phosphatase: 6.99, 5.92 and 4.96;
phosphodiesterase: 0.42, 0.41 and 0.47;
arylsulphatase: 0.25, 0.24 and 0.26.

Statistical evaluation of the analytical data showed that none of the eight soil enzyme activities was significantly different (at $P \leq 0.05$) in the rhizosphere of inoculated and non-inoculated sugar beet plants. Likewise, pH and readily available nutrients were at similar levels in the rhizosphere of both inoculated and non-inoculated plants.

It was also found that the inoculation had no negative effect on the sugar beet yield in the absence of stress due to disease.

All these results suggest that inoculation of F113Rif had no significant effect on the functioning of the studied soil ecosystem, under typical farming practices.

I.3. Soil enzyme activities as affected by inoculation of bioremediating microorganisms

I. 3.1. Inoculation of hydrocarbon-oxidising microorganisms for remediation of oil-contamined soils. Ismailov *et al.* [25] installed microplots (1 m^2 each) on a grey-brown soil (located in the dry subtropical zone of Azerbaijan) and contaminated them with 4.4 l crude oil/m^2. The control microplots were not contaminated. Then, the microplots were submitted to remediation treatments in a series of variants. N and P fertilisers were used as aqueous solutions of $(NH_4)_2SO_4$ and KH_2PO_4 at rates of 12 g N and 9 g P_2O_5/m^2, respectively. Cultures of three hydrocarbon-degrading microorganisms (*Candida guilliermondii* 916, *Pseudomonas aeruginosa* 30 and an unidentified bacterium, strain 7) were applied as inocula (0.8 g of dry microbial bimass/m^2). Two biopreparation were also tested. They were obtained from grape marc cut intro small pieces or sawdust and a suspension of *C. guilliermondii* (0.5 g of dry yeast biomass to 200 g of grape marc or to 100 g of sawdust). During the experiment (at months 8, 19 and 32), soil enzyme activities (dehydrogenase, catalase, invertase, urease and protease) were determined. The phytomass and the residual oil content were also determined periodically.

All treatments applied to oil-contamined soil led to increased activity of each enzyme, excepting protease activity which was very low in all variants. Four treatments, namely addition of biopreparation 1 (grape marc) or 2 (sawdust), mixture of the three hydrocarbon-degrading microorganisms and $(NH_4)_2SO_4$ plus KH_2PO_4, gave especially good results in increasing the enzymatic potential of oil-contaminated soil. The phytomass obtained after 32 months was 3.3 g/m^2 in oil-contaminated microplots and increased to 7.0-48.8 g/m^2 in oil-contaminated and treated microplots, but the phytomass of uncontaminated microplots (65 g/m^2) was not achieved. Oil degradation was enhanced by each treatment. In 8 months, 56-76% of hydrocarbons were degraded in the treated microplots, but only 49% in those receiving no remediation treatment. After 8 months as after 20 months, the smallest amounts of residual oil were found in microplots treated with biopreparation 1 or inoculated with the mixture of the three microbial cultures. Biodegradation of aliphatic hydrocarbons was faster than that of the other oil components.

Based on the results of this field experiment, Ismailov *et al.* [26] have carried out a laboratory experiment with the aim to verify the effect of the hydrocarbon-degrading yeast strain *Candida guilliermondii* 916 on the remediation of oil-contaminated soils. 0.5-kg samples of a grey-brown soil from the Apsheron Peninsula were contaminated with 10% (weight/weight) crude oil and inoculated with a yeast suspension (10^6 or 10^9 cells/g soil) using grape marc (cut into small

pieces) as a carrier of yeast cells. Rates of grape marc application were 0.5 and 2% (on soil weight basis). N and P fertilisers (12 g N and 9 g P_2O_5/m^2) were also added to the samples. Oil-contaminated but untreated samples served as controls. Incubation took place under optimum humidity and aeration conditions at 28^0C and lasted 60 days, during which the samples were periodically submitted to enzymological and other analyses.

Dehydrogenase activity and oil degradation were most intense in the sample treated with 10^9 yeast cells/g soil + 0.5% grape marc and their values in this sample were especially high during the first 25 days of incubation and decreased later. During the 60-day incubation, numbers of the inoculated yeast cells continuously decreased from 10^9 to ~10^3/g soil and from 10^6 to ~10^2/g soil, respectively. Finally, oil degradation was 30% in the treatment with 10^9 yeast cells/g soil + 0.5% grape marc, whereas it was only 8% in the untreated control.

For enhancing crude oil degradation in contaminated soils (leached chernozem and grey forest soil), two mixed and two pure cultures of hydrocarbon-oxidising microorganisms were tested by Zhdanova *et al.* [67] and Kireava [32]. Both mixed cultures originated from oil industrial wastewaters. They contained *Pseudomonas* sp., *Candida* sp. (or *Arthobacter* sp.) and an unidentified Gram-negative rod-shaped bacterium. In pure cultures, *Candida maltosa* and *Pseudomonas* sp. strain 6 were tested. A biopreparation consisting of buckwheat (*Fagopyrum*) chaff inoculated with one of the mixed cultures (*Pseudomonas* sp., *Candida* sp., unidentified bacterium) was also studied. Rates of oil contamination of soil samples ranged from 0.5 to 20% (weight/weight), and rates of inoculation varied between 10^6 and 10^9 microbial cells/g soil.

Each culture enhaced oil degradation, the mixed cultures being more efficient than the pure ones. The negative effects of crude oil on soil enzyme activities were partially or completely removed by the cultures. Trus, in soil samples contaminated with 5 or 10% crude oil, then treated with a mixed culture or the biopreparation mentioned above and incubated for 3 and 12 months, dehydrogenase, catalase, invertase and urease activities exhibited significantly higher values after both incubation times, in comparison with the values of the non-inoculated contaminated samples. The activity-increasing effect of the cultures was more pronounced at 5 than at 10% oil contamination, and due to the biopreparation than due to the mixed culture. Invertase and urease activities of the less contaminated samples treated with the biopreparation even exceeded after 12 months the activity levels of the uncontaminated samples.

In a field experiment, Kireeva [33] used plots (> 200 m^2) set up on a land accidentally contaminated with crude oil (due to the rupture of a pipeline in the Kaltasin district of Bashkiria, Russian Federation) and uncontaminated plots. The oil content in the upper (0-20-cm) layer of the contaminated soil (grey forest soil of heavy loam texture) was 9.6% (weight/weight). The plots were treated with Paprin. This biopreparation consists of processed biomass of hydrocarbon-oxidising

Candida and *Rhodotorula* yeasts grown on *n*-alkanes. It is rich in proteins and vitamins. The treatment was carried out with suspensions containing 5 or 50 g of Paprin/1 water. The test plants were oats (in the first year), two-row barley (in the second year) and vetch-barley mixture (in the third year).

Dehydrogenase activity determined 1, 3, 6 and 12 months after the Paprin treatment followed the order: Paprin-treated contaminated soil > uncontaminated soil > untreated contaminated soil, at each time. The activity increased with time in the Paprin-treated soil and manifested little time-dependent changes in the other two soils.

The oil content determined in soil during 3 years continuously decreased and, after 3 years, the initial 9.6% oil content was reduced to 4.95 and 1.01% in the untreated and Paprin-treated contaminated soil, respectively.

The yield of test plants was highest in the Paprin-treated plots in each year. For example, the vetch-barley yield (t/ha) was 0.66 in the untreated contaminated plots, 2.80 in the uncontaminated plots and 4.95 in the treated contaminated plots.

Khaziev [30] described a two-year field experiment, in which two commercial biopreparations (Bacispecin and Devoroil), containing hydrocarbon-oxidising microorganisms, were used for remediation of an oil-contaminated leached chernozem. Bacispecin is prepared from *Bacillus* sp. strain 739, whereas Devoroil is a mixture of several species of hydrocarbon-oxidising microorganisms. In each year, after mineral fertilization of the contaminated soil, the plots were treated with 10 g of Bacispecin or Devoroil/m^2 or remained untreated (controls). At the end of the experiment, the degree of oil degradation was found to be 71 and 60% (in the Bacispecin- and Devoroil-treated soils, respectively), which is significantly higher than the 20% oil degradation registered in the control soil. In comparison with dehydrogenase and invertase activities of the control soil, dehydrogenase activity exhibited 34 and 9% increases in the soils treated with the two biopreparation, while invertase activity showed a 17% decrease in the Bacispecin-treated soil and no changes in the Devoroil-treated one.

I. 3.2. Inoculation of bacteria for remediation of soils polluted by emissions from coking plants. Dolgova [13, 14] studied a polluted soil (chernozem) in the vicinity of a coking plant. The polluting emissions from such plants contain a large variety of organic and inorganic substances (*e.g.* phenol, thiocyanates, SO_2, H_2S, NH_3, pyridine) bound or unbound to soot. For remediation of this soil, two biopreparations (cultures of the bacteria *Pseudobacterium lacticum* strain 392 and *Pseudomonas liquefaciens* strain 399) were tested. Experimental plots were set up. Farmyard manure (7 t/ha) and mineral fertilizers (90 kg N, 60 kg P and 90 kg K/ha) were added to the plots, then they were sown with bluegrass (*Poa pratensis*) seeds previously treated with strain 392 or 399 (at a rate of 2 g of biopreparation for 20 g of seeds). Seeds not submitted to the bacterial treatment served for comparison. The phenol oxidase activity, number of phenol-oxidising microorganisms and phenol-degrading capacity of soil were determined during the vegetation period.

Each of these three indices increased in the bacterial treatments as compared with the untreated control. In addition, the bacterial preparations exhibited an increasing effect on soil catalase and invertase activities, too. Strain 399 was always more efficient than strain 392.

I. 3.3. Inoculation of bacteria for remediation of heavy metal-polluted soils. Kucharski and Niklewska [36] have carried out a pot experiment, in which 6-kg samples of a brown soil of heavy loamy sand texture were treated with Zn at rates of 0, 10, 100 and 1000 ppm (in form of $ZnSO_4 \cdot 7H_2O$), fertilised with 0.15 g N (as urea), 0.8 g P (as KH_2PO_4), 1.6 g K (as KCl + K_2SO_4) and 0.3 g Mg (as $MgSO_4 \cdot 7H_2O$), and not inoculated or inoculated with *Streptomyces variabilis, Pseudomonas* sp. and *Bacillus megaterium*, the number of the inoculated viable cells being $1.9.10^4$, $3.4.10^4$ and $2.7.10^4$/g dry soil, respectively. Finally, the soil was sown with broadbean (*Vicia faba* cv. Dino) seeds. During the vegetation period, soil moisture was maintained at 60% of water-holding capacity.

On the 14th day of growth and after harvesting the plants (at flowering stage), several plant and soil analyses were performed. Thus, dry mass of shoots, roots and root nodules and some soil enzyme activities were determined after the harvest. It was found that in the non-inoculated soil, the Zn at 10 ppm decreased only the dry mass of nodules, but addition of Zn at 100 and 1000 ppm caused the decrease of the dry mass of shoots, roots and nodules.

The inoculated bacteria attenuated the negative effect of Zn on the growth and development of broadbean, when the Zn rate was 100 ppm, but the inoculations were inefficient at 1000 ppm of Zn.

In the non-inoculated soil, dehydrogenase activity decreased in parallel with the rate of Zn addition. Urease activity was not affected by the added Zn, whereas both acid and alkaline phosphatase activities were significantly inhibited at the highest Zn rate only. The inoculated bacteria had no significant effect on the soil enzyme activities, excepting a single case: inoculation of *Streptomyces variabilis* led to increased dehydrogenase activity in the soil treated with 10 and 100 ppm of Zn.

Another pot experiment for studying the effects of Zn and bacterial inoculation was described by Kucharski [34]. A light soil (leached brown soil of loamy sand texture) was used and the test plant was yellow lupine (*Lupinus luteus* cv. Amulet). Soil samples of 2.5 kg each were treated with the following fertilisers (mg/kg soil): P (as KH_2PO_4) 250; Mg ($MgSO_4 \cdot 7H_2O$) 25; B (H_3BO_3) 2.5; Mn ($MnSO_4 \cdot 7H_2O$) 2.5; Cu ($CuSO_4 \cdot 5H_2O$) 2.5 and Mo (Na_2MoO_4) 0.25. Then, $ZnSO_4 \cdot 7H_2O$ was added to the pots at rates of 0, 2, 40 and 400 mg Zn/kg soil. *Streptomyces variabilis* and *Pseudomonas* sp. were inoculated into the soil ($2.1.10^4$ and $2.3.10^4$ viable cells/g dry soil, respectively). The soil was sown with yellow lupine seeds and its moisture content was kept constant at 60% of water-holding capacity during the vegetation period. The plants were harvested at flowering stage, then the plants and soil were submitted to different analyses.

Dry mass of shoots and roots of plants that grew in non-inoculated soil was strongly decreased by the Zn. At the highest Zn addition (400 mg/kg soil), the plants died out.

Inoculation of *S. variabilis* was beneficial for the growth and development of yellow lupine: the inoculation resulted in mitigation of the harmful effects of Zn added at rates of 2-40 mg/kg soil, but it exhibited no protective effect at the highest Zn rate. *Pseudomonas* sp. behaved similarly with the difference that this bacterium was able to exert some protective action even at the highest Zn rate.

In non-inoculated soil, dehydrogenase activity was increased at 2 and 4 mg Zn and strongly inhibited at 40 mg Zn, whereas no dehydrogenase activity was detectable at 400 mg Zn. Urease and alkaline phosphatase activities were not markedly affected by Zn addition, but acid phosphatase activity was inhibited at the highest Zn rate. Inoculation of both bacteria led to further increases in dehydrogenase activity at 2 and 4 mg Zn. At 40 mg Zn, dehydrogenase activity further increased in the *S. variabilis*-inoculated soil and was strongly inhibited in the *Pseudomonas*-inoculated soil. In the inoculated soil, as in the non-inoculated control, dehydrogenase activity was lacking at 400 mg Zn. The other enzyme activities did not show significant differences between the non-inoculated and inoculated soil.

I. 3.4. Inoculation of bacteria enhancing phytoremediation of contaminated soils. Germida and Siciliano [24] have performed a laboratory experiment, in which surface (0-15-cm) sample of a sandy loam soil (from a site in Outlook, Saskatchewan, Canada) was initially contaminated with solid 2-chlorobenzoic acid (2CBA), a model compound, because chlorinated benzoic acids are intermediates in the degradation of polychlorinated biphenyls and chlorinated herbicides. Rate of contamination was 467 mg of 2CBA/kg soil. After contamination, the soil was stored moist in metal cans for two years, then the residual extractable, "aged" 2CBA was determined and found to be 63 mg/kg soil. Subsamples (160 g/pot) of the stored soil were planted with the forage grass Daurian wild rye (*Elymus dauricus*) seeds previously inoculated with a 1:1 mixture of *Pseudomonas aeruginosa* strain R75 and *Pseudomonas savastanoi* strain CB35 (about 10^7 combined cfu/seed). The other treatments were: 1. non-planted and non-inoculated soil; 2. non-planted but inoculated soil (the seeds were autoclaved before being inoculated and planted); 3. planted but non-inoculated soil (the seeds were inoculated with autoclaved pseudomonads). The pots were placed in a growth chamber and the soil was maintained at -0.33 kPa moisture capacity. At days 14, 21, 28, 35, 49 and 56 after planting, the 2CBA level in soil was determined.

The results proved that the pseudomonad inoculants enhanced the remediation effect of the Dahurian wild rye. Thus, at day 56, the residual extractable 2CBA content (mg/kg soil) was 55 in the non-planted and non-inoculated soil and significantly lower in the other treatments, namely 47 in the non-planted but inoculated soil, 33 in the planted but non-inoculated soil and 29 in

the planted and inoculated soil. In other words, the association of plant and pseudomonads was the most efficient and the plant alone was more efficient than the pseudomonads alone in reducing the 2CBA content in soil.

At day 21 after planting, the root system was removed from some pots for extracting the proteins from the rhizosphere. The extracts were filter-sterilised and the filtrates were used for determination of their 2CBA-degrading enzyme activity and their total protein contents. The degradative enzyme activity in the rhizosphere extracts was significantly higher (P=0.001) in the planted and inoculated treatment than in the other treatments, in which the activity increased insignificantly (P > 0.05) in the order: non-planted and non-inoculated ≈ non-planted but inoculated < planted but non-inoculated. Protease treatment of the rhizosphere extracts eliminated the degradative enzyme activity. Total protein contents in the rhizosphere extracts exhibited little differences between the treatments.

Other findings will also be mentioned.

Inoculation of pseudomonads did not affect plant parameters (shoot and root dry weight and root length) during the 56-day experiment.

The 2CBA-degrading enzyme is considered to be of plant origin and its activity is increased in the presence of the inoculated pseudomonads.

The soil in each treatment was able to partially degrade 3-chlorobenzoic acid (3CBA), too. The 3CBA-degrading capacity increased significantly (P=0.05) in the treatments in the following order: non-planted and non-inoculated < planted but non-inoculated < non-planted but inoculated < planted and inoculated. Contrarily, degradation of 2,3- and 2,5-dichlorobenzoic acids was most pronounced in the non-planted and non-inoculated soil, not being significantly influenced by the remediation treatments.

In another paper, Siciliano and Germida [59] also deal with the pseudomonad inoculation-enhanced phytoremediation of chlorobenzoates in rhizosphere soil, but in this paper the enzymological aspects of the enhanced phytoremediation were not referred to.

I. 4. Soil enzymological effects of other microorganisms introduced into soil for studying fundamental aspects of the microbial inoculations

I. 4.1. Inoculation of Pseudomonas sp. RC1 rif^{100}. This bacterium is a spontaneous mutant (resistant to 100 µg of rifampicin/ml) of the *Pseudomonas* sp. strain RC1 - a rhizobacterium capable of inhibiting the root growth of winter wheat. Bolton *et al.* [4, 5] used this mutant (hereafter referred to as RC1) for inoculation of four systems: 1. ambient microcosms: intact soil-core microcosms kept in the laboratory at ambient temperature (22^0C); 2. chamber microcosms: intact soil-core microcosms kept in growth chamber with temperature fluctuations that simulated average conditions in the field; 3. field lysimeters and 4. field plots. The field site is located on the Arid Lands Ecology Reserve (in southern Washington State). The soil is of coarse-silty texture.

For all systems, the surface soil (top 15 cm) was removed, mixed, sieved, treated and then replaced. The soil was treated as follows: 1. inoculated with RC1 and amended with 1% (weight/weight) dried and chopped alfalfa; 2. inoculated but not amended; 3. not inoculated but amended and 4. not inoculated and not amended. The amended soil received $1.7.10^8$ cfu of RC1/g dry soil, while the non-amended soil received $5.5.10^7$ cfu/g dry soil. A solution of ^{15}N-labelled $(NH_4)_2SO_4$ was added to the soil of all systems for a final enrichment of 15 atom% ^{15}N, the N addition being equivalent to 100 kg N/ha. Then, the soil was brought to a final moisture content of 16%, seeded with "Daws" winter wheat, and routinely watered as the soil dried.

There were two samplings of soil and wheat, namely at the 3-leaf and boot stages of growth. The 3-leaf stage was reached at days 18 (ambient microcosms), 45 (chamber microcosms) and 144 (field lysimeters and plots) after planting; sampling depth was 0-15 cm. The boot stage sampling was carried out at days 67, 110 and 205 after planting, respectively; sampling depths were 0-15, 15-35 and 35-55 cm. The samples were analysed to determine soil and rhizosphere populations of RC1, populations of native rhizoplane bacteria, species diversity indices of heterotrophic rhizoplane bacteria as well as soil dehydrogenase activity and shoot biomass and N contents.

The population of RC1 in the surface (0-15-cm) soil layer decreased during the growth of wheat in all four systems. At the 3-leaf stage, the cfu of RC1 were higher in the amended than in the non-amended soil in all systems and presented the orders: ambient microcosm > chamber microcosm > field plot ≈ field lysimeter (in the amended soil) and chamber microcosm ≈ ambient microcosm > field plot ≈ field lysimeter (in the non-amended soil). But at the boot stage, the RC1 population size was similarly low in all systems, both amended an non-amended.

Colonisation of wheat roots by RC1 at the 3-leaf stage was not different in the systems, amended and non-amended. At the boot stage, at which, as mentioned above, three soil layers were sampled, the rhizoplane population of RC1 decreased with increasing depth in all amended and non-amended systems, excepting the chamber microcosm, where the RC1 rhizoplane population was highest at the 15-35-cm depth.

The effect of RC1 on the different groups of native rhizoplane bacteria was different. Fluorescent pseudomonas were present on the rhizoplane in significantly lower number in soil inoculated with RC1. Inoculation of RC1 did not significantly affect the total number of heterotrophs and the number of NH_4-oxidising bacteria, but led to significantly increased number of denitrifiers.

Of the species diversity indices of the rhizoplane haterotrophic bacteria, determined at the 3-leaf stage, the species richness and the Shannon-Weaver indices were not affected, whereas the evenness and equitability indices were significantly decreased by the inoculation of RC1.

Soil dehydrogenase activity (Fig. 1), determined at the 3-leaf stage, was affected significantly by RC1 in the amended ambient and chamber microcosms, but not in any of the non-amended systems. RC1 inoculation brought about a significant (P ≤ 0.05) increase of dehydrogenase activity in the ambient microcosm and a significant decrease of the activity in the chamber microcosm.

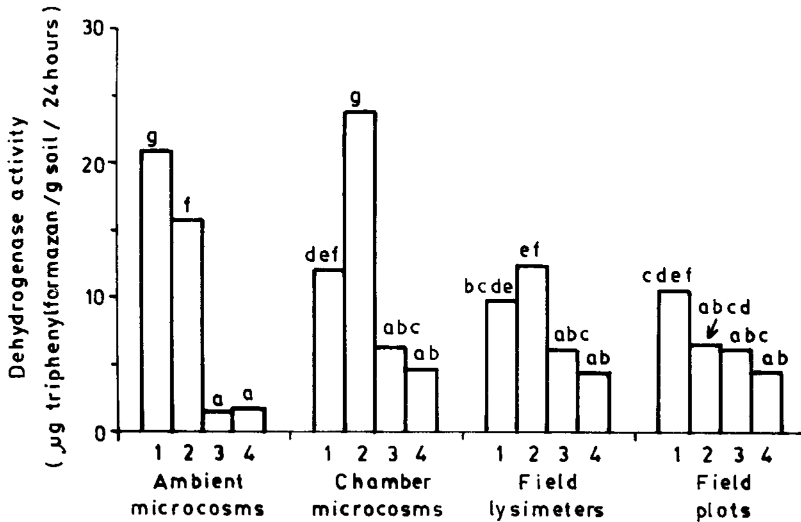

Fig. 1. *Soil dehydrogenase activity as influenced by inoculation of Pseudomonas sp. RC1 rif*^{100} *and alfalfa amendment in ambient and chamber microcosms and field lysimeters and plots at the 3-leaf stage of wheat growth* [5].

1 - Inoculated and amended. 2 - Non-inoculated but amended. 3 - Inoculated but non-amended. 4 - Non-inoculated and non-amended.

Bars labelled with different letters are significantly different at P ≤ 0.05.

Inoculation with RC1 had little influence on wheat shoot biomass at either stage of growth. Similarly, the total N and atom% ^{15}N contents of the wheat shoots, determined at the boot stage, were not influenced by inoculation of RC1.

I. 4.2. Inoculation of Flavobacterium sp. P25. The effect of this bacterium on non-rhizosphere, ectorhizosphere (=rhizosphere) soil and endorhizosphere (rhizoplane and root) enzyme activities was studied by Mawdsley and Burns [37]. *Flavobacterium* sp. P25 (hereafter referred to as P25) is a spontaneous mutant resistant to three antibiotics: streptomycin (250 µg/ml), rifampicin (100 µg/ml) and kanamycin (50 µg/ml). A calcareous grassland soil of silty loam texture, which had been the original source of P25, was used. The test

plant was winter wheat (*Triticum aestivum* var. Avalon). Soil was collected from a depth of 15-30 cm and, prior to use, it was sieved (2.88 mm). Then, soil samples (each being equivalent to 10 g dry weight) were placed in tubes and planted (at 1-cm depth) with a single seedling (germinated from surface-sterilised seed) and 1 ml of P25 suspension (1.10^9 cfu) was applied directly to the seedling, immediately after planting and before covering with soil. The experiment also comprised variants with planted but non-inoculated soil and non-planted but inoculated soil (1 ml of suspension containing 1.10^9 cfu of P25 was added to the soil surface). The tubes were kept in a growth chamber (20^0C) and the soil was maintained at 60% of water-holding capacity. At days 1, 7, 14, 21, 28 and 35, the non-rhizosphere and ectorhizosphere soil and endorhizosphere were assayed for determination of four oligase (α- and β-glucosidase, α- and β-galactosidase) and protease activities.

In the planted but non-inoculated soil, there were no significant changes in the four oligase activities of the non-rhizosphere soil, but in the ectorhizosphere the activities increased during root development so that by day 35 they were significantly ($P < 0.05$) greater (mean: +18%) than at the start of the experiment. In the endorhizosphere, α-galactosidase and α-glucosidase activities doubled over the 35-day experiment, whilst β-galactosidase activity also increased significantly ($P < 0.05$) by 37%. In contrast, β-glucosidase activity decreased by 50% by day 35.

In the non-planted but inoculated soil, no significant changes occurred in the enzyme activities.

In the planted and inoculated soil, the four oligase activities exhibited 5-18% increases in the ectorhizosphere and greater, 17-40% increases in the endorhizosphere.

Numbers of the indigenous bacteria expressing one or more of the four enzymes were also estimated in both inoculated and non-inoculated soil and compared to non-rhizosphere, ectorhizosphere and endorhizosphere enzyme activities. No relationship was found between numbers of these bacteria and the increases in enzyme activities.

Changes in the percentage of these bacteria during root development were also similar in the inoculated and non-inoculated soil. At the start of the experiment, the bacteria expressing the different enzymes were present in approximately equal numbers (about 7.10^8 cfu/g soil). But by the time the plant was 35 days old, 60-70% of bacterial isolates had α- and β-glucosidase activities and only 10-20% had α- and β-galactosidase activities. Exceptionally, the proportions of α-glucosidase-positive bacteria over the first 21 days were 20-40% greater in the inoculated than in the non-inoculated soil.

P25 inoculation had no significant effect on soil, ecto- and endorhizosphere protease activity up to day 14. However, by day 21 there was a 29% decrease in endorhizosphere protease activity which became more pronounced with time so that by day 35 it had only 55% of the activity measured in

the endorhizosphere in non-inoculated soil. But at day 35, soil and ectorhizosphere protease activity did not show significant differences between the inoculated and non-inoculated soil.

The findings that, in the P25-inoculated soil, oligase activities increased in the ecto- and endorhizospheres and protease activity decreased in the endorhizosphere may be causally related: more oligase molecules could survive as active catalysts in the ecto- and endorhizospheres due to the decreased protease activity in the endorhizosphere.

PART II. ENZYMOLOGY OF SOILS INOCULATED WITH GENETICALLY ENGINEERED MICROORGANISMS

II. 1. Inoculation of Pseudomonas putida PPO301(pRO103) and PPO301. This topic has been dealt with by Short *et al.*[58] and Doyle *et al.* [15]. *Pseudomonas putida* is a common soil bacterium. PPO301(pRO103) is a genetically engineered microorganism (GEM) - a *P. putida* strain containing plasmid-borne catabolic genes for the degradation of the herbicide 2,4-dichlorophenoxyacetate (2,4-D). The first step in the degradation pathway is oxidation of 2,4-D into 2,4-dichlorophenol (2,4-DCP) plus glyoxylate, and the last product is 2-chloromaleylacetate. This GEM can not mineralise 2,4-D, as it does not express chloromaleylacetate reductase. Plasmid pRO103 was derived from plasmid pJP4 by the insertion of the tetracycline resistance transposon Tn*1721* and a subsequent deletion of a *tfdR* regulatory gene. The deletion of the *tfdR* gene resulted in constitutive expression of plasmid pRO103.

PPO301, the homologous, plasmidless parental strain (PS) is not capable to degrade 2,4-D.

Both strains are resistant to nalidixic acid (500 μg/ml) and the GEM is also resistant to tetracycline (25 μg/ml) and Hg (25 μg/ml).

A sandy loam soil, collected from the upper 5 cm on an uncultivated aridic area in central Oregon, was used for laboratory experiments. The soil samples were adjusted to -33 kPa water tension and treated as follows: 1. amended with 500 μg of 2,4-D/g soil, oven-dry equivalent; 2. amended with 500 μg of 2,4-D/g soil plus 1% (weight/weight) glucose; 3. amended with 1% glucose and 4. left non-amended. In each treatment, the soil samples were inoculated with approximately 10^7 cfu of either GEM or PS or left non-inoculated. Subsamples of soil (50 g, oven-dry equivalent) were placed into 100-ml vials and incubated in jars at 24 ± 2^0C for 53 days. During the incubation, soil chemical, microbiological and enzymological analyses were carried out. We quote some of the results obtained.

2,4-D was degraded only in the soil inoculated with the GEM: in the studied aridic soil the indigenous microbiota did not degrade 2,4-D in contrast to the microbiota of other soils, in which, according to literature data, microbial degradation of 2,4-D takes place. Although the GEM degraded 2,4-D, there was no apparent increase in its survival relative to the PS: the number of both strains declined initially and then fluctuated between 10^5 and 10^6 cfu/g soil during the 53-day experiment.

Degradation of 2,4-D led to accumulation of 2,4-DCP. Due to this compound which exerted a toxic effect on soil fungi, in the 2,4-D-amended and GEM-inoculated soil no fungal propagules were detectable after 18 days of incubation, but in the soil that was not amended with 2,4-D but was inoculated with GEM the fungi became nondetectable only at day 53. Contrarily, the number of fungal propagules in the 2,4-D-amended and PS-inoculated soil remained high even et day 53.

Soil respiration (CO_2 evolution) in the case of soil amended with 2,4-D plus glucose and inoculated with GEM was significantly lower ($P < 0.05$) during the first 32 days and delayed relative to respiration in non-inoculated or PS-inoculated soil amended with 2,4-D plus glucose. This delayed rate of CO_2 evolution was not observed in soil amended with only glucose and inoculated with GEM. These results indicate that acumulation of 2,4-DCP caused the depression of soil respiration. However, the total amount of evolved CO_2 reached the same level after 53 days.

Accumulation of 2,4-DCP - which, as already stated, occurred only in the 2,4-D-amended and GEM-inoculated soil - affected only transiently the numbers of total heterotrophic, chitin-utilising and spore-forming bacteria.

Dehydrogenase activity increased in the glucose-amended soil, but it was suppressed by addition of 2,4-D, either with or without glucose. After day 18, the suppression of dehydrogenase activity in soil amended with both 2,4-D and glucose and inoculated with GEM was relieved as 2,4-D was degraded by GEM and 2,4-DCP had no effect on this activity. A similar relief of inhibition did not occur in soil amended with 2,4-D plus glucose and inoculated with PS or not inoculated.

Neither the GEM nor the PS had any apparent effect on the acid and alkaline phosphatase and arylsulphatase activities, irrespective of the addition of 2,4-D or/and glucose.

II. 2. Inoculation of Escherichia coli W3110(R702) and W3110. E. coli does not belong to the indigenous microbiota of soils. Therefore, its genetically engineered strain W3110(R702) was used by Doyle and Stotzky [16] as a model microorganism to detect and measure changes in microbial ecology of soil that may result from the introduction of allochthonous microorganisms.

The genetically engineered microorganism (GEM) W3110(R702) contains the plasmid R702 expressing resistance to kanamycin, tetracycline, streptomycin, sulphonamide and Hg. The homologous, plasmidless parental strain (PS) W3110 does not contain known genes conferring resistance to antimicrobials.

The soil used by Doyle and Stotzky [16] was the same aridic soil from central Oregon as that used in the experiments of Short *et al.* [58] and Doyle *et al.* [15] (see Section II.1.). The soil samples were adjusted to -33 kPa water tension with either a glucose solution yielding a final glucose concentration of 1% (weight/weight) or sterile tap water, then inoculated with GEM or PS (*ca.* 10^6 cfu/g soil, oven-dry equivalent) or left non-inoculated. Glucose-amended soil was used only for studying soil respiration. Subsamples of the soil (50 g) in 100-ml vials were placed into jars and incubated at 24 ± 2^0C for 33 days. During the incubation, a series of microbiological analyses were carried out periodically. Four enzyme (dehydrogenase, acid and alkaline phosphatase and arylsulphatase) activities were also determined. Some of the results obtained are specified below.

The number of PS was significantly lower ($P < 0.05$) than that of GEM by day 3, and PS and GEM could not be detected after days 3 and 7, respectively. The decline in added bacteria coincided with an increase in the number of protozoa. Although neither PS nor GEM was detectable after day 7, the experiment was continued to assess the changes that might only be discernible after amplification with time.

Transient, but significant differences were found in the numbers of spore-forming bacteria, fungal propagules and protozoa between soil inoculated with GEM or PS or not inoculated. But, there were no significant differences ($P > 0.05$) between the numbers of total, chitin-utilising, cellulose-utilising, nitrate-reducing and denitrifying bacteria in soil inoculated with GEM or PS or not inoculated.

Soil respiration was significantly more intense in the glucose-amended than non-amended soils when the GEM-, PS- and non-inoculated soils were considered separately. But comparison of the glucose-amended soils among each other and a similar comparison of the non-amended soils revealed no significant differences between respiration intensities of the GEM-, PS- and non-inoculated soils.

The four enzyme activities determined decreased during the first 3 days of incubation and then stabilised at relatively low levels. There were no consistently significant differences in the enzyme activities between soil inoculated with GEM or PS or not inoculated.

II. 3. Inoculation of Streptomyces lividans TK23.1 and TK23. The effects of these two strains on soil chemical, microbiological and enzymological properties were studied by Crawford *et al.* [11]. *Streptomyces lividans* TK23.1 is a GEM - a recombinant strain expressing a pIJ702.LP-encoded extracellular lignin peroxidase gene cloned from the chromosome of *Streptomyces viridosporus* T7A.

Plasmid pIJ702.LP also contains a thiostrepton resistance gene. The parental strain TK23 (PS) expresses chromosomally encoded resistance to spectinomycin.

The soil (silt-loam) used was collected from the 5-8 cm of an uncultivated field (University of Idaho Arboretum). Five-g air-dried soil samples were amended with the substrate of the extracellular enzyme produced by GEM, *i.e.* lignocellulose. Unlabelled lignocellulose, ^{14}C-lignin-labelled lignocellulose and ^{14}C-cellulose-labelled lignocellulose were prepared from poplar live cut twigs, to which unlabelled or ^{14}C-labelled lignin or cellulose precursor was added. Unlabelled and labelled plus unlabelled lignocellulose were used in a final amount of 50 mg/g soil, oven-dry equivalent. Non-amended soil samples were also used. The amended and non-amended soil samples were either not inoculated or inoculated with GEM or PS in form of spore suspension in water (approximately 10^6-10^8 cfu/g soil, oven-dry equivalent). The samples were wetted to 60% of water-holding capacity and incubated at 25^0C. During the incubation period which lasted up to 91 days, a great number of soil chemical, microbiological and enzymological analyses were performed.

A part of the results registered are summarised in the next paragraphs.

GEM and PS survived similarly in the studied soil, whether it was amended or not amended with lignocellulose. Viable counts of both strains slowly declined over a 63-day incubation period, but their populations increased to the initial inoculum levels by day 91. It is evident from these findings that the presence of lignocellulose did not give GEM an apparent selective advantage over the PS; expression of pIJ702.LP-encoded lignin peroxidase by GEM did not provide it with an environmentally advantageous phenotype.

Cumulative CO_2 evolution over a 91-day incubation period was significantly lower ($P < 0.05$) from non-amended than from lignocellulose-amended soils; in the non-amended soils, CO_2 evolution increased significantly in the GEM- and PS-inoculated soils comparative to the non-inoculated soil, whereas in the amended soils there was no significant differences in CO_2 evolution between the inoculated and non-inoculated soils.

Cumulative $^{14}CO_2$ evolution from ^{14}C-lignin-labelled lignocellulose-amended soils during 30 days of incubation increased, but insignificantly, in the order: non-inoculated < PS-inoculated < GEM-inoculated soil. During the same incubation period, the cumulative $^{14}CO_2$ evolution from ^{14}C-cellulose-labelled lignocellulose-amended soils was significantly higher in non-inoculated and GEM-inoculated soils (evolving the same amount of $^{14}CO_2$) than in the PS-inoculated soil.

In the ^{14}C-lignin-labelled lignocellulose-amended soils, the order of ^{14}C-label incorporation into humic acids after 30 days of incubation was: GEM-inoculated > PS-inoculated > non-inoculated soil. Both inoculated soils gave significantly higher values, twice that of the non-inoculated soil. But after 60 days, a reverse order was established: non-inoculated > GEM-inoculated > PS-inoculated

soil, the difference between the GEM- and PS-inoculated soils being insignificant. Incorporation of ^{14}C-label into fulvic acids showed, after 30 days, the order: non-inoculated > GEM-inoculated > PS-inoculated soil, and another order, after 60 days: GEM-inoculated > non-inoculated > PS-inoculated, the differences between soils being significant after both incubation periods.

In the experiment in which the soil was amended with ^{14}C-cellulose-labelled lignocellulose, the order of ^{14}C incorporation into humic acids was: GEM-inoculated > PS-inoculated > non-inoculated soil after 30 days (the differences between soils being significant), and GEM-inoculated > non-inoculated > PS-inoculated soil after 60 days (with no significant differences between soils). Incorporation of ^{14}C into fulvic acids followed the order: GEM-inoculated > non-inoculated > PS-inoculated soil after 30 days (with significant difference between GEM- and PS-inoculated soils), and non-inoculated > GEM-inoculated > PS-inoculated soil after 60 days (again, the difference between GEM- and PS-inoculated soils being significant).

The orders of the incorporation of ^{14}C-lignin and ^{14}-cellulose carbons of lignocellulose into humic and fulvic acids indicate that enhancement of incorporation is attributable to inoculation of GEM, but this effect is transient.

The soil microbiological analyses have shown that in general the effects of GEM on microbial populations and processes were also transient.

Soil dehydrogenase activity (Fig. 2) was higher in the lignocellulose-amended than in the non-amended soils. In the amended soils, the activity increased during the first 7 days of incubation, then continuously decreased and, thus, the lowest activity was recorded at the end of incubation period (day 35). The activity at both days 7 and 35 followed the order: GEM-inoculated > PS-inoculated > non-inoculated soil. Dehydrogenase activity in the non-amended soil was not affected by inoculation.

β-Glucosidase activity increased during the first 7 days in both amended and non-amended soils, then tended to decrease in amended soils and to increase in non-amended soils. This activity remained unaffected by inoculation during the whole incubation period (35 days).

Arylsulphatase and acid and alkaline phosphatase activities, determined after 3, 7 and 91 days of incubation, were higher in the amended than in the non-amended soils, excepting acid phosphatase activity at day 91, at which the reverse was true. The GEM-inoculated soil was significantly more enzyme-active than the other soils only in the following cases: arylsulphatase activity in amended an non-amended soils at day 3; alkaline phosphatase activity in amended soil at days 3 and 91. The significantly lowest values were registered in arylsulphatase activity of the non-amended PS-inoculated soil at day 3 and of the non-amended and non-inoculated soil at day 91. The conclusion can be drawn that the effects of GEM and PS on the activity of these three enzymes, participating in the biogeochemical cycling of S and P, are inexistent or only transient.

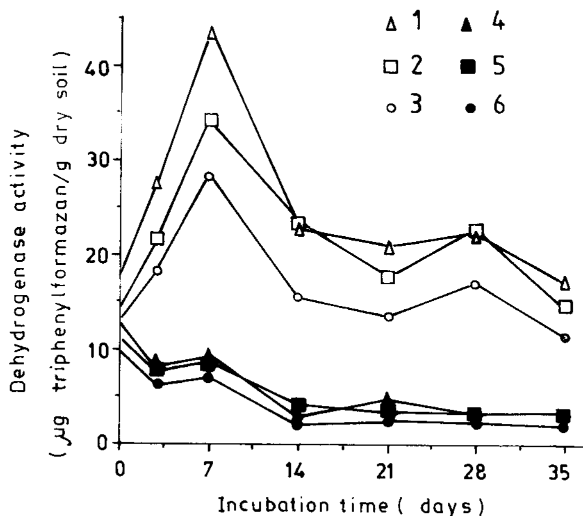

Fig. 2. *Dehydrogenase activity in soil amended with lignocellulose or non-amended and inoculated with the GEM Streptomyces lividans TK23.1 or with the parental strain S. lividans TK23 (PS) or non-inoculated* [11].

1 - Amended and GEM-inoculated. 2 - Amended and PS-inoculated.
3 - Amended but non-inoculated. 4 - Non-amended but GEM-inoculated.
5 - Non-amended but PS-inoculated. 6 - Non-amended and non-inoculated.

II. 4. Inoculation of Pseudomonas fluorescens 10586s/FAC510. This bacterial strain, studied by Meikle *et al.* [38], is a GEM containing chromosomally encoded genes for kanamycin resistance and *lux*A and B, the structural genes for luciferase, originally cloned from the bioluminescent marine bacterium *Vibrio fischeri*. This strain lacks functional *lux*C, D and E genes coding for the synthesis and recycling of the long-chain fatty aldehyde substrate of luciferase. Consequently, light emission by this strain requires addition of exogenous substrate (*n*-decyl aldehyde).

Twelve-g air-dried samples (microcosms) of a sandy loam soil were used. The soil pH, initially 6.46 (in water) and 5.38 (in $CaCl_2$ solution), was adjusted with $Ca(OH)_2$ to 7 to provide more favourable conditions for growth and activity of GEM. The microcosms were inoculated with volumes of GEM cell suspension, evenly dispersed, to enable equilibration at soil matric potentials of -30, -750 and -1500 kPa, with a final number of approximately 10^8 cells/g soil in all cases. These matric potentials were equivalent to moisture contents of 0.22, 0.070 and 0.041 ml water/g air-dried soil. A matric potential of -30 kPa was considered optimal for microbial growth and activity, -1500 kPa is wilting point and -750 kPa was chosen

as a convenient intermediate. Following inoculation, the microcosms were incubated at 25^0C for up to 3 months, and periodically sampled for estimation of the number of viable GEM cells, luminescence (measured in soil suspensions in phosphate buffer, after addition of *n*-decyl aldehyde, using an LKB 1251 Luminometer), dehydrogenase activity and substrate-induced respiration (50 μg unlabelled glucose plus U-^{14}C-glucose/g soil serving as substrate). During incubation, matric potentials were maintained by addition of water lost through evaporation. Each experiment was duplicated.

Numbers of viable GEM cells decreased during incubation and fell below detection limits in the two experiments by days 27 and 71 (at -30 kPa), 28 and 45 (at -750 kPa) and 22 and 30 (at -1500 kPa).

The experiments for estimation of luminescence (due only to the inoculated GEM), dehydrogenase activity and respiration (both due to GEM and endogenous microbiota) were terminated after 48, 70 and 49 days for -30, -750 and -1500 kPa, respectively.

Luminescence declined rapidly and was not detectable after 12 days (at -30 kPa). Decrease in luminescence were also observed at -750 and -1500 kPA, although low but measurable amounts of luminescence were maintained until days 70 and 30 at -750 and -1500 kPA, respectively.

Dehydrogenase activity initially increased then decreased to nearly constant values for the remainder of the experiment at each matric potential. However, the level of these nearly constant values was different at the three matric potentials, presenting the order: -30 kPa > -750 kPa > -1500 kPa. Similar decreases were recorded in respiration, although initial increases were less than those in dehydrogenase activity. Statistical analysis indicated that dehydrogenase activity was affected significantly by time ($P < 0.001$) and matric potential ($P < 0.01$), while respiration was only affected significantly by matric potential ($P < 0.001$).

As experiments with non-inoculated soil were not carried out, it is not possible to evaluate the contribution of the inoculated GEM to the measured soil dehydrogenase activity and respiration and to asses the effect of GEM on dehydrogenase activity and respiration of the indigenous soil microbiota.

II. 5. Inoculation of Pseudomonas fluorescens SBW 25 EeZY. The distinctive feature of the investigations performed by Naseby and Lynch [46] is that in one of the experimental variants inoculation of a GEM was combined with addition of a mixture of enzyme substrates. The studied GEM strain carries the marker genes *lac*ZY, kan^r and *xy1E.* The soil used was a silty loam taken from an agricultural site at Littlehampton (West Sussex, U. K.). Intact soil cores held in PVC tubes (60 cm in length and 15 cm in diameter) were extracted from the ground. In the glasshouse, they were placed in shallow trays filled with wet capillary matting, covered in polyethylene sheeting to reduce evaporation. Additional water was given to the top of the cores (150 ml per core every 48 hours) to ensure that the top soil did not dry out and to disseminate bacteria and

amendments through the soil cores, which were shaded by a coat of aluminium foil. Temperatures in the glasshouse were unregulated (8-32^{0}C).

Six treatments were applied:

1. control: non-amended soil cores, considered to be non-amended and non-rhizosphere soil;
2. substrate mix: soil cores were amended with a substrate mix, consisting of a solution containing 2% (weight/volume) colloidal chitin, 2% (w/v) urea and 2% (w/v) glycerophosphate; every 10 days, 100 ml of mix was added instead of water;
3. seed: soil cores were sown with wheat seeds (var. Axona);
4. seed + substrate mix;
5. seed + GEM: 10^8 cfu of GEM inoculated per seed; and
6. seed + substrate mix + GEM.

Cores were harvested after 60 days, cut open lengthways and divided into three arbitrary depth levels, 5-20, 20-35 and 35-50 cm. The soil was excavated to reveal the roots and rhizosphere soil was scraped directly from the root surface within the designated depth intervals. Non-rhizosphere soil was scraped from within the control cores from similar depths. The soil samples were submitted to enzyme extraction either at pH 5.5 or 8. The pH 5.5 extract was used for determination of β-N-acetyl-D-glucosaminidase, chitobiosidase and acid phosphatase activities, and the pH 8 extract - for determination of alkaline phosphatase, phosphodiesterase, arylsulphatase and urease activities. ATP content in the rhizosphere soil was also measured.

The number of viable GEM cells was also determined and it was found that the GEM had established as an effective population of approximately $5.8.10^5$ cells/g root in all the inoculated soil cores.

Each enzyme activity and the ATP content were evaluated in dependence of soil depth and the treatments applied.

The enzyme activities decreased significantly ($P < 0.01$) with depth, except for acid phosphatase activity, which was similar at all depths.

At the most enzyme-active soil depth (5-20 cm), the order of activity values in the six treatments was not the same for the seven enzyme activities determined.

β-N-acetyl-D-glocosaminidase activity: control < seed ≈ substrate mix < seed + GEM < seed + substrate mix + GEM < seed + substrate mix. That is, the seed + substrate mix treatment resulted in the highest activity. GEM inoculation, as compared to seed and substrate mix treatments, increased the activity but, as compared to the seed + substrate mix treatment, decreased it.

Chitobiosidase activity behaved like β-N-acetyl-D-glucosaminidase activity.

Urease activity: the order was the same as that recorded for the previous two enzyme activities, with the difference that urease activity was higher in the seed than in the substrate mix treatment.

Acid phosphatase activity did not change with depth (as already mentioned) and was not significantly affected by any of the treatments.

Alkaline phosphatase activity: seed + GEM < seed < seed + substrate mix < seed + substrate mix + GEM < control < substrate mix. This means that only the substrate mix alone led to increased activity as compared to that measured in the control, and seeding and GEM inoculation reduced it; the reduction was smaller in the seed + substrate mix + GEM treatment than in the seed + GEM treatment.

Phosphodiesterase activity was lowest in the seed + GEM treatment and had similar intensities in the other treatments.

Arylsulphatase activity: seed < control < seed + substrate mix + GEM < substrate mix < seed + GEM < seed + substrate mix. In other words, the activity-increasing effect was greatest in the seed + substrate mix treatment. GEM inoculation, as compared to seed treatment, increased and, in comparison with the seed + substrate mix treatment, decreased the activity.

ATP content, like the enzyme activities excepting acid phosphatase activity, decreased significantly ($P < 0.01$) with depth in all treatments. In the upper soil layer, ATP content presented the order: substrate mix ≈ seed + substrate mix < control ≈ seed < seed + substrate mix + GEM ≈ substrate mix + GEM. This order shows that substrate mix applied without GEM caused a reduction in the ATP content, but when applied in combination with GEM inoculation or with seed + GEM treatment, the substrate mix had an increasing effect on the ATP content.

It could be supposed that the three substrates in the mix, chitin, urea and glycerophosphate would induce the microbial synthesis of β-N-acetyl-D-glucosaminidase, chitobiosidase, urease and acid and alkaline phosphatase. But the results, as summerised above, indicate that the substrate mix did increase β-N-acetyl-D-glucosaminidase, chitobiosidase, urease and alkaline phosphatase activities and, unexpectedly, the arylsulphatase activity, too, but did not cause a significant difference in acid phosphatase and phosphodiesterase activities.

Concluding remarks. Comparison of the conditions, under which the investigations reviewed in Parts I and II were carried out, reveals a major difference: the soil enzymological effects of the inoculated natural microorganisms were studied under both laboratory and field conditions, whereas these effects of the genetically engineered microorganisms were studied only under laboratory conditions. In addition, these effects in dependence of the nature of microorganism and soil were more variable with the genetically engineered than with the natural microorganisms. These observations support the idea expressed in [15-17, 58] that the effects of the genetically engineered microorganisms on the microbial ecology of inoculated soils can be evaluated only on case-by-case basis for each genetically engineered microorganism and each soil.

REFERENCES

1. Allaberganov, Sh., Dzhumaniyazov, I. D., Allaberganov, N. A., *Vliyanie suspenzii* Chlorella vulgaris *Beijer. i* Scenedesmus obliquus (*Turp.*) *Kütz. UA-2-6 na soderzhanie organicheskikh veshchestv v oroshaemom serozeme*, in Taubaev, T. T. (Red.), *Kul'tivirovanie i Primenenie Mikrovodoroslei v Narodom Khozyaistve,* pp. 134-135, Izd. Fan, Tashkent, 1980.
2. Badr El-Din, S. M. S., Saber, M. S. M., *Effect of phosphate-dissolving bacteria on P-uptake by barley plants grown in a salt-affected calcareous soil*, "Z. Pflanzenernähr. Bodenk.", **146**, 1983, 545-550.
3. Bashan, Y., *Inoculants of plant growth-promoting bacteria for use in agriculture*, "Biotechnol. Adv.", **16**, 1998, 729-770.
4. Bolton, H., Jr., Fredrickson, J. K., Bentjen, S. A., Workman, D. J., Li, S. W., Thomas, J. M., *Field calibration of soil-core microcosms: Fate of a genetically altered rhizobacterium*, "Microb. Ecol.", **21**, 1991, 163-173.
5. Bolton, H., Jr., Fredrickson, J. K., Thomas, J. M, Li, S. W., Workman, D. J., Bentjen, S. A., Smith, J. L., *Field calibration of soil-core microcosms: Ecosystem structural and functional comparisons*, "Microb. Ecol.", **21**, 1991, 175-189.
6. Burangulova, M. N., *Fosfogidrolaznaya aktivnost' i voprosy biokhimicheskoi mobilizatsii fosfororganicheskikh soedinenii pochv*, in Girfanov, V. K. (Red.), *Azotnyi Fond i Biokhimicheskie Svoistva Pochv Bashkirii*, pp. 70-85, Inst. Biol., Ufa, 1977.
7. Burangulova, M. N., Khaziev, F. Kh., *A műtrágyák hatása a talaj foszfatáz-aktivitására*", "Agrokém. Talajtan", **14**, 1965, 101-110.
8. Burangulova, M. N., Khaziev, F. Kh., *Glubokaya obrabotka chernozemov i udobreniya kak sredstva vozdeistviya na ikh biokhimicheskuyu aktivnost'*, in Girfanov, V. K. (Red.), *Pochvy Bashkirii, Tom 2*, pp. 191-203, Inst. Biol., Ufa, 1975.
9. Burns, R. G., *Enumeration, survival, and beneficial activities of microorganisms introduced into soil*, in Huang, P. M., Berthelin, J., Bollag, J.-M., McGill, W. B., Page, A. L. (Eds.), *Environmental Impact of Soil Component Interactions. Vol. 2. Metals, Other Inorganics, and Microbial Activities*, pp. 145-164, CRC Press, Lewis Publ., Boca Raton, Florida, 1995.
10. Camprubí, A., Calvet, C., Estaún, V., *Growth enhancement of* Citrus reshni *after inoculation with* Glomus intraradices *and* Trichoderma aureoviride *and associated effects on microbial populations and anzyme activity in potting mixes*, "Plant Soil", **173**, 1995, 233-238.
11. Crawford, D. L., Doyle, J. D., Wang, Z., Hendricks, C. W., Bentjen, S.A., Bolton, H., Jr., Fredrickson, J. K., Bleakley, B. H., *Effects of a lignin peroxidase-expressing recombinant*, Streptomyces lividans *TK23.1, on biogeochemical cycling and the numbers and activities of microorganisms in soil*, "Appl. Environ. Microbiol.", **59**, 1993, 508-518.
12. Dodd, J. C., Burton, C. C., Burns, R. G., Jeffries, P., *Phosphatase activity associated with the roots and the rhizosphere of plants infected with vesicular-arbuscular mycorrhizal fungi*, "New Phytol.", **107**, 1987, 163-172.

13. Dolgova, L. G., *O fenoloksidaznoi aktivnosti pochvy v usloviyakh promyshlennogo zagryazneniya*, "Pochvovedenie", No. 9, 1973, 64-69.
14. Dolgova, L. G., *Biokhimicheskaya aktivnost' pochvy pri zagryaznenii*, "Pochvovedenie", No. 4, 1975, 113-118.
15. Doyle, J. D., Short, K. A., Stotzky, G., King, R. J., Seidler, R. J., Olsen, R. H., *Ecologically significant effects of* Pseudomonas putida *PPO301(pRO103), genetically engineered to degrade 2,4-dichlorophenoxyacetate, on microbial populations and processes in soil*, "Can. J. Microbiol.", **37**, 1991, 682-691.
16. Doyle, J. D., Stotzky, G., *Methods for the detection of changes in the microbial ecology of soil caused by the introduction of microorganisms*, "Microb. Releases", **2**, 1993, 63-72.
17. Doyle, J. D., Stotzky, G., McClung, G., Hendricks, C. W., *Effects of genetically engineered microorganisms on microbial populations and processes in natural habitats*, "Adv. Appl. Microbiol.", **40**, 1995, 237-287.
18. Dzhumaniyazov, I. D., *Vliyanie protokokkovykh vodoroslei na nakoplenie i razlozhenie gumusovykh veshchestv v oroshaemykh serozemakh*, "Trans. Int. Symp. 'Studies about Humus. Humus et Planta VI' (Praha, 1975)", 1975, 187-192.
19. Dzhumaniyazov, I. D., *Vliyanie mikrovodoroslei na biologo-biokhimicheskuyu aktivnost' pochvy i urozhainost' sel'skokhozyaistvennykh kul'tur*, "Trans. Int. Symp. 'Studies about Humus. Humus et Planta VII' (Brno, 1979)", 1979, 574-579.
20. Dzhumaniyazov, I. D., *Rol' mikrovodoroslei v povyshenii plodorodiya oroshaemogo serozema*, in Taubaev, T. T. (Red.), *Kul'tivirovanie i Primenenie Mikrovodoroslei v Narodnom Khozyaistve*, pp. 86-88, Izd. Fan, Tashkent, 1980.
21. Dzhumaniyazov, I.D., Kaziev, S.M., *Izmenenie aktivnosti mikroflory i soderzhaniya gumusa v oroshaemon serozeme pri vnesenii protokokkovykh vodoroslei*, in Taubaev, T. T. (Red.), *Kul'tivirovanie i Primenenie Mikrovodoroslei v Narodnom Khozyaistve*, pp. 82-84, Izd. Fan, Tashkent, 1980.
22. Dzhumaniyazov, I. D., Kim, S. G., Allaberganov, Sh., *K izucheniyu vliyaniya protokokkovykh vodoroslei i nekotorykh vitaminov na mikrobiologicheskuyu aktivnost' pochvy*, in Musaev, Sh. M. (Red.), *Biologiya i Selektsiya Mikroorganizmov*, pp. 138-144, Izd. Fan, Tashkent, 1980.
23. Dzhumaniyazov, I. D., Nafasov, R., Salakhitdinova, Kh., *Izmenenie mikrobiologicheskikh i biokhimicheskikh pokazatelei pochvy bogary pod vliyaniem inokulyatsii semyan pshenitsy,* "Tr. Nauchno-Issled. Inst. Bogarn. Zemledeliya Uz. SSR", No. 9, 1974, 153-155.
24. Germida, J., Siciliano, S., *The effect of pseudomonad inoculants on 2-chlorobenzoic acid degradation by* Elymus dauricus, "Poster, Symp. No. 43, 16^{e} Congr. Mondial Sci. Sol (Montpellier, 1998)".
25. Ismailov, N. M., Akhmedov, A. G., Akhmedov, V. A., *Rekul'tivatsiya neftezagryaznennykh zemel' sukhikh subtropikov Azerbaidzhana*, in Glazovskaya, M. A. (Red.), *Vosstanovlenie Neftezagryaznennykh Pochvennykh Ekosistem*, pp. 206-221, Izd. Nauka, Moskva, 1988.
26. Ismailov, N. M., Gadzhieva, V. I., Bairamova, E. M., Serebryakova, O. B., *Zondirovanie mikrobiologicheskikh protsessov v neftezagryaznennoi sero-buroi pochve uglevodorodokislyayushchimi drozhzhami*, "Izv. Akad. Nauk Az. SSR, Ser. Biol. Nauk", No.1, 1988, 97-102.

27. Jackman, R. H., Black, C. A., *Phytase activity in soils*, "Soil Sci.", **73**, 1952, 117-125.
28. Jarak, M., Protić, R., Govedarica, M., Milošević, N., Djurić, N., *Mikrobiološka aktivnost u zemljištu pod pšenicom inokulisanom diazotrofima*, "Zb. Nauč. Rad.", **3**, 1997, 39-44.
29. Khaziev, F. Kh., *O zavisimosti nukleazoi aktivnosti pochy ot pH i vliyanii na nee razlichnykh veshchestv*, "Biol. Nauki" (Moskva), No. 2, 1966, 223-227.
30. Khaziev, F. Kh. (Red.), *Pochvy Bashkortostana, Tom 2. Vosproizvodstvo Plodorodiya: Zonal'no-Ekologicheskie Aspekty*, pp. 288-299, Izd. Gilem, Ufa, 1997.
31. Kim, K. Y., Jordan, D., McDonald, G. A., Enterobacter agglomerans, *phosphate-solubilizing bacteria, and microbial activity in soil: Effect of carbon sources*, "Soil Biol. Biochem.", **30**, 1988, 995-1003.
32. Kireeva, N. A., *Biodestruktsiya nefti v pochve kul'turami uglevodorodokislyayushchikh microorganizmov,* "Biotekhnologiya", No. 1, 1996, 51-54.
33. Kireeva, N. A., *Vosstanovlenie plodorodya neftezagryaznennykh pochv*, "Neft. Gaz. Promyshlennost'", No. 7, 1996, 21-22.
34. Kucharski, J., *The effect of zinc on yellow lupine development and on activity of soil microorganisms*, "Pol. J. Soil Sci.", **25**, 1992, 177-183.
35. Kucharski, J., Niklewska, T., *The effect of manuring with straw and of introducing groups of microorganisms on the microbiological properties of light soil*, "Pol. J. Soil Sci.", **24**, 1991, 171-177.
36. Kucharski, J., Niklewska, T., *The influence of zinc on the yields of broadbean and microbiological activity of soil*, "Pol. J. Soil Sci.", **25**, 1992, 71-77.
37. Mawdsley, J. L., Burns, R. G., *Inoculation of plants with a* Flavobacterium *species results in altered rhizosphere enzyme activities*, "Soil Biol. Biochem.", **26**, 1994, 871-882.
38. Meikle, A., Amin-Hanjani, S., Glover, L. A., Killham, K., Prosser, J. I., *Matric potential and the survival and activity of a* Pseudomonas fluorescens *inoculum in soil*, "Soil Biol. Biochem.", **27**, 1995, 881-892.
39. Meikle, A., Glover, L. A., Killham, K., Prosser, J. I., *Potential luminescence as an indicator of activation of genetically-modified* Pseudomonas fluorescence *in liquid culture and in soil*, "Soil Biol. Biochem.", **26**, 1994, 747-755.
40. Morishita, T., Chiba, T., *Biological control using the capsule of* Trichiderma *sp. with C- and N-nutrients for* Pythium ultimum, "Abstr., 6th Int. Symp. on Microbial Ecology (Barcelona, 1992)", P2-06-13.
41. Muzafarov, A. M., Taubaev, T. T., Dzhumaniyazov, I. D., *Al'golizatsiya oroshaemykh zemel' protokokkovymi vodoroslyami i ee vliyanie na biologicheskuyu aktivnost' pochv i urozhainost' sel'skokhozyaistvennykh kul'tur,* "Inform. Soobshch., Inst. Mikrobiol., Akad. Nauk Uz. SSR", No. 203, 1978, 1-15.
42. Muzafarov, A. M., Taubaev, T. T., Dzhumaniyazov, I. D., *Protokokkovye vodorosli i ikh znachenie v rastenievodstve*, in Taubaev, T. T. (Red.), *Kul'tivirovanie i Primenenie Mikrovodoroslei v Narodnom Khozyaistve*, pp. 144-146, Izd. Fan, Tashkent, 1980.

43. Nafasov, R. N., *Vliyanie zamochki semyan risa suspensiei* Nostoc muscorum *na biologicheskuyu aktivnost' pochvy*, in Taubaev, T. T. (Red.), *Kul'tivirovanie i Primenenie Mikrovodoroslei v Narodnom Khozyaistve*, pp. 95-97, Izd. Fan, Tashkent, 1980.

44. Narula, N., Gupta, K. G., *Effect of ammonia-excreting* Azotobacter chroococcum *strain AC2 on enzymes of soils under cereals and legumes*, "Zentralbl. Mikrobiol.", **142**, 1987, 457-463.

45. Naseby, D. C., Lynch, J. M., *Functional impact of genetically modified microorganisms on the soil ecosystem*, in Zelikoff, J. T. (Ed.), *Ecotoxicology: Responses, Biomarkers and Risk Assessment*, pp. 419-442, SOS Publ., Fair Haven, New Jersey, 1997.

46. Naseby, D. C., Lynch, J. M., *Rhizosphere soil enzymes as indicators of perturbations caused by enzyme substrate addtition and inoculation of a genetically modified strain of* Pseudomonas fluorescens *on wheat seed,* "Soil Biol. Biochem.", **29**, 1997, 1353-1362.

47. Naseby, D. C., Moënne-Loccoz, Y., Powell, J., O'Gara, F., Lynch, J. M., *Soil enzyme activities in the rhizosphere of field-grown sugar beet inoculated with the biocontrol agent* Pseudomonas fluorescens *F113*, "Biol. Fert. Soils", **27**, 1998, 39-43.

48. Orlyanskaya, N. A., *Nekotorye fiziologo-biokhimicheskie izmeneniya v kornevoi sisteme kormovykh bobov pod vliyaniem nitragina*, in Boiko, L. A. (Red.), *Vliyanie Fiziko-Khimicheskikh Faktorov Sredy na Rasteniya*, pp. 112-118, Gos. Univ., Perm, 1978.

49. Ozhiganova, G. U., Degtyareva, I. A., Andreeva, M. G., Chernov, I. A., *Perspektivy primeneniya bakterial'nykh udobrenii pri vozdelyvanii amaranta bagyarnogo*, in *Pochvy Srednego Povolzh'ya i Urala, Teoriya i Praktika Ikh Ispol'zovaniya i Okhrany*, *Chast' 2*, pp. 165-166, Tatar. Knizhn. Izd., Kazan, 1991.

50. Rao, D. L. N., Burns, R. G., *Influence of nitrogen-fixing blue-green algae on some microbiological, biochemical and physical properties of a flooded brown earth*, "Trans. 14th Int. Congr. Soil Sci. (Kyoto, 1990)", **3**, 1990, 281-282.

51. Rao, D. L. N., Burns, R. G., *The effect of surface growth of blue-green algae and bryophytes on some microbiological, biochemical, and physical soil properties*, "Biol. Fert. Soils", **9**, 1990, 239-244.

52. Redžepović, S., Jarak, M., Milošević, N., Govedarica, M., Sikora, S., *Effect of calcification on proteolytic activity of acid soils under alfalfa*, "Proc. Abstr., 3rd Congr. Croatian Biol. (Mali Lošinj, 1987)", pp. 121-122.

53. Redžepović, M., Milošević, N., Jarak, M., Govedarica, M., Sikora, S., *Effect of calcification on cellulase activity of acid soils under alfalfa*, "Proc. Abstr., 3rd Congr. Croatian Biol. (Mali Lošinj, 1987)", pp. 122-123.

54. Sarkisyan, M. A., *Biologicheskaya aktivnost' lesnykh pochv pri vnesenii kristallonosnykh mikroorganizmov*, in Gukasyan, A. B. (Red.), *Mikroflora i Perspektivy Ee Ispol'zovaniya v Povyshenii Produktivnosti Lesov*, pp. 47-64, Inst. Lesa i Drevesiny, Sib. Otd. Akad. Nauk SSSR, Krasnoyarsk, 1978.

55. Shadmanova, N. A., Karimov, Kh. M., Ishankulova, M., *Enzimologicheskaya aktivnost' pochvy, infitsirovannoi* Verticillium dahliae *Kl., pri vnesenii trikhodermy*, "Sb. Nauch. Tr. Sredneaz. Nauchno-Issled. Inst. Zashchity Rast.", No. 12, 1978, 149-152.

56. Shadmanova, N. A., Nasretdinova, R. U., Sarymsakova, R. K., Mirzabaev, M. A., *Izmenenie biologicheskoi aktivnosti pochvy, zarazhennoi* Verticillium dahliae, *pri sovmestnom vnesenii griba trikhodermy s glaukonitom*, "Uzb. Biol. Zh.", No.2, 1977, 6-8.

57. Shatokhina, S. F., Khristenko, S. I., *Vliyanie razlichnykh sistem udobreniya i bakterial'nykh preparatov na mikrofloru, biokhimicheskie i agrokhimicheskie pokazateli chernozema tipichnogo, urozhainost' i kachestvo kukuruzy na silos*, "Agrokhimiya", No. 8-9, 1996, 3-14.

58. Short, K. A., Doyle, J. D., King, R. J., Seidler, R. J., Stotzky, G., Olsen, R. H., *Effects of 2,4-dichlorophenol, a metabolite of a genetically engineered bacterium, and 2,4-dichlorophenoxyacetate on some microorganism-mediated ecological processes in soil*, "Appl. Environ. Microbiol.", **57**, 1991, 412-418.

59. Siciliano, S. D., Germida, J. J., *Enhanced phytoremediation of chlorobenzoates in rhizosphere soil*, "Soil Biol. Biochem.", **31**, 1999, 299-305.

60. Strniša, J., Jarak, M., Jovićević, D., *Uticaj fungicida TMTD (tetramethylthiuram disulfide) na mikrobiološku aktivnost u rizosferi graška*, "Rad. Devetog Kongr. Jugoslovensk. Društva za Proučavanje Zemljišta (Novi Sad, 1997)", 1997, 487-491.

61. Tarafdar, J. C., Marschner, H., *Dual inoculation with* Aspergillus fumigatus *and* Glomus mosseae *enhances biomass production and nutrient uptake in wheat* (Triticum aestivum *L.) supplied with organic phosphorus as Na-phytate*, "Plant Soil", **173**, 1995, 97-102.

62. Taubaev, T. T., Dzhumaniyazov, I. D., Nasrullaev, B. N., Nafasov, R. N., *Vliyanie protokokkovykh vodoroslei na biologicheskuyu aktivnost' serozemnykh pochv i urozhainost' khlopchatnika*, in Taubaev, T. T. (Red.), *Vodorosli i Griby Srednei Azii*, pp. 119-128, Izd. Fan, Tashkent, 1974.

63. Van Elsas, J. D., Duarte, G. F., Rosado, A. S., Smalla, K., *Microbiological and molecular biological methods for monitoring microbial inoculants and their effects in the soil environment*, "J. Microbiol. Methods", **32**, 1998, 133-154.

64. Van Elsas, J. D., Hekman, W. E., Van Overbeek, L. S., Smit, E., *Problems and perspectives of the applications of genetically engineered microorganisms to soil*, "Trends Soil Sci.", **1**, 1991, 373-392.

65. Vladymyrova, O. V., *Hidrolitychna aktyvnist' aktynomitsetiv u hrunti*, "Mikrobiol. Zh." (Kiev), No. 3, 1970, 301-305.

66. Young, C. S., Burns, R. G., *Detection, survival and activity of bacteria added to soil,* in Bollag, J.-M., Stotzky, G. (Eds.), *Soil Biochemistry*, *Vol. 8*, pp. 1-63, Dekker, New York, 1993.

67. Zhdanova, N. V., Kireeva, N. A., Matytsina, O. I., *Nekotorye puti intensifikatsii biodestruktsii nefti v pochve*, "Neft. Gaz. Promyshlennost'", No. 5, 1994, 31-32.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, XLIV, 1-2, 1999

DIVERSITY OF SOIL NEMATODE COMMUNITIES IN THE SUATU NATURE RESERVE

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SUMMARY. - Specific and trophic structure of nematode communities in soil of the grassland in the Suatu Nature Reserve was analysed. The specific structure included 97 taxa belonging to 74 genera, some of them being found only in a specific sampling year (1997 or 1998). Most of the nematodes are ubiquitous species, often found in grasslands of the Carpathians and Transylvania. Seven species are new records for Romania: *Acrobeloides apiculatus, A. tricornis, Aglenchus fragariae, Eudorylaimus paesleri, Rotylenchus buxophilus, Takamangai balda* and *Tylencholaimellus eskei.* Six species with limited distribution were identified for the second time in Romania, namely: *Acromoldavicus skrjabini, Anatonchus hortensis, Axonchium bihariensis, Diphtherophora brevicolle, Discolaimus perplexans* and *Trophurus sculptus*. The highest density of nematode communities was found in the sod horizon. Changes in the trophic structure of nematode communities were noticed in 1998 (preponderance of omnivores) as compared to 1997 (dominance of plant feeders). The Maturity Index (MI) increased in 1998 as compared to the previous estimation, while Plant Parasite Index (PPI) had almost similar values. The PPI/MI ratio indicated an optimal use of trophic resources offered by the grassland ecosystem. These evaluations show that the nematode communities studied are in a stable equilibrium with their environment.

The increasing information on the soil fauna of natural or slightly man-affected ecosystems could provide useful insights into terrestrial habitats [10, 11, 13]. The soil nematodes are important components of all ecosystems. Their density and diversity are only surpassed by microorganisms.

The analysis of nematode community structure is now frequently used as a tool in ecological studies [1, 3, 6, 7, 9, 12, 14 - 16]; the nematode communities can be used as models and indicators to assess the quality of ecosystems [3, 8, 10, 17].

Monitoring of some protected areas by using soil nematode communities represented a point of reference for the evaluations of unprotected habitats.

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The Suatu area is a botanical nature reserve chosen for a biomonitoring programme which started in 1997. The study of soil nematode communities aimed to identify the specific and trophic structure changes of this fauna in two consecutive years of samplings.

This paper presents the first observations on soil nematode communities developed in the Suatu Nature Reserve.

Site description. The Suatu Botanical Reserve, established in 1932, on a surface of about 9.2 ha, is situated in the Transylvanian Plain (Cluj county), at an altitude of 370-430 m above sea level, closed to the village bearing the same name. This reserve shows a marked steppe character proved by the presence of xerophyllous plants, some of them being endemic (*Astragalus péterfii*) or rare (*e.g. Astragalus monspessulanus*, *Salvia nutans*, *Nepeta ucranica* etc.) for Romania. The plant association *Salvio nutantis-Festucetum rupicolae* (Zólyomi, 1937) Soó, 1964 characterises this nature reserve.

Material and methods. Soil samples were collected during the summers of 1997 and 1998, with an open corer of 15 cm in length and 2.2 cm in diameter. Five replicates, each composed of ten cores, were collected from the top 10 cm of soil. The nematodes were extracted by centrifugation method [8], fixed and preserved in 4% formaldehyde solution.

The taxonomic classification and the ordering of genera into feeding groups followed B o n g e r s [2] and Y e a t e s *et al.* [18], respectively. The Maturity Index (MI) and Plant Parasite Index (PPI) were calculated according to B o n g e r s [3] and B o n g e r s *et al.* [4].

Results and discussion. The specific structure of the soil nematode communities in the Suatu Botanical Reserve included 97 taxa belonging to 74 genera. One taxon was identified only to the family level (Table 1). There are slight differences in the structure of nematode communities identified in the two successive years (Table 1). Most of the nematodes are ubiquitous species, commonly found in grasslands of the Carpathian Mountains and Transylvania [14, 15].

Seven species are new records for the Romanian nematode fauna, namely: *Acrobeloides apiculatus, A. tricornis, Aglenchus fragariae, Eudorylaimus paesleri, Rotylenchus buxophilus, Takamangai balda* and *Tylencholaimellus eskei* (marked with ** in Table 1).

Six species with limited distribution in Romania were found here for the second time: *Acromoldavicus skrjabini, Anatonchus hortensis, Axonchium bihariensis, Diphtherophora brevicolle, Discolaimus perplexans* and *Trophurus sculptus* (marked with * in Table 1).

Table 1

Specific structure of soil nematode communities (D%) in the Suatu Nature Reserve

Taxon	Sampling year		Taxon	Sampling year	
	1997	1998		1997	1998
Acrobeles complexus	1.26	0.72	*Diploscapter coronatum*		0.06
Acrobeloides		0.38	*Discolaimus perplexans*	0.15	0.30
*A. apiculatus***	0.10		*Ditylenchus*	2.67	1.67
A. buetschli	0.05	0.10	*Dorylaimoides*	0.40	0.78
A. nanus	5.69	0.23	*D. elegans*	0.86	1.34
*A. tricornis***	0.15		*Eucephalobus*	1.01	0.17
Acrolobus emarginatus		0.24	*E. oxyuroides*	0.10	0.52
Acromoldavicus	0.25	1.50	*Eudorylaimus*	0.96	2.76
*skrjabini**			*E. carteri*	0.19	
Aglenchus agricola	2.77	0.13	*Eudorylaimus paesleri***	0.01	
*A. fragariae***	0.10		*Eumonhystera*		0.13
Alaimus	0.20	0.17	*Filenchus*	10.47	10.55
A. parvus	0.05	0.05	*Funaria*	0.05	
Amplimerlinius		0.24	*Geomohystera villosa*		0.17
Anaplectus granulosus	0.96	0.15	*Gracilacus*	0.60	0.85
*Anatonchus hortensis**		0.49	*Helicotylenchus*	1.31	
Aphelenchoides	1.81	2.06	*Heterocephalobus elongatus*	0.05	0.05
Aphelenchus avenae	4.13	2.33	*Heterodera*	0.15	
Aporcelaimellus	0.81	2.29	*Longidorella*	0.30	2.26
obtusicaudatus			*Longidorus*	0.35	0.38
Aporcelaimus	0.10		*Malenchus*		0.13
Aulolaimus		0.27	*Meloidogyne*		0.05
*Axonchium bihariensis**	1.76	3.22	*Mesocriconema*	0.86	3.22
Bastiania gracilis	0.05		*M. rusticum*	0.30	
Boleodorus thylactus	0.50	0.96	*Microdorylaimus*	0.15	
Bunonema reticulatum		0.26	*Mononchus truncatus*		0.05
B. richtersi		0.03	*Mylonchulus brachyuris*	0.50	1.56
Cephalobus	1.41	1.54	*Nygolaimus*	0.15	0.49
C. persegnis	0.25	0.05	*Panagrolaimus rigidus*	0.25	0.51
Cervidellus	0.10		*Paramphidelus*	0.10	0.16
Chiloplacus	1.26	0.56	*P. dolichurus*	0.70	0.68
Coslenchus costatus	5.89	4.65	*P. uniformis*	0.05	
Criconemoides informis	1.86	0.08	*Paraphelenchus*	0.35	0.13
Cylindrolaimus	0.05		*pseudoparietinus*		
Diphtherophora		1.15	*Paratylenchus*	7.55	3.09
*D. brevicolle**	1.31		*Paraxonchium laetificans*	0.15	0.10
D. communis	0.35	0.49	*Pelodera*		0.03

Table 1 (continued)

Taxon	Sampling year 1997	1998	Taxon	Sampling year 1997	1998
Plectus	0.81	0.55	*T. ettersbergensis*	0.96	
P. acuminatus	0.45	0.15	*Tripyla*		0.05
P. parvus		0.27	*Tripylina arenicola*	0.60	1.43
Pratylenchus	0.86	0.28	*Trophurus sculptus**		0.05
Prionchulus punctatus		1.18	*Tylenchollaimellus eskei***	0.25	2.04
Prismatolaimus intermedius	0.75	0.20	*Tylencholaimus*	0.45	8.06
			T. minimus	1.06	
Rhabditidae		0.12	*T. stecki*	0.05	
Rotylenchulus borealis	1.51	0.37	*Tylenchorhynchus dubius*	3.98	5.52
Rotylenchus	5.58	17.66	*Tylenchus*	2.37	1.02
*R. buxophilus***	12.00		*Tylocephalus auriculatus*	0.55	0.87
Steinernema	0.10	0.49	*Wilsonema otophorum*	0.45	0.17
*Takamangai balda***	0.50	2.38	*Ypsylonellus vexilliger*	2.37	0.76

Note: * Species with limited distribution; ** New records for the Romanian fauna.

The dominant nematode taxa were *Rotylenchus, R. buxophilus, Filenchus, Paratylenchus, Coslenchus costatus, Acrobeloides nanus* and *Tylenchorhynchus.*The genera *Filenchus* and *Rotylenchus* are eudominant. *R. buxophilus* had the highest contribution to the nematode communities.

The highest densities of nematodes were found in the sod horizon (1043 ind./100 g dry soil and 3839 ind./100 g dry soil, in 1997 and 1998, respectively).

The trophic structure of the nematode communities showed the dominance of plant feeders followed by bacterial feeders, hyphal feeders and omnivores in samples collected in 1997 (Table 2). Changes in this structure took place during 1998, when the decreasing order of feeding groups was: plant feeders > omnivores > hyphal feeders > bacterial feeders > predators. A high contribution of the omnivorous nematodes in 1998 was in defavour of plant feeding and bacterial feeding ones.

The values of Maturity Index (MI) increased in 1998 in comparison to 1997, while those of Plant Parasite Index (PPI) were almost similar in both estimations (Table 2). These values reflected a stable equilibrium of soil nematode communities in this grassland.

Values of the ratio PPI/MI indicated an optimal using of trophic resources offered by the grassland ecosystem [5].

Table 2

Trophic structure (%), Maturity Index (MI), Plant Parasite Index (PPI) and PPI/MI ratio of soil nematode communities in the Suatu Nature Reserve

	Sampling year	
	1997	1998
Bacterial feeding	19.83	11.50
Hyphal feeding	12.23	15.80
Plant feeding	59.03	49.40
Omnivores	7.40	16.0
Predators	1.41	5.60
Insect parasites	0.10	0.50
MI	2.68	3.35
PPI	2.50	2.57
PPI/MI	0.96	0.77

Conclusions. 1. A relatively high specific diversity of nematode communities was found in the soil of grassland in the Suatu Nature Reserve (97 taxa belonging to 74 genera). Most of the nematodes are ubiquitous species, frequently found in grasslands of the Carpathians and Transylvania.

2. Seven species are new records for the Romanian fauna, namely: *Acrobeloides apiculatus, A. tricornis, Aglenchus fragariae, Eudorylaimus paesleri, Rotylenchus buxophilus, Takamangai balda* and *Tylencholaimellus eskei.* Six nematodes with limited distribution in Romania were found here for the second time, namely: *Acromoldavicus skrjabini, Anatonchus hortensis, Axonchium bihariensis, Diphtherophora brevicolle, Discolaimus perplexans* and *Trophurus sculptus*.

3. Changes in the trophic structure of nematode communities were noticed in 1998 as compared to 1997: the plant feeding group had significant contributions in 1997 and the omnivores in 1998.

4. The values of the Maturity Index (MI) increased in 1998 as compared to those from the previous year, while the Plant Parasite Index (PPI) had almost similar values. Both values reflect a stable equilibrium of the nematode communities with their environment. The PPI/MI ratio indicated an optimal use of trophic resources offered by the grassland ecosystem.

REFERENCES

1. B o n g e r s, M., H a r r i s o n, A., *Nematode fauna of a British atlantic heathland*, in D e G o e d e, R.G.M., B o n g e r s, T. (Eds.), *Nematode Communities of Northern Temperate Grassland Ecosystems*, pp.15-20, Focus, Giessen , 1998.
2. B o n g e r s, T., *De nematoden van Nederland,* KNNV Bibliotheekuitgave, No. 46, Pirola, Schoorl, 1988.
3. B o n g e r s, T., *The Maturity Index: an ecological measure of environmental disturbance based on nematode species composition,* "Oecologia", **83**, 1990, 14-19.
4. B o n g e r s, T., de G o e d e, R. G. N., K o r t h a l s, G., Y e a t e s, G. W., *Proposed changes of c-p classification for nematodes*, "Russ. J. Nematol.", **3**, 1995, 61-62.
5. B o n g e r s, T., v a n d e r M e u l e n, H., K o r t h a l s, G., *Inverse relationship between the nematode maturity index and plant parasite index under enriched nutrient conditions*, "Appl. Soil Ecol.", **6**, 1997, 195-199.
6. D e G o e d e, R. G. M., *Terrestrial nematodes in a changing environment,* Ph. D. Thesis, CIP - Koninklijke Bibliotheek, Haag, 1993.
7. D e G o e d e, R. G. M., B o n g e r s, T., *Nematode community structure in relation to soil and vegetation characteristics*, "Appl. Soil Ecol.", **1**, 1994, 29-44.
8. D e G r i s s e, A. T., *Redescription ou modifications de quelques techniques utilisées dans l'étude des nématodes phytoparasitaires*, "Meded. Fac. Landbouww. Gent", **34**, 1969, 351-370.
9. F r e c k m a n, D. W., *Bacterivorous nematodes and organic matter decomposition*, "Agric., Ecosyst. Environ.", **24**, 1988, 195-217.
10. H o d d a, M., W a n l e s s, F. R., *Nematodes from an English chalk grassland: species distribution*, "Nematologica", **40**, 1994, 116-132.
11. H o d d a, M., W a n l e s s, F. R., *Nematodes from an English chalk grassland: population ecology*, "Pedobiologia", **38**, 1994, 530-545.
12. J i m e n e z M i l a n, F., G o m e z B a r c i n a, A., *Nematode communities from a coast-mountain transect in Sierra Nevada, Spain*, in D e G o e d e, R. G. M., B o n g e r s, T. (Eds.), *Nematode Communities of Northern Temperate Grassland Ecosystems*, pp. 147- 150, Focus, Giessen, 1998.
13. N o r t o n, D. C., N i b l a c k, T. L., *Biology and ecology of nematodes*, in N i c k l e, W. R. (Ed.), *Manual of Agricultural Nematology*, pp. 85-102, Dekker, New York, 1991.

14. P o p o v i c i, I., *Structura şi dinamica comunităţilor de nematode* (Nematoda), in P o p o v i c i, I. (Ed.coord.), *Parcul Naţional Retezat-Studii Ecologice*, pp. 200-214, West Side, Braşov, 1993.

15. P o p o v i c i, I., *The use of soil nematodes to assess the quality of technogenic soil rehabilitation*, "Stud. Univ. Babeş-Bolyai, Biol.", **38** (1-2), 1993, 43-53.

16. P o p o v i c i, I., *Structure of nematode communities in mountain grasslands from Romania*, in D e G o e d e, R. G. M., B o n g e r s, T. (Eds.), *Nematode Communities of Northern Temperate Grassland Ecosystems*, pp. 221-240, Focus, Giessen, 1998.

17. S a m o i l o f f, M. R., *Nematodes as indicators of toxic environmental contaminants,* in V e e c h, J. A., D i c k s o n, D. W. (Eds.) *Vistas on Nematology*, pp. 433-439, Painter Printer Co., DeLeon Springs, Florida, 1987.

18. Y e a t e s, G. W., B o n g e r s, T., D e G o e d e, R. G. M., F r e c k m a n, D. W., G e o r g i e v a, S. S., *Feeding habits in nematode families and genera - an outline for soil ecologists*, "J. Nematol.", **25**, 1993, 315 - 331.

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DIPLOPODE DE PE VALEA ARIEŞULUI

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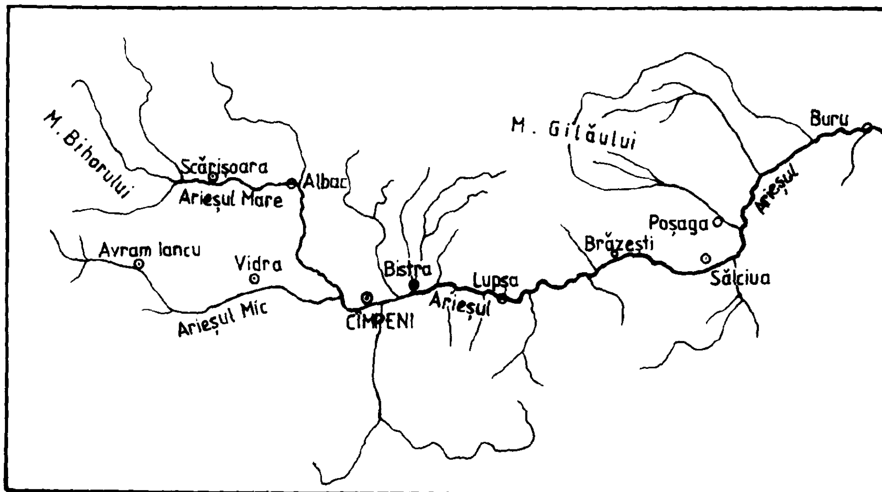
SUMMARY. - Diplopods from the Arieş Valley. A number of 25 species of diplopods belonging to 8 families and 5 orders were collected from the Arieş Valley within the Alba county. We emphasise the occurrence of *Karpatophyllon dacicum* Ceuca, 1964 which is endemic to the Apuseni Mountains.

Râul Arieş îşi are izvoarele în Munţii Bihorului din Masivul Apusenilor, în final vărsându-se în Mureş. Au fost colectate numeroase exemplare de diplopode începând de la valea Bistrei, în amonte şi până lângă comuna Poşaga, în aval. Pentru colectarea materialului au fost amplasate numeroase capcane "Barber" de către un colectiv al Catedrei de zoologie din Cluj-Napoca, în diferite staţiuni din spaţiul mai sus menţionat. Capcanele au fost îngropate în diferite ecosisteme, ca: lunci cu salcâmi şi plopi, păduri de foioase, de amestec şi de conifere, începând de la Poşaga, Sălciua, Brezeşti, Bistra şi valea Bistrei (Fig. 1).



F i g. 1. *Harta râului Arieş, de la izvoare până la comuna Buru (numai pe parcursul jud. Alba).*

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Ord. *GLOMERIDA* Leach, 1815
Fam. *Glomeridae* Leach, 1815

Glomeris pustulata Latreille, 1804

Poşaga. 7♂♂+13♀♀, 9.VIII.1997. *Sălciua.* 15♂♂+2♀♀, 22.IX.1998. Specie ce preferă biotopuri, mai mult sau mai puţin deschise, sub pietre, sub lemne putrezite, în păduri sub frunzar, sau chiar sub pernele de muşchi. Este o specie central-europeană rară; la noi se mai cunoaşte de la Cluj-Napoca.

Glomeris hexasticha Brandt, 1833

Sălciua. 1♀, 23.IX.1998. *Bistra.* 8♂♂ + 4♀♀, 22.IX.1998. Preferă, mai cu seamă, marginile de păduri, crângurile, tufişurile, sub pietre, sub trunchiuri căzute şi, bineînţeles, sub frunzar. Este o specie central-europeană, care are o largă răspândire şi în ţara noastră [2].

Glomeris connexa C.L. Koch, 1847

Bistra. 3♂♂+2♀♀, 1.VII.1998. *Valea Bistrei.* 3♂♂, 22.IX.1998. *Poşaga.* 3♂♂+10♀♀, 22.IX.1998. Se întâlneşte în frunzarul pădurilor de amestec, sau chiar în cele de conifere, sau sub trunchiuri căzute, uneori în litieră, rareori sub pietre. Este şi aceasta tot o specie central-europeană.

Glomeris prominens Attems, 1903

Bistra. 4♂♂+3♀♀, 2.VI.1998. Se găseşte în frunzarul pădurilor de foioase, uneori în humusul de sub acestea, la adâncimi de câţiva cm. A fost descrisă de la Tuşnad şi se pare că este o specie endemică, la noi găsindu-se mai cu seamă în Transilvania.

Ord. *POLYDESMIDA* Leach, 1815
Fam. *Polydesmidae* Leach, 1815

Polydesmus complanatus (Linnaeus, 1761)

Poşaga. 3♂♂+1♀, 9.VII.1997; 5♂♂+5♀♀+23 juv., 2.VI.1998. *Sălciua.* 1♂, 2.VI.1998. *Brezeşti.* 2♂♂, 2.VI.1998. Trăieşte mai cu seamă în pădurile de foioase, uneori şi în cele de conifere. Nu rareori ajunge prin zăvoaiele cu arini, prin crânguri şi pe la margini de păduri; rareori în locuri deschise, sub bucăţi de lemne sau sub pietre, şi în parcuri neîngrijite. Pe verticală poate ajunge până la 2000 m altitudine. Fiind o specie troglofilă, poate fi întâlnită şi în peşteri. Este o specie europeană.

Polydesmus montanus Daday, 1889

Valea Bistrei. 3♂♂, 22.IX.1998. *Poşaga.* 2♂♂, 9.VII.1997. Se pare că are aceleaşi preferinţe ca şi *P. complanatus* în Carpaţii noştri. Se întâlneşte mai frecvent în pădurile de conifere, mai rar în cele de foioase. Şi această specie pătrunde uneori în peşteri. Descrisă iniţial de la noi, acum se mai cunoaşte şi din Ucraina şi Polonia.

Polydesmus schässburgensis Verhoeff, 1889

Poşaga. 11♂♂+1♀, 9.VII.1997. *Brezeşti.* 27♂♂+16♀♀, 2.VII.1997. Este tot o specie de păduri de foioase, aflându-se mai cu seamă pe la liziere. Descrisă din fauna ţării noastre, ea a mai fost menţionată şi din Ungaria şi Iugoslavia.

Fam. *Paradoxosomatidae* Daday, 1889

Strongylosoma stigmatosum (Eichwald, 1830)

Sălciua. 1♂, 1.VI.1998. *Bistra.* 1♂+1♀, 2.VI.1998. Această specie preferă regiunile calcaroase din pădurile de foioase, rareori mai poate fi întâlnită prin livezi sau prin peşteri. Este o specie tipic central-est-europeană.

Ord. *CHORDEUMATIDA* C.L. Koch, 1847
Fam. *Mastigophorophyllidae* Verhoeff, 1899

Karpatophyllon dacicum Ceuca, 1964

Brezeşti. 4♂♂+5♀♀+5 juv., 23.IX.1998. Este o specie montană întâlnită în frunzarul pădurilor de foioase, cu trunchiuri căzute, mai mult sau mai puţin putrezite; rareori, în cele de conifere. Specie endemică în Munţii Apuseni [1].

Este necesar de menţionat următoarele aspecte referitoare la conformaţia gonopodelor anterioare la exemplarele colectate aici. Dacă ne referim la porţiunile distale ale gonopodelor anterioare, se observă ca, la un exemplar (Fig. 2-A), ramura internă sau lobul medial este mult lăţit, iar vârful telopoditului are formă ovală. La alt exemplar, din aceeaşi populaţie (Fig. 2-C), se vede că ramura internă, sau lobul medial, este normal dezvoltat (asemănător cu cel de la forma tipică) (Fig. 2-B), pe când vârful telopoditului este alungit, cu aspect de frunză (Fig. 2-C). Referitor la gonopodele posterioare se poate menţiona că ramura internă este curbată mult posterior, la toţi indivizii studiaţi (Fig. 2-E), restul aspectului acestor gonopode este mai mult sau mai puţin asemănător cu forma tipică (Fig. 2-D).

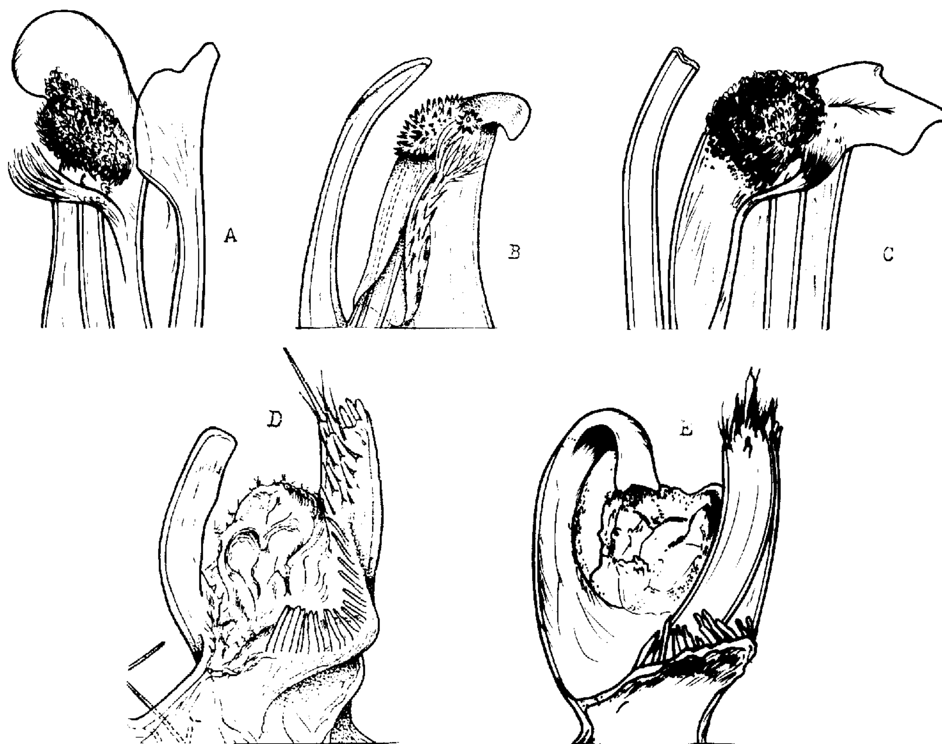
Mastigona transsilvanicum Verhoeff, 1897

Brezeşti. 4♂♂+17♀♀+8 juv., 1.VI.1998. *Sălciua.* 1♂+5♀♀+2 juv., 1.VI.1998. *Bistra.* 2♂♂, 22.IX.1998. Specie ce poate fi întâlnită în pădurile cu lemne căzute şi putrezite, uneori sub scoarţa copacilor; deseori poate fi găsită şi sub pietrele şi cărbunii din focurile relativ recent stinse de la margini de păduri. Se mai cunoaşte şi din Ungaria şi Bulgaria.

Fam. *Craspedosomidae* Gray, 1843

Craspedosoma transsilvanicum Verhoeff, 1897

Brezeşti. 1♂+1♀, 2.VI.1998. *Poşaga.* 1♂+9♀♀+5 juv., 9.VII.1997. *Sălciua.* 2♂♂+1♀, 1.VI.1998. *Bistra.* 3♂♂+12♀+63 juv., 25.IX.1998. Se întâlneşte în pădurile de foioase, mai cu seamă la marginile lor. Nici sub pietre nu este rară. De asemenea, ca şi specia precedentă, poate fi găsită printre cărbunii stinşi din focurile relativ recent făcute. Este o specie central-europeană.



F i g. 2. *Jumătatea distală a gonopodelor anterioare la* Karpatophyllon dacicum *Ceuca, 1964.*

A - Porţiunea terminală a gonopodului anterior drept, văzut posterior, al unui exemplar mascul de la Brezeşti.

B - Porţiunea terminală a unui gonopod stâng anterior, văzut posterior, al exemplarului tipic de la Scărişoara.

C - Aceeaşi porţiune a gonopodului stâng anterior, văzut posterior, al altui exemplar de la Brezeşti.

D - Gonopodul posterior stâng, văzut posterior, al exemplarului de la Scărişoara.

E - Gonopodul posterior stâng, văzut posterior, al altui exemplar de la Brezeşti.

Fam. *Chordeumatidae* C.L. Koch, 1847

Melogona transsilvanicum Verhoeff, 1897

Sălciua. 1♂, 1.VI.1998. 1♂, 23.IX.1998. Această mică şi sprintenă specie este relativ frecventă în frunzarul pădurilor de foioase şi în grădinile şi parcurile neîngrijite. Poate fi întâlnită primăvara, dar nici vara nu este rară. Frecventă în Transilvania. Probabil să existe şi în Ungaria [3].

Ord. *JULIDA* Leach, 1814

Fam. *Julidae* Leach, 1814

Cylindroiulus luridus (C.L. Koch, 1847

Sălciua. 2♂♂+2♀♀, 1.VI.1998. *Brezeşti.* 9♂♂+3♀♀, 1.VI.1998. *Bistra.* 6♂♂+7♀♀, 23.IX.1998. Este, poate, cea mai răspândită specie dintre diplopodele din ţara noastră. Se întâlneşte unde găseşte condiţii favorabile de viaţă, în frunzar, sub trunchiuri căzute, sub pietre. Pe verticală poate ajunge până la 1500 m altitudine. Este o specie central-vest-europeană.

Enantiulus nanus (Latzel, 1884)

Sălciua. 1♂, 1.VI.1998. *Brezeşti.* 3♂♂+3♀♀, 2.VI.1998. Specie care preferă cu predilecţie frunzarul umed al pădurilor de foioase. De obicei, pe câte o suprafaţă, relativ mică, dacă se insistă, pot fi colectaţi mai mulţi indivizi. Este o specie vest-central-europeană.

Haplophyllum mehelyi (Verhoeff, 1897)

Brezeşti. 1♂+1♀, 2.VI.1998. Specie tipică de frunzar care, se pare, că preferă solul pietros, urcând, uneori până aproape de 2000 m altitudine. Este o specie central-vest-europeană.

Leptoiulus trilobatus (Verhoeff, 1894)

Poşaga. 2♂♂+2♀♀, 9.VII.1997. *Sălciua.* 6♂♂+3 juv., 1.VI.1998. *Brezeşti.* 7♂♂+1♀, 2.VI.1998. *Bistra.* 16♂♂+9♀♀+3 juv., 3.VI.1998. Este o specie de frunzar de foioase şi de păduri de conifere. Poate fi întâlnită şi sub trunchiuri căzute sau sub pietre. Pe verticală poate ajunge până la 2000 m. Specie central-europeană.

Allopodoiulus verhoeffi (Jawlowski, 1931)

Brezeşti. 3♂♂, 2.VI.1998. *Sălciua.* 1♀, 1.VI.1998. *Bistra.* 2♂♂+6♀♀, 2.VI.1998. *Valea Bistrei.* 1♀+1 juv., 2.IX.1998. Specie care trăieşte în frunzarul umed. Rareori se poate găsi sub pietre sau sub trunchiuri căzute şi putrezite. Specie central-europeană.

Unciger transsilvanicus (Verhoeff, 1899)

Sălciua. 2♂♂, 1.VI.1998. *Bistra.* 1♂+2 juv., 22.IX.1998. *Valea Bistrei.* 1♂+1♀, 1.VI.1998. Este o specie care nu este legată de un anumit biotop; ea poate fi întâlnită atât în frunzarul pădurilor de foioase, sub trunchiuri căzute, cât şi în parcuri, grădini, cimitire şi chiar în locuri deschise. Specie central-europeană.

Megaphyllum peltyurum (Latzel, 1884)

Poşaga. 2♂♂+2♀♀, 9.VII.1997. *Bistra.* 1♂+1♀, 22.IX.1998. De obicei, această specie este întâlnită în pădurile de conifere, mai cu seamă sub trunchiuri căzute şi putrezite. Mai rar, poate fi întâlnită în pădurile înalte de foioase şi chiar sub pietre. Poate fi întâlnită în Apuseni, Carpaţii sudici şi Iugoslavia.

Megaphyllum imbecillum (Latzel, 1884)

Poşaga. 2♂♂+2, 9.VII.1997. *Brezeşti.* 2♂♂, 2.VI.1998. *Bistra.* 2♂♂+1♀, 23.IX.1998. De obicei, această mică specie populează frunzarul pădurilor de foioase, uneori în număr mare de exemplare, pe câte o suprafaţă, relativ mică, cu umiditate accentuată. Este o specie central-europeană.

Megaphyllum unilineatum (C.L. Koch, 1838)

Poşaga. 5♂♂+9♀♀, 1.VI.1998. *Sălciua.* 2♂♂+2 juv., 1.VI.1998. *Bistra.* 1♂+2 juv., 23.IX.1998. Specie de joasă altitudine care poate fi întâlnită la margini de păduri, crânguri, în pădurile de salcâmi din terenurile nisipoase, sub pietre sau în lunci. Este o specie central-europeană.

Megaphyllum transsilvanicum (Verhoeff, 1897)

Sălciua. 1♂+5♀♀, 2.VI.1998. Specie frecventă în pădurile de foioase, găsindu-se, uneori, şi în frunzarul celor de conifere. Este o specie central-europeană.

Megaphyllum projectum dioritanum (Verhoeff, 1907)

Sălciua. 2♂♂+3♀♀, 1.VI.1998. *Brezeşti.* 3♂♂+1♀, 2.VI.1998. *Poşaga.* 3♂♂+2♀♀, 9.VII.1997. *Bistra.* 4♂♂+10♀♀+11 juv., 23.IX.1998. *Valea Bistrei.* 1♂+1♀, 1.VI.1998. Este o formă ce poate fi întâlnită pretutindeni, atât în păduri de foioase cât şi în cele de conifere şi chiar mai sus. Este o formă central-europeană.

Ommatoiulus sabulosus (Linnaeus, 1758)

Poşaga. 4♂♂+12♀♀+4 juv., 1.VI.1998. *Sălciua.* 4♀♀+6 juv., 1.VI.1998. *Brezeşti.* 5♂♂+6♀♀, 2.VI.1998. *Bistra.* 1♂♂+2♀♀+12 juv., 23.IX.1998. Specie tipic euritopă, care poate fi întâlnită aproape peste tot unde

se găsesc diplopode, atât în câmp deschis, sub pietre sau lemne putrezite, cât şi în păduri de foioase sau chiar de conifere (de preferinţă la marginile lor). Pe verticală poate fi întâlnită de la nivelul mării până la 2000 m altitudine. Se cunoaşte din Spania până în sudul Uralilor [4].

Ord. *POLYZONIDA* Gervais, 1844
Fam. *Polyzonidae* Gervais, 1844

Polyzonium germanicum Brandt, 1831

Sălciua. 1♂+1, 1.VI.1998. *Brezeşti.* 1♂, 1.VI.1998. *Bistra.* 7♂♂+12♀♀+3 juv., 23.IX.1998. Această specie trăieşte în frunzarul pădurilor de foioase, aflându-se în sol, sub detritusul de sub pătura de frunze; rareori poate fi întâlnită în pădurile de conifere şi chiar sub pătura de muşchi. Femela are grijă de pontă, înrulându-se în jurul ei. Este o specie europeană, din Franţa până în Urali.

Deci, s-au colectat de pe valea Arieşului 476 de indivizi încadraţi în 25 de specii şi o subspecie, cuprinse în 5 ordine şi 8 familii.

BIBLIOGRAFIE

1. Ceuca, T., *Genurile* Karpatophyllon *şi* Stenophyllum *în fauna de Diplopode a României*, "Stud. Univ. Babeş-Bolyai, Biol.", **34** (1), 1989, 52-56.
2. Ceuca, T., *Genul* Glomeris *Latr. 1892 în fauna de Diplopode a României (cu câteva aspecte teratologice)*, "Stud. Univ. Babeş-Bolyai, Biol.", **34** (2), 1989, 49-55.
3. Schubart, O., *Tausendfüssler oder* Myriapoda. I. Diplopoda, Fischer, Jena, 1934.
4. Stojalowska, W., *Krocionogi* (Diplopoda) *Polski*, Pol. Akad. Nauk, Warszawa, 1961.

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FAUNISTIC AND ECOLOGICAL STUDIES ON THE CICADA POPULATIONS (*INSECTA*, *HOMOPTERA*, *AUCHENORRHYNCHA*; *CICADELLIDAE* AND *CERCOPIDAE*) ALONG THE SUPERIOR COURSE OF THE SOMEŞUL CALD RIVER (ROMANIA)

VALENTIN POPA* and ROXANA COJOCNEANU*

SUMMARY. - In the studied area we have identified 37 cicada species belonging to 2 families, 9 subfamilies and 31 genera. The ecological analysis shows that the greatest biodiversity is found in the meadow at Smida.The biodiversity index decreases from Smida to the gorge area according to the increase in altitude. The most similar ecosystems are the spruce forest at Doda Pilii and the spruce forest on the right slope in the gorge. Concerning the different types of ecosystems, the highest similarity index is between the swampland at Doda Pilii and the pasture at Smida.

In the biological literature there are few data concerning the cicada fauna in the Someşul Cald basin, and this determined us to carry out such a research. In our country studies have been performed in similar ecosystems from other mountainous regions, such as the Bucegi Massif [1, 2] and the Retezat Massif [3].

The zone studied by us is situated in the Bihor Mountains, between the Vlădeasa Massif and the Gilău Mountains, at an altitude between 1200-1700 m above sea level. Here, the spruce forms more or less consistent forests, due especially to extensive deforestation. Besides spruce, other tree species, such as the birch (*Betula pubescens*), found especially in the swampland in association with *Sphagnum* sp., willow (*Salix purpurea*), mountain alder (*Alnus incana*) also grow in this area.

The geological substratum of the area is varied. In the sector between the Fântânele Reservoir and the confluence with Valea Firei, there are cropping out substantial metamorphic formations from the Precambrian belonging to the Bihor Autochthonous. Frequently, layers of sedimentary rocks of Mesozoic age may cover them, extending predominantly at the springs of the fluvial network mentioned before. Thus, the relief of the zone wears the mark of a lithological puzzle structure with the individualisation of some specific metamorphic forms. On the other hand, there are other totally different forms developed on the soluble rocks (Fig. 1).

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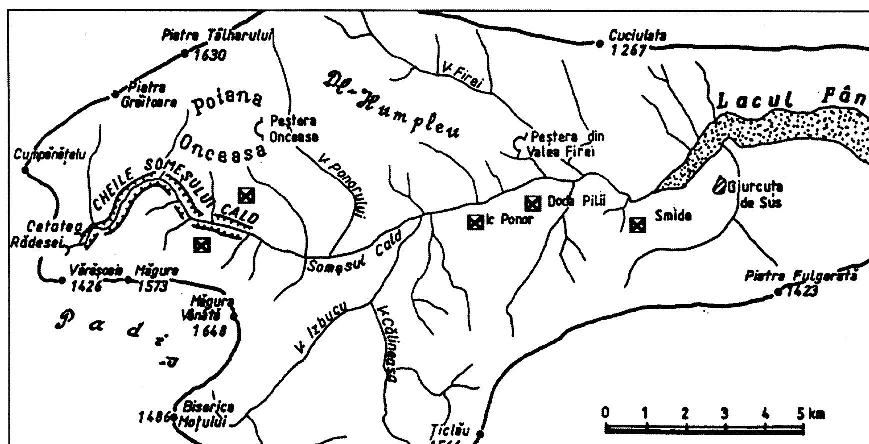


Fig. 1. *The Someşul Cald - Ic Ponor zone with the sampling sites.*

In 1998 we have collected biological material from an area extending between the narrow valley of the Someşul Cald gorge and the end of the Smida Reservoir (Fântânele Lake). The samplings had in view collecting the biological material from varied ecosystems, in order to study the biodiversity of the species in the studied area. Also, we have collected material from ecosystems with resembling biotopes, in order to compare the fauna composition of these ecosystems.

The stations chosen are specified below.

Station I. Smida

At this station we have collected many samples, because of the great number of ecosystems and habitats present here.

Spruce forest - situated on the right bank of the Someşul Cald river, near the end of the reservoir. The area, located at an altitude of approximately 960 m above sea level displays small channels and microdepressions, heavily supplied with subterraneous water, which has stimulated the abundant growth of the peat moss *Sphagnum* sp. Aside from *Sphagnum* sp., here we can also find the birch tree (*Betula pubescens*).

Combinations of mesophilous and mesohygrophilous grasslands are found in the meadow on the right bank of the Someşul Cald river, at approximately 965 m above sea level, in an enclosed area which occupies 2 ha. Here we have chosen a few sampling sites, described below.

Meadow with *Festuca rubra* - here we could find *Festuca rubra* and *Nardus sticta*, with a dominance of efficient fodder species (*Agrostis tenuis, Anthoxanthum odoratum, Trifolium pratense*).

Salicetum purpureae association - a *Salix purpurea* isle in the meadow.

Area with hygrophilous vegetation - situated very close to the river bank, where the humidity is high.

Sphagnetum 1 - situated in close vicinity of the grasslands, near the fringe of a swampy spruce forest. The area is completely covered by a layer of *Sphagnum* sp. We have also collected material in the proximity of this sector, to check the existence in this biotope of a mixture of species from the neighbouring meadow.

Sphagnetum 2 - situated upstream from the mesohygrophilous meadow complex (pastures) in a microdepression with water excess in spring and autumn.

Pasture - a land where the herbivorous animals of the inhabitants graze. From phytocoenological point of view, the composition of this land resembles that of the downstream mesohygrophilous meadow complex.

Station II. Doda Pilii

Spruce forest on the place called "la cuptoare" - situated on the southwestern slope of the mountain and on the small terrace on the left bank of the Someşul Cald valley. At the foot of the mountain, on a flat area (at an altitude of 970 m above sea level), there can be found a typical *Sphagno-Piceetum* association, with a well developed herb layer dominated by the peat moss, but with a small number of species and with many specimens of dried spruce.

Swampland - a community formed by species of *Carex* and *Juncus*, on grounds of high relative humidity, as a result of the great number of springs found here.

Station III. Ic Ponor

Mixed forest - the predominant tree species are *Fagus sylvatica* and *Picea abies*. The samples in this biotope have been collected especially from the low herbaceous and shrubby vegetation.

Coppice - a community of *Alnus incana* populates this ecosystem. It is situated at 1000 m above sea level, on a flat land, but with small secondary riverbeds. A great floral diversity and a luxuriant development of the herb layer characterise this ecosystem.

Station IV. The Gorge

At the entrance of the gorge - on the left side we have sampled biological material from the herbaceous vegetation and from the trees situated at the fringe of the spruce forest on the left slope of the mountain.

The left side of the gorge - here we have sampled biological material from the spruce forest, which covers the northern slope of the mountain. Among the herbaceous plants that populate this spruce forest we mention the endemic species: *Pulmonaria rubra, Symphytum cordatum* and *Cardamine glanduligera*.

The right side of the gorge - the sampling has also been made from the spruce forest, which covers the southern mountainside, but the composition of the herb and shrubby layers is different from that of the opposite side of the gorge. Here we mainly find communities of *Vaccinium myrtillus* and *Luzula sylvatica.*

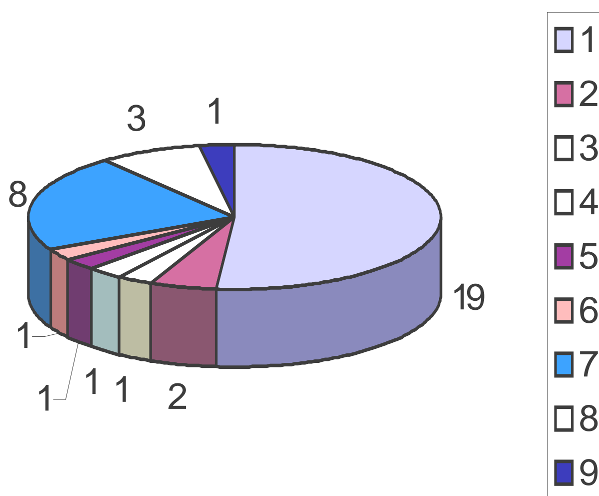
Material and methods. The samples of the biological material have been collected using an entomological net when collecting from herbaceous vegetation, and by using the umbrella net, when collecting from the trees. We have collected both qualitative and quantitative samples. At all sampling sites, we have taken samples of the same size (100 mows). The biological material collected was kept in recipients containing 70% alcohol. The determination of species was carried out based on the morphological features, especially of the genital system, according to the literature [4, 6, 7]. The components of the genital system have been preserved as permanent slides using Canada balm, and are kept in the collection of the Department of Zoology.

We have calculated the relative abundance (R.A.%) for each species, using the formula: R.A.% = NiX100/N, where Ni - number of individuals of the species i and N - total number of individuals in a biocoenosis.

In the ecological study of the cicada populations we have used the following ecological indices: the numeric dominance index: D = nX100/Nt, where n - the number of individuals of the species i; Nt - the total number of individuals from all the ecosystems in the studied area; the Shannon - Weaver diversity index, calculated using the formula: H'= -Σ piXlog pi, where pi is the proportion of individuals of species i; log - decimal logarithm; the equitability index, calculated with the formula: e = H'/Hmax; Hmax = logS, where S represents the total number of species in a biocoenosis and the Morisita ecological similarity index, calculated with the formula: Idm = 2Σxi X yi/ (l1 + l2) X N1 X N2; l1 = Σxi X (xi - 1)/N1 X (N1 - 1); l2 = Σyi X (yi - 1)/N2 X (N2 - 1), where xi - number of individuals of species i in locality 1; yi - number of individuals of species i in locality 2; N1 - total number of individuals in locality 1; N2 - total number of individuals in locality 2.

Results and discussion. The results of our research are included in Table 1. The material collected in the area totals 1713 individuals, belonging to 37 species, 2 families, 9 subfamilies and 31 genera. From systematical point of view, the most represented family is *Cicadellidae* (Latreille, 1825), with 7 subfamilies and 33 species, compared to *Cercopidae* (Leach, 1815), with 4 species. This family distribution is absolutely normal. The small number of species from the family *Cercopidae* is not due to its weak representation in this area, but to the fact that the number of species belonging to this family is much smaller compared to the family *Cicadellidae*. In the Central European fauna only 24 species of *Cercopidae* have been recorded [5].

Regarding the distribution of species in subfamilies, the best represented in number of species is the subfamily *Deltocephalinae* (Fieber, 1896), with 19 species, followed by the subfamily *Typhlocybinae* (Kbm. 1868), with 8 species, the other subfamilies being less represented (Fig.2). The explanation is similar to that regarding the families.



F i g. 2. *Distribution of the cicada species in subfamilies.*

1 – Deltocephalinae. 2 – Macropsinae. 3 – Evacanthinae.
4 – Cicadellinae. 5 - Aphrodinae. 6 – Dorycephalinae.
7 – Typhlocybinae. 8 – Aphrophorinae. 9 - Cercopinae.

Table 1

Cicada species (Homoptera, Auchenorrhyncha, Cicadellidae and Cercopidae) identified along the superior course of the Someşul Cald river

Species number	Taxa	Number of individuals	Stations and sampling sites
	Family Cicadellidae Latr. 1825		
	Subfam. Deltocephalinae Fieb. 1896		
	Tribe Balcluthini Baker, 1915		
	Genus *Balclutha* Kirk. 1891		
1	*Balclutha punctata* Thnb. 1782	2 M, 2 F	Smida, meadow with *Festuca rubra*
		1 M	Gorge entrance, the fringe of the spruce forest
		10 M,12 F	Right slope of the gorge, spruce forest
		1 F	Smida, *Salicetum purpureae* ass.
		1 F	Smida, *Sphagnetum* 1
		4 M, 5 F	Doda Pilii, spruce forest at "la cuptoare"
		1 F	Smida, meadow with hygrophilous vegetation
		1 F	Ic Ponor, mixed forest
		1 F	Ic Ponor, coppice
2	*Balclutha saltuella* Kbm. 1868	1 F	Smida, meadow with *Festuca rubra*
	Tribe Athysanini van Duzee, 1892		
	Genus *Euscelis* Brullé, 1832		
3	*Euscelis obsoletus* Kbm. 1858	1 M, 1 F	Smida, meadow with hygrophilous vegetation
	Genus *Streptanus* Rib. 1942		
4	*Streptanus sordidus* Zett. 1828	1 M	Smida, meadow with *Festuca rubra*
	Genus *Speudotwttix* Rib. 1942		
5	*Speudotettix subfusculus* Fall. 1806	1 M	Ic Ponor, mixed forest
	Genus *Elymana* De Long, 1936		
6	*Elymana sulphurella* Zett. 1828	6 M, 6 F	Smida, *Sphagnetum 2*
		12 M, 10 F	Smida, meadow with *Festuca rubra*
		4 M, 1 F	Smida, meadow with hygrophilous vegetation
		1 F	Ic Ponor, coppice
		1 F	Smida, pasture
	Genus *Cicadula* Zett. 1840		

Table 1 (continued)

7	*Cicadula quadrinotata* F. 1794	114 M, 32 F	Smida, *Sphagnetum* 2
		100 M, 34 F	Smida, meadow with *Festuca rubra*
		7 M, 2 F	Smida, *Sphagnetum* 1, border area
		25 M, 12 F	Smida, meadow with hygrophilous vegetation
		48 M, 6 F	Doda Pilii, swampland
		204 M, 50 F	Smida, pasture
		1 F	Smida, *Sphagnetum* 1
	Genus *Rhopalopyx* Rib. 1939		
8	*Rhopalopyx preyssleri* H. S. 1839	1 M	Smida, *Sphagnetum* 1, border area
		9 M, 5 F	Smida, *Sphagnetum* 2
		1 M	Doda Pilii, spruce forest at "la cuptoare
		1 F	Smida, pasture
		4 M, 2 F	Smida, meadow with *Festuca rubra*
	Genus *Idiodonus* Ball. 1936		
9	*Idiodonus cruentatus* Pnz. 1799	2 F	Smida, *Sphagnetum* 2
	Tribe Deltocephalini Fieb. 1869		
	Genus *Deltocephalus* Burm. 1838		
10	*Deltocephalus pulicaris* Fall. 1806	4 M, 6 F	Smida, *Sphagnetum* 2
	Tribe Doraturini Rib. 1952		
	Genus *Doratura* J. Shlb. 1871		
11	*Doratura stylata* Boh. 1847	5 M, 15 F	Smida, meadow with *Festuca rubra*
		11 M, 13 F	Smida, *Sphagnetum* 2
		1 M, 1 F	Smida, meadow with *Festuca rubra*
		1 F	Smida, pasture
	Tribe Macrostelini Kirk. 1906		
	Genus *Macrosteles* Fieb. 1866		
12	*Macrosteles sexnotatus* Fall. 1806	2 F	Smida, meadow with *Festuca rubra*
	Genus *Sagatus* Rib. 1948		
13	*Sagatus punctifrons* Fall. 1826	2 M, 1 F	Smida, *Salicetum purpureae* ass.
	Tribe Paralimnini Distant, 1908		
	Genus *Verdanus* Rib. 1947		
14	*Verdanus abdominalis* F. 1803	57 M, 57 F	Smida, *Sphagnetum* 2
		29 M, 55 F	Smida, meadow with *Festuca rubra*
		9 M, 11 F	Smida, *Sphagnetum* 1, border area

Table 1 (continued)

		1 M, 1 F	Smida, *Sphagnetum* 1
		2 M, 8 F	Smida, meadow with hygrophilous vegetation
		13 M, 22 F	Smida, pasture
		1 M, 1 F	Doda Pilii, swampland
	Genus *Arthaldeus* Rib. 1947		
15	*Arthaldeus pascuellus* Fall. 1826	2 M, 6 F	Smida, *Sphagnetum* 2
		36 M, 15 F	Smida, meadow with *Festuca rubra*
		4 M, 1 F	Smida, *Sphagnetum* 1, border area
		10 M, 12 F	Smida, meadow with hygrophilous vegetation
		7 M, 9 F	Smida, pasture
		1 M, 2 F	Smida, *Sphagnetum* 1
	Genus *Sorhoanus* Rib. 1947		
16	*Sorhoanus xanthoneurus* Fieb. 1869	23 M, 34 F	Smida, *Sphagnetum* 2
		4 M, 1 F	Smida, *Sphagnetum* 1, border area
		43 M, 32 F	Smida, pasture
		28 M, 5 F	Doda Pilii, swampland
		1 F	Smida, meadow with *Festuca rubra*
		1 F	Smida, *Sphagnetum* 1
	Genus *Turrutus* Rib. 1947		
17	*Turrutus socialis* Fl. 1861	1 M, 6 F	Smida, *Sphagnetum* 2
		10 M, 13 F	Smida, meadow with *Festuca rubra*
	Genus *Jassargus* Zachv. 1934		
18	*Jassargus (Arrailus) alpinus* Fieb.1869	5 M, 3 F	Smida, *Sphagnetum* 1, border area
		11 M, 15 F	Smida, *Sphagnetum* 2
		2 F	Smida, meadow with hygrophilous vegetation
		3 M,4 F	Doda Pilii, spruce forest at "la cuptoare"
		7 M, 13 F	Smida, *Sphagnetum* 1
		3 F	Smida, pasture
	Genus *Pasammotettix* Haupt, 1929		
19	*Psammotettix confinis* Dhlbm. 1850	2 M, 1 F	Smida, meadow with *Festuca rubra*
	Subfam. Macropsinae Evans, 1935		
	Genus *Macropsis* Lewis, 1834		
20	*Macropsis marginata* H.S. 1836	5 F	Smida, *Salicetum purpureae* ass.
21	*Macropsis planicollis* Thms. 1870	1 M, 2 F	Smida, *Salicetum purpureae* ass.
	Subfam. Evacanthinae Crumb, 1911		
	Genus *Evacanthus* Le P.S. 1827		

Table 1 (continued)

22	*Evacanthus interruptus* L. 1758	3 F	Right slope of the gorge, spruce forest
		1 M	Doda Pilii, spruce forest at " la cuptoare"
		9 M, 22 F	Gorge entrance, herbaceous vegetation
		8 M, 10 F	Ic Ponor, mixed forest
	Subfam. Cicadellinae Latreille, 1825		
	Genus *Cicadella* Latreille, 1817		
23	*Cicadella viridis* L. 1758	3 M, 1 F	Smida, meadow with *Festuca rubra*
		1 M, 1 F	Smida, *Sphagnetum* 1, border area
		2 M, 3 F	Smida, *Sphagnetum* 1
		1 M, 5 F	Smida, meadow with hygrophilous vegetation
		22 M, 4 F	Doda Pilii, swampland
		5 M,4 F	Smida, pasture
	Subfam. Aphrodinae Haupt, 1927		
	Genus *Strogyllocephalus* Fl. 1861		
24	*Strogyllocephalus livens* Zett. 1838	1 M	Doda Pilii, spruce forest at "la cuptoare"
	Subfam. Dorycephalinae Oman, 1943		
	Genus *Eupelix* Germ. 1821		
25	*Eupelix cuspidata* F. 1755	1 M	Smida, meadow with hygrophilous vegetation
		2 F	Smida, meadow with *Festuca rubra*
	Subfam. Typhlocybinae Kbm. 1868		
	Genus *Empoasca* Walsh, 1862		
26	*Empoasca* (s.str.) *vitis* Gothe, 1875	2 M, 1 F	Doda Pilii, spruce forest at "la cuptoare"
27	*Empoasca* (s. str.) *pteridis* Dhlbm.1850	3 M,1 F	Doda Pilii, swampland
		7 M, 4 F	Smida, *Sphagnetum* 1
28	*Empoasca (Kybos) butleri* Edw. 1908	3 M, 17 F	Smida, *Salicetum purpureae* ass.
	Genus *Erythria* Fieb. 1866		
29	*Erythria montandoni* Put. 1880	1 M, 6 F	Left slope of the gorge, spruce forest
30	*Erythria manderstjernii* Kbm. 1868	1 M	Right slope of the gorge, spruce forest
	Genus *Forcipata* De Long & Caldwell, 1936		

Table 1 (continued)

31	*Forcipata citrinella* Zett. 1828	1 F	Right slope of the gorge, spruce forest
	Genus *Eupteryx* Curtis, 1833		
32	*Eupteryx aurata* L . 1758	3 M, 3 F	Ic Ponor, coppice
33	*Eupteryx* sp. (?)	2 M, 1 F	Ic Ponor, mixed forest
	Family Cercopidae Leach, 1815		
	Subfam. Aphrophorinae A.&S. 1843		
	Genus *Aphrophora* Germ. 1821		
34	*Aphrophora alni* Fall. 1805	2 F	Ic Ponor, coppice
	Genus *Philaenus* Stal, 1864		
35	*Philaenus spumarius* L..1758		
	Var. *xanthocephala*	2 F	Ic Ponor, coppice
		1 F	Right slope in the gorge, spruce forest
		6 M, 3 F	Left slope in the gorge, spruce forest
		1 M, 2 F	Doda Pilii, spruce forest at " la cuptoare"
		2 M	Ic Ponor, coppice
		1 M	Ic Ponor, mixed forest
	Var. *lateralis*	1 M, 1 F	Smida, *Sphagnetum* 1
	Var. *leucocephala*	1 M	Left slope in the gorge, spruce forest
		1 M, 1 F	Smida, *Sphagnetum* 1, border area
	Genus *Neophilaenus* Haupt, 1935		
36	*Neophilaenus lineatus* L. 1758	1 M, 1 F	Smida, *Sphagnetum* 2
		15 M, 23 F	Smida, *Sphagnetum* 1
		1 M, 1 F	Doda Pilii, spruce forest at " la cuptoare"
		4 F	Smida, meadow with hygrophilous vegetation
		1 F	Ic Ponor, coppice
	Subfam. Cercopinae Leach, 1815		
	Genus *Cercopis* Fabr. 1775		
37	*Cercopis sanguinolenta* Scop. 1763	1 F	Doda Pilii, spruce forest at " la cuptoare"

The 37 species identified here are spread differently in the studied ecosystems and habitats, as shown in Table 2.

Analysing Table 2, it can be observed that the greatest number of species (25) in the studied six ecosystems was recorded in the meadow, due to its richer and more diversified trophic basis, compared to the other types of ecosystems. The cicada feed especially on the species of *Poaceae*. The importance of the trophic basis for the cicada communities also results from the structure of the coenosis in a *Sphagnetum* ass. situated in a spruce forest

(*Sphagnetum* 1), and a *Sphagnetum* ass. situated in a pasture (*Sphagnetum* 2). In the pasture on *Sphagnetum* ass., a number of 13 species were identified, while in the spruce forest on *Sphagnetum* ass. only 10 species were observed. In the pasture on *Sphagnetum* ass., the cicada move easily outside the area covered with *Sphagnum* sp., to feed. The cicada do not feed on *Sphagnum* sp., but in the *Sphagnetum* ass. there are intercalations of *Carex* and *Juncus*, especially in the pasture on *Sphagnetum* 2. The abundance of these species is greater than in *Sphagnetum* 1, situated in the spruce forest. In the meadow at Smida we have sampled biological material also from the *Salicetum purpureae* association, which forms a small isle inside the ecosystem. The number of species collected here is small (5 species); they are monophagous, strictly related to the trophic basis: *Sagatus punctifrons, Macropsis marginata, Macropsis planicollis* and *Empoasca butleri*. In the pasture, the number of species is more reduced compared to the meadow, due to the intense grazing. The ecosystem with the smallest number of species (4) is the spruce forest on the left bank of the gorge, with northern aspect, where the herbaceous vegetation is less abundant. Comparing the faunistic richness in each station of the spruce forests, we have found that the number of species is greatest at Smida, followed by the station Doda Pilii and station Ic Ponor, the less abundant region in number of species being the gorge area. This decrease in faunistic abundance is positively correlated with the increase in altitude between the station Smida and the gorge area. Regarding the spreading of species in ecosystems, it can be concluded that there is a great number of species located in a single ecosystem (stenobiont species): *Balclutha saltuella, Conosanus obsoletus, Streptanus sordidus, Idiodonus cruentatus, Macrosteles sexnotatus, Turrutus socialis, Psammotettix confinis, Macropsis marginata, Macropsis planicollis, Empoasca butleri.* All of these species are praticolous and, thus, strongly related to the trophic basis of the meadow, whereas *Speudotettix subfusculus, Strogyllocephalus livens, Erythria montandoni, Erythria manderstjernii, Forcipata citrinella, Eupteryx aurata, Eupteryx* sp. (?) are sylvan species, rarely surpassing the forest limits. *Aphrophora alni* and *Cercopis sanguinolenta* are euribiont species, but in the studied area we have found them in small numbers. The species that are spread in two ecosystems are *Empoasca vitis* and *Doratura stylata*. *Empoasca vitis* has been collected from ecosystems with distinct nonbiotic and biotic conditions (spruce forest, meadow) which indicates that the ecological valence of this species is large. *Doratura stylata* is a praticolous species, which is indicated by its spread only in meadow and pasture. The other species inhabit more types of ecosystems, and have a wide ecological valence. From faunistic point of view, the presence of *Eupteryx* sp.(?) is very interesting because of its penis shape. This species is closely related to *Eupteryx stachydearum* (Hardy, 1846) but its penis shape is different.

Table 2

Distribution of the cicada species and the number of inviduals on ecosystems and stations

Species	Type of ecosystem: Spruce forest					Mixed forest		Coppice		Meadow		Pasture		Swampland		
	Smida	Doda Pilii	R.B*	L.B.*	R.A. % **	Ic Ponor	R.A. % **	Ic Ponor	R.A. % **	Smida	R.A. % **	Smida	R.A. % **	Doda Pilii	R.A. % **	D % ***
Balclutha punctata	1	9	22	1	0.13	1	4.16	1	7.69	5	0.6					2.335085
Balclutha saltuella										1	0.1					0.058377
Conosanus obsoletus										2	0.2					0.116754
Streptanus sordidus										1	0.6					0.058377
Speudotettix subfusculus						1	4.16									0.058377
Elymana sulphurella								1	7.69	34	4.1	1	0.2			2.101576
Cicadula quadrinotata	10				4					280	33.8	254	62.7	54	36.48	34.90952
Rhopalopyx preyssleri	1	1			0.8					20	2.4	1	0.2			1.342674
Idiodonus cruentatus										2	0.2					0.116754
Deltocephalus pulicaris										16	1.9	3	0.7			1.109165
Doratura stylata										46	5.5	1	0.2			2.743724
Macrosteles sexnotatus										2	0.2					0.116754
Sagatus punctifrons										3	0.3					0.175131
Verdanus abdominalis	22				8.8					198	23.9	35	8.6	2	1.35	15.00292
Arthaldeus pascuellus	8				3.2					59	7.1	16	3.9			4.845301
Sorhoanus xanthoneurus	6				2.4					58	7	75	18.5	34	22.97	10.09924
Turrutus socialis										30	3.6					1.751313
Jassargus alpinus	28	7			14					28	3.3	3	0.7			3.85289
Psammotettix confinis										3	0.3					0.175131
Macopsis marginata										5	0.6					0.291886
Macropsis planicollis										3	0.3					0.175131
Evacanthus interruptus		1	3	31	14	18	75									3.093987
Cicadella viridis	7				2.8					4	0.4	8	1.9	26	17.56	2.62697
Strogyllocephalus livens		1			0.4											0.058377
Eupelix cuspidata	7	1			3.2					4	0.4	8	1.9	26	17.56	2.685347
Empoasca vitis		3			1.2						0.2					0.291886
Empoasca pteridis	11	1			4.8									4	2.7	0.934034
Empoasca butleri										2	2.4					1.167542
Erythria montandoni				7	2.8											0.40864
Erythria mandjerstjemii			1		0.4											0.058377
Forcipata citrinella			1		0.4											0.058377
Eupteryx aurata								6	46.15							0.350263
Eupteryx sp. (?).						3	12.5									0.175131
Aphrophora alni								2	15.38							0.116754
Philaenus spumarius	4	3	1	10	7.2	1	4.16	2	15.38							1.225919
Neophilaenus lineatus	38	2			16			1	7.69	2	0.2			2	1.35	2.62697
Cercopis sanguinolenta		1			0.4											0.058377
Number of species	**12**	**11**	**5**	**4**		**5**		**6**		**25**		**11**		**7**		

* Right bank of the gorge, left bank of the gorge.
**Relative abundance.
*** Numeric dominance.

Concerning the relative abundance (R.A.%) (Table 2), it has been observed that the highest value is for *Neophilaenus lineatus* (R.A.% = 16) in the spruce forest. In this ecosystem we have found two other species with high relative abundance values: *Jassargus alpinus* and *Evacanthus interruptus* (R.A.%= 14). In the spruce forest the lowest relative abundance value is attained by *Balclutha punctata* (R.A.% = 0.13). From the mixed forest we mention *Evacanthus interruptus* (R.A.% = 75), and *Eupteryx* sp. (?) (R.A.% = 12.5). In the coppice the most abundant species is *Eupteryx aurata* (R.A.% = 46.15), followed by *Aphrophora alni* and *Philaenus spumarius* (R.A.% = 15.38), which are hygrophilous species. In the meadow, the most abundant species are *Cicadula quadrinotata* (R.A.% = 33.8) and *Verdanus abdominalis* (R.A.% = 23.9), and the less abundant species is *Balclutha saltuela* (R.A.% = 0.1). *Cicadula quadrinotata* shows the highest values of the relative abundance both in pastures and in swamplands, which proves that it is a eurihygric species. In the literature it is mentioned as a rare species for the Transylvanian fauna [1].

Calculating the numeric dominance we have found that *Cicadula quadrinotata* (34.9%), *Verdanus abdominalis* (15%), and *Sorhoanus xanthoneurus* (10.09%) dominate all the ecosystems in the studied area.

The values of the ecological diversity and equitability indices are presented in Table 3. The highest ecological diversity index is recorded for the Smida meadow (0.92), followed by the spruce forest at Smida (0.91) and Doda Pilii (0.87). The lowest diversity index is recorded for the spruce forest in the gorge area (0.38). High values of the diversity index are found in the coppice and swampland (0.66). The spruce forest accommodates fewer species of cicada. Compared to the meadow, the pasture at Smida has a lower diversity index (0.52), and the species found here have a few number of individuals (e = 0.2). Concerning the degree of the representation of each species in biocoenosis (equitability), the richest species in individuals are those that inhabit the coppice (e = 0.59). There are few species that live in this ecosystem, but these species are very exigent regarding the humidity factor. Swampland is also an ecosystem with high relative humidity, but here the equitability is lower. This fact is probably due to trophic bases with poorer quality compared to the coppice, the interspecific competition being much more accentuated. The lowest equitability index has been recorded for the pasture at Smida. This proves that the cicada populations are strongly affected by grazing.

Table 3

Values of the Shannon-Weaver diversity index (H') and equitability index (e) in the studied ecosystems and stations

	Type of ecosystem								
	Spruce forest				Mixed forest	Coppice	Meadow	Pasture	Swampland
Stations	Smida	Doda Pilii	R.B. *	L.B. *	Ic Ponor	Ic Ponor	Smida	Smida	Doda Pilii
H'	0.91286	0.87816604	0.34127722	0.42187161	0.379116706	0.66217201	0.924658	0.528251	0.66478523
e	0.423256	0.59444821	0.2357982	0.2495858	0.274637681	0.59430829	0.316655	0.202608	0.30596258
Number of species	12	11	5	4	5	6	25	11	7

* Right bank of the gorge.

* Left bank of the gorge.

The Morisita ecological simila-rity indices between the same types of ecosystems from dif-ferent stations, as well as between different ecosystems are presented in Tables 4 and 5. Comparing the values of the similarity indices recorded in the same type of ecosystem (spruce forest) (Table 4), it is noticeable that the highest degree of resem-blance (0.63) is found between the spruce forest at the station Doda Pilii and the station on the right slope of the gorge. The high degree of resemblance between the two stations is due to the high relative humidity, which determines the presence of hygrophilous species, both in the phytocoenoses and the cicada communities. The simila-rity index between the spruce forests at Smida and Doda Pilii is 0.49. The resemblance between the two spruce forests consists especially in the compo-sition of the herb and shrubby layers, which is important for the cicada communities. Great eco-logical differences can be obser-ved between the spruce forests found on the right and left slopes of the gorge (0.17). These considerable ecological differences are due to the distinct aspect of the two slopes. The right slope, having southern aspect, is more exposed to the sun, and has a more diversified floristic composition, compared to the left slope, which has northern aspect.

The similarity index of the various ecosystems (Table 5) shows that the highest degree of resemblance (0.82) is between the swampland at Doda Pilii and the pasture at Smida. Probably, this great similarity is due to the fact that in the pasture there are many isles with *Sphagnum* sp., in which as in the swampland, there are also found *Carex* sp. and *Juncus* sp. A high similarity index (0.79) is also recorded between the pasture and the meadow. The similarity index between the meadow and spruce forest at Smida is 0.44, which is higher than the indices for the other spruce forests. This may be explained by the spatial proximity of the two ecosystems at Smida, the cicada being able to easily move from the meadow into the forest, and into the opposite direction.

Table 4

Values of the Morisita similarity index between different stations of the same ecosystem

	Spruce forest			
Stations	Smida	Doda Pilii	R.B.*	L.B.*
Smida				
Doda Pilii	0.49			
R.B.	0.09	0.63		
L.B.	0.01	0.01	0.17	

*Right bank of the gorge. *Left bank of the gorge.

Table 5

Values of the Morisita similarity index between different types of ecosystems and stations

Type of ecosystem		Coppice	Mixed forest	Meadow	Pasture	Swampland
	Stations	Ic Ponor	Ic Ponor	Smida	Smida	Doda Pilii
Spruce forest	Smida	0.13	0.004	0.44	0.24	0.3
	Doda Pilii	0.23	0.11	0.06	0.008	0.03
	R.B. *	0.15	0.19	0.01	0	0
	L.B. *	0.09	0.95	0.0003	0	0
Mixed forest	Ic Ponor	0.02				
Meadow	Smida	0.0003	0.0006		0.79	0.66
Pasture	Smida	0	0			0.82
Swampland	Doda Pilii	0	0			

* Right bank of the gorge. * Left bank of the gorge.

Conclusions.1. Along the superior course of the Someşul Cald river, from the Ic Ponor - Gorge area, we have identified 37 species of cicada, belonging to 2 families, 9 subfamilies and 31 genera.

2. Most of the species belong to the family *Cicadellidae*.

3. The most abundant cicada fauna lives in the meadow (25 species). In spruce forests, mixed forests and the coppice, the number of species, as the number of individuals, is more reduced.

4. In the gorge area, where the average temperature is much lower compared to Doda Pilii and Smida, the numbers of species and individuals are very reduced.

5. More abundant populations in the gorge area are represented by *Evacanthus interruptus* and *Philaenus spumarius*, which proves that these species are adapted to lower average temperatures.

6. The spread of the species in the studied zone may be influenced directly by climatic factors and also indirectly by the trophic basis, which is less abundant in the gorge area compared to Smida and Doda Pilii, where the Someş valley is wider and the herbaceous vegetation is more abundant and diversified.

7. The greatest relative abundance has been recorded for *Neophilaenus lineatus* in the spruce forest, for *Evacanthus interruptus* in the mixed forest, while *Cicadula quadrinotata* is abundant in more ecosystems.

8. The dominant species in the studied zone are: *Cicadula quadrinotata*, *Verdanus abdominalis* and *Sorhoanus xanthoneurus*.

9. The highest ecological diversity index has been recorded for the Smida meadow and spruce forest, and the lowest one for the spruce forest on the right bank of the gorge.

10. The highest ecological similarity index has been registered between the spruce forest at Doda Pilii and the spruce forest on the right slope of the gorge, where the humidity is higher, favourable to hygrophilous species, and the temperature is relatively low. The highest ecological similarity index between different ecosystems has been noted between the swampland at Doda Pilii and the pasture at Smida, due to the high resemblance of the trophic basis in these two ecosystems.

REFERENCES

1. C a n t o r e a n u, M., *Contribuţii la cunoaşterea faunei de cicadine din Sinaia şi împrejurimi(I)*, "An. Univ. C.I. Parhon, Ser. Şti. Nat., Biol.", **9**, 1958, 95-104.
2. C a n t o r e a n u, M., *Contribuţii la cunoaşterea faunei de cicadine din Sinaia şi împrejurimi(II),* "An. Univ. C.I. Parhon, Ser. Şti. Nat., Biol.", **9**, 1958, 135-147.
3. C a n t o r e a n u, M., *Structura comunităţilor de cicadine* (Homoptera, Auchenorrhyncha) *din Retezat*, in P o p o v i c i, I. (Ed. coord.), *Parcul Naţional Retezat - Studii Ecologice*, pp. 251-253, West Side, Braşov, 1992.
4. D e l l a G i u s t i n a, W., *Homoptères* Cicadellidae, "Faune France", **73** (3), 1989, 1-350.
5. H o l z i n g e r, E. W., F r ö h l i c h, W., G ü n t h a r t, H., L a u t e r e r, P., N i c k e l, H., O r o s z, A., S c h e d l, W., R e m a n e, R., *Vorläufiges Verzeichnis der Zikaden Mitteleuropas* (Insecta: Auchenorrhyncha*)*, "Beitr. Zikadenk.", **1**, 1997, 43-62.
6. R i b a u t, H., *Homoptères Auchénorhyques. I (*Typhlocybidae*)*, "Faune France", **31**, 1936, 1-228.
7. R i b a u t, H., *Homoptères Auchénorhyques. II (*Jassidae*)*, "Faune France", **57**, 1952, 1-472.

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OMALIUM IMITATOR LUZE, 1906 (*COLEOPTERA: STAPHYLINIDAE, OMALINAE*), A RARE SPECIES IN ROMANIA'S FAUNA

ADALBERT BALOG*

SUMMARY. - *Omalium imitator* is recorded for the first time in the Făgăraş Montains. This record is the second one on the occurrence of *O. imitator* in Romania's fauna. Depiction of the aedeagus of this species is a novelty for the coleopterological literature of our country. Some aspects concerning morphology and distribution of *O. imitator* are presented.

Omalium imitator was described by Luze in 1906 from the Alps. Occurrence of this species in our country was firstly recorded by Tóth in 1982 [4]. The species was found in the Maramureş Mountains. Other data on the presence of this species in Romania's fauna are not available. *O. imitator* is not mentioned at all or is referred to only rarely in the European coleopterological publications [1-5].

In June 1998, I took part, together with the zoologists K. Manfred (Austria) and K. Horst (Germany), in a field research in the Făgăraş Mountains. In the area called Saua Caprei (situated at 1200 m above sea level), I caught, by means of Reiter sieve, a male staphylinid specimen which, following its preliminary examination, seemed to belong to the species *Omalium imitator*. Detailed laboratory examination of the body and of the microscopic preparation of genitalia of the specimen indicated that its preliminary identification as *O. imitator* was correct. Correctness of the identification was confirmed by experts of the Natural History Museum in Budapest.

Description of the species. *Omalium imitator* is 2.5-3 mm in length. The head is quite long, flattened in dorso-ventral plane, with no pubescence, it is smaller than the prothorax (Fig. 1). The ocelli are well developed, the short mandibulae are asymmetrical (the left one is simple, whereas the right one has an interior protuberance). The elytrae are longer than the prothorax, which is sloping. The posterior angle is 90° and the longitudinal ditches are scarcely shiny. A dense and fine punctuation is present on the elytrae. The head is black,

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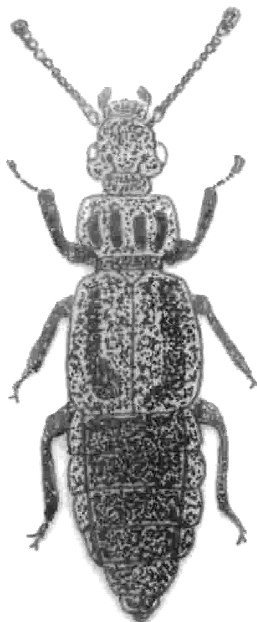
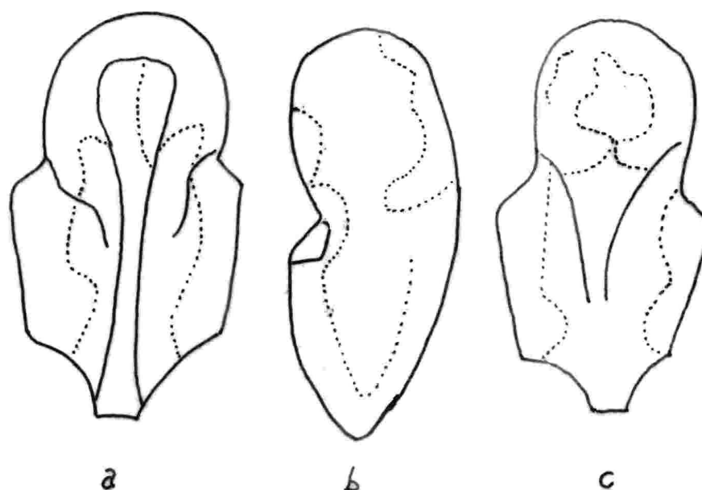
but the body is reddish-brown with yellowish-brown legs and antennae. The aedeagus has a characteristic form: it is round in the superior part, cubic in the middle part and has two excavations in the inferior part (Fig. 2).



F i g. 1. *Male of* Omalium imitator.



F i g. 2. *Aedeagus of* Omalium imitator.
a - Dorsal view. b - Lateral view. c - Ventral view.

Up to now, *O. imitator* was recorded mostly from the south-eastern part of Central Europe. It occurs in areas situated at 1200 m above sea level or at higher altitudes. It can be found in 5-10-cm thick snow spots.

Conclusions. 1. *O. imitator*, a rare species in the coleopterological fauna of Romania and of the other European countries, was recorded for the first time in the Făgăraş Mountains. According to present knowledge, these mountains are the southern boundary of the occurrence of *O. imitator.*

2. Depiction of the *O. imitator* aedeagus is a novelty for the coleopterological literature of our country.

REFERENCES

1. P a t k ó, F., *Staphylinide noi pentru fauna României*, "Bul. Inf. Soc. Lepidopterol. Rom.", **5** (1), 1994, 77-80.
2. R ă i a n u, L., *Studii asupra unor specii noi sau puţin cunoscute în fauna României aparţinând genului* Gabrius *Curt.* (Coleoptera: Staphylinidae), "An. Univ. Al. I. Cuza, Iaşi, Secţ. II, Şti. Nat., Biol.", No. 1, 1968, 111-114.
3. S z é k e s i, V., *Fauna Hungariae.* Staphylinidae, I, Akad. Kiadó, Budapest, 1963.
4. T ó t h , L., *Fauna Hungariae.* Staphylinidae, II, Akad. Kiadó, Budapest, 1982.
5. Z e r c h e, L., *Monographie der Paläarktischen Coryphiini* (Coleoptera: Staphylinidae, Omalinae), "Beitr. Entomol.", **43**, 1993, 320-373.

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REPRODUCEREA ŞI COMPORTAMENTUL FEROMONAL LA *OSTRINIA NUBILALIS* HBN. (*LEPIDOPTERA: CRAMBIDAE*) ÎN CONDIŢII DE LABORATOR. 6. DURATA DE VIAŢĂ A ADULŢILOR, OVIPOZITAREA ŞI FECUNDITATEA PENTRU DIFERITE GENERAŢII LA DOUĂ LINII DE CREŞTERE

ALEXANDRU CRIŞAN[*] şi GHEORGHE STAN[**]

SUMMARY. - Reproduction and Pheromonal Behaviour of Ostrinia nubilalis Hbn. (Lepidoptera: Crambidae) under Laboratory Conditions. 6. Adult Longevity, Oviposition and Fecundity in Different Generations of Two Strains. Two strains (ON-2C and ON-7G) of the *Ostrinia nubilalis* phenotype Z were reared under laboratory conditions for 4 and 16 generations, respectively. The adult longevity, oviposition behaviour and female fecundity were investigated in the populations of these strains. The female longevity was greater than that of the males and the virgin adults lived longer than the mated ones. In the generations 0 to 3 (G0-G3) the mean adult longevity gradually decreased. The circadian period of oviposition was localised in the first part of the scotophase and it was comparatively examined with the periodicity of the mating and pheromonal behaviour, under laboratory and field conditions. In the G0-G3 of the ON-2C strain we recorded a gradual diminution of the egg masses laid/female/day (2.1-0.9) and of the total egg masses laid/female (12.7-5.6), but in the ON-7G strain the variation interval was between 2.0-0.9 and 20.4-2.1, respectively. The values increased from G4-G5 and in the G10-G16 they were close to those recorded in G0. For the two strains, ON-2C and ON-7G, the mean number of eggs/egg masses varied on the intervals of 22.6-28.9 (with mean fecundity of 161.2-286.7 eggs) and 21.7-36.2 (with mean fecundity of 52.7-420.9 eggs), respectively. In the two strains the mean length of the oviposition period was 15.0 and 15.9 days, respectively, and a very narrow interval of variation was recorded for all generations. A positive, significant correlation was recorded between fecundity and the number of egg masses/female in the G0-G3 and G10-G16. Excepting G0-G3 of the ON-2C strain, the same type of correlation was obtained between fecundity and female longevity. But, in the G4-G9 a negative correlation was recorded between these parameters. The obtained data suggest the existence of three phases in the growth of *Ostrinia nubilalis*: acclimatization (G0-G3), accomodation (G4-G9) and stabilization (over G10). In the first phase the rearing techniques and laboratory conditions have an important role to ensure a good quality of the laboratory population.

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Ostrinia nubilalis Hbn. (sfredelitorul porumbului) este o specie dăunătoare cu areal larg, pe mai multe continente, practic semnalată peste tot, acolo unde se cultivă porumbul, principala plantă gazdă. Cercetări referitoare la biologia şi ecologia acestei specii o semnalează ca dăunător şi la alte specii de plante cultivate, cum sunt: ardei, cartofi, tomate, ceapă, fasole, măcriş, cânepă, bumbac [1, 3, 7, 8, 16]. Inițial a fost considerată o specie mono-oligofagă, strict legată de porumb, este practic o specie polifagă, fiind întâlnită frecvent şi pe buruieni. Atacul este eurimer, cu excepția rădăcinii, fiind afectate practic toate părțile plantei.

Datorită importanței sale economice, majoritatea cercetărilor au urmărit studiul comportamentului feromonal şi secvențele comportamentului de reproducere la această specie. Aceste aspecte au fost investigate şi pentru rasa feromonală din Transilvania [3-5, 18, 19]. Mult mai puțin s-a analizat influența factorilor interni şi externi asupra creşterii, dezvoltării şi reproducerii în condiții naturale [7, 16]. Cercetări privind influența unor factori asupra reproducerii, în condițiile creşterii controlate în laborator, sunt relativ puține [6, 10]. Unele din aceste studii se referă la performanțele reproductive, asociate în special cu durata de viață a adulților sau comportamentul de ovipozitare [9, 14], care a fost analizat în special în câmp [8].

Lucrarea prezintă rezultatele obținute în studiul ovipozitării, fecundității şi longevității adulților de *Ostrinia nubilalis* pentru diferite generații, din două suşe crescute în condiții controlate de laborator.

Material şi metode. Datele studiului au la bază cercetări efectuate în perioada 1986-1990. Materialul biologic a provenit din populații de *O. nubilalis* aclimatizate în condițiile creşterii în laborator, pe dietă artificială, pe o suşă cu 4 generații (ON-2C) şi o alta cu 16 generații (ON-7G). In condiții de laborator au mai fost crescute şi alte suşe provenind de la populații din Transilvania sau din sudul țării. Condițiile pentru creşterea larvelor şi experimentarea adulților au fost: temperatura de 24 ± 1°C; regimul fotoperiodic de 17:7 ore (lumină: întuneric); intensitatea luminii > 950 lx. în fotofază şi 0,5-2 lx. în scotofază; umiditatea relativă > 75%. După împupare, pupele s-au sexat şi s-au menținut separate, în condiții doar puțin modificate fată de cele de creştere (întuneric continuu; 22 ± 1°C; UR > 50%). Emergența s-a înregistrat zilnic, iar adulții s-au pus în vase de sticlă, în care a existat hârtie de filtru ca suport şi s-au hrănit cu soluție de glucoză sau fructoză 10%. Din aceste vase s-au constituit perechile de adulți pentru studiul ovipozitării.

In studiul ovipozitării s-au folosit una sau mai multe perechi de adulți, iar depunerea pontelor a fost urmărită permanent, la intervale de 15 minute, pe toată durata scotofazei. S-a înregistrat periodicitatea zilnică de depunere a pontei şi ritmul circadian, la nivelul diferitelor generații.

Durata de viață a fost urmărită separat pe sexe, la adulți virgini, sau pe perechi de adulți în cazul celor împerecheați.

Datele au fost prelucrate statistic. A fost estimată valoarea coeficientului de corelație, iar diferențele dintre variante s-au apreciat prin testul de semnificație Duncan's New Multiple Range Test (D'sNMRT; P=0,01-0,05). Durata de viată la nivel populațional s-a apreciat cu ajutorul relației mediei ponderate, pentru fiecare sex, în toate generațiile:

$$n = \frac{\sum n_i \cdot x_i}{N_T}$$

(n = durata medie de viață; n_i = numărul de zile corespunzătoare intervalului duratei de viață a indivizilor generației studiate; x_i = numărul de indivizi care au trăit n_i zile; N_T = numărul total de indivizi din generația respectivă).

Rezultate şi discuții. *Durata de viață a adulților*. Longevitatea adulților este un indice biologic cu influență directă asupra potențialului de reproducere a speciei.

Pentru suşa ON-2C datele obținute au prezentat o variație caracteristică între sexe şi între generații, în funcție de statutul reproductiv (Tabel 1). Adulții virgini au trăit în medie mai mult decât cei împerecheați, iar femelele mai mult decât masculii.

Tabel 1

Durata de viață a adulților virgini şi împerecheați de Ostrinia nubilalis la primele 4 generații ale suşei ON-2C, în condițiile creşterii controlate în laborator

Generația	Durata de viață ($\bar{x}$ zile)*									
	Adulți împerecheați**					Adulți virgini**				
	♂♂	♀♀	Media	Min.	Max.	♂♂	♀♀	Media	Min.	Max.
G0	8,5a	9,8a	9,2a	3 (3)	15 (15)	9,6a	10,8a	10,2a	7 (6)	18 (19)
G1	5,9b	8,9b	7,4b	3 (3)	17 (12)	8,2b	9,8b	9,0b	5 (5)	14 (18)
G2	5,1b	8,0c	6,6c	2 (4)	10 (15)	6,6c	9,5b	8,1c	5 (6)	15 (14)
G3	5,9b	8,3b	7,1b	2 (2)	15 (14)	8,7b	10,1a	9,4b	6 (5)	20 (24)

* Aceeaşi literă indică diferențe nesemnificative în cadrul aceleiaşi grupe (Duncan'sNMRT; P=0,05).

** Pentru minim şi maxim, prima cifră reprezintă masculii, iar cifra din paranteză - femelele.

Datele obţinute implică câteva constatări. In primul rând, ele arată că aclimatizarea unei suşe noi la creşterea în condiţii de laborator determină o diminuare a comportamentului şi a adaptării in primele generaţii. De fapt, în cercetări preliminare, atât masculii cât şi femelele de *O. nubilalis* pentru primele generaţii din laborator au avut o durată de viaţă medie mai mică decât a adulţilor din câmp. Depăşirea şocului de colonizare este diferită la fiecare specie în funcţie de biologia, ecologia şi comportamentul acesteia, dar la majoritatea speciilor studiate şi crescute în laborator, în primele 3-4 generaţii vitalitatea se reduce, creşte mortalitatea (mai ales la nivelul stadiului de larvă) şi se diminuează comportamentul de reproducere, fecunditatea şi fertilitatea. Cele mai profunde modificări se manifestă însă la nivelul stadiului de larvă, asociate fiind şi cu acceptarea unei noi surse de hrană. Intervine astfel şi gradul de fagie al speciei în funcţie de complexitatea relaţiei cu planta gazdă. In general, speciile polifage sunt mai puţin pretenţioase la adaptarea în laborator, dar modelul diminuării vitalităţii şi comportamentului în primele generaţii este caracteristic la toate speciile, durata fiind cea diferită, în funcţie de combinaţia reuşită între valorile optime ale factorilor interni şi externi. La specia *Mamestra brassicae*, de exemplu, perioada critică este pentru primele 5 generaţii [17]. Tehnica de creştere prezintă pentru unele specii o importanţă deosebită, dar nu în aşa măsură încât să explice modelul de creştere şi dezvoltare în aceste prime generaţii. Chiar la *O. nubilalis* o diminuare a umidităţii în cabinetele termostatate a determinat o reducere a duratei de viaţă, a nivelului de împerechere şi a fecundităţii. Această "tatonare" a valorilor optime în primele generaţii poate afecta modelul comportamental. Intervin aici o serie de aspecte privind adaptarea, în relaţie cu influenţa selecţiei, aclimatizării sau "domesticirii" [11].

Pentru *O. nubilalis* datele din Tabel 1 arată o aparentă revigorare a populaţiei începând cu G3, fenomen parţial confirmat şi pentru suşa ON-7G. Aceste date sunt asemănătoare cu cele obţinute de alţi cercetători, pentru suşe din sudul ţării sau din Asia [1, 15], dar la acestea nu a existat o monitorizare atât de precisă a evoluţiei factorilor care influenţează creşterea şi dezvoltarea şi a valorilor parametrilor prin care acestea se pot aprecia. Datele noastre privind longevitatea adulţilor se referă numai la cei hrăniţi. La aceştia, durata de viaţă a crescut semnificativ faţă de cei nehrăniţi. O hrană pe bază de hidraţi de carbon este deosebit de importantă la această specie, prezenţa ei determinând o creştere a duratei de viaţă de 2,4 ori şi a ovipozitării de 3,5 ori, comparativ cu adulţii nehrăniţi. Pentru cei hrăniţi numai cu apă distilată diferenţele au fost mai mici [20]. Un model asemănător a fost obţinut şi în cercetări similare efectuate la populaţii din SUA [9].

In cazul suşei ON-7G modelul de supravieţuire a adulţilor pentru primele 4 generaţii a fost similar cu cel înregistrat pentru cealaltă şusă. Mai mult,

pentru generația a 3-a (G3) şocul aclimatizării a avut impactul cel mai redus din toate cele 16 generații. Pentru G4-G5 modelul se aseamănă cu cel din G1, se realizează apoi o creştere în medie a procentului de supraviețuire care s-a menținut până în G14 cam la nivelul G0. In G15 a avut loc a nouă scădere a duratei medii de viață, după care în G16 din nou s-a atins, aproape, nivelul din G3. Deosebirile dintre cele două suşe au fost semnalate în special la durata maximă de viață a adulților, pentru cea de-a doua suşa aceasta a fost mai mare la nivelul tuturor generațiilor. De asemenea, după G10 procentajul maxim de supraviețuire s-a inregistrat până la adulții de 5-7 zile. Explicăm aceste aspecte în special prin perfecționarea metodei de creştere şi de control a factorilor de mediu.

Evoluția duratei de viață pentru cele 16 generații, la adulții împerecheați şi virgini este prezentată în Tabel 2.

Tabel 2

Durata de viață a adulților virgini şi împerecheați, în relație cu generația la Ostrinia nubilalis pentru suşa ON-7G cu 16 generații (1989-1991)

Generația	Durata de viață ($\overline{x}$ zile)*									
	Adulți împerecheați**					Adulți virgini**				
	♂♂	♀♀	Media	Min.	Max.	♂♂	♀♀	Media	Min.	Max.
G0	10,6a	11,2a	10,9a	2(4)	19 (23)	10,4a	12,2a	11,3a	3(4)	20(26)
G1	7,9b	10,8b	9,4b	1(2)	20 (21)	8,4b	11,1b	9,8b	3(4)	21(23)
G2	5,4c	7,8c	6,6c	2(3)	10 (20)	7,3c	8,4c	7,9c	4(3)	17(19)
G3	11,4d	16,6d	14,0d	2(4)	27 (28)	11,6d	13,4d	12,5d	4(6)	26(29)
G4	7,3b	9,4f	8,4e	2(2)	17 (17)	9,2e	11,2b	10,2b	5(4)	16(22)
G5	8,7e	10,3b	9,5b	2(2)	19 (19)	10,4a	12,1a	11,3a	4(5)	21(19)
G6	8,2e	9,5bf	8,9be	1(2)	15 (17)	9,9a	12,3a	11,1a	3(4)	18(22)
G7	9,3f	12,2a	10,8a	2(2)	16 (20)	10,6a	13,4d	12,0d	3(2)	19(27)
G8	10,4a	12,8e	11,3a	3(3)	20 (21)	11,3d	12,9f	12,1d	4(5)	23(28)
G9	10,2a	11,5a	10,9a	3(4)	17 (18)	11,8d	13,1df	12,5d	5(6)	20(19)
G10	11,3d	12,6ae	11,9f	3(7)	19 (19)	12,6f	14,5e	13,6e	7(8)	24(27)
G11	11,2d	12,2a	11,7f	4(5)	16 (19)	12,4f	14,4e	13,4e	6(6)	26(29)
G12	9,6af	10,0b	9,8b	5(4)	14 (15)	12,3f	12,8f	12,6d	5(7)	18(19)
G13	10,6a	12,9e	11,8f	6(7)	20 (21)	12,8f	13,7d	13,3e	8(7)	26(24)
G14	9,5af	12,6a	11,1af	3(4)	22 (21)	13,5g	14,8e	14,2f	6(6)	24(24)
G15	7,6b	9,2f	8,4e	3(3)	17 (19)	10,4a	11,6d	11,0a	5(5)	21(23)
G16	12,1g	15,1d	13,6g	3(6)	20 (24)	11,8d	14,9e	13,4e	7(6)	25(23)

* Aceeaşi literă indică diferențe nesemnificative în cadrul aceleiaşi grupe (Duncan'sNMRT; P=0,05).

** Pentru minim şi maxim, prima cifră reprezintă masculii, iar cifra din paranteză - femelele.

Se poate considera că la *O. nubilalis* şocul de colonizare acţionează deci numai la nivelul primelor 3 generaţii după care a existat o stabilizare a duratei medii de viaţă în jurul valorilor de 10,6 zile pentru adulţii acuplaţi (11,6 zile la femele; 9,7 zile la masculi) şi 12,4 zile pentru cei virgini (13,2 zile la femele; 11,5 zile la masculi). Aceste valori sunt în medie mai mici decât cele înregistrate pentru adulţii altor populaţii ale acestei specii [14]. Analizând datele din tabelul de mai sus se poate afirma că odată depăşit şocul de colonizare, specia s-a adaptat la creşterea în condiţii de laborator. Cu cât numărul de generaţii este mai mare, calitatea populaţiilor este mai bună la nivelul parametrilor biologici, dar este afectat comportamentul. Pe de altă parte, modificări mai pronunţate sunt la nivelul stadiului de larvă şi mai puţin evidente sunt modificările referitoare la durata de viaţă a adulţilor. Suşa de *O. nubilalis* a avut doar 16 generaţii, număr prea mic pentru a desprinde concluzii cu valoare informaţională certă. Comparând longevitatea adulţilor acuplaţi cu cei virgini, a existat o corespendenţa între datele de la nivelul fiecărei generaţii, durata medie de viaţă a celor acuplaţi fiind în medie mai mică decât a celor virgini.

Comportamentul de ovipozitare. In condiţii de laborator, la *O. nubilalis*, secvenţa de acuplare durează între 1-4 ore, perioadă în care are loc transferul spermatoforului. După acuplare, cele două sexe se despart şi rămân astfel, în poziţii retrase, până la sfârşitul scotofazei. Nu a avut loc un comportament de ovipozitare în aceeaşi scotofază în care a avut loc acuplarea. Depunerea pontei s-a iniţiat în scotofaza imediat următoare. *O. nubilalis* depune ouăle sub formă de grupe de ouă (ponte), echivelentul ooplăcilor de la speciile de noctuide, incluse într-o masă cleioasă translucidă care se solidifică după ovipozitare. In acest fel ouăle sunt protejate pe perioadă de maturare.

Ritmul circadian al ovipozitării este prezentat în Fig. 1. Nu au fost diferenţe în modelul de ovipozitare în funcţie de generaţia din care a provenit femela observată. Totuşi s-a observat că odată cu creşterea numărului de generaţii perioada circadiană a fost relativ mai scurtă la nivel populaţional, rezultat al adaptării la noile condiţii din laborator unde ritmicitatea regimului fotoperiodic şi delimitarea netă dintre scotofază şi fotofază au imprimat această caracteristică.

In Fig. 1 sunt prezentate sintetic şi alte date obţinute în studiul comportamentului de reproducere a speciei *O. nubilalis*, în condiţii de laborator şi câmp. Perioada de ovipozitare este localizată în prima parte a scotofazei, perioadă care se suprapune cu curba de zbor a masculilor şi femelelor. Perioada de zbor a masculilor este mai mare, iar în partea de mijloc a scotofazei se suprapune cu zborul de curtare. După iniţierea scotofazei se declanşează activitatea locomotoare care este deci în relaţie cu ovipozitarea (pentru femelele acuplate anterior), dar şi cu zborul comportamental în relaţie cu maturizarea sexuală şi hrănirea. Această activitatea locomotoare de la începutul scotofazei nu este în legătură cu reproducerea (chemare, curtare, acuplare).

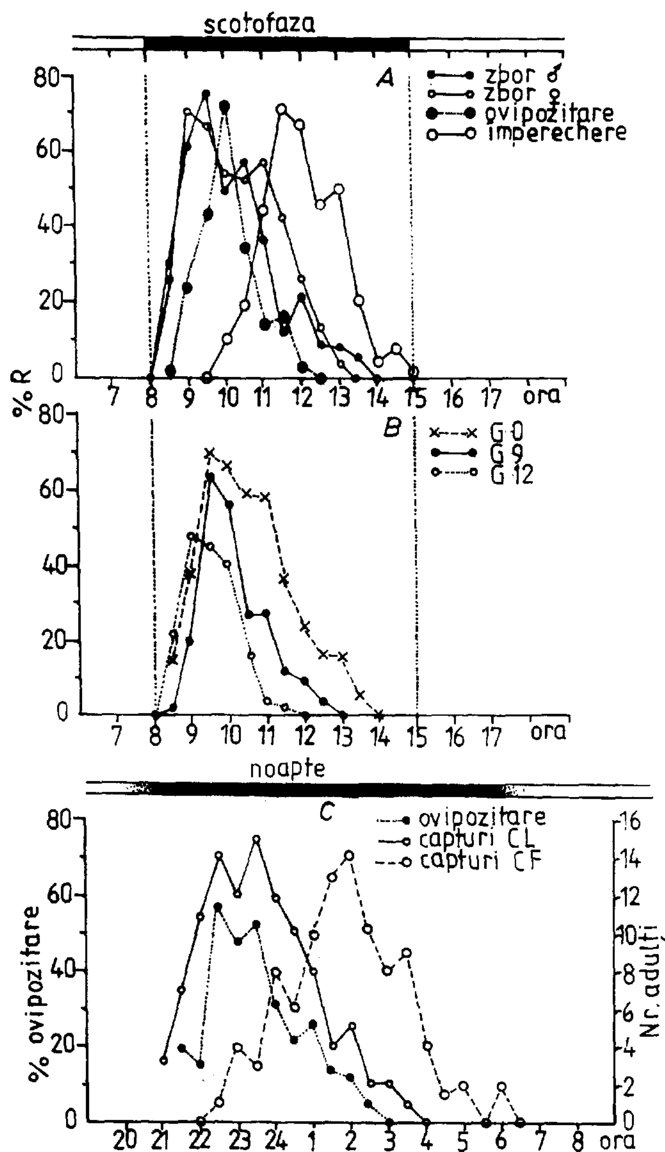

Fig. 1. *Ritmul circadian al comportamentului de ovipozitare la* Ostrinia nubilalis, *evaluat ca procentaj de răspuns (%R) din numărul total de femele observate.* *A* - In condiţii de laborator în relaţie cu zborul şi împerecherea. *B* - In laborator în funcţie de generaţie. *C* - In câmp comparativ cu periodicitatea capturării la capcana luminoasă (CL) şi la capcanele feromonale (CF).

In condiții de câmp, perioada de capturare a masculilor şi femelelor de *O. nubilalis* în capcana luminoasă (Fig. 1 C) se suprapune astfel cu perioada de zbor din laborator, în timp ce perioada de captură a masculilor la capcanele cu feromon sexual sintetic şi cu femele virgine se suprapune cu perioada de împerechere din laborator. Datele noastre confirmă un comportament similar observat la populațiile speciei din Bulgaria [12] sau din SUA [16].

Ritmicitatea de depunere a pontei pe toată durata de viață a femelei nu a scos în evidența deosebiri marcante între generații, mai ales pe intervalul G5-G16, doar că începând cu G8 a crescut numărul mediu de ponte depuse zilnic. Deosebirile au fost similare la ambele suşe pe intervalul G0-G3, dar marcate de o variație care nu a fost caracterizată de corespondență între aceleaşi generații. Fenomenul poate fi pus în legătură cu aclimatizarea diferită a celor două suşe şi unele discordanțe în ceea ce priveşte valorile absolute ale factorilor din laborator.

Aşadar, pentru suşa ON-2C datele obținute au evidențiat o similaritate a modelului pentru cele 4 generații, dar se poate constata că pe linia G0-G3 s-a obținut o modificare a trendului curbei generale, dependent de evoluția duratei medii de viață a adulților. In rest, aspectul cel mai interesant este legat de vitalitatea generației. Astfel, pentru G2-G3 care reprezintă generații cheie în aclimatizarea în laborator, odată cu îmbătrânirea femelelor s-a diminuat progresiv şi numărul mediu de ponte/femelă, depuse zilnic. Se pare însă că acest aspect nu este o caracteristică a speciei. Mai mult, este deosebit de interesant comportamentul "reținere a pontei", astfel neexistând diferențe mari (în ceea ce priveşte numărul mediu de ponte depuse zilnic), între femelele de 2-4 zile şi cele de 15-16 zile. Considerăm că acest model comportamental este specific şi are o deosebită valoare adaptativă pentru *O. nubilalis* care este o specie cu fecunditate redusă. Acest model de ovipozitare combinat cu strategia de încorparare a ouălor într-o masă de protecție, capacitatea de împerechere a masculului în mai multe nopți (fiecare femelă se împerechează o singură dată pe noapte şi dominant o singură dată pe durata de viață) şi durata de viață relativ lungă, suplinesc prolificitatea mai mică şi numărul redus al împerecherilor multiple (nu au existat diferențe privind ovipozitarea la femelele cu un singur spermatofor şi cele cu 3 spermatofori în bursa copulatoare).

Datele referitoare la comportamentul de împerechere [6] au evidențiat că la *O. nubilalis* a predominat net un singur spermatofor/femelă, atât pentru adulții din laborator cât şi pentru cei din câmp. In laborator, maxim 8-15,5% din femele au prezentat 2 spermatofori şi 1,4% au avut 3 spermatofori. Rezultatele concordă cu cele obținute pentru femelele din câmp, capturate la capcanele luminoase [7].

In ceea ce priveşte ritmicitatea numărului de ponte depuse zilnic, pe toată durata de viață, datele arată o corelație cu evoluția mediei longevității, la nivelul fiecărei generații.

Pentru suşa ON-2C datele sunt prezentate în Tabel 3. In cadrul primelor 4 generaţii se observă o diminuare progresivă a numărului mediu de ponte/femelă, pentru G0-G3, la fel ca şi în cazul evoluţiei longevităţii. Intre longevitatea femelelor şi numărul mediu de ponte pentru primele 3 generaţii a existat o corelaţie pozitivă relativ strânsă (r = 0,8648). O corelaţie similară a existat şi între media longevităţii şi media celui mai mare număr de ponte/femelă (r = 0,8928).

Tabel 3

Ritmicitatea depunerii pontelor fertile şi numărul total de ponte depuse de femelele de Ostrinia nubilalis în condiţii de laborator, pe toată durata de viaţă, pentru primele 4 generaţii, la suşa ON-2C (1987-1988)

Generaţia	Număr mediu de ponte/femelă/zi			Număr total de ponte/♀***			Număr mediu de zile de ovipozitare
		Interval**			Interval**		
	Media*	Min.	Max.	Media*	Min.	Max.	
G0	2,1a	0,3	3,4	12,7a	0,7	28,9	15
G1	1,5b	0,2	3,2	6,7b	0,8	17,2	18
G2	0,8c	0,2	2,0	9,6c	2,6	15,6	14
G3	0,9c	0,2	1,8	5,6d	1,8	12,4	13

* Aceeaşi literă indică diferenţe nesemnificative în cadrul aceleiaşi grupe (Duncan'sNMRT; P=0,01).
** Min., Max. - Media pentru valorile minime, respectiv maxime, din 5 repetiţii/generaţie (30 femele/repetiţie).
***Numărul total de ponte depus de o femelă pe toată durata de viaţă.

La nivelul suşei ON-7G, modelul comportamental nu a fost semnificativ diferit pentru primele generaţii (Tabel 4). In schimb, începând cu G7 se constată o creştere semnificativă a numărului mediu de ponte/femelă, deci, implicit, un număr mai mare de femele care depun mai multe ponte. Creşterea fecundităţii, odată cu creşterea numărului de generaţii succesive în condiţii de laborator, confirmă existenţa unei calităţi optime a populaţiei hrănite în laborator.

In ceea ce priveşte numărul total de ponte depuse de o femelă pe toată durata de viaţă, datele prezentate în Tabelele 3 şi 4 evidenţiază existenţa unui interval larg de variaţie, atât ca diferenţe între indivizi cât şi în funcţie de generaţie. Datele noastre sugerează existenţa unor populaţii de o calitate de categoria medie-bună. B ă r b u l e s c u [1] a obţinut în medie, pentru o şusă din sudul ţării crescută în laborator pe alt tip de dietă artificială, între 8,1 şi 11,5 ponte/femelă, valori apropiate de cele obţinute de noi. In schimb rezultatele nu confirmă observaţiile altor autori care, în condiţiile creşterii de generaţii

succesive în laborator, au înregistrat o scădere progresivă a numărului de ponte/femelă [13]. Neexistenţa unei gradaţii (crescătoare sau descrescătoare) sugerează că generaţia nu a influenţat calitatea ovipozitării, iar valorile obţinute sunt dependenţe de calitatea creşterii la nivelul fiecărei generaţii. Luând datele în ansamblu se poate spune chiar că a existat o creştere a numărului de ponte/femelă, odată cu creşterea numărului de generaţii. Aceste rezultate concordă cu cele obţinute şi la alte specii, dar trebuie precizat că este necesară o creştere dirijată.

Tabel 4

Ritmicitatea depunerii pontelor fertile şi numărul total de ponte depuse de către femelele de Ostrinia nubilalis în condiţii de laborator, pe toată durata de viaţă, la suşa ON-7G cu 16 generaţii (1989-1991)

Generaţia	Număr mediu de ponte/femelă/zi			Număr total de ponte/♀***			Număr mediu de zile de ovipozitare
	Media*	Interval**		Media*	Interval**		
		Min.	Max.		Min.	Max.	
G0	1,52a	0,8	2,4	11,4a	0,8	24,8	20
G1	1,14b	0,2	1,6	7,5b	0,9	14,4	18
G2	1,04be	0,3	2,6	2,1c	1,7	9,2	13
G3	0,92e	0,4	1,6	10,6a	4,3	12,8	25
G4	1,05be	0,3	1,9	7,0bh	1,8	10,8	16
G5	0,94e	0,3	1,8	5,1d	2,7	16,4	15
G6	1,11be	0,3	2,0	7,8b	3,5	24,2	15
G7	0,99e	0,7	2,0	6,6h	4,8	22,6	15
G8	1,66a	1,0	2,4	7,7b	7,2	18,6	14
G9	1,36a	0,2	2,8	10,3ai	4,4	26,4	15
G10	1,73c	0,2	2,4	13,1e	8,2	30,4	16
G11	1,96cd	1,0	2,9	12,4f	10,4	22,8	13
G12	1,70ac	1,0	2,4	9,2i	7,5	16,4	10
G13	1,64a	0,6	2,7	14,4e	6,2	26,5	16
G14	1,33a	0,4	2,8	10,5ai	4,2	22,8	16
G15	1,45a	0,4	2,2	7,1b	3,1	28,4	15
G16	2,02d	0,8	3,8	20,4g	8,4	34,2	18

* Aceeaşi literă indică diferenţe nesemnificative în cadrul aceleiaşi grupe (Duncan'sNMRT; P=0,01).
** Min., Max. - Media pentru valorile minime, respectiv maxime, din 5 repetiţii/generaţie (30 femele/repetiţie).
*** Numărul total de ponte depus de o femelă pe toată durata de viaţă.

Fecunditatea femelelor. Termenul este acceptat conform cercetărilor moderne, ca număr total de ouă/femelă (ouă depuse şi ouă rămase în ovare la moartea femelei). In acest studiu ne referim numai la ouăle depuse, încorporate în ponte.

Pentru suşa ON-2C datele evidenţiază o fecunditate cuprinsă în medie între 160-300 ouă/femelă (Tabel 5). Fecunditatea cea mai mare s-a înregistrat pentru femelele din G0, înregistrându-se apoi o diminuare pentru următoarele 3 generaţii. Pentru primele 4 generaţii ale acestei linii de creştere a existat o strânsă corelaţie pozitivă doar între fecunditate şi numărul de ponte/femelă (r = 0,9472), iar corelaţia dintre fecunditate şi longevitatea femelelor a fost nesemnificativă.

Tabel 5

Fecunditatea femelelor de Ostrinia nubilalis
în condiţii de laborator la suşa ON -2C

Generaţia	Număr de ouă/pontă ($\bar{x}$)*			Număr de ponte/♀ ($\bar{x}$)	Număr total de ouă/♀ ($\bar{x}$)
	Media	Min.**	Max.**		
G0	22,63a	13,7	31,5	12,7	287,40
G1	28,52b	21,6	35,4	6,7	191,08
G2	26,83c	15,4	38,2	9,6	257,57
G3	28,95b	15,3	42,6	5,6	162,12

* Aceeaşi literă indică diferenţe nesemnificative în cadrul aceleiaşi grupe (Duncan'sNMRT; P=0,01).
** Min., Max. - Media pentru valorile minime, respectiv maxime, din 5 repetiţii/generaţie (30 femele/repetiţie).

Pentru suşa ON-7G (Tabel 6) datele privind fecunditatea arată o diminuare progresivă doar pentru primele trei generaţii, iar pentru G3 se confirmă vitalitatea populaţiei din laborator, datele fiind în concordanţă cu longevitatea mare a adulţilor şi numărul de ponte depuse. Pentru primele 4 generaţii a existat o strânsă corelaţie pozitivă între fecunditate şi numărul de ponte/femelă (r = 0,9614), sau între fecunditate şi longevitatea femelelor (r = 0,9532). Pe intervalul G4-G9, corelaţia a fost negativă şi nesemnificativă între aceşti parametri. Datele confirmă existenţa unei faze de "iniţiere a acomodării" [2, 11] la noile condiţii de creştere în laborator. După această perioadă, pe intervalul G10-G16 între parametrii de mai sus a existat din nou o corelaţie pozitivă, dar cu valori mai reduse (r = 0,8434, respectiv, r = 0,7866). Pe de altă parte, începând cu G10, valoarea medie a fecundităţii/femelă a atins valori peste 300 şi 400 ouă/femelă. Aceste date nu confirmă rezultatele obţinute de R a u n [13], iar modelul de dezvoltare la *O. nubilalis* este asemănător cu cel al speciei *M.brassicae*. Pentru asigurarea heterogenităţii este însă nevoie de o împrospătare cu material biologic din natură sau efectuarea unei selecţii după modelul realizat la specia *M. brassicae* [17].

Tabel 6

Fecunditatea femelelor de Ostrinia nubilalis în condiţii de laborator la suşa ON -7G

Generaţia	Număr de ouă/pontă ($\bar{x}$)*			Număr de ponte/♀ ($\bar{x}$)	Număr total de ouă/♀ ($\bar{x}$)
	Media	Min.**	Max.**		
G0	22,16a	16,78	27,54	11,4	252,62
G1	23,52a	17,23	29,81	7,5	176,40
G2	25,09c	19,02	31,16	2,1	52,69
G3	36,16d	32,08	40,24	10,6	383,29
G4	30,40eg	26,08	34,72	7,0	212,80
G5	32,34be	28,46	36,18	5,1	164,83
G6	26,42c	23,30	29,54	7,8	206,08
G7	27,24c	24,74	31,74	6,6	179,78
G8	21,74af	16,14	27,34	7,7	167,39
G9	24,58ac	19,64	29,52	10,3	253,17
G10	23,94a	21,70	26,18	13,1	313,61
G11	26,04c	20,92	31,16	12,4	322,89
G12	25,09c	20,90	29,28	9,2	230,83
G13	28,54g	22,62	34,46	14,4	410,98
G14	23,76a	20,72	26,80	10,5	249,48
G15	20,78f	14,54	27,02	7,1	147,54
G16	20,63f	16,72	24,54	20,4	420,85

* Aceeaşi literă indică diferenţe nesemnificative în cadrul aceleiaşi grupe (Duncan's NMRT; P=0,01).
** Min., Max. - Media pentru valorile minime, respectiv maxime, din 5 repetiţii/generaţie (30 femele/repetiţie).

Cunoaşterea detaliilor referitoare la longevitatea adulţilor, corelată cu capacitatea lor reproductivă oferă informaţii asupra populaţiilor obţinute în laborator şi direcţia în care ele pot fi folosite în studiul complex al speciei. Aceste date devin cu atât mai importante cu cât sunt obţinute de la specii importante din punct de vedere economic.

Concluzii. 1. La ambele linii de creştere, durata medie de viaţă a femelelor a fost mai mare decât a masculilor, iar adulţii virgini au trăit în medie mai mult decât cei împerecheaţi.

2. Perioada circadiană de ovipozitare a fost localizată în prima parte a scotofazei, iar durata ei s-a redus odată cu creşterea numărului de generaţii. Pentru primele 4 generaţii, la ambele linii, s-a înregistrat o diminuare progresivă a numărului de ponte depuse de o femelă/zi şi a numărului total de ponte depuse. Durata medie a perioadei de ovipozitare a fost de 15,0 zile la suşa ON-2C şi 15,9 zile la suşa ON-7G şi a variat în limite foarte înguste în funcţie de generaţie.

3. Pentru primele 4 generații s-a înregistrat o diminuare progresivă a numărului de ponte depuse zilnic şi a numărului total de ponte depuse pe toată durata de viață. Incepând cu generațiile 4-5 valorile au crescut, iar pe intervalul G10-G16 s-au apropiat de cele din G0.

4. Numărul de ouă/pontă şi fecunditatea femelelor au variat pe intervale mai înguste fără dependență de generație sau susă. Totuşi, linia de creştere cu 16 generații a înregistrat valori absolute mai mari la nivelul tuturor parametrilor, explicația fiind asociată cu stabilirea unor valori optime pentru factorii care influențează creşterea.

5. În obținerea unei calități optime a unei populații de laborator pentru *O. nubilalis,* datele obținute sugerează existența a 3 faze: aclimatizare (G0-G3), acomodare (G4-G9) şi stabilizare (peste G10).

BIBLIOGRAFIE

1. B ă r b u l e s c u, A., *Contribuții la creşterea sfredelitorului porumbului* (Ostrinia nubilalis *Hbn.*) *pe diete artificiale*, "An. Inst. Cercet. Cereale Plante Tehn." (Fundulea), **44**, 1979, 377-381.
2. B o l l e r, E. F., C h a m b e r s, D. L. , *Quality aspects of mass-reared insects*, în R i d g w a y, R. L., V i n s o n, S. B. (Eds.), *Biological Control by Augmentation of Natural Enemies*, p. 219-235, Plenum Press, New York, 1977.
3. C r i ş a n, A., R o m a n, M. C., S t a n, G., C o r o i u, I., O n i ş o r, A., C h i ş, V., C i u p e, H., O p r e a n, I., *Field researches on the behaviour of the European corn borer*, Ostrinia nubilalis *Hbn*. (Lepidoptera: Pyralidae) *in relation to synthetic pheromone blends*, "Stud. Univ. Babeş-Bolyai, Biol.", **33** (2), 1988, 49-60.
4. C r i ş a n, A., S t a n, G., *Reproducerea şi comportamentul feromonal la* Ostrinia nubilalis *Hbn*. (Lepidoptera: Crambidae) *în condiții de laborator. 1.Comportamentul de chemare al femelei*, "Bul. Inf. Soc. Lepidopterol. Rom.", **5** (3-4), 1994, 245-255.
5. C r i ş a n, A., S t a n, G., C h i ş, V., *Reproducerea şi comportamentul feromonal la* Ostrinia nubilalis *Hbn*. (Lepidoptera: Crambidae) *în condiții de laborator. 3.Testarea olfactometrică a răspunsului masculilor la feromonul sexual natural şi sintetic*, "Bul. Inf. Soc. Lepidopterol. Rom.", **6** (3-4), 1995, 291-304.
6. C r i ş a n, A., S t a n, G., R o m a n, M.C., *Reproducerea şi comportamentul feromonal la* Ostrinia nubilalis *Hbn.* (Lepidoptera: Crambidae) *în condiții de laborator. 5. Observații preliminare privind influența unor factori asupra comportamentului de împerechere*, "Bul. Inf. Soc. Lepidopterol. Rom.", **7** (1-2), 1996, 41-58.
7. E l l i o t t, W. M., *Mating frequency of the female European corn borer,* Ostrinia nubilalis (Lepidoptera: Pyralidae), *in southwestern Ontario*, "Can. Entomol.", **109**, 1977, 117-122.

8. E l l i o t t, W. M., D i r k s, V. A., *Postmating age estimates for female European corn borer moths,* Ostrinia nubilalis (Lepidoptera: Pyralidae), *using time-related changes in spermatophores*, "Can. Entomol.", **111**, 1979, 1325-1335.
9. L e a h y, T. C., A n d o w, D. A., *Egg weight, fecundity, and longevity are increased by adult feeding in* Ostrinia nubilalis (Lepidoptera: Pyralidae), "Ann. Entomol. Soc. Amer.", **87**, 1994, 342-349.
10. L o u g h n e r, G. E., B r i n d l e y, T. A., *Mating success of the European corn borer*, Ostrinia nubilalis, *as influenced by environmental factors*, "Ann. Entomol. Soc. Amer.", **64**, 1971, 1091-1094.
11. O c h i e n g - O d e r o, J. P. R., *Does adaptation occur in insect rearing systems, or is it a case of selection, acclimatization and domestication ?*, "Insect Sci. Appl.", **15**, 1994, 1-7.
12. P a v l o v, A. K., M u t l a k, H. A., *Studies on the population dynamics of* Ostrinia nubilalis *Hbn*. (Lepidoptera: Pyralidae) *by using pheromone, light traps and the cage methods*, în Proc. Symp. "Noctuid Sex Pheromones Communication. Theory and Application" (Sofia, September 12-16, 1994), 1994, 17-18.
13. R a u n, E. S., *European corn borer*, în S m i t h, E. D. (Ed.), *Insect Colonization and Mass Production*, p. 321-337, Acad. Press, New York, 1966.
14. R o y e r, L., M c N e i l, J. N., *Male investment in the European corn borer* Ostrinia nubilalis (Lepidoptera: Pyralidae): *impact on female longevity and reproductive performance,* "Funct. Ecol.", **7** (2), 1993, 209-215.
15. S a l a m a - S a m ir, A., *Rearing the corn borer*, Ostrinia nubilalis *Hbn. on a semiartificial diet*, "Z. Angew. Entomol.", **65**, 1970, 216-218.
16. S h o w e r s, W. B., R e e d, G. L., R o b i n s o n, J. F., D e r o z a r i, M. B., *Flight and sexual activity of the European corn borer*, "Environ. Entomol.", **5**, 1976, 1099-1104.
17. S t a n, G., C h i ş, V., M o r a r, M., *Studiul capacităţii reproductive la* Mamestra brassicae *L.* (Lepidoptera: Noctuidae): *Influenţa vârstei asupra împerecherii în relaţie cu generaţia, la două linii de creştere în laborator*, "Bul. Inf. Soc. Lepidopterol. Rom.", **7** (1-2), 1996, 61-79.
18. S t a n, G., C r i ş a n, A., *Reproducerea şi comportamentul feromonal la* Ostrinia nubilalis *Hbn*. (Lepidoptera: Crambidae) *în condiţii de laborator. 2.Comportamentul de curtare*, "Bul. Inf. Soc. Lepidopterol. Rom.", **6** (1-2), 1995, 51-66.
19. S t a n, G., C r i ş a n, A., C h i ş, V., *Reproducerea şi comportamentul feromonal la* Ostrinia nubilalis *Hbn*. (Lepidoptera: Crambidae) *în condiţii de laborator. 4.Influenţa vârstei masculilor şi a perioadei de testare asupra comportamentului de răspuns la feromonul sexual natural şi sintetic*, "Bul. Inf. Soc. Lepidopterol. Rom.", **7** (1-2), 1996, 23-39.
20. S t a n, G., C r i ş a n, A., C h i ş, V., S t a n, G. G., *Influenţa hranei asupra fecundităţii, fertilităţii şi duratei de viaţă a adulţilor de* Mamestra brassicae *L.* (Lepidoptera: Noctuidae) *şi* Ostrinia nubilalis *Hbn.* (Lepidoptera: Crambidae), "Stud. Cercet. Biol., Ser. Biol. Anim.", 1999 (sub tipar).

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EFECTUL NaCl ASUPRA METABOLISMULUI CELULELOR DE *DIGITALIS LANATA* CULTIVATE *IN VITRO*

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SUMMARY.- Effect of NaCl on the Metabolism of Digitalis lanata Cells Cultivated in Vitro. Two cell lines of *Digitalis lanata*, 18 and 6-C100, were grown in high-level NaCl media. They were isolated on usual media - the cell line 18, or on media containing cholesterol (as screening agent) - the cell line 6-C100. Both cell lines were cultivated on Murashige-Skoog's medium with or without NaCl (8.5, 17, 34, 55 and 68 mM). We studied the effect of salt stress on cell growth, water content of cells (FW/DW), proline accumulation, peroxidase activity, as well as on the biosynthesis of cardenolide (digitoxin), depending on the NaCl concentration and on the cell culture age. High NaCl concentrations (55 and 68 mM) determined changes of cell metabolism, irrespectively of the cell culture age and treatment length. Thus, both cell growth and proline synthesis were inhibited in both cell lines. The cell water content strongly decreased under the salt stress, especially in the cell line 18. The cell line 6-C100 was more tolerant to NaCl as compared to cell line 18. An intensification of the peroxidase activity was also observed, especially in the cell line 6-C100. As regards the cardenolide accumulation, NaCl had the same effect as the abiotic elicitors. Addition of 8.5 mM NaCl to the cell culture medium strongly stimulated the digitoxin biosynthesis. Thus, the cell line 18 accumulated 20.7 μg digitoxin/g DW (43 times more than the control), and the cell line 6-C100 accumulated 12.6 μg digitoxin/g DW (17 times more than the control), after 12 days of treatment.

Pentru obţinerea unor cantităţi sporite de metaboliţi secundari valoroşi pentru industria farmaceutică prin intermediul culturilor de celule vegetale, este necesară ameliorarea condiţiilor de cultură. Între aceste condiţii se înscriu compoziţia mediului nutritiv şi elicitorii biotici şi abiotici.

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În urma atacului agenţilor patogeni (bacterii sau fungi) asupra plantelor, compuşii (denumiţi şi elicitori biotici) produşi de astfel de agenţi pot să inducă modificări în manifestarea anumitor gene care vor conduce în final la apariţia mecanismelor de apărare. Dintre aceste mecanisme face parte şi sinteza de fitoalexine şi, în unele cazuri, şi a altor metaboliţi secundari [7]. Din numeroase date rezultă că şi alţi factori de stres cum sunt: modificări ale pH-lui, ale presiunii osmotice, căldura ori frigul, razele UV, precum şi săruri ale metalelor grele, pot să inducă răspunsuri asemănătoare, ei încadrându-se în aceeaşi clasă a elicitorilor, fiind denumiţi elicitori abiotici. Mărirea sintezei metaboliţilor secundari, ca răspuns la diferite tipuri de stres, de exemplu prin elicitare, pare a fi o caracteristică generală a culturilor celulare vegetale [10, 11].

Suspensiile celulare de *Digitalis* nediferenţiate din punct de vedere morfologic produc în general cantităţi foarte mici de cardenolide şi, după un anumit interval, pot să-şi piardă această capacitate [15]. După autorii respectivi, manifestarea metabolismului secundar al culturilor celulare de *Digitalis* este integrată într-un program de exprimare a genelor care conduce la specializarea celulară. Totuşi, biosinteza de cardenolide poate să aibă loc şi în culturi celulare de *Digitalis* nediferenţiate [6, 8].

Liniile celulare de *Digitalis lanata* selectate de noi [8] au sintetizat diferite cantităţi de cardenolide (digoxină şi digitoxină). Unele dintre ele, după doi ani de subcultivări succesive pe acelaşi tip de mediu, şi-au pierdut complet capacitatea de a biosintetiza astfel de compuşi, iar altele au acumulat, în cantităţi neînsemnate, doar digitoxină [9].

Prin modificarea compoziţiei mediilor nutritive ale suspensiilor celulare de *Digitalis*, respectiv a balanţei hormonale [19] sau a nivelului calciului [6], s-a reuşit o sporire semnificativă a acumulării de cardenolide. Cu toate că în literatura de specialitate nu am întâlnit date care să ateste utilizarea elicitorilor abiotici în culturi celulare de *Digitalis lanata*, primele experimente, efectuate de noi cu sulfat de cupru, au dat rezultate promiţătoare [9].

Clorura de sodiu este utilizată în majoritatea cazurilor pentru elucidarea unor aspecte care privesc rezistenţa celulei vegetale faţă de stresul salin. Până în prezent ea nu a fost întrebuinţată ca şi agent de inducere sau de stimulare a proceselor metabolismului secundar în culturi de suspensii celulare vegetale.

În lucrarea de faţă am investigat influenţa stresului salin asupra mai multor procese fiziologice (creştere celulară, viabilitate celulară, activitate peroxidazică, precum şi sinteza de prolină şi de cardenolide) la două linii celulare, 18 şi 6-C100, de *Digitalis lanata*, în funcţie de concentraţia NaCl şi de vârsta culturilor celulare.

Material şi metode. *a) Culturile celulare*. Liniile celulare 18 şi 6-C100 de *Digitalis lanata*, obţinute anterior [8], au fost crescute într-un mediu lichid Murashige-Skoog (MS)[17], cu 1 mg/l 2,4-D şi 2 mg/l BA, în vase Erlenmeyer de 300 ml ce conţineau 50 ml mediu nutritiv. Ele au fost subcultivate la un interval de 14 zile, raportul dintre inocul şi mediul proaspăt fiind de 1:6. Vasele de cultură au fost menţinute pe un agitator rotativ orizontal (100 rpm) la o fotoperioadă de 16 ore şi la o temperatură de 25°C. Pentru testarea efectului clorurii de sodiu, s-au realizat două tipuri de experimente. În primul experiment, în vasele cu suspensii celulare aflate în ziua a 10-a de cultură, s-a introdus câte 1 ml dintr-o soluţie de NaCl, de o anumită concentraţie, astfel ca în aceste vase concentraţiile de sare să ajungă la un nivel de 17, 34 şi 68 mM. În vasele martor s-a introdus 1 ml de apă distilată. În al doilea experiment, NaCl a fost adăugată la mediul nutritiv înainte de autoclavare, în concentraţii de 8,5, 17, 34 şi 55 mM.

În scopul determinării creşterii celulare şi a raportului substanţă proaspătă/substanţă uscată (S.p./S.u.), utilizat ca un indice al conţinutului celular în apă, s-a recoltat (prin filtrare) şi cântărit biomasa celulară (proaspătă şi uscată). Probele respective au fost utilizate şi pentru analizele biochimice. Viabilitatea celulară s-a determinat prin metoda de colorare cu albastru Evans [2].

b) Analize biochimice. Activitatea guaiacol-peroxidazică a fost determinată spectrofotometric după metoda dată de Bouchet şi colab. [4]. Prolina a fost extrasă într-o soluţie apoasă de acid sulfosalicilic şi s-a dozat spectrofotometric [1].

Extracţia şi dozarea cardenolidelor s-a realizat după metoda dată de Jones şi Veliky [14]. Cardenolidele au fost extrase din substanţa uscată, cu 20 ml etanol (70%) prin refluxare la 70°C timp de 20 min. Identificarea glicozidelor cardiotonice s-a realizat prin compararea cromatografică a extractelor cu probe etalon de digoxină, digitoxină şi lanatozida C. Pentru determinarea cantitativă, plăcile cromatografice au fost citite la 380 nm cu un densitometru DESAGA CD 60. Curba etalon a fost realizată pentru concentraţii de 0,2 - 0,6 μg digitoxină/spot.

Toate experimentele şi analizele au fost repetate de trei ori.

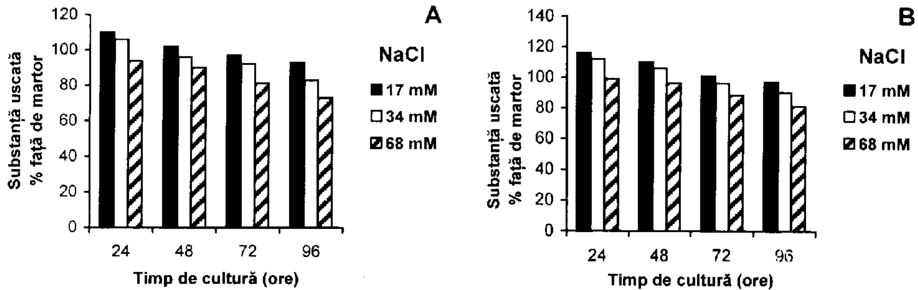
Rezultate şi discuţii. Aşa cum arătam şi mai sus, după doi ani de subcultivări succesive pe acelaşi tip de mediu, suspensiile celulare de *Digitalis lanata* au încetat să acumuleze cardenolide, respectiv digoxină [9]. Singurul glicozid cardiotonic care mai este sintetizat de majoritatea suspensiilor celulare este digitoxina, dar în cantităţi extrem de mici (sub 1 μg/g S.u.).

Creşterea celulară

Liniile celulare alese de noi fac parte din două grupuri diferite care au fost izolate fie de pe medii fără agent de selecţie - linia 18, fie de pe medii cu colesterol (ca agent de selecţie) - linia 6-C100. Ele etalează anumite caracteristici fiziologice, aşa cum sunt creşterea celulară, conţinutul celular în apă şi acumularea de digitoxină, care le deosebesc net una de alta [9]. În ceea ce priveşte creşterea, linia 18 atinge faza staţionară în ziua 14-a de cultură, zi în care ea acumulează cantitatea maximă de

biomasă celulară (10,08 g/l S.u.). Linia 6-C100 atinge faza respectivă în ziua a 10-a (8,4 g/l S.u.), în a 14-a zi aflându-se în plin stadiu de declin al ciclului celular (6,78 g/l S.u.) [9].

Adăugarea clorurii de sodiu la culturile aflate în ziua a 10-a de cultură a avut ca efect general inhibiţia creşterii celulare care a depins atât de linia celulară, deci implicit şi de stadiul de dezvoltare a ei, cât şi de concentraţia de NaCl (Fig.1). Se observă astfel că linia 18 este mult mai sensibilă la acţiunea stresului salin în comparaţie cu linia 6-C100. Biomasa celulară a acesteia a scăzut cu peste 20% faţă de martor, atunci când celulele au fost crescute în medii nutritive cu 68 mM NaCl pentru o perioadă de 24 sau 98 de ore (Fig. 1A). La linia 6-C100, deşi în momentul introducerii NaCl suspensiile celulare se aflau în stadiul staţionar, acest proces este mult mai atenuat, după primele două zile de tratament constatându-se chiar o uşoară stimulare a creşterii celulare la concentraţii ale NaCl de 17 şi 34 mM (Fig. 1B).



F i g. 1. *Efectul NaCl asupra creşterii celulare în culturi de suspensii celulare de* Digitalis lanata.

NaCl a fost adăugată în mediul suspensiilor în ziua a 10-a de cultură, iar celulele au fost recoltate după 24, 48, 72 şi 96 de ore.

A - Linia celulară 18. Valoarea absolută a martorului (g/l S.u.): - 8,45 (24 ore - ziua a 11-a); 9,22 (48 ore - ziua a 12-a); 9,85 (72 ore - ziua a 13-a); 10,08 (96 ore - ziua a 14-a).

B - Linia celulară 6-C100. Valoarea absolută a martorului (g/l S.u.): - 8,30 (24 ore - ziua a 11-a); 7,88 (48 ore - ziua a 12-a); 7,30 (72 ore - ziua a 13-a); 6,78 (96 ore - ziua a 14-a).

Inhibiţia creşterii celulare este mult mai puternică atunci când suspensiile celulare de *D. lanata* sunt cultivate de la început în medii nutritive cu adaos de NaCl (Tabel 1), timp de 12 zile. Şi în acest caz, tot linia celulară 18 s-a dovedit a fi mai sensibilă, cantitatea de substanţă uscată scăzând cu 60% în prezenţa a 55 mM de NaCl. La linia 6-C100, în aceeaşi variantă, biomasa celulară s-a diminuat cu 40%. La ambele linii celulare, inhibiţia creşterii celulare a fost proporţională cu mărirea concentraţiei de NaCl.

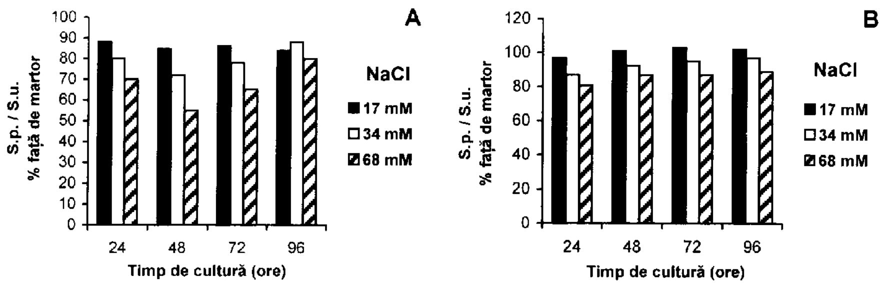
Procesul de inhibiție este şi mai accentuat în cazul conținutului celular în apă (S.p./S.u.), în special la linia 18, indiferent de momentul şi de concentrația în care NaCl a fost adăugată la mediul de cultură al suspensiilor (Fig. 2 şi Tabel 1). De fapt, sub influența stresului salin se produce o deshidratare a celulelor, deshidratare ce are loc în paralel cu creşterea concentrației de NaCl. Ea este mai puțin evidentă în cazul suspensiilor celulare ale liniei celulare 6-C100, la care, chiar şi în condiții normale de cultură, raportul S.p./S.u. este mult mai redus pe întreg ciclu celular, față de linia 18. Inhibiții ale creşterii celulare sau scăderea conținutului celular în apă, ca şi rezultat al stresului salin, s-au constatat şi la alte culturi de țesuturi şi celule vegetale [3, 16, 21, 27]. Dar, în experimentele respective s-au utilizat întotdeauna linii celulare, a căror toleranță față de NaCl a fost indusă prin cultivări repetate ale celulelor respective pe medii cu adaos de sare. În contrast cu ele, la linia celulară 6-C100 de *D. lanata* este posibil ca acest caracter de toleranță să se fi imprimat ca urmare a creşterii celulelor pe medii în care agentul de selecție a fost colesterolul [8], deoarece ele nu au fost cultivate anterior pe medii cu NaCl.

Tabel 1

Efectul concentrației de NaCl şi al duratei de tratament asupra activității peroxidazice şi a viabilității celulare la două linii celulare (18 şi 6-C100) de Digitalis lanata

Durata de tratament* (ore)	NaCl (mM)	Peroxidază (µg guaiacol/g S.p.)		Numărul celulelor vii (%)	
		18	6-C100	18	6-C100
24	0	10,84	17,28	89,7	80,2
	17	11,27	17,80	81,6	76,2
	34	13,55	25,92	68,2	69,0
	68	7,37	14,86	49,3	52,9
48	0	13,73	19,69	88,4	76,1
	17	19,50	26,34	82,2	72,3
	34	22,24	40,37	72,5	63,9
	68	18,40	32,69	61,9	57,8
72	0	16,88	22,76	83,5	68,8
	17	24,31	20,87	77,6	65,4
	34	30,89	32,32	69,3	57,8
	68	21,94	28,53	58,5	49,5
96	0	20,09	24,94	78,7	63,4
	17	24,11	24,19	71,6	59,0
	34	30,14	27,43	59,8	50,7
	68	16,07	20,45	47,2	39,3

* Soluția de NaCl a fost introdusă în ziua a 10-a de cultură, iar celulele au fost recoltate după 24, 48, 72 şi 96 de ore.



F i g. 2. *Efectul NaCl asupra conținutului celular în apă (S.p./S.u.) în culturi de suspensii celulare de* Digitalis lanata.

NaCl a fost adăugată în mediul suspensiilor în ziua a 10-a de cultură, iar celulele au fost recoltate după 24, 48, 72 şi 96 de ore.

A - Linia celulară 18. Valoarea absolută a martorului: - 20,61 (24 ore - ziua a 11-a); 21,82 (48 ore - ziua a 12-a); 23,15 (72 ore - ziua a 13-a); 24,47 (96 ore - ziua a 14-a).

B - Linia celulară 6-C100. Valoarea absolută a martorului: - 16,78 (24 ore - ziua a 11-a); 17,57 (48 ore - ziua a 12-a); 18,61 (72 ore - ziua a 13-a); 19,15 (96 ore - ziua a 14-a).

Activitatea peroxidazică şi conținutul în prolină

Glicofitele, pentru a contracara efectul prezenței în mediu a unor ioni toxici cum sunt cei de Na^+ şi Cl^-, trebuie să dețină anumite mecanisme de adaptare care să facă față stresului osmotic şi toxic [18]. În acest sens, merită să fie reținuți doi markeri ai stresului salin şi anume: activitatea peroxidazică [12] şi nivelul prolinei [26]. Amândoi sunt legați de efectele secundare ale stresului aşa cum sunt: hiperacumularea peroxidului de hidrogen (H_2O_2), a radicalilor superoxidici ($O^•$) şi a radicalilor hidroxil ($OH^•$) ce sunt responsabili de un larg evantai de perturbări celulare [24]. Peroxidazele, prezente în toate compartimentele celulare, catalizează descompunerea H_2O_2 [23], iar prolina înlătură radicalii hidroxil [25].

În contextul celor arătate mai sus este explicabilă sporirea activității peroxidazice a celor două linii celulare de *Digitalis lanata* ca urmare a cultivării lor în medii cu sare (Tabel 2). Un proces asemănător s-a observat şi la alte culturi celulare, aşa cum sunt cele de tomate [22], crescute în condiții similare. Totuşi, în cazul suspensiilor celulare de *D.lanata*, se mai remarcă faptul că intensitatea activității peroxidazice scade atunci când cele două linii sunt cultivate în medii cu concentrații de NaCl mai mari de 34 mM. Fenomenul este explicabil, deoarece, la acest nivel de salinitate (68 mM NaCl), viabilitatea scade marcant la ambele linii celulare aproximativ în aceeaşi proporție, deşi numărul de celule vii ale liniei 6-C100 este permanent mai mic decât cel al liniei 18 (Tabel 2). Diferența se datorează în special decalajului de fază în care se găsesc cele două linii pe parcursul ciclului lor celular. Astfel, în timp ce linia 6-C100 intră din ziua 11 în faza de declin, linia 18 se

Tabel 2

Influența concentrației de NaCl asupra creşterii celulare şi a conținutului celular în apă (S.p./S.u.) la două linii celulare (18 şi 6-C100) de Digitalis lanata, după 12 zile de tratament

NaCl (mM)	Substanță uscată (g/l)		S.p./S.u.	
	18	6-C100	18	6-C100
0	9,22	8,30	21,82	17,57
8,5	8,99	8,27	19,16	16,53
17	7,75	7,22	16,19	14,65
34	6,47	6,63	12,66	12,51
55	4,33	5,21	8,73	10,72

află în aceeaşi perioadă de timp încă în stadiul linear de creştere şi ajunge în faza staționară numai în a 14-a zi de cultură. De asemenea, trebuie subliniat şi faptul că linia 6-C100 prezintă în mod constant o activitate peroxidazică mai mare decât linia 18, chiar şi atunci când este crescută în condiții normale de cultură. Este posibil ca activitatea crescută a enzimelor respective să constituie încă un factor de toleranță pentru linia 6-C100 față de stresul salin, având în vedere că peroxidazele, printre altele, modifică proprietățile peretelui celular. Ele pot cataliza legarea intercatenară a extensinei şi a pectinei, ceea ce va conduce în final, alături de alte procese, la rigidizarea peretelui celular [5].

Un alt proces care s-a observat la celule de tutun este micşorarea dimensiunii lor [3], care se datorează, după opinia autorilor, pierderii extensibilității peretelui celular ca şi rezultat al adaptării lor la sare. Deşi celulele liniei 6-C100 de *D.lanata* nu sunt adaptate la condiții de salinitate, ele au totuşi dimensiuni mai mici decât cele ale liniei 18 (date nepublicate). Probabil că şi această particularitate, care imprimă celulelor o rezistență sporită la anumiți factori de stres, a fost dobândită tot ca urmare a creşterii lor pe medii cu colesterol.

În ceea ce priveşte acumularea de prolină este de remarcat nivelul ei mult mai ridicat în celulele liniei 6-C100 față de linia 18, cultivate în condiții normale. Totuşi, cantitatea acestui compus scade proporțional cu mărirea concentrației de NaCl, în ambele linii celulare (Tabel 3). În general, la multe linii celulare adaptate la condiții de salinitate se constată că în urma cultivării lor pe medii cu concentrații mărite de NaCl are loc o acumulare sporită de prolină, în comparație cu liniile sensibile [13, 18]. Astfel că este puțin probabil ca prolina să constituie, în cazul suspensiilor celulare de *D.lanata*, unul din mecanismele de apărare față de stresul salin. În acest sens există şi alte exemple în care nivelul prolinei nu este aşa de ridicat pentru a juca un rol semnificativ în mecanismul de apărare a plantelor împotriva stresului salin [20].

Tabel 3

Efectul concentraţiei de NaCl şi al duratei de tratament asupra acumulării de prolină în două linii celulare (18 şi 6-C100) de Digitalis lanata

NaCl (mM)	Prolină (µM/g S.p.)			
	48 ore de tratament*		12 zile de tratament**	
	18	6-C100	18	6-C100
0	10,85	15,29	10,85	15,29
17	9,74	15,29	8,73	14,74
34	9,33	13,94	7,42	12,95
55	8,27	13,36	6,49	11,27

* Soluţia de NaCl a fost introdusă în suspensiile celulare aflate în ziua a 10-a de cultură. Celulele au fost recoltate după 48 de ore.

** NaCl a fost adăugată în mediul de cultură înainte de autoclavare. Celulele au fost recoltate după 12 zile de cultură.

Sinteza de cardenolide

După cum se observă din Tabelul 4, cele două linii se deosebesc una de alta şi prin cantităţile de digitoxină acumulate de celulele cultivate pe medii fără sare. Astfel, linia 6-C100 acumulează nivele mai ridicate din acest compus. Deşi adaosul de NaCl în ziua a 10-a de cultură a avut ca efect o stimulare puternică a sintezei de digitoxină la ambele linii, totuşi, la linia 18 se remarcă o acumulare mai mare a acestui cardenolid (de 9 ori mai mare decât la martor), faţă de linia 6-C100 (de 7 ori mai mare decât la martor). Această stimulare a avut loc după 72 de ore de la introducerea clorurii de sodiu şi a scăzut apoi în paralel cu creşterea concentraţiei de NaCl şi cu durata de tratament. Scăderea a fost mult mai intensă la linia 6-C100 la care nivelele NaCl de 68 mM (după 72 de ore) şi, respectiv, de 34 şi 68 mM (după 96 de ore) au produs inhibiţia totală a sintezei de digitoxină.

Introducerea clorurii de sodiu de la început în mediul celor două linii celulare a avut un efect oarecum similar cu cel descris anterior. Astfel, după 12 zile de tratament tot celulele liniei 18 acumulează cele mai mari cantităţi de digitoxină, în comparaţie cu cele ale liniei 6-C100. Intensitatea stimulării biosintezei de digitoxină a fost însă cu mult mai mare decât în primul experiment, linia 18 acumulând 20,7 µg digitoxină/g S.u. (de 43 ori mai mult decât martorul), iar linia 6-C100 - 12,6 µg digitoxină/g S.u. (de 17 ori mai mult decât martorul). De asemenea, se remarcă aceeaşi influenţă negativă a concentraţiilor sporite de NaCl asupra sintezei de cardenolide. În acest sens se ştie, dacă NaCl este considerată elicitor, că elicitorii nu stimulează întotdeauna biosinteza metaboliţilor secundari, dar pot să inducă sau să înlăture represia lor.

Tabel 4

Efectul concentrației NaCl şi al duratei de tratament asupra sintezei de digitoxină la două linii celulare (18 şi 6-C100) de Digitalis lanata

NaCl (mM)	Digitoxină (µM/g S.u.)							
	Durata de tratament (ore şi ziua de cultură)							
	48 ore* (ziua 12)		72 ore* (ziua 13)		96 ore* (ziua 14)		288 ore** (ziua 12)	
	18	6-C100	18	6-C100	18	6-C100	18	6-C100
0	0,48	0,71	0,70	0,88	0,74	0,92	0,48	0,71
8,5	-	-	-	-	-	-	20,70	12,40
17	0,48	0,80	6,76	6,00	4,51	5,43	2,20	0,98
34	0,42	0,65	5,25	4,65	3,05	0,00	0,32	0,00
55	-	-	-	-	-	-	0,00	0,00
68	0,33	0,58	3,16	0,00	0,00	0,00	-	-

* Soluția de NaCl a fost adăugată la suspensii aflate în ziua a 10-a de cultură, iar celulele au fost recoltate după 48, 72 şi 96 de ore.
** NaCl a fost introdusă în mediul nutritiv înainte de autoclavare.

La acest nivel al experimentelor efectuate de noi este extrem de dificil de a explica modul în care NaCl poate să producă stimulări aşa de puternice ale sintezei unui cardenolid şi anume a digitoxinei, la liniile celulare de *Digitalis lanata* care erau aproape lipsite de această capacitate. Dificultatea este sporită şi de cunoştințele extrem de reduse asupra modului în care un semnal extracelular este convertit într-un semnal intracelular care este apoi transmis la nucleu, precum şi asupra modificării exprimării genelor responsabile de manifestarea metabolismului secundar. De asemenea, nu putem să facem nici o corelație între diferitele procese ale metabolismului primar, cu cele ale metabolismului secundar, respectiv cu sinteza de cardenolide. Singura relatare, pe care o deținem despre testarea efectului NaCl asupra unor suspensii celulare ce sintetizează şi anumiți metaboliți secundari, se referă doar la L-DOPA. Acest compus este de fapt un aminoacid catecolic şi este precursor al mai multor metaboliți secundari, aşa cum sunt alcaloizii [28].

La fel de dificil este de răspuns şi la întrebarea: de ce linia 18, mai slab productivă, sintetizează ca răspuns la stresul salin cantități mai mari de digitoxină față de linia 6-C100 ? Ceea ce putem afirma însă cu certitudine este că acest agent de stres, NaCl, a acționat ca un elicitor abiotic veritabil asupra ambelor linii celulare de *Digitalis lanata*.

Concluzii. Modul în care cele două linii celulare, 18 şi 6-C100 de *Digitalis lanata* au răspuns la stresul salin a depins şi de particularităţile lor morfo-fiziologice. Ele au fost probabil dobândite în urma creşterii celulelor pe medii cu sau fără agent de selecţie (colesterol), medii de pe care au fost izolate. Unele dintre aceste proprietăţi, aşa cum sunt raportul mic dintre substanţa proaspătă şi cea uscată (conţinutul celular în apă), precum şi activitatea peroxidazică crescută, se pare că i-au conferit liniei 6-C100, selectată de pe mediu cu colesterol, o toleranţa sporită la stresul salin. Sub influenţa NaCl multe procese metabolice suferă modificări. Astfel, este inhibată creşterea celulară, scade conţinutul celular în apă, se măreşte activitatea peroxidazică şi se micşorează cantitatea de prolină. Însă aspectul cel mai important este legat de constatarea că NaCl, în concetraţii mici şi moderate, indiferent de momentul în care este adăugată în mediul de cultură, a stimulat puternic biosinteza de digitoxină la ambele linii celulare, comportându-se ca un elicitor abiotic veritabil.

BIBLIOGRAFIE

1. Bates, L.S., Waldren, R.P., Teare, I.D., *Rapid determination of free proline for water stress studies*, "Plant Soil", **39**, 1973, 205-207.
2. Bhojwani, S.S., Razdan, M.K., *Plant Tissue Culture: Theory and Practice*, Elsevier, Amsterdam, 1983.
3. Binzel, M.L., Hasegawa, P.M., Hanada, A.K., Bressan, R.A., *Adaptation of tobacco cells to NaCl*, "Plant Physiol.", **79**, 1985, 118-125.
4. Bouchet, M., Joassin, L., Schwind, F., Gaspar, T., *Effets à court et à long terme d'une variation de température et d'un traitement chimique sur les peroxidases de la betterave sucrière*, "Med. Fac. Landbouww. Rijksuniv. Gent", **42**, 1977, 1671-1679.
5. Brett, C., Waldron, K., *Control of cell wall extensibility*, în Black, M., Chapman, J. (Eds.), *Physiology and Biochemistry of Plant Cell Walls*, p.101-113, Unwin Hyman, London, 1990.
6. Cacho, M., Morán, M., Tárago, F.J., Corchete, P., *Calcium restriction induces cardenolide accumulation in cell suspension cultures of* Digitalis thapsi *L.*, "Plant Cell Reports", **14**, 1995, 786-789.
7. Darvill, A.G., Albersheim, P., *Phytoalexins and their elicitors. A defense against microbial infection in plants*, "Annu. Rev. Plant Physiol.", **35**, 1984, 243-275.
8. Deliu, C., Bota, C., Munteanu-Deliu, C., Cristea, V., Mocan, S., *Sinteza de cardenolide în culturi celulare de* Digitalis lanata, "Stud. Univ. Babeş-Bolyai, Biol.", **43** (1-2), 1998, 78-84.

9. Deliu, C., Bota, C., Munteanu-Deliu, C., Keul-Butiuc, A., Mocan, S., Cristea, V., Halmagyi, A., *Increased accumulation of digitoxin by some cell lines of* Digitalis lanata *in response to addition of cupric sulfate*, "Contrib. Bot." (Cluj-Napoca), 1999 (în curs de publicare).

10. DiCosmo, F., Misawa, M., *Eliciting secondary metabolism in plant cell cultures*, "Trends Biotechnol.", **3**, 1985, 318-322.

11. DiCosmo, F., Towers, G.H.N., *Stress and secondary metabolism in cultured plant cells*, în Timmermann, T.B., Steelink, C., Loewus, F.A. (Eds.), *Phytochemical Adaptation to Stress*, p. 95-175, Plenum Press, New York, 1984.

12. Gaspar, T., Penel, C., Castillo, F.J., Greppin, H., *A two-step control of basic and acidic peroxidases and its significance for growth and development*, "Physiol. Plant.", **64**, 1985, 418-423.

13. Guerrier, G., Delumeau, O., Renard, M., Laval-Martin, D., *Proline metabolism and NAD kinase activity in soybean calli during short- and long-term exposures to light and NaCl*, "Biol. Plant.", **40**, 1997/1998, 1-9.

14. Jones, A., Veliky, I.A., *Biotransformation of cardenolides by plant cell cultures*, "Planta Med.", **42**, 1981, 160-166.

15. Luckner, M., Diettrich, B., *Formation of cardenolides in cell and organ cultures of* Digitalis lanata, în Newmann, K.H., Barz,W., Reinhard, E. (Eds.), *Primary and Secondary Metabolism of Plant Cell Cultures*, p.154-163, Springer, Berlin, 1985.

16. Luts, S., Guerrier, G., *Peroxidase activities of two rice cultivars differing in salinity tolerance as affected by proline and NaCl*, "Biol. Plant.", **37**, 1995, 577-586.

17. Murashige, T., Skoog, F., *A revised medium for rapid growth and bioassay with tobacco tissue cultures*, "Physiol. Plant.", **15**, 1962, 473-497.

18. Olmos, E., Hellin, E., *Mechanism of salt tolerance in cell line of* Pisum sativum*: biochemical and physiological aspects*, "Plant Sci.", **120**, 1996, 37-45.

19. Palazon, J., Bonfill, M., Cusido, R.M., Pinol, M.T., Morales, C., *Effects of auxin and phenobarbital on morphogenesis and production of digitoxin in* Digitalis *callus*, "Plant Cell Physiol.", **36**, 1995, 247-252.

20. Perez-Alfocea, E., Estan, M.T., Caro, M., Guerrier, G., *Osmotic adjustment in* Lycopersicon esculentum *and* L.pennelli *under NaCl and polyethylene glycol 6000 isoosmotic stresses*, "Physiol. Plant.", **87**, 1993, 493-498.

21. Prakash, S.N., Widholm, J.M., *Comparison of tissue culture and whole plant responses to salinity in potato*, "Plant Cell, Tissue Organ Cult.", **33**, 1993, 273-280.

22. Sancho, M.A., Forchetti, S.M., Pliego, F., Valpuesta, V., Quesada, M.A., *Peroxidase activity and isoenzymes in the culture medium of NaCl adapted tomato suspension cells*, "Plant Cell, Tissue Organ Cult.", **44**, 1996, 161-167.

23. Siegel, B.Z., *Plant peroxidases - an organismic perspective*, "Plant Growth Regul.", **12**, 1993, 303-312.

24. Smirnoff, N., *The role of active oxygen in the response of plants to water deficit and desiccation*, "New Phytol.", **125**, 1993, 27-58.

25. Smirnoff, N., Cumbes, Q.J., *Hydroxyl radical scavenging activity of compatible solutes*, "Phytochemistry", **17**, 1978, 1521-1522.

26. Steward, G.R., Lee, J.A., *The role of proline accumulation in halophytes*, "Planta", **120**, 1974, 279-289.

27. Trivedi, S., Galiba, G., Sankhla, N., Erdei, L., *Responses to osmotic and NaCl stress of wheat varieties differing in drought and salt tolerance in callus cultures*, "Plant Sci.", **73**, 1991, 227-232.

28. Wichers, H.J., Visser, J.F., Huizing, H.J., Pras, N., *Occurrence of L-DOPA and dopamine in plants and cell cultures of* Mucuna pruriens *and effects of 2,4-D and NaCl on these compounds*, "Plant Cell, Tissue Organ Cult.", **33**, 1993, 259-263.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, XLIV, 1-2, 1999

RELATIONS BETWEEN GROWTH, CHLOROPHYLL CONTENT AND NITRATE DISTRIBUTION IN *ZEA MAYS* GROWING UNDER NITROGEN LIMITATION

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SUMMARY.- The utilization and translocation of nitrogen were investigated in exponentially growing, nitrogen-limited *Zea mays* L. plants. The plants were supplied with nitrogen (N) daily at exponentially increasing, suboptimal, relative nitrogen addition rates (RAR). Contents of starch, glucose, fructose and the relative growth rate (RGR) were measured. The distribution of nitrate in the plant organs was also determined. At a growth rate ranging from optimal to 50%, the carbohydrates accumulated. Growth below 50% of optimal N concentration in the medium caused decreased contents of glucose and fructose and increased levels of starch. The chlorophyll content increased in parallel with the RGR. At low levels of nitrogen added in the medium, more than 60% of the dry matter and nitrate accumulated in the roots. As the nitrogen content of the medium was increased, the dry matter and the nitrate were redistributed in the plant. Thus, at 35% nitrogen introduced in the medium, 22.15% and 26.08% of the dry matter and the nitrate, respectively, were found in the roots, while 64.39 and 66.3%, respectively, were concentrated in the leaves.

The quantitatively most important mineral elements required by the plants are nitrogen and phosphorus. Limitation of either of these two elements results in extensive changes in the CO_2 assimilation rate, and especially in the rate of carbohydrate utilisation. The overall consequence is that the carbohydrates accumulate. However, other types of nutrient deficiencies can also lead to the carbohydrate accumulation, as shown for magnesium [17] and potassium [7] deficiencies. The general view is that the processes associated with carbon utilisation (especially growth and respiration) are more sensitive to these types of nutrition deficiencies than is photosynthesis.

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Plant hormones play a dominant role in the regulation of growth and development, including source-sink relationships and plant yield. Environmental factors such as light, temperature, humidity and nutrients can influence the synthesis, degradation and action of hormones and it is likely that the primary responses of plants are due to changes in hormonal balance [9]. Plants subjected to different degrees of nutrient limitation may not only show quantitative differences in growth rate, but they present differences in chemical composition [18] and morphology, *e.g.* in shoot/root ratio [3], as well.

Although hormonal control of carbohydrate partitioning is primarily exerted on sink-related processes, the activity of source processes is also to some extent under hormonal control. Stomatal conductance and photosynthesis are often decreased by application of ABA, but there is considerable interspecific variation in this respect [12]. IAA has been reported to enhance photosynthesis and stomatal opening [1]. Enhanced photosynthesis was, however, not found when Robinson *et al.* [13] studied the effect of IAA on protoplasts. Kinetin and GA have also been reported to enhance photosynthesis, *e.g.* in the experiments conducted by Waring *et al.* [18] on maize leaves.

The intention of this work was to investigate the process of acquisition and utilisation of nitrate in plants adapted to conditions of controlled, growth-limiting nitrogen supply. Special attention was paid to: 1. adaptation of growth to growth-limiting nitrate availability, 2. distribution of nitrate in the plant organs according to nitrogen limitation and 3. the effect of nitrogen limitation on accumulation of carbohydrates and on chlorophyll content.

Materials and methods. Maize plants were cultivated in a sterile K medium [8] in Erlenmeyer flasks, under conditions of 12 hours of light, 21°C and 70% humidity. The plants were grown in open glass vessels. Contamination by algae or bacteria was negligible. Growth rate was determined as fresh weight increase. The relative growth rate, RGR, was expressed as percentage fresh weight increase /day, calculated from the formula:

$$RGR = 100(\ln W_2 - \ln W_1)/(t_2 - t_1)$$

where W_2 and W_1 are the fresh weights at start (t_1) and harvest (t_2), respectively.

Two different treatments were used to induce N deficiency. In one experiment, multiple series of exponentially growing cultures were maintained at different suboptimal rates determined by the daily dose of N. In the other experiment, cultures with sufficient N were transferred to N-free media in a study over time.

1. Effects of suboptimal N supply. Multiple series of exponentially growing cultures were maintained at different suboptimal rates determined by the daily dose of N. Thereafter, growth-limiting amounts of N, together with ample amounts of all other essential nutrients, were added daily at rates that were increased exponentially with time. The relative rate of N addition (RAR) was adjusted in a series of cultures to 5, 15, 25 and 35% /day according to the formula:

$$RAR = 100(\ln N_2 - \ln N_1)/(t_2 - t_1)$$

where $\ln N_2 - \ln N_1$ represents the increase in N bound in new biomass during the period $t_2 - t_1$. The cultures were harvested once a week. Part of the harvest was analysed, and the rest was used to start a new set of suboptimals. Before having been used in the experiment, the cultures were allowed to adapt several weeks to their specific RAR and corresponding RGR.

2. Effects of N starvation over time. Plants were precultured for 1 week in a N-sufficient medium, thereafter they were transferred to N-free but otherwise complete medium [6]. The medium was completely renewed every 24 hours and the RGR determined. Aliquots of 1.5 g fresh weight were harvested for determination of carbohydrate and chlorophyll contents.

For determination of starch, the plant material, immediately after harvesting, was dried at 60°C for 24 hours. The starch content was determined by quantification of glucose before and after cleavage of the polysaccharide to glucose by amyloglucosidase. The principle of the glucose assay is that, in the presence of hexokinase, glucose is converted to glucose 6-phosphate, which reduces $NADP^+$ to NADPH. The amount of NADPH formed is stoichiometric with the amount of glucose and is determined spectrophotometrically at 340 nm. Each sample (25-50 mg) was dissolved in a mixture of 5 ml dimethylsulphoxide and 1 ml 8M HCl and shaken in a 60°C water bath for 40 min. The solutions were then cooled to room temperature and their pH was adjusted to 5 with 5M NaOH and their volumes were completed to 25 ml. The extracts were filtered and 0.2 ml of the extract was used for the assay, which was carried out according to [2].

For determination of glucose and fructose, the dry and ground samples were dissolved in 20 ml distilled water in 50-ml Erlenmeyer flasks and shaken in a water bath at 60°C for 2 hours. The samples were then cooled to room temperature and their volumes completed to 25 ml. After filtration, 0.5 ml of the extracts were used for the quantitative analysis of glucose and fructose according to [2]. Glucose was determined as described for the starch assay. Fructose was phosphorylated by hexokinase and the concentration of glucose 6-phosphate was determined before and after conversion of fructose 6-phosphate to glucose 6-phosphate by phosphoglucose isomerase.

The chlorophyll content, from an alcoholic extract, was determined photocolorimetrically at 660 nm.

The distribution of dry matter and nitrogen in the plant organs was monitored for plants supplied with insufficient nitrogen.

The nitrate content in aqueous extracts was determined photocolorimetrically with phenol-disulphonic acid.

Results and discussion. As can be seen in Table 1, all measured carbohydrates showed a significant negative correlation with the RGR ($r = -0.893$, $P = 0.01$). The chlorophyll content increased in parallel with the RGR (Table 2). When optimally growing plants were transferred to a N-free but otherwise complete medium, starch, glucose and fructose began to accumulate after 24 hours. At high levels of starch accumulated in the cell, the chlorophyll content decreased. The relationships between RGR values and levels of glucose, fructose and starch were similar in the two sets of experiments, as were the relationships between chlorophyll content and starch levels.

Table 1

Starch, glucose and fructose contents in dry weight (DW) of maize plants grown at different relative addition rates (RAR) of N in the culture medium

RAR (% / day)	RGR (% / day)	Starch (mg/g DW)	Glucose (mg/g DW)	Fructose (mg/g DW)
5	4.4	140	12	14.2
15	16.8	125	10.3	15.9
25	22.0	89	8.5	13.5
35	32.5	58	6.5	11.2

Table 2

Chlorophyll content and dry weight (DW) percentage in fresh weight (FW) of maize plants grown at different relative addition rates (RAR) of N in the culture medium

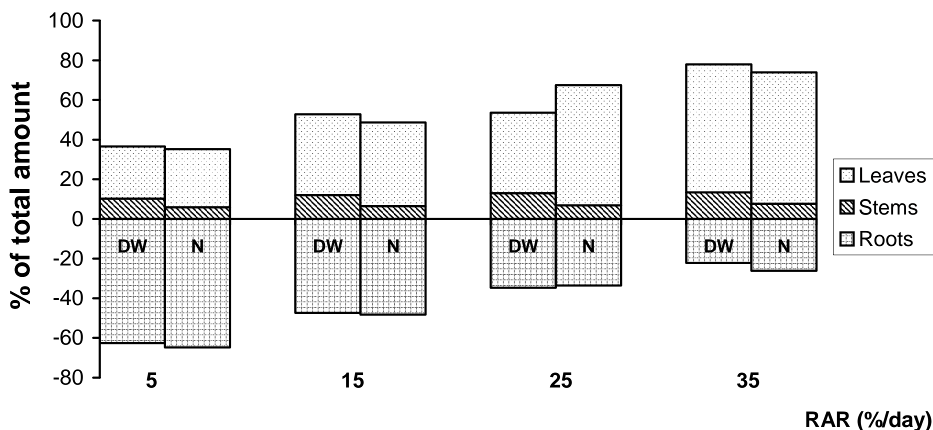
RAR (%/day)	RGR (% / day)	Chlorophyll content		DW (% of FW)
		mg/g FW	mg/g DW	
5	4.4	0.28	3.0	9.0
15	16.8	0.43	4.8	8.3
25	22.0	0.48	7.8	7.6
35	32.5	0.58	9.9	5.8

The relative growth decrease in response to N deficiency inducing starch accumulation in the chloroplast is in accordance with earlier reports of Thorsteinsson and Tillberg [16]. The results obtained from the two different approaches also matched each other consistently.

The increases in glucose and fructose levels are due to sucrose hydrolysis, since they accumulated in a synchronised manner (although in different amounts) [19].

The changes in the levels of glucose, fructose and starch in the differently N-stressed plants indicate that these carbohydrate pools may be involved in the synchronisation of the photosynthesis and the carbon utilisation, indirectly measured as RGR.

Fig.1 shows the distribution of dry matter and nitrate contents in organs of maize plants supplied with insufficinet nitrogen; at low levels of nitrogen added in the medium, more than 60 % of the dry matter and nitrate accumulated in the roots. As the nitrogen content of the medium was increased, dry matter and nitrate are redistributed in the plant. Thus, at 35% nitrogen introduced in the medium, 22.15 % and 26.03 % of the dry matter and the nitrate, respectively, were found in the roots, while 64.39% and 66,3%, respectively, were concentrated in the leaves.



F i g. 1. *Distribution of dry matter (DW) and nitrate (N) contents (% of total amount) in organs of maize plants grown at different relative addition rates (RAR) of N in the culture medium.*

The present knowledge regarding the regulation of sucrose synthesis pathway and the regulation of starch synthesis [7, 11] impels the following hypothetical sequence of events in the first 70 hours of starvation. The decreased assimilate utilisation in the cytosol (lowered RGR) causes accumulation of glucose

and fructose. When the assimilate demands decrease, it has been reported that the activity of the sucrose phosphate synthase (SPS) decreases [7]. The mechanism for this response is still obscure. The decrease in SPS activity also leads to increased levels of the sucrose phosphate precursors and triose phosphate (TP) as described by Stitt and Heldt [15]. Alternatively, a similar effect could be obtained by an increase in hexose-phosphate pools via phosphorylation of glucose and fructose by nucleotide triphosphate-dependent kinases [5]. Increased levels of fructose 6-phosphate contribute indirectly to inhibition of fructose-1,6-biphosphatase (FBPase) activity and to elevation of the TP content. Since TP can move freely across the chloroplast membrane in exchange for phosphorus (P_i), increased cytosolic TP levels induce a corresponding elevation in the chloroplastic TP levels. The increased chloroplastic TP/P_i ratio that follows stimulates the activity of ADP glucose pyrophosphorylase, thereby inducing starch synthesis [11].

The decrease in the glucose and fructose levels after the starvation period is possibly due to a decrease in the pathway of sucrose synthesis either at the sucrose phosphate synthase(SPS) or the FBPase-controlled reactions. Alternatively, the decreased glucose and fructose levels could be due to an extensive accumulation of TP or other sugar phosphates.

In both experimental models used, the plants showed a strong negative correlation between the chlorophyll content and starch level. The physiological significance of such negative correlation has been debated in the literature. Some authors have found negative correlation [10, 16], while others have not [4]. Inconsistencies reported in the literature in the negative correlation between CO_2 assimilation rate and leaf starch content might be due to interspecific differences in TP threshold levels for the activation of the starch synthesis pathway in different species and tissue maturation stages. For example, leaves with high threshold levels (and poor starch accumulation) might show a better negative correlation between CO_2 assimilation rate and soluble sugar levels rather than starch, as has been shown in the wheat [7]. Sawada *et al.* [14] suggested, for soybean leaves, an alternative explanation: the photosynthetic apparatus of the leaf might differ in sensitivity to starch concentration depending on the species and on the maturation stages.

Conclusions. 1. N deficiency, developed in both of the two culturing methods employed, caused a decrease in assimilate utilisation with accompanying elevations in glucose, fructose and starch contents. A severe deficiency can even inhibit the export of sugars from the chloroplast and/or decrease the activity of the pathway of sucrose synthesis. The accumulation of starch is accompanied by a corresponding decrease in the chlorophyll content. The results support the hypothesis that the photosynthesis can be regulated by end product inhibition in response to a N deficiency.

2. The nitrate distribution in the plant organs is directly related to the nitrogen content of the culture medium. At low levels of nitrogen, the nitrate accumulates in the roots, while at higher suboptimal levels the nitrate concentrates in the leaves.

REFERENCES

1. B i d w e l l, R. G. S., L e w i n, W. B., T a m a s, I.A., *The effects of auxin on photosynthesis and respiration*, in W i g h t m a n n, F., S e t t e r f i e l d, G. (Eds.), *Biochemistry and Physiology of Plant Growth Substances,* pp. 361-376, Acad. Press, London, 1986.
2. B M G m b H B i o c h e m i c a, *Methods of Enzymatic Food Analysis. Instructions for Analysis in* Research, *Routine Analysis and Official Control,* pp.51-52, Mannheim, 1984.
3. C l a r k s o n, D. T., H a n s o n, J. B., *The mineral nutrition of higher plants*, "Annu. Rev. Plant Physiol.", **31**, 1980, 239-298.
4. C l u a s s e n,W., B i l l e r, E., *The significance of sucrose and starch contents of the leaves for regulation of net photosynthetic rates*, "Z.Pflanzenphysiol.", **81**, 1977, 189-198.
5. D e n n i s, D.T., G r e y s o n, M. F., *Fructose 6-phosphate metabolism in plants,* "Physiol. Plant.", **69,** 1987, 395-404.
6. E r i c s s o n, T., L a r s s o n, C., T i l l b e r g, E., *Growth responses of* Lemna *to different levels of nitrogen limitation,* "Z. Pflanzenphysiol.", **105,** 1982, 331-340.
7. H u b e r, S. C., *Inter- and intra-specific variation in photosynthetic formation of starch and sucrose,* "Z. Pflanzenphysiol.", **101,** 1981, 49-54.
8. M a e n g, J., K h u d a i r i, A., *Studies on the flowering mechanism in* Lemna*. Amino acid changes during flower induction,* "Physiol. Plant.", **28**, 1973, 264-270
9. M a r s c h n e r, N., *Mineral Nutrition of Higher Plants,* pp.115-154, Acad. Press, London, 1986.
10. M u a n e y J. R., G u i n n, K. E., F r y, K. E., H e s k e t h, J. D., *Correlation of photosynthetic carbon dioxide uptake and carbohydrate accumulation in cotton, soybean, sunflower and sorghum,* "Photosynthetica", **13,**1979, 260-266.
11. P r e i s s, J., *Starch, sucrose biosynthesis and partitioning of carbon in plants are regulated by orthophosphate and triose-phosphates,* "Trends Biochem. Sci.", **9,** 1984, 24-27.
12. R a s c h k e, K., H e d r i c h, R., *Simultaneous and independent effects of abscisic acid on stomata and photosynthetic apparatus in whole leaves,* "Planta", **163,** 1985, 105-118.

13. R o b i n s o n, S. P., W i s k i c h, J.T., P a l e g, L.G., *Effects of indole acetic acid on CO_2 fixation, electron transport and phosphorylation in isolated chloroplasts,* "Aust. J. Plant Physiol.", **5,** 1978, 425-431.
14. S a w a d a, S., H a y a k a v a, T., F u k u s h i, K., K a s a i, M., *Influence of carbohydrates on photosynthesis in single rooted soybean leaves used as source-sink model,* "Plant Cell Physiol.", **27,** 1986, 591-600.
15. S t i t t, M., H e l d t, H. W., *Control of photosynthetic sucrose synthesis by fructose 2,6-biphosphate,* "Plant Physiol.", **79,** 1985, 599-608.
16. T h o r s t e i n s s o n, B., T i l l b e r g, J., *Carbohydrate partitioning and growth in* Lemna gibba *L. The effects on nitrogen limitation*, "Physiol. Plant.", **71**, 1989, 264-270.
17. V e s k, M., P o s s i n g h a m, V., M e r c e r, F.V., *The effect of mineral nutrent deficiency on the structure of the leaf cells of tomato, spinach and maize,* "Aust.J. Bot.", **14,** 1986, 1-8.
18. W a r i n g, R. H., M c D o n a l d, A. J. S., L a r s s o n, S., E r i c s s o n, T., W i r e n, A., A r w i n d s s o n, E., E r i c s s o n, A., L o h a m m a r, T., *Differences in chemical composition of plants grown at constant relative growth rates with stable mineral nutrition,* "Oecologia", **66,** 1985, 157-160.
19. W h i t t i n g h a m, C. P., K e y s, A. J., B i r d, I. F., *The enzymology of sucrose synthesis in leaves,* in G i b b s, M., L a t z k o, E. (Eds.), *Encyclopedia of Plant Physiology,* Vol. 6, pp.313-326, Springer, Berlin,1979.

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HISTOLOGICAL AND ULTRASTRUCTURAL MODIFICATIONS INDUCED BY TWO ALKYLATING AGENTS ON THE WISTAR RAT KIDNEY

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SUMMARY. – The alkylating agents are widely used in the therapy of many types of cancer. Carboplatin and Cyclophosphamide belong to that family of drugs, the anticancer activity of which is due to their capacity of interacting with DNA, RNA, altering their structural and functional integrity and blocking the DNA, RNA and protein synthesis.

Our investigations tried to evaluate comparatively the histological and ultrastructural modifications induced by similar doses of Carboplatin and Cyclophosphamide on the Wistar rat kidney.

The microscopic examination demonstrated the appearance of certain histological alterations, the intensity, gravity and extension of which were different, depending on the type of cytostatic and the moment of the sacrification.

Carboplatin induced an oedema of the Bowman's space correlated with the appearance of a polymorphous granular material inside it; the uriniferous tubules appeared dilatated, with necrosis processes of the epithelial cells, and full of a hyaline material.

Cyclophosphamide had a less severe nephrotoxicity, consisting of the appearance of a glomerular stasis, mesangial oedema, zonal tubulonephrosis correlated with the presence of a hyaline material inside the tubules.

By electron mycroscopy, it could be noticed that both cytostatics induced grave nuclear alteration (the nucleus had a peculiar shape and an obvious pycnosis tendency), the appearance of an apical cytoplasm completely vacuolised, and wide areas of intracellular lysis. The mitochondria were affected, too, they being swollen and having a rarefied matrix. Some uriniferous tubules appeared partially or completely affected by a necrosis process. In some cells, the brush border was affected, the microvilli being swollen and destroyed here and there, and the basal infoldings appeared hypertrophied. All these modifications were graver in the group treated with Carboplatin.

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Alkylating agents are drugs widely used nowadays in the anticancer therapy. They have the capacity of interacting with DNA and RNA, altering their structural and functional integrity. The antineoplasic activity of these cytostatics is based on the alkylating reaction with the NH_2 groups in the structure of the nucleotide residues of the nucleic acids. As a consequence of this kind of chemical reactions, the drugs bind to DNA and RNA, finally inducing their fragmentation [3, 11]. The alkylating reactions block both the reading of the genetic message and the replication of DNA [8].

Carboplatin and Cyclophosphamide belong to this family of anticancer drugs. They are successfully used in the chemotherapy of many types of malignant diseases, and, owing to their immunosuppressive activity, in transplantations of tissues and organs. Although the chemotherapy with these two cytostatics tried only to obtain a remission of the malignant tumours in different organs, their action not being selective, efficient doses of these cytostatics induce many and very grave complications in most of the tissues and organs. The most affected are the germinal and hematopoietic tissues, the digestive mucosa and the organs involved in their metabolisation and eliminations. According to previous studies, the kidney is the principal organ which eliminates the two cytostatics and their metabolites. Therefore, our studies tried to establish the histological and ultrastructural modifications induced by equivalent doses of Carboplatin and Cyclophosphamide, administered in a single therapeutic dose on the Wistar rat kidney.

Material and methods. Our experiments were carried out with the following nine groups of healthy adult male Wistar rats, weighing 190 $\pm$ 10 g and maintained under bioclimatic laboratory conditions, with no food for 18 hours before the treatment, but having water *ad libitum*:

-group U – untreated (control) group;

-group C_1, C_2, C_3 and C_4 treated i.v. with 400 mg Carboplatin /m^2 body surface and sacrificed 24 hours, 4, 11 and 18 days after the treatment;

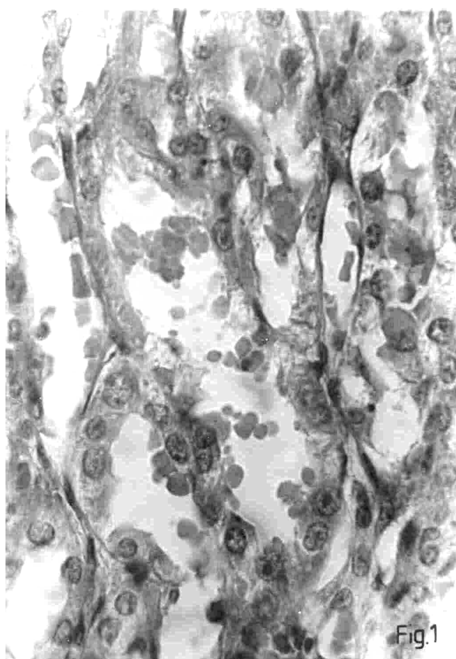
-group P_1, P_2, P_3 and P_4 treated i.v. with 40 mg Cyclophosphamide /kg body weight and sacrificed 24 hours, 4, 11 and 18 days after the treatment.

The animals were not fed for 18 hours before the sacrification. Having sacrificed the animals, we took fragments from the kidney. For microscopic examination the fragments were fixed in 10% neutral formol, processed by the paraffin technique and the sections of 6 μm were stained by the hematoxylin-eosin, Masson-Goldner trichrome and PAS methods [10]. For ultrastructural investigations fragments of kidney were prefixed in 2.7% glutaraldehyde solution and postfixed in 2% osmic acid solution. The fragments were dehydrated in acetone and then embedded in Vestopal W. The ultrathin sections were obtained using an LKB III ultramicrotome and were contrasted with uranyl acetate. Examination of the sections were performed in a TESLA-BS-500 electron microscope [1, 7, 12].

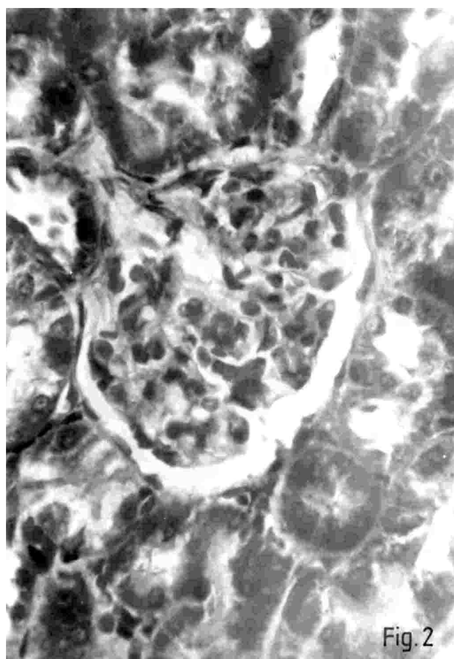
On the stained and contrasted sections we studied by microscopic and electronomicroscopic examination the histological and ultrastructural modifications induced by the two drugs in concordance with the moment of the sacrification.

Results and discussion. The microscopic and electronomicroscopic examination of the kidney sections obtained from the rats treated with the two alkylating drugs showed the appearance of certain histological and ultrastructural modifications, the intensity, gravity and extension of which were different, depending on the type of cytostatic and the moment of the sacrification.

Thus, at the level of groups treated with Carboplatin some discrete changes already appeared 24 hours after the treatment, both in the cortex and medulla of the kidney, but they had a zonal character, the affected areas being limited. In some renal corpuscles a PAS-negative polymorphous granular material appeared, demonstrating a slight alteration of the ultrafiltration of the plasma with the appearance of the proteins in urine. In some renal tubules, especially in the proximal convoluted tubules in the cortex, incipient necrosis processes of the tubular cells appeared and, in addition, a PAS-negative hyaline granular material was also evident (Fig. 1).

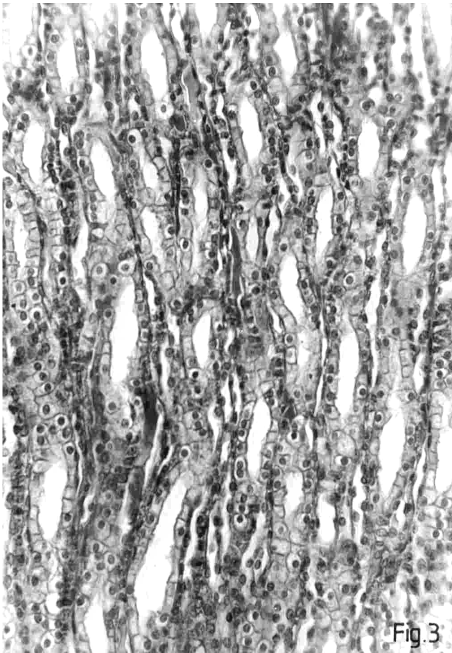


F i g. 1. *Granular material inside the proximal convoluted tubules* (× 480).

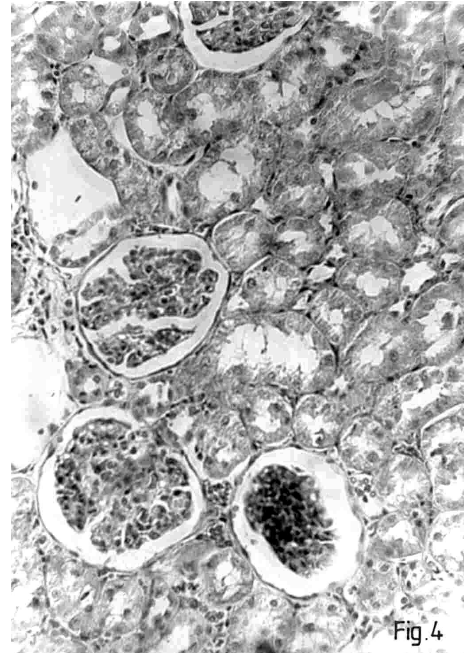


F i g. 2. *Glomerular stasis correlated with diffuse intravascular coagulation phenomena* (× 480).

In group C_2, sacrificed 4 days after the treatment, it could be noticed that the granular material in the Bowman's space had increased slightly, the amount of this material not being very significant. Besides, an obvious glomerular and interstitial stasis correlated with diffuse intravascular coagulation phenomena appeared (Fig. 2). Such phenomena of diffuse intravascular coagulation appeared in the capillaries in the medulla, too (Fig. 3). Big PAS-positive hyaline thrombi could be seen, especially in the tubules between the cortex and medulla.



F i g. 3. *Phenomena of diffuse intravascular coagulation in capillaries in the medulla* (× 180).

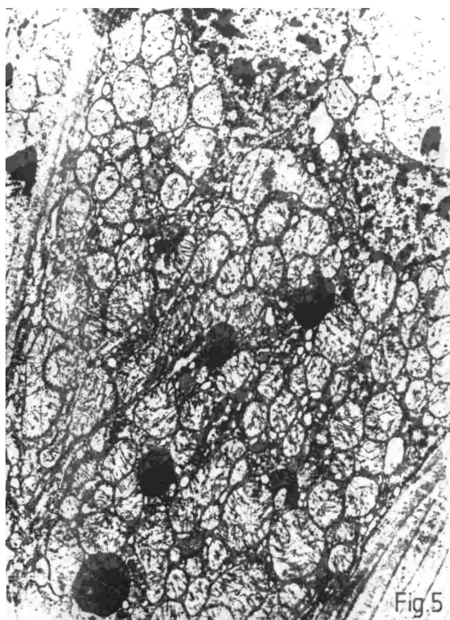


F i g. 4. *Two lobated renal corpuscles and one atrophied corpuscle in the cortex* (× 180).

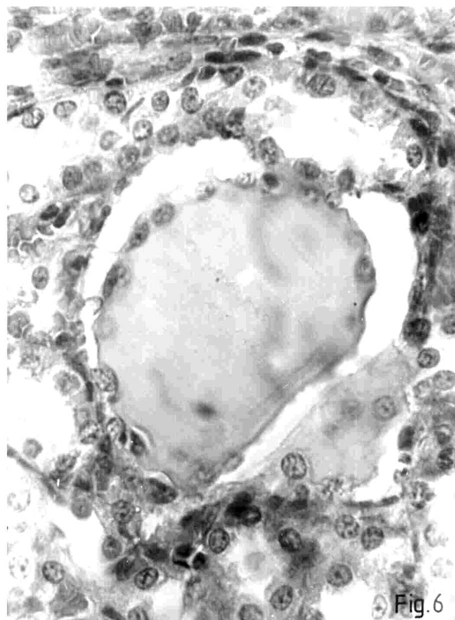
After 11 days, the intravascular coagulation phenomena had disappeared, but it could be noticed a mesangial oedema and, in some areas, a mesangial hypercellularity correlated with some necrobiosis processes at the level of endothelial and mesangial cells. Most of the renal corpuscles appeared lobated and a few of them were atrophied (Fig. 4).

The areas occupied by affected renal tubules were not significantly larger than in the group sacrificed after 4 days from the chemotherapy, but some tubules were a little dilatated, as a consequence of the urinary stasis, and the epithelium of these tubules was more affected.

The electron microscope investigations of the kidney in this group demonstrated the presence of some ultrastructural modifications which consisted in the appearance of some nuclei with an irregular contour and the chromatin condensed to the periphery. The cytoplasm contained many vesicles and large areas of intracellular lysis disposed especially in the upper cytoplasm. Many mitochondria had suffered a process of swelling, their matrix becoming less dense, rarefied (Fig. 5). Besides, the number of lysosomes decreased.



F i g. 5. *Nuclei with peculiar shape and mitochondria with rarefied matrix in the tubular cells* (× 8820).

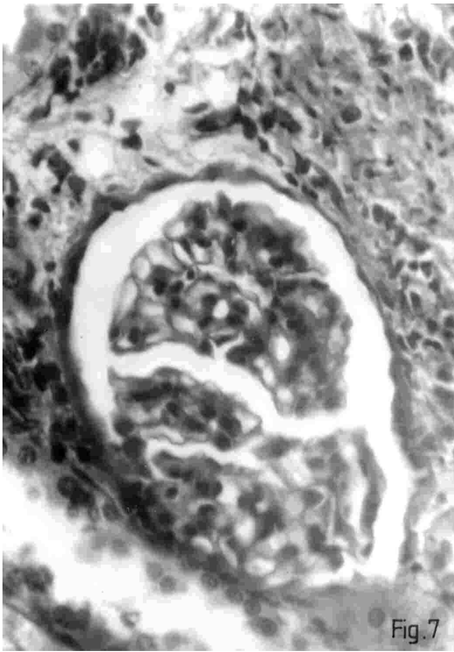


F i g. 6. *Big hyaline thrombus which includes the tubular epithelium* (× 480).

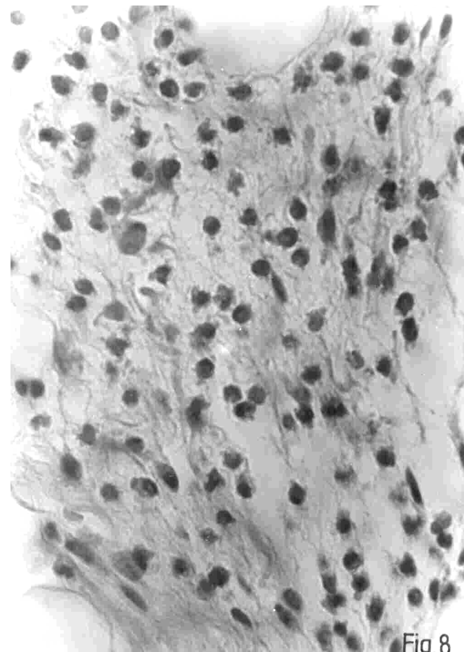
After 18 days from the chemotherapy (group C_4) it could be noticed a sclerosis of many glomerules correlated with an alteration of their contour, with the appearance of some interstitial lymphohistiocyte infiltrations, with sclerosis processes at the level of arterioles and glomerules and interstitial nephritis. The cylindric thrombi inside the renal tubules appeared hyaline, some of them including the tubular epithelium as a consequence of the necrosis processes of the cells (Fig. 6). The PAS reaction showed the presence of a PAS-positive material inside the mesangium, the glomerules appearing retracted and lobated. The Bowman's capsule was irregulate, thickened with PAS-positive material, and in many renal corpuscles it had a "double outline" aspect.

Concerning the groups treated with Cyclophosphamide, the first histological modifications appeared 24 hours from the treatment.Thus, at the level of renal corpuscles it could be seen a discrete stasis correlated with a mesangial oedema, the glomerules having a swollen aspect. In some proximal convoluted tubules proteins appeared in urine as a consequence of the alteration of the ultrafiltration. Such phenomena of proteic tubulonephrosis could be noticed only in the cortex of the kidney and had a zonal character.

In group P_2, sacrificed 4 days after the treatment, the mesangial oedema, glomerular stasis and tubulonephrosis processes persisted and had a zonal character (Fig. 7).



F i g. 7. *Mesangial oedema which induced a lobated aspect of the renal corpuscles* (× 480).

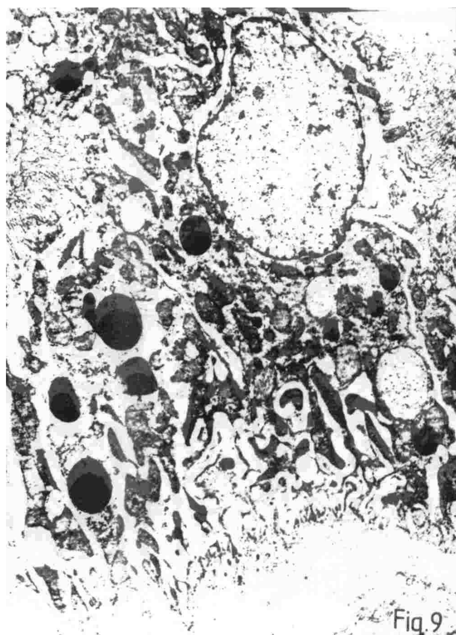


F i g. 8. *Interstitial lymphohistiocyte infiltration* (× 480).

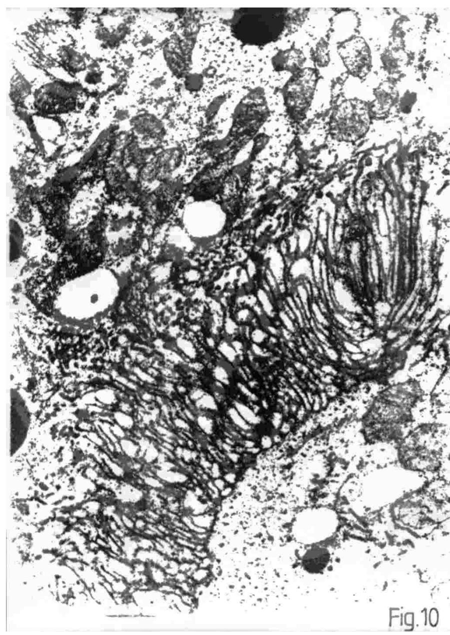
After 11 days, all the modifications had a decreased intensity, and here and there some phenomena of lymphohistiocyte interstitial nephritis appeared (Fig. 8).

The electron microscope examination showed the appearance of some nuclear modifications concerning the shape (pycnosis tendency). In some cells the nucleus had a peculiar aspect, this phenomenon being less pronounced than in groups treated with Carboplatin. The mitochondria were swollen, having a

rarefied matrix. The basal infoldings of some cells appeared significantly swollen (Fig. 9), this aspect being correlated with the swelling of the microvilli (Fig. 10). A few cells in the proximal convoluted tubules were vacuolised in their upper cytoplasm and had their brush border partially or completely destroyed, the cellular components being discharged into the lumen of the renal tubule, where it contributed to the formation of the thrombi.



F i g. 9. *Basal infoldings of some tubular cells appear significantly swollen* (× 8820).



F i g. 10. *Swollen microvilli of the tubular cells in the proximal convoluted tubules* (× 17640).

In group P_4, sacrificed after18 days from the treatment, the histological aspect was comparable with that which appeared in the control group.

These histological and ultrastructural modifications presented demonstrate and confirm the nephrotoxicity of these two alkylating agents. The gravity, extension and dynamics of the lesions were different. Thus, the modifications induced by Cyclophosphamide were less grave, affecting small areas, and they did not aggravate significantly during the 18 days of the experiment, on the contrary, they decreased. These results are in concordance with previous studies concerning the nephrotoxicity of Cyclophosphamide, according to which this cytostatic has a glomerular and tubular toxicity and

induces significant structural and functional modifications only when it is administered in high dose or a very long period of time [2, 13, 15].

In the groups treated with Carboplatin, the lesions were much more serious, affecting wide areas and they progressively and significantly aggravated during the experiment. According to the previous investigations, the nephrotoxicity of this anticancer drug is minimal when it is administered in low doses, while high doses induce a glomerular and tubular toxicity which could affect the function of the kidney [2, 4-6, 9, 14].

Our results demonstrate that the two cytostatics have a nephrotoxic effect, they affecting both the structures involved in the ultrafiltration process (arterioles, capillaries, mesangium, Bowman's capsule) and the epithelium of the uriniferous tubules. But, Cyclophosphamide has a less severe nephrotoxicity than Carboplatin.

Conclusions 1. Both Carboplatin and Cyclophosphamide have a nephrotoxic effect which is more increased in the case of Carboplatin.

2. From the point of view of nephrotoxicity Cyclophosphamide is better tolerated than Carboplatin.

3. The nephrotoxic effects consist in the alteration both of the structures involved in the ultrafiltration process and the epithelium of the uriniferous tubules.

4. Cyclophosphamide has a moderate nephrotoxicity, the histological and ultrastructural modifications not being very grave during the experiment.

5. Carboplatin is more nephrotoxic, the lesions induced by it being grave enough, affecting significantly the structure and function of the kidney.

REFERENCES

1. B r e n d a, S. W., *A Beginner's Handbook in Biological Transmission Electron Microscopy,* Butler & Tanner, London ,1981.
2. B r o c k, N., P o h l, J., S t e k a r, J., *Detoxification of urotoxic oxazaphosphorines by sulfhydryl compounds,* "J.Cancer Res. Clin. Oncol.", **10**, 1981, 311-320.
3. C a l v e r t, A. H., *Dose optimisation of carboplatin in adults,* "Anticancer Res.", **14**, 1994, 2273-2278.
4. D a i c o v i c i u, D., C h i r i c u ţ ă, I., *Complicaţiile provocate de tratamentul citostatic,* in *Cancerul Chimioterapie*, Vol.3, pp. 53-56, Inst. Oncol., Cluj-Napoca, 1983.

5. E t t i n g e r, L. J., K r a i l o, M. D., G a y n o n, P. S., H a m m o n d, G. D., *A phase I study of carboplatin in children with acute leukemia in bone marrow relapse. A report from the children's cancer group,* "Cancer", **72**, 1993, 917-922.
6. F r e n k e l, J., K o o l, G., K r a k e r, J., *Acute renal failure in high dose carboplatin chemotherapy,* "Med. Pediatr. Oncol.", **25**, 1995, 473-477.
7. G r i g g, A., S z e r, J., S k o v, K., B a r n e t t, M., *Multi-organ dysfunction associated with high-dose carboplatin therapy prior to autologous transplantation,* "Bone Marrow Transplantation", **17**, 1996, 67-74.
8. K a y, D., *Techniques for Electron Microscopy*, Alden & Mowbray , Oxford, 1967.
9. K n o x, R. J., F r i e d l o s, F., L y d a l l, D. A., R o b e r t s, J. J., *Mechanism of cytotoxicity of anticancer platinum drugs: evidence that cis-diamminedichloroplatinum (II) and cis-diammine- (1, 1-cyclobutanedicarboxylato)platinum (II) differ only in the kinetics of their interaction with DNA,* "Cancer Res.", **46**, 1979, 1972-1979.
10. M i l l w a r d, M. J., W e b s t e r, L. K., T o n e r, G. C., B i s h o p, J. F., Rischin, D., S t o k e s, K. H., J o h n s t o n, V. K., H i c k s, R., *Carboplatin dosing based on measurement of renal function - experience at the Peter MacCallum Cancer Institute,* "Cancer Res.", **26**, 1996, 372-379.
11. M u r e ş a n, E., G a b o r e a n u, M., B o g d a n, A. T., B a b a , A. I., *Tehnici de histologie normală şi patologică*, Ed. Ceres, Bucureşti, 1974.
12. P ă u n, R., U r s e a, U., L u c a, N., C o c u l e s c u, M., L u c a, R., *Agenţii alchilanţi,* in *Terapia imunosupresivă*, pp. 107-116, Ed. Med., Bucureşti, 1972.
13. P l o a i e, P. G., P e t r e, Z., *Introducere în microscopia electronică cu aplicaţii la biologia celulară şi moleculară*, Ed. Acad. R.S.R , Bucuresti, 1979.
14. S k r e t k o w i c z, J., S e k u l s k a, M., D a n i e l w i c z, M., W a g r o w s k a - D a n i e l w i c z, M., P o l a k o w s k i, P., *Effect of some anticancer drugs and combined chemotherapy on renal toxicity,* "Cancer", **5**, 1996, 51-58.
15. S l a v u t s k y, I., C a m p o s, E., G o n z a l e s C i d, M., L a r r i p a, I., *Micronuclei induction by carboplatin in human lymphocyte subpopulation,* "Anticancer Drugs", **6**, 1995, 758-762.
16. T a l a r, W. C., H i j a z i, Y. M., M c C l e l l a n, H. W., L i n e h a m, W. M. C., H a l l a n a n, W., L u b e n s k y, I., K e r r, G. S., H o f f m a n, G. S., F a u c i, A.S., S n e l l e r, M. C., *Cyclophosphamide-induced cystitis and bladder cancer in patients with Wegener granulomatosis,* "J. Clin. Oncol.", **124**, 1996, 477-484.

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ATTENUATION OF THE FLUOCINOLONE-ACETONID-N INDUCED HISTOLOGICAL ALTERATION OF HYPOTHALAMIC-PITUITARY-ADRENAL AXIS BY PROPRANOLOL IN WHITE WISTAR RATS

ERIKA KIS[*], CONSTANTIN PUICĂ[**], CRISTINA PAŞCA[*], VICTORIA DOINA SANDU[*] and IOSIF MADAR[**]

SUMMARY. - The hypothalamic-pituitary-adrenocortical (HPA) system and sympathoneural and adrenomedullary systems are major effector systems that serve to maintain homeostasis during stress. Corticotropin-releasing hormone (CRH) in the paraventricular nucleus (PVN) of the hypothalamus, a determinant of both HPA and autonomic responses to stress, is under the control of many neurotransmitters and neuropeptides. Norepinephrine (NE) and epinephrine (E) potently stimulates CRH neurons in the PVN. Acetylcholine, serotonin, interleukines, tumour necrosis factor-α and prostaglandins all have been shown to stimulate and γ-aminobutyric acid and substance P to inhibit either CRH or ACTH secretion. The hypothalamus is generally believed to be site of the negative feed-back mechanism by which glucocorticoids counterregulate neuroendocrine responses to stressors; NE and E are thought to be the potent endogenous stimulators of CRH in the paraventricular nucleus of the hypothalamus. The presence of noradrenergic and adrenergic synapses on CRH cells in the PVN suggests close anatomical and functional interactions between central adrenergic and noradrenergic activity and HPA function. In the present study in mature male Wistar rats histological alteration of HPA axis manifested by an intensification of the neurosecretory activity in PVN, an increase of cellular basophilia at the level of adenohypophysis, and an enhancement of adrenal secretory activity were produced by Fluocinolone-acetonid-N treatment. Fluocinolone-acetonid-N is a topical dermocorticosteroid widely used in the treatment of some skin diseases (dermatoses). Clinical and experimental data clearly demonstrate that, due to percutaneous absorption capacity of epicutaneously applied topical glucocorticosteroids, there appear different systemic adverse side-effects besides their beneficial actions. When daily dermocorticosteroid treatment for three days was associated with Propranolol administration (50 μg s.c./100 g b.w/day) in rats, the above-mentioned parameters were significantly attenuated as compared to the corresponding dermocorticosteroid-treated controls. It is concluded that β-adrenoreceptor stimulation significantly participates in stress-induced alterations of the HPA axis.

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Stress-induced activation of the hypothalamic-pituitary-adrenocortical axis is attended by release of corticotropin-releasing hormone (CRH) from paraventricular nucleus of the hypothalamus, followed by adrenocorticotropin (ACTH) and glucocorticoid release. Among many possible neurotransmitters released in the PVN during exposure to various stressors, norepinephrine (NE) [11] and epinephrine (E) [2, 9] are thought to be potent stimulators of CRH neurons in the PVN. NE and E in the PVN are derived from medullary noradrenergic and adrenergic cells and, to a minor extent, from locus ceruleus. Noradrenergic and adrenergic terminals in the PVN synapse on CRH neurons, administration of NE and E into PVN substantially increase expression of mRNA for CRH in the PVN and for proopiomelanocortin (POMC) in the anterior part of pituitary gland and plasma ACTH in dose-dependent manner [4].

It is well established that β-adrenoreceptors are activated to the same degree by epinephrine and norepinephrine, and Propranolol is a specific β -adrenoreceptor blocking agent without sympathomimetic activity [2,7,8].

In order to test the possible role of β-adrenoreceptor-mediated effects of Fluocinolone-acetonid-N upon hypothalamic neurosecretory, pituitary and adrenal secretor activity, in the present study the dynamics of these parameters was compared in dermocorticosteroid-treated (stressed) group and Propranolol-treated stressed groups.

Materials and method. The experiments were carried out on mature (60-day-old) male Wistar rats. The animals were kept under standardized bioclimatic conditions and fed on a common rat chow, with water *ad libitum.*

Commercial Fluocinolone-N ointment ("Antibiotice" S.A., Iaşi), containing 25 mg Fluocinolone-acetonid-N/100 g excipient, was applied topically to the skin at 2 cm^2, for three consecutive days, by smearing 50 mg ointment/100 g b.w. on the inguinal region, the daily dose of Fluocinolone-acetonid-N being equal to 12.5 μg/100 g b.w.

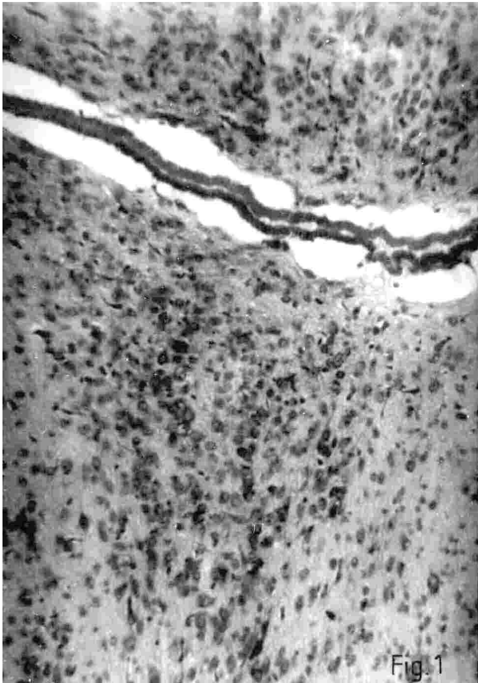
Propranolol pharmaceutical solution ("Sicomed" S.A. Bucharest) was utilized by injecting s.c. daily doses of 50 μg from this β-adrenoreceptor blocking agent/100 g b.w./3 days, simultaneously with applying daily epicutaneous treatment with Fluocinolone-N ointment.

After 16 hours of fasting and 24 hours following the cessation of treatments, the animals (Fluocinolone-treated group, Fluocinolone+Propranolol-treated group), together with controls (Untreated group, Propranolol-treated group) were sacrificed by exsanguination.

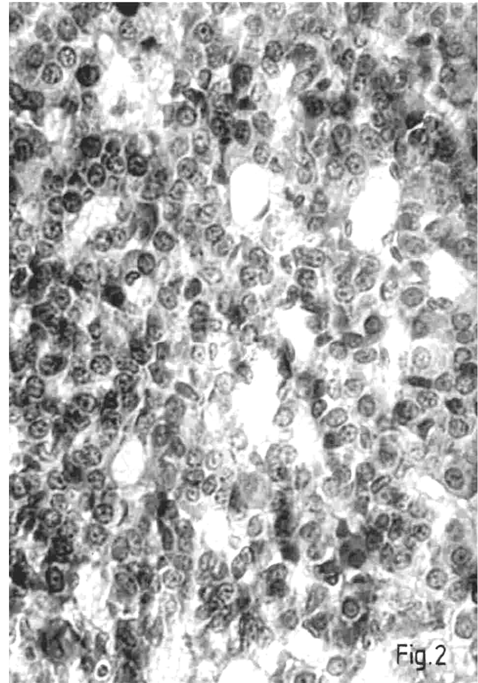
The brain, the pituitary gland and the adrenal of slaughtered animals were fixed in Bouin liquid and afterwards processed in view of being embedded in paraffin. The fragments were sectioned at the Reichert-Austria type microtome with a thickness of 7 μ. For rendering evident the hypothalamic neurosecretion, the staining of the sections was made by means of the Kluver–

Barrera method, the staining of consecutive cells of the pituitary gland was carried out by the method of Hurduc and co-workers and the staining of adrenal gland was made by means of hematoxylin-eosin method [10]. The histological preparations obtained were examined on the $IORC_4$ photonic microscope.

Results and discussions. In the Fluocinolone–treated group PVN shows an intense neurosecretory activity, respectively a marked accumulation of neuropeptides in the cellular pericaryon both in parvocellular neurons and magnocellular ones (Fig.1) and, at the same time, an intense loading of nervous fibres with neurosecretion can also be seen. The cellular body and nuclei are intensely hypertrophied. Anterior pituitary gland shows aspects of hypertrophy and hyperplasia, respectively an increase in glandular basophilia, which results in an alteration of the relation between cells, in favour of the basophil ones. Certain beta-cells show intensely vacuolized cytoplasma, with a foamy aspect. These are the so-called Crooke's cells (Fig.2), which are present under conditions of the organism's exposure to various factors of stress. In our case they are due to an excess of glucosteroids.



F i g. 1. *Intense neurosecretory activity in parvocellular and magnocellular neurons of PVN* (× 270).



F i g. 2. *Crooke's cells in anterior pituitary gland* (× 900).

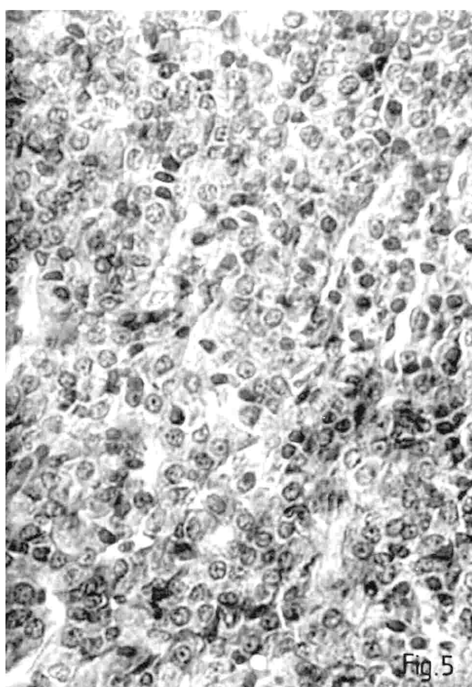
F i g. 3. *Atrophy of fasciculata zone and of the reticularis one, hypertrophy of neurons secreting cathecolamines* (× 270).

F i g. 4. *PVN with a normal or reduced neurosecretory activity* (× 270).

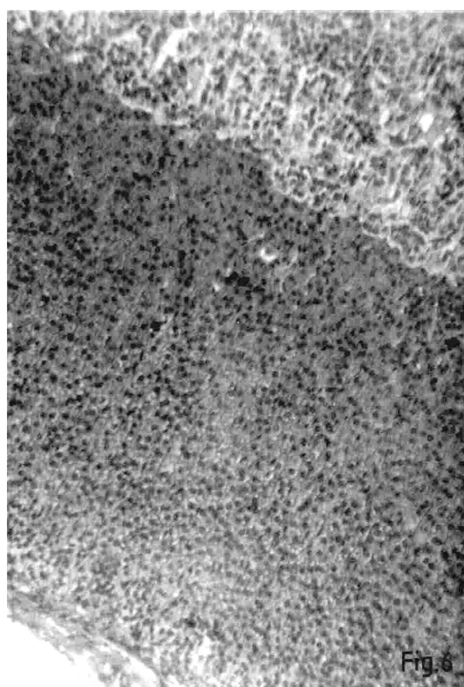
The examination of the histological aspect of the adrenal gland in Fluocinolone-treated group allows us to notice the structural alterations induced by this ointment. Compared to the untreated group, in stressed rats, remarkable decrease in width of adrenal cortex is noticed and it seems to be due to an important loss of parenchymal cells from the fasciculata zone and reticularis one (Fig.3). The fasciculata zone is narrower but more compact than in the control group. Although a decrease of the cortical zone as against the medullary one takes place, its compact structure suggests an intense secretory activity. Under normal conditions, the increase in plasma concentration of glucocorticoids determines the inhibition of hormonal secretion by a negative feed-back mechanism, but under the conditions of our experiment the glucocorticoids show a reactivity specific of both the stressing factors, determining an activation of the axis indifferent of the plasma level of glucocorticoids, and the endogenous glucocorticoids which, in high concentrations, determine an atrophy of the fasciculata zone and of the

reticularis one. In the medullary zone an intense secretory activity takes place, which manifests itself by dilatation of blood vessels associated with an increase in volume of neurons secreting cathecolamines (Fig.3).

The administration of Propranolol to Fluocinolone-treated rats seems to be sufficient to prevent almost all these morphophysiological alterations. In PVN, neurons with a normal or reduced neurosecretory activity are visualized (Fig.4), beside neurons with a cell body full of neurosecretory granules, moderate or slightly increased quantity of neurosecretory granules being present along the nervous fibres. Anterior pituitary gland presents an aspect which is closer to that of the group of controls, noticing, however, a slight hyperplasia of beta basophil cells (Fig.5), the Crooke's cells not being present. At the same time, in Fluocinolone-treated group a Propranolol treatment normalizes the relative adrenal weights and the histological structure of the gland is almost normal (Fig.6).



F i g. 5. *Slight hyperplasia of basophil pituitary cells* (× 900).



F i g. 6. *The normal histological structure of the adrenal* (× 270).

Literature data [13] as well as our results have revealed that the excess of glucocorticoids, determined either by a treatment with glucocorticoid-based drugs, or based on the action of different factors of stress, induces histophysiological alterations of the hypothalamic-hypophyseal-adrenal axis.

Under normal conditions, the main CRH producers are the parvocellular neurons of the paraventricular nucleus [5]. The neurosecretion reaches the median eminence by paraventriculo-infundibular fibres, where they discharge into the primary capillaries of the hypothalamic-hypophyseal portal system arriving at last in adenohypophysis [1, 12] . Data of the bibliography [3] as well as our results show that under conditions of stress an intense discharge of neurohormones takes place and, implicitly, of CRH, indifferent of the plasma concentration of glucocorticoids, which is reflected by a volume increase of secretory neurons from the paraventricular nucleus, the glucocorticoids acting in this way as factors of stress. Morever, an increase of plasma concentration of cortisol, determined either by administration of glucocorticoids or by the action of some factors of stress, ascertains the appearance of Crooke's cells. These cells are ACTH-secreting basophil cells, with an excentric nucleus and foamy cytoplasma full of granules. Their occurrence corresponds to the adenohypophysis exhausting phase.

At the level of the adrenal gland, an intensification of the catecholamine-secreting activity is observed, reflected by the hypertrophy of the secretory cells in the medullary zone of adrenal gland. This explains the strong reaction of paraventricular hypothalamic nuclei which present a rich catecholamine innervation. Experimentally, it was demonstrated that under conditions of stress a strong discharge of adrenaline and noradrenaline takes place, stimulating the CRH secretion, and acting on the corticotropic cells by the agency of β-adrenergic receptors, fact demonstrated by the synthesis blockage after administration of Propranolol which is beta-blocking [7,8]. The present findings suggest the possibility that under dermocorticosteroid treatment conditions in mature rats the β-adrenoreceptor stimulation plays a major conditioning role in HPA axis hyperactivity of excessively released corticosterone and catecholamines. In fact, there is evidence that β-adrenoreceptor blockade with Propranolol reduces the hyperactivity of the HPA axis in human subjects, while adrenaline administration stimulates the ACTH and cortisol release in man, and intracerebroventricular infusion of adrenaline [9] and noradrenaline [11] enhances the release of CRH into the hypophysial portal circulation. This is consistent with the recent findings that α_1-adrenoreceptor blockade prevents the immobilization-induced CRH release from the median eminence [6]. Unilateral or bilateral surgical or chemical lesions of the ventral noradrenergic bundle or of the brain stem between the

locus ceruleus and rostral portion of medulla decrease NE concentration and tyrosine hydroxylase and CRH immunoreactivity in the PVN and decrease CRH levels in hypophysial-portal blood. Beside, it has been reported that adrenaline exerts direct stimulatory effects on steroidogenesis in primary cultures of bovine adrenocortical cells [9].

Conclusions. 1. In mature male Wistar rats β-adrenoreceptor activity *in vivo* plays an evaluable facilitating role in intensification of HPA axis activity, elicited by short-term epicutaneous treatment with Fluocinolone-N ointment.

2. β-Adrenoreceptor blockade *in vivo* with Propranolol, applied s.c. simultaneously with epicutaneous Fluocinolone-acetonid-N treatments, suppresses the HPA axis structural and functinal disorders, elicited by transcutaneous absorption of epicutaneously applied dermocortosteroid.

3.The results obtained demonstrate the antistress effect of Propranolol, which supports the recommendation of using this drug in combination with dermocorticosteroid treatment.

REFERENCES

1. B u m a, P., N i e u w e n h u y s, R., *Ultrastructural characterization of exocytotic release sites in different layers of the median eminence of the rat,* "Cell Tissue Res.", **252**, 1988, 107-114.
2. C a p u a n o, C. A., L e i b o w i t z, S. F., B a r r, G. A., *The pharmaco-ontogeny of the perifornical lateral hypothalamic β_2–adrenergic and dopaminergic receptor systems mediating epinephrine- and dopamine-induced suppression of feeding in the rat,* "Dev. Brain Res.", **70**, 1992, 1-7.
3. H a r b u z, M. S., J e s s o p, D. S., L i g h t m a n, S. L., C h o w d r e y, H. S., *The effects of restraint of hypertonic saline stress on corticotropin-releasing factor, arginine vasopressin and proenkephalin A mRNAs is the CFY, Spargue-Dawley and Wistar strains of rat,* "Brain Res.", **667**, 1994, 6-12.
4. I t o i, K., S u d a, T., T o z a w a, F., D o b a s h i, I., O h m o r i, N., S a k a i, Y., A b e, K., *Microinjection of norepinephrine into the paraventricular nucleus of the hypothalamus stimulates CRH gene expression in conscious rats,* "Endocrinology", **135**, 1994, 2177-2182.
5. K a t h l e e n, M. O., L e e, S., R i v i e r, C., *Role of arginine vasopressin and corticotropin-releasing factor in mediating alcohol-induced adrenocorticotropin and vasopressin secretion in male rats bearing lesions of the paraventricular nuclei*, "Brain Res.", **744**, 1997, 83-95.

6. K i s s, A., A g u i l e r a, G., *Participation of α_1-adrenergic receptors in the secretion of hypothalamic corticotropin-releasing hormome during stress,* "Neuroendocrinology", **56**, 1992, 153-160.
7. M a d a r, J., C r ă c i u n, C., G i u r g e a, R., B o r d a, D., R o m a n, I., Crăciun, V., I l y é s, I., *Antiglucocorticoid effects of propranolol on thymolysis and on thymus metabolic and ultrastructural disorders induced by percutan-absorbed hydrocortisone-17-butyrate in young rats. ,* in C r ă c i u n, C., A r d e l e a n, A. (Eds.), *Current Problems in Cellular and Molecular Biology,* pp. 254-263, Ed. Risoprint, Cluj-Napoca, 1998**.**
8. M a d a r, J., G i u r g e a, R., R o m a n, I., B o r d a, D., *Age-related role of beta-adrenoreceptor activity during clobetasol-induced thymolysis in male Wistar rats,* in C r ă c i u n, C., A r d e l e a n, A. (Eds.), *Current Problems in Cellular and Molecular Biology,* pp.254-263, Ed. Risoprint, Cluj-Napoca, 1998**.**
9. M a d a r, J., R u s u, V.M., Ş i l d a n, N., I l o n c ă, A**.,** *Attenuation of the stress-induced hyperglycemia, thymolysis,* *adrenal hypertrophy and liver adenylate cyclase activity by propranolol in immature and mature young rats,* "Rev. Roum. Biol. Anim.", **34,** 1989, 15-19.
10. M u r e ş a n, E., G a b o r e a n u, M., B o g d a n, A. T., B a b a, A. I., *Tehnici de histologie normală şi patologică,* Ed. Ceres, Bucureşti, 1974.
11. P a c á k, K., P a l k o v i t s, M., K v e t n a n s k y, R., M a t e r n, P., H a r t, K., K o p i n, I. J., G o l d s t e i n, S.D., *Cathecolaminergic inhibition by hypercortisolemia in the paraventricular nucleus of conscious rats*, "Endocrinology", **136**, 1995, 4814-4819.
12. T i m o t h y., G. D*., Serotonin and the regulation of hypothalamic-pituitary-adrenal axis function, "*Life Sci.", **58**, 1996,1683-1694.
13. Y e h u d a, R., *Stress and glucocorticoid* , "Science", **275**, 1997, 1662-1663.

INTRAHYPOTHALAMIC INJECTION OF INSULIN DOES NOT ALTER THE PLASMA GLUCOSE LEVEL IN STREPTOZOTOCIN-DIABETIC RATS

CRISTIAN SEVCENCU[*] and NINA ŞILDAN[**]

SUMMARY. - Insulin receptors are widely distributed in the brain and one of the regions with high insulin content is the lateral hypothalamus (LH).We have previously reported that the injection of 0.2 µl of insulin into the LH leads to a significant decrease in plasma glucose level. The aim of the present study was to establish if this hypoglycemic effect still appears if insulin is injected into the LH of streptozotocin (STZ)-diabetic rats. Male albino Wistar rats were treated with STZ (4 mg/100 g, i.p.) and, after three days, 0.2 µl of insulin was injected into the LH of the animals. Blood samples were taken from the caudal vein 5, 15 and 30 minutes after the injection of insulin, and plasma glucose concentrations were determined. In our experiments, after a small and insignificant hyperglycemic effect produced by the implantation of the needle into the LH, the plasma glucose levels tended to return to the initial values after the injection of insulin. The conclusion is that the hypoglycemic effect of the insulin injected into the LH is blocked by a pre-treatment with STZ.

Early studies on glucose uptake in the nervous tissue postulated that insulin is not required for brain glucose metabolism.

This early perception of the brain as an insulin-insensitive tissue is no longer valid. It is now well-established that insulin and its receptors are widely distributed in various brain areas. Regions having high concentrations of extractable insulin closely correspond to those having high content in insulin receptors. These are the cerebral cortex, olfactory bulbs and hypothalamus [14]. More recently, Garcia-De Lacola *et al.* [4] have established that proliferative chick embryonic neuroretina expresses hybrids of insulin receptors/IGF receptors with high affinity for insulin, which can mediate the effects of proinsulin and insulin in the proliferative stage of neurogenesis. However, the roles played by insulin in the brain and the mechanisms involving the brain insulin receptors remain still unclear.

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In 1992, Schwartz *et al.* [14] proposed a key role for insulin as a signal for the central nervous system (CNS) in the regulation of energy balance and body weight. A strong support for this hypothesis is the observation that insulin induces a state of negative energy balance leading to weight loss when it is administered into the brain [8, 20].

Consistent with previous observations, McGowan *et al.* [9] reported a decrease in body weight after intrahypothalamic infusions of insulin.

Establishing that CNS infusions of insulin to diabetic rats reduced both hyperphagia and over-expression of hypothalamic NPY mRNA, Sipols *et al.* [17] concluded that a deficiency of insulin in the brain is an important cause of diabetic hyperphagia. However, Rizk *et al.* [13] showed that, in obese mice, the hypothalamic expression of NPY mRNA was significantly higher, whereas the expression of insulin receptors remained unaffected.

Another effect of intrahypothalamic administration of insulin is a decrease in plasma glucose level. Iguchi *et al.* [6] reported a significant hypoglycemic effect after the injection of insulin into the ventromedial hypothalamic nucleus (VMN).

In concordance with these results, we have also obtained a significant decrease in plasma glucose level after the injection of the insulin in the lateral hypothalamus (LH) [12].

According to their observations, Iguchi *et al.* [6] suggested that both VMN and LH are insulin responsive glucoregulator centres and these structures regulate blood glucose concentrations by controlling the hepatic glucose metabolism.

In the present experiments, in order to verify a possible pancreatic involvement in this effect, we blocked the B-pancreatic islets with streptozotocin (STZ), before the intrahypothalamic injection of insulin, to see if a hypoglycemic effect still occurs.

Material and methods. Male albino Wistar rats (200-300 g) were used. The animals had free access to food and water until the day of the stereotaxic operation.

Three days before the injection of insulin into the LH, the rats were treated with STZ (4 mg/100 g, i.p.). The plasma glucose level from the initial samples of the experimental series (230.8 mg/100 ml on the average) confirmed that the animals were diabetic.

Before the experiments, the rats were anaesthetised with Nembutal (35 mg/kg) and then placed into the stereotaxic apparatus.

After a small hole was made in the skull, the needle of a microsyringe was implanted into the brain according to the following coordinates [1]: 2 mm posterior to bregma, 1.3 mm lateral to the midline and 8 mm ventral to the horizontal zero plane. Then, 0.2 µl of insulin (0.008 IU) was injected into the LH, and blood samples were taken from the caudal vein, 5, 15 and 30 minutes after the injection.

The plasma glucose concentrations were determined enzymatically, using the method of Werner *et al.* [19].

Following the experiments, the animals were killed by perfusing their hearts with 40% neutral formalin. The fixed brains were cut for anatomical control, following the same coordinates as those used for the injection.

Results. As we reported previously [12], in untreated animals the implantation of the needle induced a small but significant decrease in plasma glucose level (Fig. 1).

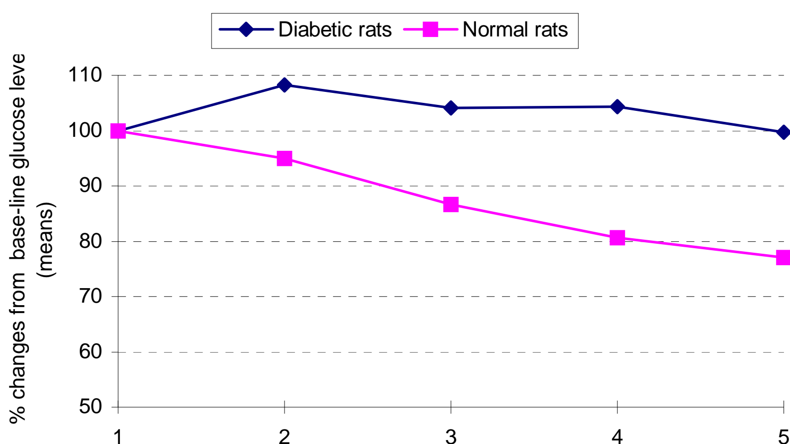

Fig. 1. *Plasma glucose concentrations after stereotaxic injection of 0.2 µl of insulin into the LH.*
1 - Base-line glucose level. 2 - Sample at 5 minutes after the implantation of the needle. 3, 4, 5 - Samples at 5, 15 and 30 minutes after the injection of insulin.

After the application of insulin, the glycemia continues to decrease, and 30 minutes after the injection we registered a marked hypoglycemic effect, which was not an extension of the mechanical stimulation produced by the needle.

As can be seen in Fig. 1, in STZ-diabetic rats, after a small hyperglycemic effect produced by the implantation of the needle, the plasma glucose level tends to return to the initial value. Since the changes of glycemia are transient and insignificant, we may state that in STZ-diabetic animals the intrahypothalamic injection of insulin does not produce the hypoglycemic effect observed in the untreated animals.

Discussion. In the nondiabetic state, insulin produces an increase in plasma insulin concentrations inducing a decrease in plasma glucose levels when infused into the brain [7].

In the present experiments, the hypoglycemic effect of insulin injected into the LH of the normal rats, an effect probably produced through the activation of some parasympathetic fibres innervating the pancreas [5, 11] is blocked by a pre-treatment with STZ.

In good agreement with our results, there is evidence that in STZ-diabetic rats, the intracerebroventricular infusion of insulin at a dose of 3 mU/day for 6 days does not alter circulating insulin and glucose levels [15].

Although the major effect of STZ is to induce a diabetic state, mainly by the reduction of pancreatic insulin content and the decrease of serum insulin concentration, there is also evidence for a direct involvement of the autonomic nervous system (ANS) in potentiation of the STZ-induced hyperglycemia. Thus, between the factors that contribute to the pathogenesis of the diabetes, Dunbar *et al.* [3] mention an increase in the activity of the sympathetic components of ANS.

Part of the mechanisms underlying the control of plasma glucose levels are undoubtedly related to the activity of the ANS. This control is focussed both on the regulation of the pancreatic production of insulin and glucagon and on the hepatic glucose metabolism.

Consistent with ANS influences on the hepatic glucose metabolism, Shimazu *et al.* [16] mention that chemical and electrical stimulation of both VMN and peripheral sympathetic nerves innervating the liver leads to hepatic glucose output by rapid activation of the key glycogenolytic enzyme, glycogen phosphorylase. This results in hyperglycemia and a marked reduction in liver glycogen content. In contrast, the stimulation of LH and of the parasympathetic vagal pathways innervating the liver leads to hepatic glycogenesis by activation of the key enzyme, glycogen synthetase, resulting in a decrease of plasma glucose level.

Corroborating these data with the observation of Dunbar *et al.* [3] that the diabetes is expressed by a decrease in the activity of ANS parasympathetic components, one could state that, beside the destructive effect on the β-pancreatic cells STZ might also affect, in one way or another, the control of LH on the activity of hepatic glycogen synthetase.

Studies by Dunbar *et al.* [3] reveal that, in diabetic state, a defect in the sensitivity of the hypothalamus to glucose and insulin may occur, and this defect might be consistent with our hypothesis on STZ-induced alteration of LH.

Indeed, there are several lines of evidence which indicate an analogous mechanism of STZ action on pancreatic islets and on nerve cells, by means of depleting intracellular NAD^+ and inhibiting cellular functions [18].

On the other hand, studies on ultrastructural and morphometric changes in different hypothalamic nuclei of STZ-diabetic rats showed severe alterations in neuronal profiles, such as: accumulation of glycogen, axonal and dendritical changes, dilated and fragmented endoplasmic reticulum and loss of neuronal organelles [2].

Taking into consideration all these functional and structural changes induced by STZ treatment, the fact that the hypoglycemic effect of insulin injected into LH is blocked by a pre-treatment with STZ may be evaluated from at least two points of view.

First, it is known that the stimulation of LH leads to pancreatic insulin secretion and that insulin has an excitatory local effect on neuronal discharge when it is applied in the LH [10], probably transmitted to the pancreas through parasympathetic fibres [5, 11]. Thus, it is logical to presume that at least part of the hypoglycemic effect induced by insulin injected into the LH is due to its stimulating effects on this structure, reflected in an increased pancreatic insulin production. Our present experimental results seem to support this interaction between insulin, LH and the pancreatic production of insulin, since a previous alteration of cellular functions both in LH and in pancreas by STZ treatment blocks the hypoglycemic effect of the insulin injected into LH.

Second, taking into consideration the interactions between LH and the hepatic glucose metabolic pathways mentioned above, we can also suggest that part of the STZ inhibition of the hypoglycemic effect of insulin injected into the LH might be due to the suppression of some neuronal functions in LH produced by STZ and, hence, to the elimination of the LH control on the hepatic glucose metabolism.

This last opinion is a hypothesis that has to be verified by further experiments.

As a **conclusion** of the present study, we may state that, since the hypoglycemic effect of the insulin injected into the LH is blocked by a pre-treatment with STZ, the endocrine pancreas is one of the target organs for the hypothalamic signals generated in response to the injected insulin.

REFERENCES

1. Bures, I., Petran, M., Zachar, J., *Electrophysiological methods in biological research*, p. 397, Acad. Sci., Prague, 1962.
2. Dheen, S.T., Tay, S.S.W., Wong, W.C., *Ultrastructural changes in the hypothalamic paraventricular nucleus of the streptozotocin-induced diabetic rat*, "Acta Anat.", **149**, 1994, 291-299.
3. Dunbar, J. C., Colemen, E. R., Shaffer, L. R., Marzouq, L., *Central adrenergic suppression augments the insulin and glucagon secretory, and the glycogenolytic responses in streptozotocin-diabetic rats*, "Hormone Res.", **36**, 1991, 80-85.
4. Garcia-De Lacola, M., Alarcon, C., De La Rosa, E. J., De Pablo, F., *Insulin/insulin-like growth factor-I hybrid receptors with high affinity for insulin are developmentally regulated during neurogenesis*, "Endocrinology", **140**, 1999, 233-243.
5. Havel, P. J., Valverde, C., *Autonomic mediation of glucagon secretion during insulin-induced hypoglycemia in rhesus monkeys*, "Diabetes", **45**, 1996, 960 - 966.
6. Iguchi, A., Burleson, P.D., Szabo, A.J., *Decrease in plasma glucose concentrations after microinjection of insulin into VMN*, "Am. J. Physiol.", **240**, 1981, E95-E100.
7. Marfaing-Jallat, P., Levacher, C., Calando, Y., Picon, L., Penicaud, L., *Glucose utilization and insulin binding in discrete brain areas of obese rats*, "Physiol. Behav.", **52**, 1992, 713-716 .
8. McGowan, M. K., Andrews, K. M., Kelly, J., Grossman, S. P., *Effects of chronic intrahypothalamic infusions of insulin on food intake and diurnal meal patterning in the rat*, "Behav. Neurosci.", **104**, 1990, 373- 385.
9. McGowan, M. K., Andrews, K. M., Kelly, J., Grossman, S. P., *Chronic intrahypothalamic infusions of insulin or insulin antibodies alter body weight and food intake in the rat*, "Physiol. Behav.", **51,** 1992, 753- 766.
10. Oomura, Y., Kita, H., *Insulin acting as a modulator of feeding through the hypothalamus*, "Diabetologia", **20**, 1981, 290-298.
11. Pilo, B., George, J. K., *Endocrine pancreas and cholinergic control of metabolic regulation in pigeon*, "Pavo", **35**, 1997, 143-150.
12. Pop, M., Sevcencu, C., Şildan, N., *Recepteurs insuliniques au niveau de l'hypothalamus latéral et les conséquences de la vagotomie bilatérale sur la résponse de leur stimulation adéquate*, "Rev. Roum. Morphol., Embryol. Physiol.", **24**, 1987, 105-109.
13. Rizk, N. M., Li, L. S., Juergen, E., *Hypothalamic expression of neuropeptide-Y in the New Zealand obese mouse*, "Int. J. Obesity", **22**, 1998, 1172-1177.

14. Schwartz, M. W., Figlewicz, D. P., Baskin, D. G., Woods, S. C., Porte, D., Jr., *Insulin in the brain: A hormonal regulator of energy balance*, "Endocrine Rev.", **13,** 1992, 387-414.
15. Schwartz, M. W., Figlewicz, D. P., Baskin, D. G., Woods, S. C., Porte, D., Jr., *Insulin and the central regulation of energy balance*: *Update 1994,*"Endocrine Rev.", No. 2, 1994, 109-112.
16. Shimazu, T., Sudo, M., Minokoshi, Y., Takahashi, A., *Role of the hypothalamus in insulin-independent glucose uptake in peripheral tissues,* "Brain Res. Bull.", **27**, 1991, 501-504.
17. Sipols, A. J., Baskin, D. G., Schwarts, M. W., *Effect of intracerebroventricular insulin on diabetic hyperphagia and hypothalamic neuropeptide gene expression*, "Diabetes", **44**, 1995, 147-151.
18. Takasawa, S., Nata, K., Yonecura, H., Okamoto, H., *Cyclic ADP-ribose in insulin secretion from pancreatic B-cells*, "Science", **259**, 1993, 370-373.
19. Werner, W., Rey, H. G., Wielinger, R. H., *Properties of a new chromogen for determination of glucose in blood, according to GOD/POD method,* "Z. Anal. Chem.", **252**, 1970, 224-228.
20. Woods, S. C., Stein, L. J., McKay, L. D., Porte, D., Jr., *Suppression of food intake by intravenous nutrients and insulin in the baboon*, "Am. J. Physiol.", **247**, 1984, R393-R401.

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ELECTROLYTIC LESIONING OF THE LATERAL HYPOTHALAMUS BLOCKS THE HYPOGLYCEMIC EFFECT OF INTRAHYPOTHALAMIC INJECTION OF INSULIN

CRISTIAN SEVCENCU*

SUMMARY.- The roles played by insulin in the brain and its interactions with hypothalamic structures are still subjects of debate. Intracerebral administration of insulin induces, among other effects, a decrease in plasma glucose concentration. In one of our previous reports, we have mentioned that, 30 minutes after the injection into the lateral hypothalamus (LH), insulin induced a significant decrease of the glycemia. The present study aimed to determine whether that effect strictly involved the LH, or the hypoglycemia still appears when the LH is electrolytically destroyed. In our experiments, the electrocoagulation itself had a hyperglycemic effect and the injection of insulin into the destroyed area was not followed by a significant decrease in plasma glucose concentration. Thus, we may conclude that the LH contributes to the regulation of plasma glucose level and is undoubtedly involved in the hypoglycemic effect mentioned above.

The presence of insulin in the brain and its ability to cross the blood-brain barrier have been clearly demonstrated [23]. There are also several lines of evidence which support the concept that insulin, or at least insulin-related molecules, are produced by neurons in the adult brain [5, 7, 11].

Intracerebral insulin acts on brain insulin receptors which have been extensively studied in order to better understand the specific functions of insulin in the central nervous system (CNS) [1, 21]. Beside the "classical" CNS insulin receptor, a recent study mentions a new class of insulin/insulin-like growth factor I hybrid receptors expressed by proliferative chick embryonic neuroretina cells, and probably involved in the action of the locally produced proinsulin[11].

Although the current understanding of the roles played by insulin in the brain is not complete, there are many experimental data which suggest that insulin is involved in crucial processes in the CNS. Thus, insulin has been shown to stimulate biosynthetic activities, neural cell and fibre outgrowth, and neuron-specific enzyme levels [2, 10, 20]. It also stimulates glycolytic key enzymes in cerebral cortex, such as hexokinase and phosphofructokinase [8].

Inhibiting norepinephrine release from nerve terminals [26], as well as its neuronal reuptake [24], insulin clearly acts as a neuromodulator. Consistent

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with these findings, it has also been shown that insulin acts on peripheral sensory nerves [4], and, in the CNS, increasing the uptake of tryptophan in the brain cell, it interferes with serotoninergic pathways [30].

Recently, it has been proposed a key role for insulin as a signal for CNS in the regulation of the energy balance and body weight, and the mechanisms underlying this process seem to involve central effectors of feeding, such as serotonin, bombesin, galanin, norepinephrine and NPY [15, 22, 23, 27].

In concordance with this hypothesis, the most suggestive action of insulin is the reduction of food intake and body weight when it is infused into the brain, particularly in hypothalamic regions [13, 14]. At the same time, and probably part of the mechanisms underlying these anorexic effects, intrahypothalamic injection of insulin leads to a decrease in plasma glucose level [9].

We have also previously reported that 0.2 µl of insulin (0.008 IU) injected into the lateral hypothalamus (LH) induced a 28% decrease in plasma glucose level, 30 minutes after the injection [19].

The present experiments were designed to provide further information about LH involvement in that effect.

Material and methods. Male albino Wistar rats (200-300 g) were used. They had free access to food and water until the day of the stereotaxic operations.

The animals were anaesthetised with Nembutal (35 mg/kg) and then placed into the stereotaxic apparatus.

After a small hole was made in the skull, the electrode (0.2 mm coated diameter size) was implanted into the LH according to the following coordinates [3] : 2 mm posterior to bregma, 1.3 mm lateral to the midline and 8 mm ventral to the horizontal zero plane. For the electrolytic lesioning of the LH, a 2 mA, 15-second anodal current was used.

Five days after this operation, the needle of a microsyringe was implanted into the destroyed hypothalamic area and 0.2 µl insulin (0.008 IU) was injected.

Blood samples were taken from the caudal vein, 5, 15 and 30 minutes after the injection.

The plasma glucose concentrations were determined enzymatically, using the method of Werner *et al* [29].

Following the experiments, the animals were killed by perfusing their hearts with 40% neutral formalin. The fixed brains were cut for anatomical

control, following the coordinates used for the implantation of the electrode and the needle.

Results. As we reported previously [19], in normal rats the implantation of the needle induced a small but significant decrease in plasma glucose level (Fig. 1).

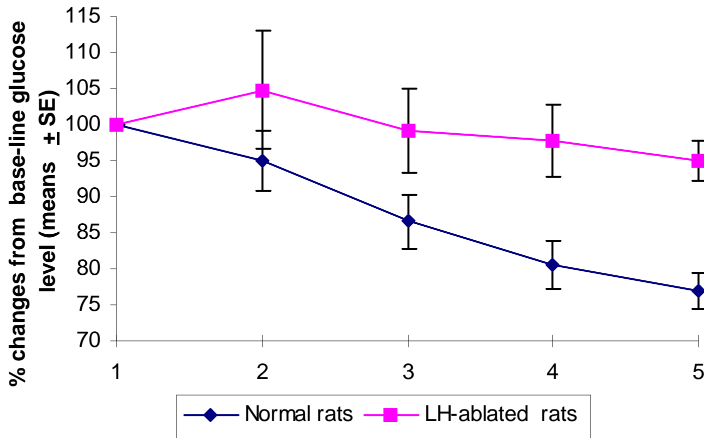

Fig. 1. *Effects of insulin injected into the LH area of LH-ablated rats comparatively to those obtained after the injection of insulin into the same area of normal animals.*

After application of insulin, the glycemia continues to decrease, and 30 minutes after the injection we registered a marked hypoglycemic effect, which was not an extension of the mechanical stimulation produced by the needle.

As can be seen in Fig. 1, the implantation of the needle produced an opposite effect in the rats subjected to electrolytic lesioning of the LH. The injection of insulin in the destroyed area induced an insignificant decrease ($p>0.05$) in plasma glucose level. Thus, we may conclude that intrahypothalamic injection of insulin in LH-lesioned animals does not produce the hypoglycemic effect observed in normal rats.

In the present experiments, we have also observed that the electrolytic lesioning itself produced a significant increase of the peripheral glycemia. This hyperglycemic effect is illustrated in Fig. 2.

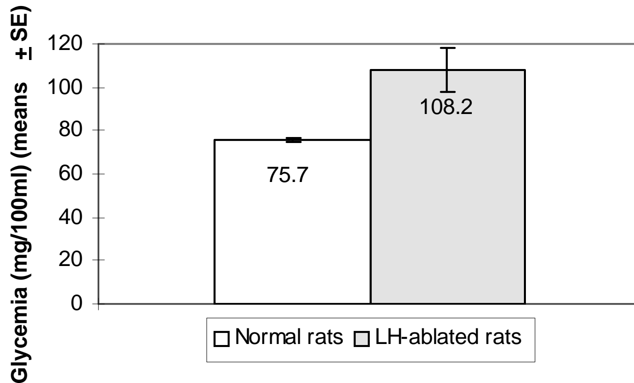

Fig. 2. *Effect of the LH lesioning on plasma glycemia.*

Discussion. As we have already mentioned, in one of our previous articles [19] we reported a significant decrease in plasma glucose level 30 minutes after an injection of insulin into the LH.

These findings are consistent with other reports [1, 12, 28].

The aim of the present experiments was to establish if the hypoglycemic effect mentioned above is due to the action of insulin on this hypothalamic area, or the hormone injected into LH diffuses and exerts its actions on some other hypothalamic structures known to be involved in the control of energy balance.

The fact that the injection of insulin into the electrolytically destroyed LH region is not followed by a significant decrease in plasma glucose concentration leads us to the conclusion that the LH is undoubtedly related to the hypoglycemic effect of insulin injected into this area.

This conclusion is consistent with the observations of Iguchi *et al.* [9] who obtained similar hypoglycemic effects as we did, after the injection of insulin both in the LH, and in the ventromedial hypothalamic nucleus (VMN). Making an attempt to interpret the lowering of plasma glucose concentration after the injection of insulin into the LH, they demonstrated the preservation of the hypoglycemic effect of insulin injected into the LH of the VMN-ablated rats. Their first conclusion was that the effects were not due to the diffusion of insulin from the LH into a biologically active VMN receptor area, and the second one that the "insulin sensitive glucoregulator" postulated by them consists of at least two components - VMN and LH.

Our present results confirm the role of LH as a glucoregulator centre, and there are also some other lines of evidence which support this hypothesis.

Thus, electrical and chemical stimulation of LH leads to hepatic glycogenesis by activation of the key enzyme, glycogen sythetase [25].

Furthermore, iontophoretic applications of insulin, glucose or glucose-insulin mixture alter the electrical activity of a significant proportion of the neurons in the LH. When insulin is applied alone, it has an excitatory effect on the neuronal discharge [16, 17].

It has also been demonstrated that the infusion of insulin into the third cerebral ventricle leads to an increase in plasma insulin concentration inducing a decrease of plasma glucose level [28] via stimulation of the vagal nerves [6, 18, 28].

Taking together, these data represent an experimental support which explains both the hypoglycemic effect of insulin injected into the LH and the lack of the response when insulin is applied into the electrolytically destroyed LH region. Thus, if insulin is a signal for CNS [23], then one site of its action is the LH, since it stimulates the neuronal discharge in this structure. This excitatory effect would induce the activation of glycogen synthetase, as well as an increase in plasma insulin concentration, both of these actions leading to a decrease in plasma glucose concentrations. When the LH is destroyed, none of these effects occur, and the glycemia remains stable.

If we accept the hypothesis that the LH has glucoregulatory functions and its stimulation induces hypoglycemic effects, then it is obvious that its destruction would determine an increase in plasma glucose concentrations, and our results seem to demonstrate this effect.

Finally, it can be **concluded** that the LH contributes to the regulation of plasma glucose level and is undoubtedly responsible for the hypoglycemic effect of insulin injected into this brain region.

REFERENCES

1. Adamo, M., Raizada, M. K., LeRoith, D., *Insulin and insulin-like growth factor receptors in the nervous system*, "Mol. Neurobiol.", **3**, 1989, 71- 100.
2. Bath, N. R., *Insulin dependent neurite outgrowth in cultured embryonic mouse brain cells*, "Dev. Brain Res.", **11**, 1983, 315-318.
3. Bures, I., Petran, M., Zachar, J., *Electrophysiological methods in biological research*, p. 397, Acad. Sci., Prague, 1962.
4. Delaney, C. A., Murchie, K. J., Westerman, R. A., Maximilian, P. C., *Rapid actions of insulin on sensory nerve function*, "Neuroreport", **9**, 1998, 2775-2779.

5. Garcia-De Lacola, M., Alarcon, C., De La Rosa, E. J., De Pablo, F., *Insulin/insulin-like growth factor-I hybrid receptors with high affinity for insulin are developmentally regulated during neurogenesis*, "Endocrinology", **140**, 1999, 233-243.
6. Havel, P. J., Valverde, C., *Autonomic mediation of glucagon secretion during insulin-induced hypoglycemia in rhesus monkeys*, "Diabetes", **45**, 1996, 960-966.
7. Heindenreich, K., *Insulin in the brain. What is its role?*, "Endocrinol. Metabol.", **2**, 1991, 9-12.
8. Hoyer, S., Prem, L., Sorbi, S., Amaducci, L., *Stimulation of glycolytic key enzymes in cerebral cortex by insulin*, "Neuroreport", **4**, 1993, 991-993.
9. Iguchi, A., Burleson, P. D., Szabo, A. J., *Decrease in plasma glucose concentration after microinjection of insulin into VMN*, "Am. J. Physiol.", **240**, 1981, E95-E100.
10. Kyriakis, J. M., Hausman, R. E., Peterson, S. W., *Insulin stimulates cholin acetyltransferase activity in cultured embryonic chicken retinal neurons*, "Proc. Natl. Acad. Sci. USA", **84**, 1987, 7463-7471.
11. LeRoith, D., Adamo, M., Shemer, J., Waldbilig, R., Lesniak, M. A., DePablo, F., Hart, C., Roth, J., *Insulin-related materials in the nervous system of vertebrates and non-vertebrates: Possible extrapancreatic production*, "Hormone Metabol. Res.", **20**, 1988, 411-420.
12. Marfaing-Jallat, P., Levacher, C., Caladano, Y., Picon, L., Penicaud, L., *Glucose utilization and insulin binding in discrete brain areas of obese rats*, "Physiol. Behav.", **52**, 1992, 713-716.
13. McGowan, M. K., Andrews, K. M., Grossman, S. P., *Role of intrahypothalamic insulin in circadian patterns of food intake, activity, and body temperature*, "Behav. Neurosci.", **106**, 1992, 380-385.
14. McGowan, M. K., Andrews, K. M., Grossman, S. P., *Chronic intrahypothalamic infusions of insulin or insulin antibodies alter body weight and food intake in the rat*, "Physiol. Behav.", **51**, 1992, 753-766.
15. McKibbin, P. E., McCarthy, H. D., Shaw, P., Williams, G., *Insulin deficiency is a specific stimulus to hypothalamic neuropeptide Y: A comparison of the effects of insulin replacement and food restriction in streptozotocin- diabetic rats*, "Peptides", **13**, 1992, 721-727.
16. Oomura, Y., Kita, H., *Insulin acting as a modulator of feeding through the hypothalamus*, "Diabetologia", **20**, 1981, 290-298.
17. Oomura, Y., Ono, T., Ooyama, H., Wayner, M. J., *Glucose and osmosensitive neurons of the rat hypothalamus*, "Nature", **222**, 1969, 282- 284.
18. Pilo, B., George, J. K., *Endocrine pancreas and cholinergic control of metabolic regulation in pigeon*, "Pavo", **35**, 1997, 143-150.
19. Pop, M., Sevcencu, C., Şildan, N., *Recepteurs insuliniques au niveau de l'hypothalamus latéral et les conséquences de la vagotomie bilatérale sur la résponse de leur stimulation adéquate*, "Rev. Roum. Morphol., Embryol. Physiol.", **24**, 1987, 105-109.

20. Recio-Pinto, E., Rechler, M. M., Ishii, D. N., *Effects of insulin, insulin-like growth factor II, and nerve growth factor on neurite formation and survival in cultured sympathetic and sensory neurons*, "J. Neurosci.", **6**, 1986, 1211-1219.
21. Sartori, C., Stefanini, S., Bernardo, A., Augusti-Tocco, G., *Insulin receptor in mouse neuroblastoma cell line N18TG2: Binding properties and visualization with colloidal gold*, "Int. J. Dev. Neurosci.", **10**, 1992, 281-289.
22. Schwartz, M. W., Bergman, R. N., Kahn, S. E., Taborsky, G. J., Fisher, L. D., Sipols, A. J., Woods, S. C., Steil, G. M., Porte, D., Jr., *Evidence for entry of plasma insulin into cerebrospinal fluid through an intermediate compartment in dogs. Quantitative aspects and implications for transport*, "J. Clin. Invest.", **88**, 1991, 1272-1281.
23. Schwartz, M. W., Figlewicz, D. P., Baskin, D. G., Woods, S. C., Porte D., Jr., *Insulin in the brain: A hormonal regulator of energy balance*, "Endocrine Rev.", **13,** 1992, 387-414.
24. Schwartz, M. W., Figlewicz, D. P., Baskin, D. G., Woods, S. C., Porte, D., Jr., *Insulin and the central regulation of energy balance*: *Update 1994*, "Endocrine Rev.", No. 2, 1994, 109-112.
25. Shimazu, T., Sudo, M., Minokoshi, Y., Takahashi, A., *Role of the hypothalamus in insulin-independent glucose uptake in peripheral tissues,* "Brain Res. Bull.", **27**, 1991, 501-504.
26. Shimosawa, T., Ando, K., Ono, A., Takahashi, K., Isshiki, M., Kanda, M., Ogata, E., Fujita, T., *Insulin inhibits norepinephrine overflow from peripheral sympathetic nerve ending*, "Biophys. Res. Commun.", **188**, 1992, 330-335.
27. Sipols, A. J., Baskin, D. G., Schwartz, M. W., *Effect of intracerebroventricular insulin on diabetic hyperphagia and hypothalamic neuropeptide gene expression*, "Diabetes", **44**, 1995 , 147-151 .
28. Taborsky, G. J., Bergman, R. N., *Effect of insulin, glucose, and 2-deoxyglucose infusion into the third cerebral ventricle of conscious dogs on plasma insulin, glucose, and free fatty acids*, "Diabetes", **29**, 1980, 278-283.
29. Werner, W., Rey, H. G., Wielinger, R. H., *Properties of a new chromogen for determination of glucose in blood, according to GOD/POD method,* "Z. Anal. Chem.", **252**, 1970, 224-228.
30. Wurtman, R. J., Wurtman, J. J., *Serotoninergic mechanism and obesity*, "J. Nutr. Biochem.", **9**, 1998, 511-515.

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ESEU ASUPRA EVOLUȚIEI LA NIVEL MOLECULAR

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SUMMARY. - Essay on the Evolution at Molecular Level. Although all organisms are highly complex and the evolution of different components can be followed, the most exciting data were revealed by the analysis of DNA, RNA and protein molecules. This essay is mainly focussed on two questions: 1) how and to what rate did DNA sequences evolve and 2) how may changes at the level of DNA affect organism properties.

Different genes evolved with various rates and the rate of sequence evolution is greater for the DNA that lacks function. Through transposition and unequal recombination families of related genes have arised. Transposons can affect the phenotype of the organism by causing mutations, which are mostly not advantageous to the organism. Transposable elements persist merely by their capacity to transpose ("selfish DNA"), rather than by changing organism adaptability. Enzymes with new functions may arise through DNA sequence fusions or internal duplications.

The new insights provided by DNA, RNA and protein analysis are currently incorporated in population genetics and evolution.

Introducere. Analiza structurii, organizării şi funcționării materialului ereditar dă o nouă dimensiune diversității vieții. Materialul genetic reprezintă un subiect de studiu fascinant, el reprezentând, în ultimă instanță, baza informațională a tuturor caracteristicilor unui organism. Deşi organismele vii au o compoziție complexă, fiind alcătuite din numeroase tipuri de molecule cum ar fi lipide, hormoni steroizi, alcaloizi, glucide şi altele, care au făcut şi fac obiectul unor studii evolutive comparative, datele cele mai interesante sunt furnizate de analiza macromoleculelor de ADN, ARN şi proteine [13, 18].

Astfel, studiul electroforetic al proteinelor a adus date interesante pentru genetica populațiilor privind fluxul de gene, variabilitatea genetică şi selecția naturală. Prin secvenționarea ADN sau cu ajutorul enzimelor de restricție s-au obținut date privind polimorfismul la nivelul nucleotidelor. Studiile la nivel molecular au adus informații în plus privind relațiile filogenetice dintre specii.

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În acest eseu vor fi abordate, în mod special, două probleme: a) care este viteza de evoluție a secvențelor de ADN şi b) cum anume modificările evolutive ale secvențelor de ADN pot afecta proprietățile organismului.

Variabilitatea secvențelor de ADN

Analiza ADN cu ajutorul enzimelor de restricție, deşi relativ grosieră, a evidențiat o variabilitate intraspecifică considerabilă la nivelul nucleotidelor. Astfel, Langley şi colab. [12] au utilizat 8 enzime de restricție pentru a evidenția variabilitatea unui fragment de ADN de 12 Kb (kilobaze) la drosofila; fragmentul respectiv includea şi locusul pentru enzima alcooldehidrogenază. Pentru 18 cromozomi analizați, autorii menționați au identificat 4 situsuri polimorfe. Extrapolând aceste date la întregul genom autorii au ajuns la concluzia că un individ de drosofila ar trebui să prezinte, în medie, 4 din 1000 de situsuri în stare heterozigotă. Un studiu mai detaliat a fost realizat de către Kreitman [11], care a determinat întreaga secvență a bazelor azotate pentru locusul enzimei alcooldehidrogenază, incluzând secvențele de control adiacente, pentru 11 gene individuale izolate de la *Drosophila melanogaster,* colectată de pe patru continente diferite. Electroforetic s-au distins două alele notate Adh^F şi Adh^S. Aceste două alele au prezentat o frecvență clinală paralelă în funcție de latitudine şi determină sinteza a 2 izoenzime cu activitate diferită. Cele două alele diferă în privința unei singure nucleotide, care determină înlocuirea aminoacidului treonină de către lizină. În plus, secvența analizată a prezentat un polimorfism silențios abundent şi o mare variabilitate la nivelul intronilor. Alela Adh^F a prezentat o variabilitate mai mare decât alela Adh^S. Analiza secvenței acestor alele l-a determinat pe Kreitman să presupună că alela Adh^F a luat naştere din alela Adh^S printr-o mutație punctiformă, înlocuind codonul pentru treonină cu cel pentru lizină. Presupunerea lui Kreitman este susținută şi de faptul că la alte două specii de *Drosophila* este prezentă numai alela Adh^S. Analiza acestor alele l-a determinat pe Kreitman să presupună că, cel puțin de două ori în istoria acestor gene, au avut loc recombinări intragenice. Prin analize ulterioare, Hudson şi Kaplan [9] au sugerat că au avut loc cel puțin 5 evenimente de recombinare intragenică, fiind posibile chiar 150.

Viteza de evoluție a secvențelor de ADN şi proteine

Analiza secvențelor de aminoacizi şi nucleotide pentru diferite gene omoloage la diferite specii a evidențiat faptul că unele secvențe de ADN evoluează cu viteză mai mare decât altele.

În mod oarecum surprinzător, secvențele unor proteine nefuncționale evoluează mai rapid, iar modificarea codonilor sinonimi este mai frecventă. Aceste date susțin aşa-numita teorie neutralistă a evoluției. Pentru exemplificare, redăm, în Fig. 1, harta determinată cu ajutorul enzimelor de restricție pentru secvența netranscrisă, de spațiere (NTS), a genelor ARNr, comparativ la om şi maimuțe.

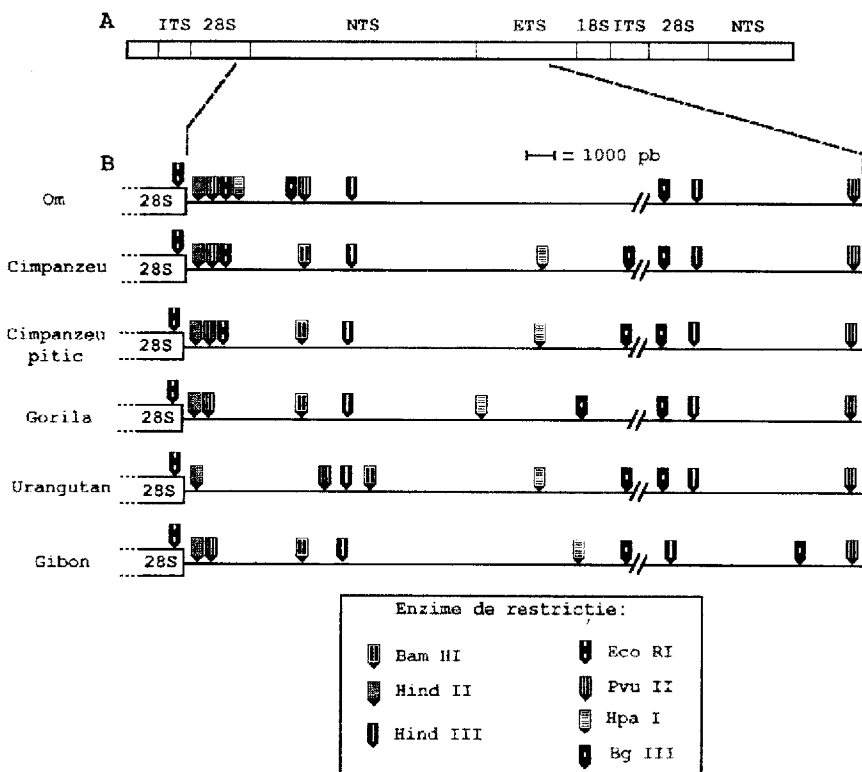


Fig. 1. *Harta evidențiată cu ajutorul enzimelor de restricție pentru secvența netranscrisă, de spațiere (NTS), a genelor ARNr, comparativ la om și maimuțe: A) organizarea secvențelor repetate conținând secvențe transcrise pentru ARNr 18S și 28S, ITS = introni, ETS = exoni; B) prezentarea comparativă a situsurilor de recunoaștere pentru 7 enzime de restricție, a NTS, la om și maimuțe; zona întreruptă reprezintă o porțiune care nu a fost analizată* ([7] *modificat).*

Unele secvențe de ADN au rămas neschimbate o perioadă lungă de timp. De exemplu, o parte a secvenței genei care controlează ritmul circadian la drosofila a fost regăsită ca secvență multiplă, repetată în ADN-ul păsărilor și mamiferelor, dar funcția acestor secvențe la vertebrate nu este cunoscută. O parte dintre genele care afectează segmentarea la drosofila (Ultrabitorax, Antennapedia ș.a.) prezintă o secvență codificând 60 de aminoacizi, care a fost identificată și în ADN-ul broaștelor și omului [8].

Un exemplu interesant este reprezentat de situsurile de inițiere, acele regiuni ale genelor unde este inițiată transcripția de către ARN polimerază. ARN polimeraza I transcrie numai secvențele de ADN care codifică ARNr. Situsul de inițiere a

genelor ARNr este foarte diferit în privința secvențelor de nucleotide la drosofila, om şi *Xenopus*. ARN polimeraza II transcrie numeroase gene care codifică ARNm, deci este de aşteptat ca situsul de inițiere al acestor gene să nu varieze prea mult. S-a identificat, întradevăr, o secvență "consens" a situsului de inițiere pentru diferite gene, atât la aceeaşi specie, cât şi la organisme foarte diferite. Conservarea funcției acestor situsuri de inițiere a fost demonstrată şi experimental, sistemul de transcripție a ADN uman fiind capabil să transcrie *in vitro* gena pentru proteina constitutivă a firului de mătase de la *Bombyx mori*.

Modificări evolutive ale localizării şi numărului de gene

Relațiile de linkaj dintre gene evoluează prin rearanjamente cromozomiale cum ar fi inversiile, translocațiile, fuziunile sau ruperile (fisiunile) de cromozomi. Cantitatea totală de ADN creşte foarte mult prin poliploidizare. Dar, prin studiul ADN repetitiv s-au identificat şi alte mecanisme care pot modifica localizarea şi numărul de secvențe de ADN. La toate eucariotele s-au identificat familii de gene, cu secvențe de nucleotide identice sau foarte similare. Numărul de copii într-o familie de gene variază între 2 şi 10^6; în genomul uman familia Alu prezintă mai mult de 500000 de copii, între care există doar diferențe minore ale bazelor azotate. Numărul de familii de gene per genom este, adesea, de câteva sute, iar copiile unei familii pot fi grupate pe un singur cromozom sau dispersate pe mai mulți cromozomi. Redăm, mai jos, familia de gene pentru globine umane distribuită pe trei cromozomi (Fig. 2).

Unitatea alcătuită din genele pentru ARNr 18S şi 28S, împreună cu secvențele de spațiere (NTS), reprezentate schematic mai sus, se repetă în tandem de câteva sute de ori pe un cromozom la *Xenopus*, pe când la om, această familie de gene este dispersată pe 5 cromozomi diferiți. Diferențele privind numărul de copii ale secvențelor repetitive sunt, în cea mai mare parte, cauza diferențelor de mărime a cromozomilor la diferite specii. În cadrul genului *Plethodon* speciile diferă în ceea ce priveşte cantitatea de ADN per celulă, uneori de 3 ori, aceste diferențe fiind însoțite de diferențe corespunzătoare ale dimensiunilor cromozomilor.

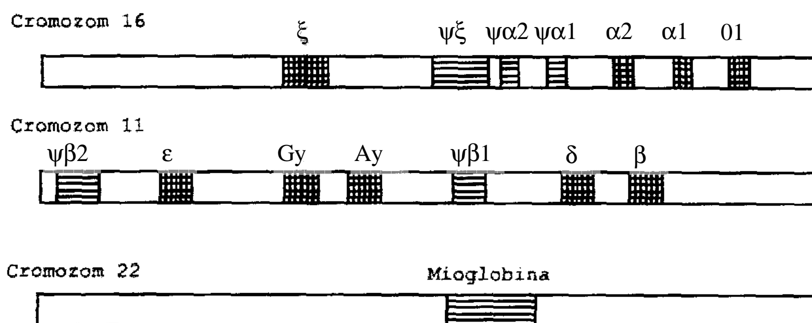


Fig.2. *Reprezentarea schematică a distribuției cromozomiale a familiei de gene pentru globine umane* ([7] *modificat*).

Existența familiilor de gene ridică o serie de întrebări: cum se modifică numărul de copii ale unei gene în evoluție? Cum sunt transferate unele copii în noi situsuri în genom? Care sunt efectele numărului de copii? Care sunt mecanismele care guvernează asemănarea secvențelor genelor membre ale unei familii?

Crossing-overul inegal şi evoluția genelor duplicate

Crossing-overul inegal între cromozomi omologi face ca un cromozom să poarte o deleție, iar celălalt gena duplicată. Dacă crossing-overul inegal apare între două secvențe deja duplicate, rezultatul va fi o copie pe un cromozom şi alte trei copii pe cromozomul omolog. Cu cât numărul de copii, situate în tandem, este mai mare, cu atât devine mai probabil crossing-overul inegal. Un cromozom care poartă un anumit număr de copii poate deveni fixat într-o populație prin drift genetic sau prin selecție naturală. Pe de altă parte, genele duplicate pot evolua divergent ca urmare a mutației, driftului genetic sau selecției naturale. Filogenia genelor poate fi diferită de cea a speciilor care le poartă. De exemplu, analiza secvenței de aminoacizi a indicat că mioglobina şi câteva din lanțurile hemoglobinei vertebratelor au luat naştere prin duplicarea succesivă a genei unei globine ancestrale (Fig. 3).

Alături de genele funcționale, familia de gene pentru globine conține şi gene non-funcționale sau pseudogene (Ψ). Pseudogenele prezintă, comparativ cu genele funcționale, numeroase substituții incluzând codonii terminali, care previn translația în polipeptide funcționale. Pseudogenele din familia de gene pentru globine umane prezintă secvențe de nucleotide omoloage, atât cu intronii, cât şi cu exonii genelor funcționale.

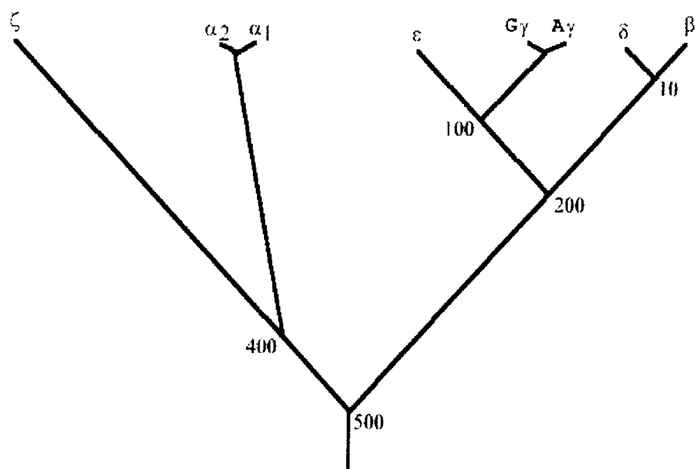
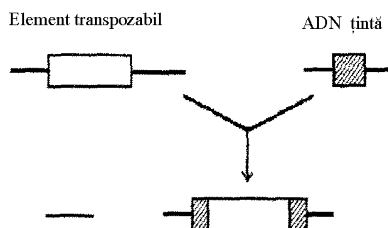


Fig. 3. *Reprezentarea arborelui filetic al genelor pentru globine umane (cifrele reprezintă momentul duplicării, în milioane de ani, conform estimărilor bazate pe "ceasul molecular")* ([7] *modificat*).

Numeroase gene structurale prezintă pseudogene. Unii cercetători au calculat, estimativ, perioada de când anumite gene au devenit non-funcționale. Astfel, pseudogena $\Psi\alpha2$ de la șoarece și $\Psi\alpha1$ de la om se pare că au devenit non-funcționale după o perioadă de aproximativ 4 milioane de ani după ce au apărut prin duplicare, iar $\Psi\beta2$ de la șobolan a devenit silențioasă imediat după apariția sa.

Poliploidizarea, asemănător duplicării, crește numărul copiilor unei gene, copii care pot prelua funcții divergente. De exemplu, peștii din familia *Salmonidae* (păstrăvul, somonii etc.) și cei din familia *Catostomidae* provin din strămoși comuni care au devenit tetraploizi cu aproximativ 50 milioane de ani în urmă. La ambele familii unele din genele duplicate au funcții divergente.

1. Transpoziție conservativă:



2. Transpoziție replicativă:

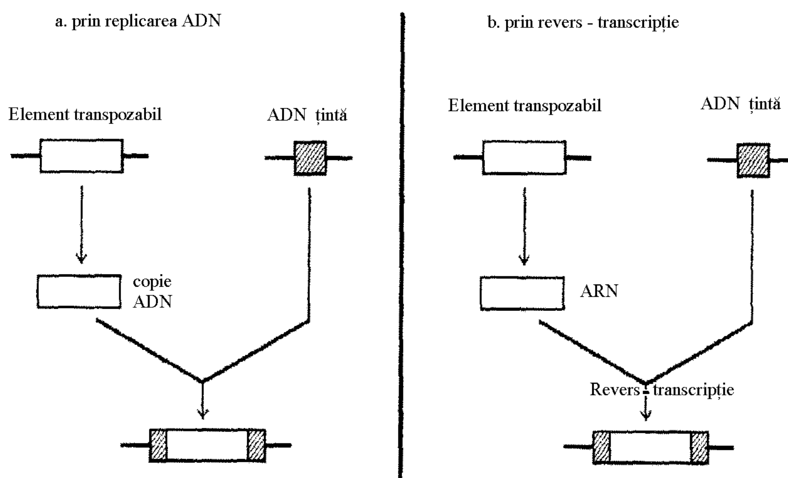


Fig.4. *Reprezentarea schematică a celor trei modele teoretice de realizare a transpoziției.*

Elementele genetice mobile

Descoperirea cea mai uimitoare a geneticii moleculare a fost cea referitoare la existența unor secvențe de ADN care se pot duplica şi apoi reinsera în alte situsuri ale genomului. Aceste elemente mobile [2, 6, 20] includ: epizomii (acele plasmide care au capacitatea de a se integra în cromozomul bacterian şi care se pot replica şi atunci când nu sunt inserate) şi transpozonii (= elemente transpozabile), care se replică numai când sunt inserate în cromozom. Prezența elementelor transpozabile se recunoaşte prin existența unor secvențe scurte, direct repetate ("direct repeats"), de o parte şi de alta a transpozonului în ADN țintă. Secvențele inserate sunt excizate cu o rată foarte scăzută de numai 10^{-10} - 10^{-9}/ gamet, viteza transpoziției fiind, în general, de aproximativ 10^{-5} - 10^{-4} /gamet (Fig.4). În anumite condiții viteza transpoziției creşte, considerabil. De exemplu, generația F1, rezultată din încrucişarea femelelor de drosofila linia M (care nu prezintă elemente P în cromozomi), cu masculi ai liniei P (care prezintă elemente P), va fi alcătuită din indivizi care au în celulele lor citoplasmă de tip M şi cromozomi purtători de elemente P. La aceşti descendenți, viteza de transpoziție a elementelor P este de 20 de ori mai mare, decât la muştele care prezintă citoplasmă de tip P. Aceste date experimentale sugerează că liniile P dispun de anumite mecanisme citoplasmatice de reglare a vitezei transpoziției. La procariote s-a demonstrat că unele elemente transpozabile, o dată inserate în genom, pot inhiba (represa) inserția altor elemente în imediata lor vecinătate. Un astfel de mecanism este, probabil, prezent şi la eucariote. Fenomenul a fost denumit imunitate de transpoziție. Când inhibiția afectează situsurile unui singur cromozom, ea poartă denumirea de **represie de tip cis**. Atât la procariote, cât şi la eucariote s-a descris şi o **represie de tip trans** ("trans-acting repression"), atunci când secvențele inserate scad viteza de inserție a altor secvențe transpozabile, în altă parte în genom.

La o anumită specie, diversele familii de elemente transpozabile pot reprezenta 10% din ADN total, sau chiar mai mult. *Drosophila melanogaster* prezintă cel puțin 30 de familii de elemente transpozabile, care reprezintă aproximativ 50% din ADN mediu repetitiv [7].

Elementele transpozabile care se replică prin revers-transcripție (transcripție inversă) se numesc şi retrotranspozoni (v. schema redată în Fig. 4b) [1, 19]. Asemănător retrovirusurilor, cu care se aseamănă, retrotranspozonii se replică prin revers-transcripția ARN în prezența reverstranscriptazei. Gena pentru enzima reverstranscriptază, notată "pol", a fost identificată, atât la retrovirusuri, cât şi în elementele transpozabile Ty de la drojdii; o secvență similară face parte din elementul "copia" de la drosofila. De asemenea, secvențele de nucleotide ce alcătuiesc numeroase familii de ADN repetitiv, cum ar fi familia Alu de la mamifere, indică formarea acestora prin revers-transcripție.

O serie de date experimentale sugerează că există numeroase situsuri de inserție a elementelor transpozabile, pe care acestea le ocupă la întâmplare. Totuşi, unele experimente indică că unele regiuni ale cromozomilor sunt mai susceptibile la inserția transpozonilor, decât altele.

Care sunt efectele transpozabile din perspectiva evoluției?

Unele elemente transpozabile pot purta gene cu efecte fenotipice adaptative. De exemplu, genele care conferă rezistență la medicamente (îndeosebi antibiotice) şi cele care permit metabolizarea unui substrat nou sunt frecvent purtate de epizomii bacteriilor. Se pare că aceste gene sunt, de fapt, gene ale gazdei care au fost "capturate" de elementele transpozabile. Majoritatea elementelor transpozabile nu poartă, însă, informație genetică care să afecteze fenotipul organismului. Singura informație pe care o poartă pare a fi cea necesară propriei lor replicări. Deoarece numărul de copii ale unui transpozon poate creşte prin transpoziție, proporția genomului alcătuit din familii de elemente transpozabile poate creşte, aparent, fără nici o limită. Varianta (elementul transpozabil) care este tranpozată cu frecvență mai mare va alcătui cea mai mare parte a familiei de elemente transpozabile şi, deci, se poate spune că prezintă un avantaj adaptativ. Acesta este primul exemplu cunoscut de acțiune a selecției naturale la nivel molecular (şi nu la nivelul organismului întreg). Aceste secvențe nu sunt utile organismului, dimpotrivă, ele există deoarece se propagă autonom şi, de aceea, au fost denumite **ADN egoist** ("selfish DNA") [3, 15]. ADN-ul egoist poate fi considerat ca parazit al genomului în care rezidă. ADN egoist este diferit de ADN-ul lipsit de funcție, care se replică împreună cu genele funcționale, dar nu creşte în proporție la nivelul genomului; D o v e r [4] a propus pentru acesta din urmă termenul de ADN ignorant.

Elementele transpozabile pot afecta organismul prin efectul lor mutagen. Ele pot purta secvențe promotoare ("promoter") determinând reactivarea unor gene represate, situate după locul lor de inserție. Transpozonii pot, de asemenea, să stopeze funcționarea unor gene prin întreruperea fie a secvenței structurale, fie a unei secvențe de control (de reglare). Astăzi, se ştie că multe din mutantele clasice descrise la drosofila, cum ar fi white, bitorax, sunt, de fapt, cauzate de inserția unor transpozoni. Rearanjamentele cromozomiale (inversii, deleții) sunt frecvent determinate de recombinarea între doi membri ai unei familii de elemente transpozabile. Redăm, în Fig. 5, modelul teoretic care ar putea explica un astfel de efect. Segmentele pe care le pot transporta transpozonii, prin mecanismul redat schematic în Fig. 5, sunt adesea de câteva mii de baze azotate; dacă un astfel de segment, împreună cu transpozonul, se inseră într-o altă genă, este clar că va aboli funcția genei țintă. De exemplu, multe din inserțiile în locusul white, la drosofila, au o lungime de 14 Kb (kilobaze). Asemănător majorității mutațiilor, şi cele cauzate de transpozoni nu sunt favorabile organismului, dar în culturile bacteriene menținute în chemostat s-au descris şi mutații avantajoase.

Un alt aspect interesant este cel al aşa-numitelor pseudogene procesate, care se pare că au luat naştere prin revers-transcripția transpozonilor (supoziție susținută de absența intronilor). Astfel de pseudogene procesate s-au descris la om, şoarece şi la alte mamifere. Ele sunt situate în altă parte în genom, în timp ce pseudogenele obişnuite sunt adiacente copiilor funcționale.

Elementele transpozabile se pot multiplica în genom şi apoi se pot răspândi în populațiile gazdei, extrem de rapid. De exemplu, elementele P lipsesc din

populațiile de drosofila colectate în anii cincizeci, ai acestui secol, şi menținute de atunci în laborator. În schimb, toate populațiile naturale, din întreaga lume, poartă aceste elemente. Datele experimentale şi teoria matematică sugerează că aceste elemente nu s-au pierdut în populațiile menținute în laborator. Dacă elementele P ar fi reprezentat o trăsătură ancestrală, atunci ele ar trebui să fie prezente şi la alte specii înrudite cu *Drosophila melanogaster*, cum ar fi de exemplu *D. simulans*. S-a demonstrat, însă, că elementele P lipsesc la aceste specii înrudite. Un grup de specii, mai îndepărtate din punct de vedere filogenetic, poartă elementele P şi se bănuieşte că acestea au reprezentat sursa de "infecție" pentru *D. melanogaster*.

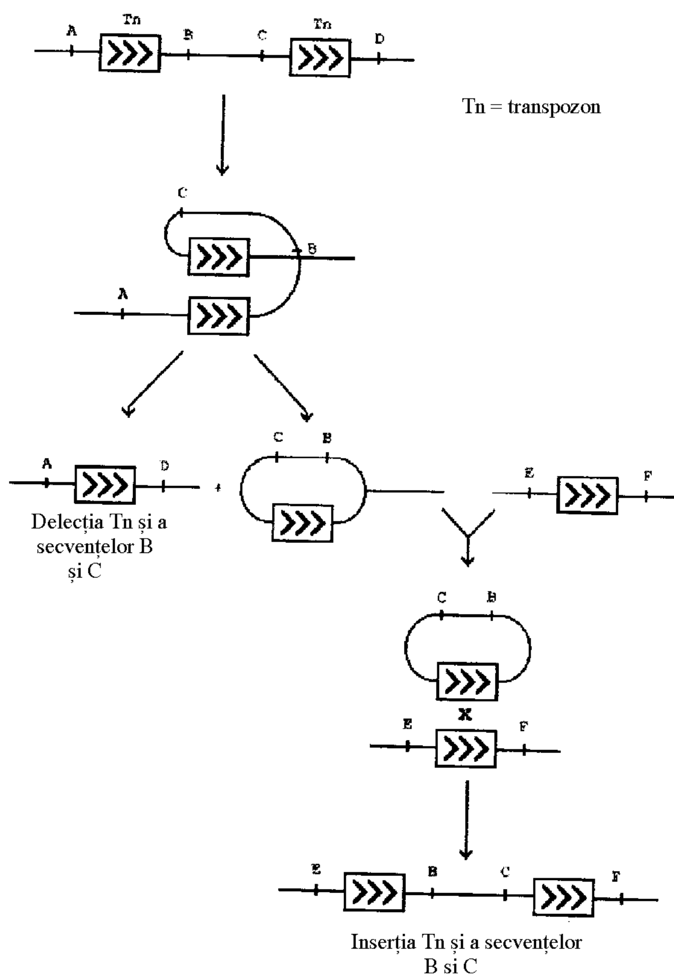


Fig. 5. *Modelul teoretic al recombinării a doi dintre membrii unei familii de elemente transpozabile* [7].

Dacă elementele transpozabile se pot multiplica aşa de repede, există oare factori care pot limita numărul de copii? Dacă da, care sunt aceşti factori? Vom încerca să răspundem acestor întrebări, în paragrafele care urmează.

Evoluția mărimii genomului

Cantitatea de ADN per genom haploid variază foarte mult la diferite organisme, existând diferențe mari chiar şi între speciile înrudite. Cantitatea de ADN nu influențează fenotipul organismelor, dar afectează mărimea celulelor şi viteza diviziunii celulare. Atât durata interfazei, cât şi cea a mitozei cresc o dată cu creşterea cantității de ADN. Speciile care au valoarea C (= cantitatea de ADN per genom haploid) mare se dezvoltă mai încet decât speciile care au valoarea C mică. Plantele efemere au valoarea C mică, în timp ce speciile perene au valoarea C mare. Specia de salamandre *Plethodon vehiculum*, care are valoarea C de aproximativ două ori mai mare decât *P. cinereus*, atinge la maturitate aceeaşi dimensiune cu *P. cinereus*, dar numărul de celule este redus la jumătate. Acest aspect şi implicațiile lui în evoluție au fost puțin investigate. Se pare că acțiunea selecției naturale asupra vitezei de dezvoltare afectează evoluția cantității de ADN.

Este de presupus că diferențele dintre populațiile unei specii în privința numărului şi localizării secvențelor repetitive de ADN pot reduce împerecherea cromozomilor omologi la hibrizi, reducând astfel fertilitatea acestora şi ducând, deci, la speciație. Există, totuşi, destul de puține date care să susțină această ipoteză; dimpotrivă, hibrizii între unele specii înrudite de ierburi, care diferă între ele cu valori de până la 50% din cantitatea de ADN, prezintă o împerechere normală a cromozomilor, formarea de chiasme şi segregare normală. S-a presupus că, în acest caz, ADN omolog din cromozomi se împerechează, în timp ce secvențele repetitive interstițiale, care au lungimi diferite, formează răsuciri colaterale (Fig. 6).

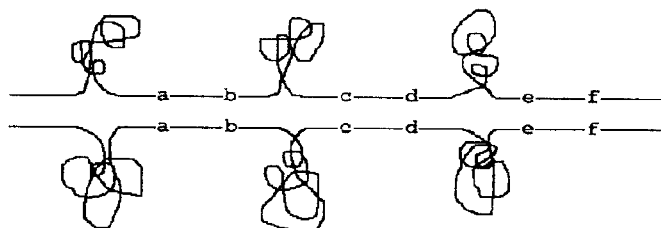


Fig. 6. *Modelul care ar putea explica împerecherea normală a unor cromozomi care diferă în privința cantității de ADN; în timp ce secvențele omoloage se recunosc, zonele neomoloage formează răsuciri laterale* [7].

Amplificarea numărului de copii ale unei gene se pare că afectează capacitatea de adaptare pe seama variabilității cantitative a produsului genei respective, de exemplu a unei enzime. Dacă produsul genei, aflat în exces, reduce capacitatea de adaptare, selecția naturală va favoriza mecanisme de compensare a dozei, care vor reduce activitatea uneia sau a mai multor copii ale genei. Există dovezi că, la unele specii tetraploide de pești cum este crapul (*Cyprinus carpio*), cantitățile de ARN şi enzime sunt reglate sub nivelul celor de la specii diploide înrudite. ARNr, de exemplu, este degradat mai repede la speciile tetraploide. Dimpotrivă, selecția naturală va favoriza creşterea numărului de copii ale unei gene al cărei produs este necesar în cantități mari, cum este ARNr pentru care există în genom sute de gene.

Modele ale evoluției numărului de copii ale unei gene

Dacă excesul de copii ale unei gene reduce adaptabilitatea, frecvența în populație a unui cromozom cu *n* copii va fi afectată de viteza de apariție prin crossing-over inegal şi de viteza de eliminare prin acțiunea selecției naturale. Dacă efectul numărului de copii este aşa de mic, încât poate fi ignorat, cum este cazul ADN satelit care nu codifică proteine, unul sau altul dintre cromozomi, purtând un număr diferit de copii ale genei, se va fixa în populație ca urmare a acțiunii driftului genetic. Crossing-overul inegal generează un număr variabil de copii cu probabilitate mai mică în cazul genelor unice, comparativ cu familiile multigenice, deci este mai probabil ca o populație să rămână la stadiul unei singure copii, pentru o perioadă mai îndelungată. Prin urmare, pentru acele regiuni ale cromozomilor care au o rată crescută de recombinare, copiile unice ale genelor sunt mai probabile decât secvențele repetate, dacă acestea din urmă nu sunt favorizate de selecția naturală. Secvențele înalt repetitive apar cu o probabilitate mai mare în acele zone ale cromozomilor care au o rată de recombinare scăzută. Aceasta ar putea explica abundența ADN mediu şi înalt repetitiv în apropierea centromerilor, la capetele cromozomilor şi pe cromozomul Y, toate având o rată scăzută a crossing-overului.

Mărimea familiilor de gene care "proliferează" via elemente transpozabile este determinată de ratele de transpoziție şi deleție. Walsh [21] a modelat matematic modificarea numărului de pseudogene procesate, de tipul celor care sunt inserate prin revers-transcripție, dar nu pot să-şi formeze propriile copii în continuare. Walsh a calculat procentul reprezentat de pseudogenele procesate din ADN total şi a constatat că ele pot reprezenta aproximativ 9% din ADN-ul total.

Controlul ADN egoist

După cum am văzut, elementele transpozabile pot fi considerate paraziți ai ADN gazdă. Cum poate fi prevenită multiplicarea lor nelimitată? Creşterea numărului de copii ale transpozonilor are două componente: creşterea în cadrul genomului şi răspândirea lor în populație (de la părinți la urmaşi). La organismele cu reproducere asexuată, evoluția numărului de copii presupune modificarea

frecvenței unor clone care prezintă un număr diferit de copii. Dacă elementele transpozabile scad adaptabilitatea gazdei, este mai probabilă fixarea unei clone ce conține mai puține copii ale transpozonului. Probabil din această cauză, procariotele prezintă mai puțin ADN mediu repetitiv decât eucariotele. La organismele cu înmulțire sexuată, recombinarea aduce în același genom inserții provenind de la indivizi diferiți, de aceea distribuția numărului de copii în populație este asemănătoare unui caracter cu determinism poligenic. Dacă elementele transpozabile nu afectează adaptabilitatea, se poate demonstra matematic că genomul va deveni saturat cu transpozoni. Totuşi, în general, elementele transpozabile au efecte negative asupra organismelor, datorită mutațiilor pe care le induc. Dacă adaptabilitatea gazdei scade o dată cu creşterea numărului de copii ale transpozonilor în genom, numărul mediu de copii va tinde spre echilibru între rata de apariție prin transpoziție şi rata de eliminare prin selecție. Echilibrul va fi stabil, cu excepția situației în care efectul nefavorabil per element creşte o dată cu numărul de elemente. Numărul de elemente transpozabile per genom poate atinge o valoare de echilibru scăzută, dacă rata de transpoziție a fiecărui element este reglată, de exemplu dacă rata de apariție scade pe măsură ce numărul de elemente creşte. La nivel genic, selecția va favoriza, dimpotrivă, elementele cu o rată de transpoziție mai mare. Imunitatea de transpoziție, în configurație cis, poate fi înțeleasă, în acest context, ca o competiție între transpozoni pentru anumite zone ale cromozomilor (asemănător competiției teritoriale între animale). Un element mutant, care poate exclude forma non-mutantă, va prezenta un avantaj selectiv, dar valoarea acestui avantaj este foarte scăzută (după unele calcule 10^4). Dacă o secvență mutantă reduce transpoziția în întregul genom (atât în configurație cis cât şi trans), atât a transpozonilor competitori cât şi a celor asemănători acestora, probabilitatea ca frecvența acestei secvențe să crească este foarte mică.

Selecția la nivelul întregului organism favorizează nu numai elementele transpozabile care reduc frecvența transpoziției, dar şi orice altă proprietate a organismului care are efect represor. În acelaşi timp, selecția la nivel genic va favoriza ADN-ul cel mai egoist. S-ar părea că, la nivelul organismului, se dă o luptă evolutivă între genomul gazdă şi elementele transpozabile, asemănătoare "cursei înarmării" coevolutive în care sunt antrenate prada şi prădătorul, parazitul şi gazda. Se poate spune că, în această luptă, ADN egoist este în avantaj.

Evoluția familiilor de gene

Caracteristica cea mai remarcabilă a familiilor de gene este aceea a uniformității membrilor lor, familii având mii de membri fiind foarte omogene în ceea ce priveşte secvențele de nucleotide. De exemplu, secvența NTS, redată anterior (Fig. 1), din gena pentru ARNr 18S şi 28S este dispusă la om pe 5 cromozomi diferiți, şi este foarte omogenă. Omogenitatea este valabilă numai pentru copiile prezente într-un organism individual. Divergența evolutivă a speciilor este însoțită de evoluția concertată a membrilor unei familii de gene. În cadrul unei specii, genele membre ale unei familii nu evoluează independent. Acest

fapt se poate explica prin aceea că toți membrii unei familii de gene, de la fiecare specie, descind dintr-o secvență de nucleotide ancestrală. Mecanismele intraspecifice, de omogenizare a membrilor unei familii de gene, sunt: transpoziția, crossing-overul inegal şi conversia genelor.

Evoluția concertată prin traspoziție

Acțiunea omogenizatoare a transpoziției este ilustrată de pseudogenele procesate, care nu mai sunt capabile să formeze copii adiționale. Toate pseudogenele procesate, apărute prin transpoziție, vor fi omogene. Familiile de gene procesate prezintă o diferență a secvențelor de nucleotide de numai 5-15%. În schimb, copiile multiple ale elementelor transpozabile cresc şansa mutațiilor, deci ele vor varia mai mult în secvența de nucleotide, cu cât numărul lor este mai mare.

Evoluția concertată prin schimb inegal

Crossing-overul inegal şi conversia genelor sunt mecanisme care pot determina evoluția concertată a familiilor de gene. Nu se ştie aproape nimic despre efectul acestora asupra adaptabilității, a variabilității secvențelor pentru care familiile de de gene devin omogene; dar, este de aşteptat ca mutațiile unor astfel de gene să afecteze adaptabilitatea şi unele caractere fenotipice. D o v e r [5] a introdus termenul de derivă moleculară pentru fixarea în populație a unei variante anume dintr-o familie de gene. Autorul menționat a presupus că mecanismele de omogenizare pot face ca specia să evolueze divergent în privința trăsăturilor fenotipice. Deriva moleculară ar putea determina speciația, dacă într-o populație se fixează o variantă dintr-o familie de gene care cauzează sterilitatea hibrizilor.

Evoluția adaptativă din perspectivă moleculară

S-au făcut puține legături între evoluția genomului şi evoluția caracterelor fenotipice. Majoritatea aspectelor legate de evoluția genomului (substituții silențioase, evoluția pseudogenelor, evoluția secvențelor netranscrise) nu au efecte fenotipice. Pentru a analiza evoluția la nivel molecular în legătură cu evoluția caracterelor fenotipice, este necesar să se analizeze evoluția genelor structurale şi a secvențelor lor reglatoare. O descriere la nivel molecular a evoluției necesită analiza modului în care evoluează structura şi funcția proteinelor, a modului în care evoluează organizarea lor în căi biochimice, a reglării expresiei lor în diferite țesuturi şi în diferite momente ale dezvoltării ontogenetice.

Evoluția genelor structurale şi a proteinelor

Majoritatea proteinelor sunt alcătuite din câteva sau mai multe subunități, regiuni continue, compacte ale macromoleculei care se disting spațial unele de altele. Adesea, subunitățile au funcții diferite sau au aceeaşi funcție care se desfăşoară, mai mult sau mai puțin, independent. Pentru multe proteine există o corespondență între subunități şi exonii care alcătuiesc gena respectivă. S-a sugerat

că, în acest caz, probabil gene separate inițial au fuzionat ulterior, în sensul că sunt transcrise împreună în ARNm. În acest proces, au putut să apară funcții enzimatice noi.

Duplicarea genelor, în totalitate sau numai pe anumite porțiuni, poate asigura proteinelor structuri şi funcții noi. Multe proteine conțin secvențe repetate de aminoacizi, ceea ce se poate interpreta ca fiind rezultatul alungirii genei prin duplicații interne succesive. Imunoglobulinele vertebratelor sunt molecule complexe care recunosc şi leagă molecule străine, denumite antigene. Recunoaşterea antigenului este funcția subunităților "variabile" ale proteinei, în timp ce subunitățile constante au rol în legarea antigenului. Omologia secvențelor acestor două tipuri de subunități indică probabilitatea apariției lor prin duplicarea unei gene primordiale.

Evoluția divergentă a funcției proteinelor

Majoritatea vastului repertoriu biochimic al organismelor vii pare să fi luat naştere prin divergența structurală şi funcțională a unor gene duplicate succesiv. De exemplu, majoritatea proteazelor la eucariote au secvențe de aminoacizi destul de similare pentru a sugera omologia lor. Aceste enzime includ proteazele digestive: tripsină, chemotripsină, carboxipeptidază, fosfolipază; în plus, câteva enzime cu rol în coagularea sângelui şi dizolvarea cheagurilor de sânge au secvențe înrudite cu proteazele digestive (Fig. 7).

Evoluția activității enzimelor

Adaptarea biochimică se poate realiza fie prin modificarea structurii unei enzime, fie prin modificarea activității acesteia, fie pe ambele căi. Se ştie, încă, destul de puțin despre evoluția activității enzimelor. Importanța cunoaşterii acestei evoluții se poate exemplifica prin cazul adaptării insectelor erbivore la compuşii toxici prezenți în plantele cu care se hrănesc. De exemplu, unele specii de plante din familia *Apiaceae* conțin furanocumarine liniare toxice, cum ar fi xantotoxina. Aceste plante sunt consumate de specii specializate de insecte, cum sunt larvele lepidopterului *Papilio polyxenes*. Această specie degradează rapid xantotoxina ingerată în compuşi lipsiți de toxicitate. O altă specie, *Spodoptera frugiperda*, care nu este adaptată la xantotoxină, poate degrada şi ea această moleculă, dar cu viteză mult mai mică. Activitatea de detoxificare a xantotoxinei este prezentă în țesuturile ambelor specii, dar este mult mai mare la nivelul tractului digestiv al larvelor de *Papilio polyxenes*. Specializarea trofică a acestei specii a fost însoțită de modificarea activității şi a specificității de țesut a unui mecanism biochimic care pare a fi larg răspândit la lepidoptere.

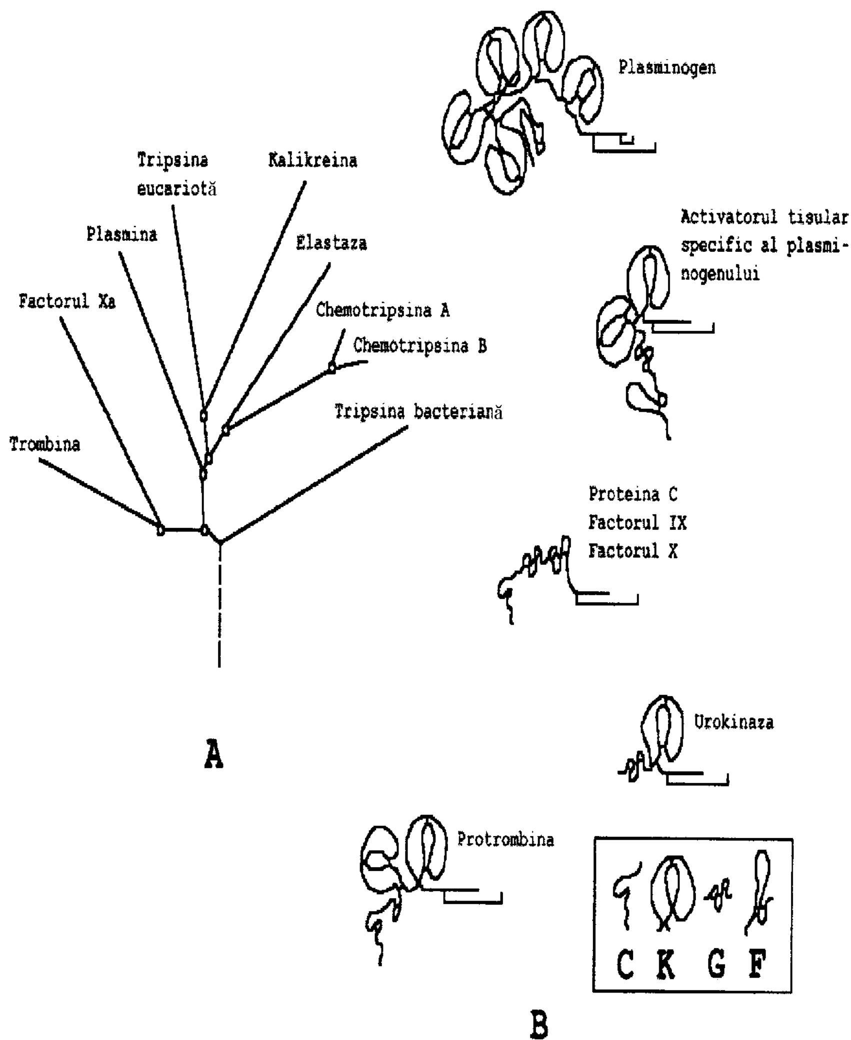


Fig. 7. *Reprezentarea arborelui filogenetic probabil al proteazelor (A) şi structura unor proteine în care subunitatea proteazică (reprezentată ca o bară) este asociată cu subunități neproteazice de tip C, K, G şi F (B). Aceste subunități apar în diferite combinații, sugerând că genele care le controlează s-au format prin asamblarea diferitelor tipuri de exoni* ([7] *modificat*).

Transferul orizontal de gene

Cu excepţia fluxului de gene care are loc prin hibridarea speciilor strâns înrudite, modificările genetice care apar la o anumită specie sunt, arareori, transmise la alte specii. Fluxul de informaţie genetică are loc vertical (în interiorul speciei şi prin speciaţie). Totuşi, unele date moleculare, mai recente, sugerează că, cel puţin ocazional, a avut loc pe parcursul evoluţiei şi un transfer de informaţie genetică între taxoni îndepărtaţi din punct de vedere filogenetic, deci pe orizontală [17]. În etapele timpurii ale evoluţiei, în stadiul apariţiei şi diversificării organismelor unicelulare un astfel de transfer trebuie să fi jucat un rol important. Fuziunea celulară şi înglobarea celulelor mai mici de către cele mai mari, prin acţiunea unor substanţe chimice sau a unor agenţi fizici (câmp electric), asemănătoare celor utilizate astăzi în experimentele de inginerie genetică, ar fi putut reprezenta mecanismele unui astfel de transfer [16]. Astfel, în istoria evolutivă a celulei eucariote un pas deosebit de însemnat a fost incorporarea de celule procariote ca şi simbionţi intracelulari (endosimbionţi), luând, astfel, naştere cloroplastele şi mitocondriile [14]. Asocierea devine completă, atunci când o parte sau întregul genom al endosimbiontului se integrează în genomul celulei gazdă. Aşa este cazul virusurilor şi plasmidelor. Virusurile şi plasmidele pot purta gene care afectează fenotipul gazdei şi, uneori, acţionează ca vectori de transfer între specii neînrudite. Un vector natural de gene este şi bacteria *Agrobacterium tumefaciens*, bacterie folosită ca vector şi în experimentele de transformare genetică (genică) a plantelor. Cazul cel mai uimitor de transfer al unei gene între specii diferite este cel al genei pentru leghemoglobină, moleculă caracteristică soiei (*Glycine max*) şi altor leguminoase. Raportul introni - exoni şi o mare parte a secvenţei de aminoacizi sunt aşa de asemănătoare cu hemoglobinele vertebratelor şi cele ale unor nevertebrate, încât pare posibil ca gena să fi fost transferată de la animale la plante [10]. Deocamdată, este greu de apreciat dacă acest transfer de informaţie genetică între specii a avut un rol important în evoluţie sau, dimpotrivă, este doar o curiozitate a evoluţiei. În schimb, transferul orizontal de gene generează temeri legate de eliberarea în mediul natural a organismelor transformate genetic în laboratoarele de inginerie genetică, prin posibila migrare a transgenelor de la o specie la alta.

Concluzii. Secvenţele de ADN variază, în cadrul populaţiilor, în privinţa situsurilor care alterează produsul final al genelor şi a celor care nu alterează acest produs.

Viteza de evoluţie a genelor este diferită, fiind mai mare pentru secvenţele de ADN lipsite de funcţie.

Familiile de gene iau naştere prin transpoziţie şi prin recombinare inegală; prin aceste mecanisme, precum şi prin conversie genică, familiile de gene evoluează frecvent în mod concertat.

Elementele transpozabile afectează organismul, îndeosebi, prin inducerea mutațiilor. Transpozonii se mențin în genom prin capacitatea lor transpozabilă (ADN egoist) şi nu prin conferirea unui avantaj organismului.

Fuziunea şi duplicarea internă a secvențelor de ADN pot da naştere la enzime cu funcții noi.

Modificarea mutațională, adaptativă, a structurii şi activității enzimelor este o caracteristică importantă a evoluției.

Alături de transferul de gene pe verticală, s-a demonstrat şi existența unui transfer pe orizontală, între specii. Importanța acestui transfer pentru evoluție nu este, încă, pe deplin lămurită.

Noile date de biologie moleculară au fost deja incorporate în teoriile evoluționiste.

BIBLIOGRAFIE

1. Baltimore, D., *Retroviruses and retrotransposons: the role of reverse transcription in shaping the eukaryotic genome*, "Cell", **40**, 1985, 481-482.
2. Campbell, A., *Transposons and their evolutionary significance*, în Nei, M., Koehn, R.K. (Eds.), *Evolution of Genes and Proteins*, p. 258-279, Sinauer Associates, Sunderland, Massachusetts, 1983.
3. Doolittle, W.F., Sapienza, C., *Selfish genes, the phenotypic paradigm and genomic evolution*, "Nature", **284**,1980, 601-603.
4. Dover, G.A., *Ignorant DNA?* "Nature", **285**, 1980, 618-620.
5. Dover, G.A., *Molecular drive: a cohesive mode of species evolution*, "Nature", **299,** 1982, 111-117.
6. Finnegan, D.J., Will, B.H., Bayev, A.A., Bowcock, A.M., Brown, L., *Transposables DNA sequences in eukaryotes*, în Dover, G.A., Flavell, R.B. (Eds.), *Genome Evolution*, p. 29-40, Acad. Press, New York, 1982.
7. Futuyma, D.J., *Evolutionary Biology*, Sinauer Associates, Sunderland, Massachusetts, 1986.
8. Gehring, W.J., *The homeo box: a key to the understanding of development?* "Cell", **40**, 1985, 3-5.
9. Hudson, R.R., Kaplan, J.L., *Statistical properties of the number of recombination events in the history of a sample of DNA sequences*, "Genetics", **111**, 1985, 147-164.
10. Jeffreys, A.J., *Evolution of globin genes*, în Dover, G.A., Flavell, R.B. (Eds.), *Genome Evolution,* p. 157-176, Acad. Press, New York, 1982.
11. Kreitman, M., *Nucleotide polymorphism at the alcohol dehydrogenase locus of* D. melanogaster, "Nature", **304**, 1983, 412-417.
12. Langley, C.H., Montgomery, E., Quattlebaum,W.F., *Restriction map variation in the Adh region of* Drosophila, "Proc. Natl. Acad. Sci. USA", **79**, 1982, 5631-5635.

13. Li, W.-H., Graur, D., *Fundamentals of Molecular Evolution*, Sinauer Associates, Sunderland, Massachusetts, 1991.
14. Margulis, L., *Symbiosis in Cell Evolution: Life and Its Environment on the Early Earth*, Freeman, San Francisco, 1981.
15. Orgel, L.E., Crick, F.H.C., *Selfish DNA: the ultimate parasite*, "Nature", **284**, 1980, 604-606.
16. Rákosy-Tican, L., Coman, N., *Cell fusion and its possible relevance for evolution*, "Evol. Adapt." (Cluj), **5**, 1995, 55-65.
17. Rákosy-Tican, L., Soran, V., Dragoş, N., *Gene and genome transfer during evolution*. "Evol. Adapt." (Cluj), **3**, 1988, 31 - 37.
18. Ridley, M., *Evolution*, Blackwell Sci. Publ., Boston, 1993.
19. Rubin, G.M., *Dispersed repetitive DNAs in* Drosophila, în Shapiro, J.A. (Ed.), *Mobile Genetic Elements*, p. 329-361, Acad. Press, New York, 1983.
20. Shapiro, J.A. (Ed.), *Mobile Genetic Elements*, Acad. Press, New York, 1983.
21. Walsh, J.B., *How many processed pseudogenes are accumulated in a gene family?* "Genetics", **110**, 1985, 345-364.

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ACTIVITATEA DEHIDROGENAZICĂ ÎN SOL CA TEST ECOTOXICOLOGIC PENTRU POLUANȚI ANORGANICI ŞI ORGANICI

ALIONA POPA*

SUMMARY. - Dehydrogenase Activity in Soil as Ecotoxicological Test for Inorganic and Organic Pollutants. In a laboratory experiment, samples of an alluvial soil were treated with salts of bivalent heavy metals (Hg^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+}, Co^{2+} and Cu^{2+}) and organic substances (the detergents "Rex" and "Ariel", the herbicide 2,4-D as well as with fuel oil and phenol) at the following three rates (per 100 g air-dry soil): 0.001, 0.01 and 0.1 g heavy metal; 0.1, 1 and 2 g detergent; 0.001, 0.01 and 0.1 g 2,4-D; 0.1, 1 and 5 ml fuel oil; and 0.001, 0.01 and 0.1 ml phenol. Untreated samples were the controls. After 24 hours of incubation at room temperature, actual and potential dehydrogenase activities of the samples were determined with the TTC reduction method.

The results have shown that both actual and potential dehydrogenase activities were inhibited by the heavy metals. Exceptionally, Zn^{2+} at its low and medium rates stimulated the actual activity. Hg^{2+} was the strongest inhibitor of both activities. The degree of inhibition correlated with the rate of heavy metal addition. The detergents at their low and medium rates stimulated both dehydrogenase activities; "Ariel", manufactured with addition of enzymes, was more stimulatory than "Rex" containing no added anzymes. At the high rate, "Rex" manifested an inhibitory effect, whereas "Ariel" had no effect on the two activities. 2,4-D and phenol behaved like the detergent "Rex". The fuel oil was inhibitory at its each rate and the degree of inhibition increased with increasing rate of fuel oil addition.

The conclusion has been drawn that dehydrogenase activity is a sensitive indicator of soil pollution and, consequently, it may be used as an ecotoxicological test for both inorganic and organic pollutants.

Activitatea dehidrogenazică a solului, considerată drept un indicator global al activității biologice a microorganismelor, a fost deja folosită ca test ecotoxicologic pentru evaluarea efectelor poluanților asupra microbiotei solului (v. de ex. lucrările de sinteză [1, 2, 4-23]).

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În prezenta lucrare ne-am propus să contribuim, prin date experimentale noi, la demonstrarea valorii activității dehidrogenazice a solului ca test ecotoxicologic pentru poluanți anorganici şi organici. Într-un experiment de laborator, am modelat poluarea solului prin tratarea probelor de sol cu săruri de metale grele bivalente (Hg^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+}, Co^{2+} şi Cu^{2+}), cu detergenții "Rex" şi "Ariel", ierbicidul 2,4-diclorfenoxiacetic (2,4-D), precum şi cu motorină şi fenol.

Material şi metode. Experimentul a fost efectuat cu un sol aluvial, nisipos, slab înțelenit (pH/H_2O=7.5; pH/KCl=7.0) de la Şodorât (Cluj). Probele de sol colectate de la adâncimea de 5-15 cm au fost lăsate să se usuce la aer, apoi au fost cernute (printr-o sită cu ochiuri de 2 mm). S-au cântărit câte 100 g sol la care s-au adăugat sărurile metalelor grele sub formă de soluții: $HgCl_2$, $Cd(NO_3)_2 \cdot 4H_2O$, $ZnCl_2$, $(CH_3COO)_2Pb \cdot 3H_2O$, $CoSO_4 \cdot 6H_2O$ şi $CuSO_4 \cdot 5H_2O$. Au fost preparate soluții în trei concentrații (0,001%, 0,1% şi 1%) din fiecare sare. Variantele experimentale au fost tratate cu câte 10 ml soluție din cele trei concentrații ale sărurilor metalelor grele prezentate în Tabelul 1.

Tabel 1

Schema variantelor experimentale realizate în vederea urmăririi activității dehidrogenazice în sol ca test ecotoxicologic pentru poluanți anorganici

Varianta	Sol (g)	Ionul metalic	Concentrația finală a ionului metalic (g/100 g sol)
V1	100		0,001
V2	100	Hg^{2+}	0,01
V3	100		0,1
V4	100		0,001
V5	100	Cd^{2+}	0,01
V6	100		0,1
V7	100		0,001
V8	100	Zn^{2+}	0,01
V9	100		0,1
V10	100		0,001
V11	100	Pb^{2+}	0,01
V12	100		0,1
V13	100		0,001
V14	100	Co^{2+}	0,01
V15	100		0,1
V16	100		0,001
V17	100	Cu^{2+}	0,01
V18	100		0,1
V19	100	-	-

Din fiecare substanță organică studiată au fost preparate soluții apoase în trei concentrații. Eşantioanele de sol au fost tratate cu câte 10 ml soluție din cele trei concentrații ale substanțelor organice, încât în variantele experimentale s-a ajuns la concentrațiile finale ale substanțelor organice prezentate în Tabelul 2. Cele trei cantități de motorină au fost dizolvate în câte 10 ml acetonă cu care s-a tratat solul. După evaporarea acetonei, eşantioanele cercetate au fost completate cu câte 10 ml apă distilată.

Tabel 2

Schema variantelor experimentale realizate în vederea urmăririi activității dehidrogenazice în sol ca test ecotoxicologic pentru poluanți organici

Varianta	Sol (g)	Substanța organică	Concentrația finală a substanței organice (g sau ml/100 g sol)
V1	100		0, 1 g
V2	100	Detergent "Rex" (fără enzime)	1 g
V3	100		2 g
V4	100		0, 1 g
V5	100	Detergent "Ariel" (cu enzime)	1 g
V6	100		2 g
V7	100		0,001 g
V8	100	2,4-D	0,01 g
V9	100		0,1 g
V10	100		0,1 ml
V11	100	Motorină	1 ml
V12	100		5 ml
V13	100		0,001 ml
V14	100	Fenol	0,01 ml
V15	100		0,1 ml
V16	100	Martor	-

Eşantioane de sol netratate au servit ca martori.

Variantele experimentale astfel realizate au fost incubate la temperatura laboratorului, timp de 24 ore, după care s-au făcut analizele enzimatice. Activitatea dehidrogenazică (actuală şi potențială) a fost determinată prin metoda reducerii TTC [3] şi se exprimă în mg formazan/3 g sol (substanță uscată)/24 ore la 37^0C.

Rezultate. Rezultatele efectului poluanților anorganici asupra activității dehidrogenazice în sol sunt prezentate în Fig. 1 şi 2. Se evidențiază formarea formazanului în majoritatea amestecurilor de reacție care au conținut TTC. Excepție face varianta de sol aluvial tratată cu concentrația maximă (0,1%) a ionului Hg^{2+}. În acest caz este consemnată o inhibiție totală a activității dehidrogenazice actuale. Este evident efectul negativ al ionilor metalici (în cele trei concentrații studiate) asupra activității dehidrogenazice actuale şi potențiale în sol.

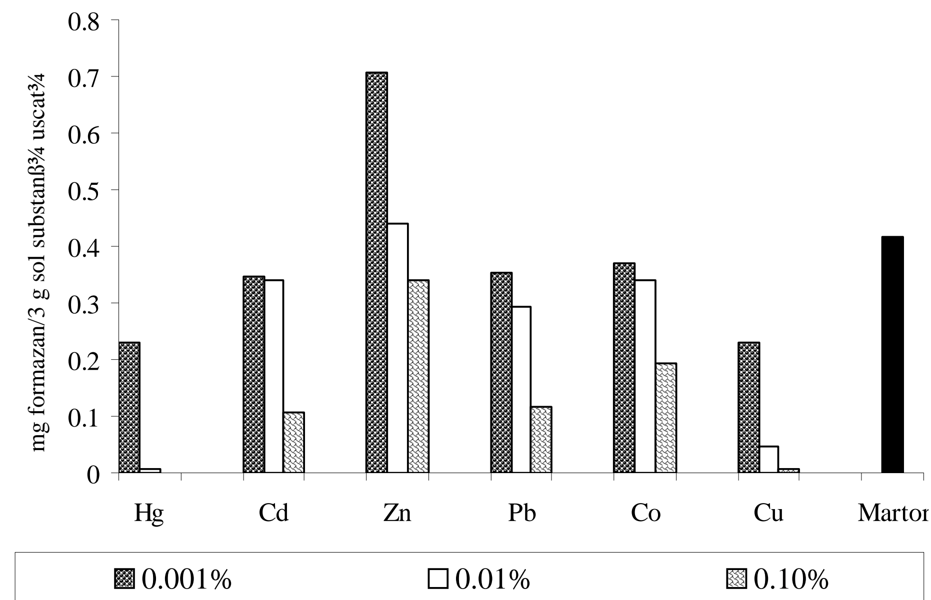

Fig. 1. *Activitatea dehidrogenazică actuală determinată prin metoda TTC - test ecotoxicologic pentru poluanți anorganici într-un sol aluvial.*

După cum se observă în Fig. 1, cel mai toxic ion s-a dovedit a fi Hg^{2+}, activitatea dehidrogenazică actuală fiind puternic inhibată de concentrația 0,01% a acestuia şi total inhibată de concentrația 0,1%. În ordinea descrescătoare a toxicității față de activitatea dehidrogenazică în solul studiat urmează ionul Cu^{2+}. La concentrații ale acestuia de 0,01 şi 0,1%, activitatea dehidrogenazică actuală a fost abia decelabilă. Excepție de la efectul inhibitor al ionilor metalelor grele asupra activității dehidrogenazice în sol face ionul Zn^{2+}. La concentrația 0,01% a ionului se înregistrează o stimulare nesemnificativă a activității dehidrogenazice actuale, iar la concentrația 0,001%, activitatea dehidrogenazică actuală a crescut de 2,1 ori față de martor.

Se poate afirma că adaosul de glucoză a stimulat activitatea microorganismelor care sintetizează dehidrogenazele, indiferent de adaosul ionilor metalici. Adică valorile activității dehidrogenazice potențiale sunt mult mai mari decât acelea ale activității dehidrogenazice actuale.

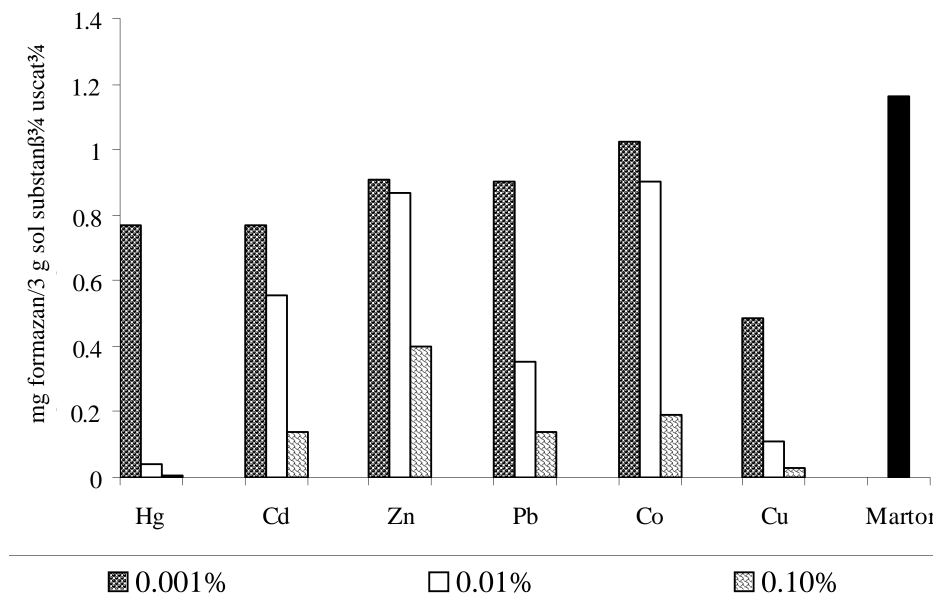

Fig. 2. *Activitatea dehidrogenazică potențială determinată prin metoda TTC - test ecotoxicologic pentru poluanți anorganici într-un sol aluvial.*

Comparativ cu martorul, în fiecare eşantion tratat cu unul din cei 6 ioni testați, activitatea dehidrogenazică potențială a atins un nivel inferior. Cele mai mici valori au fost consemnate la variantele tratate cu concentrațiile 0,01 şi 0,1% ale ionilor Hg^{2+} şi Cu^{2+}. Ionul Co^{2+} s-a dovedit a fi cel mai puțin toxic, la concentrațiile 0,01 şi 0,001% înregistrându-se cele mai ridicate valori ale activității dehidrogenazice potențiale dintre eşantioanele tratate cu ioni metalici.

La toate variantele de sol studiate se constată existența unei corelații negative lineare între concentrația ionului de metal şi intensitatea activității dehidrogenazice, atât actuale, cât şi potențiale. Valorile coeficienților de corelație sunt cuprinse între $r = -0,590$ ($p > 0,05$) la variantele tratate cu Zn^{2+} şi $r = -0,985$ ($0,05 > p > 0,01$) la variantele tratate cu Co^{2+}.

Rezultatele prezentate în Fig. 1 şi 2 ne îndreptăţesc să afirmăm că testul dehidrogenazic poate fi folosit cu succes la estimarea calităţii biologice a solurilor, la aprecierea gradului lor de poluare cu substanţe anorganice.

Patru dintre cele 5 substanţe organice testate (detergenţii "Rex" şi "Ariel", 2,4-D şi fenolul), în concentraţiile mici şi mijlocii folosite, stimulează activitatea dehidrogenazică actuală şi potenţială în solul aluvial studiat. Concentraţiile cele mai mari ale celor patru substanţe organice inhibă în toate cazurile ambele tipuri de activitate dehidrogenazică (Fig. 3 şi 4).

Dintre cei doi detergenţi, după cum se observă, "Ariel" are efectul stimulator cel mai puternic. Această observaţie denotă biodegradabilitatea mai mare a detergentului "Ariel", probabil datorită adaosului de enzime la conţinutul acestui detergent. Chiar dacă nu se cunoaşte natura acestor enzime, fără îndoială că este vorba despre hidrolaze, produşii rezultaţi în urma activităţii lor oferind o sursă suplimentară de nutrienţi pentru microorganismele din sol responsabile de activitatea dehidrogenazică a acestuia.

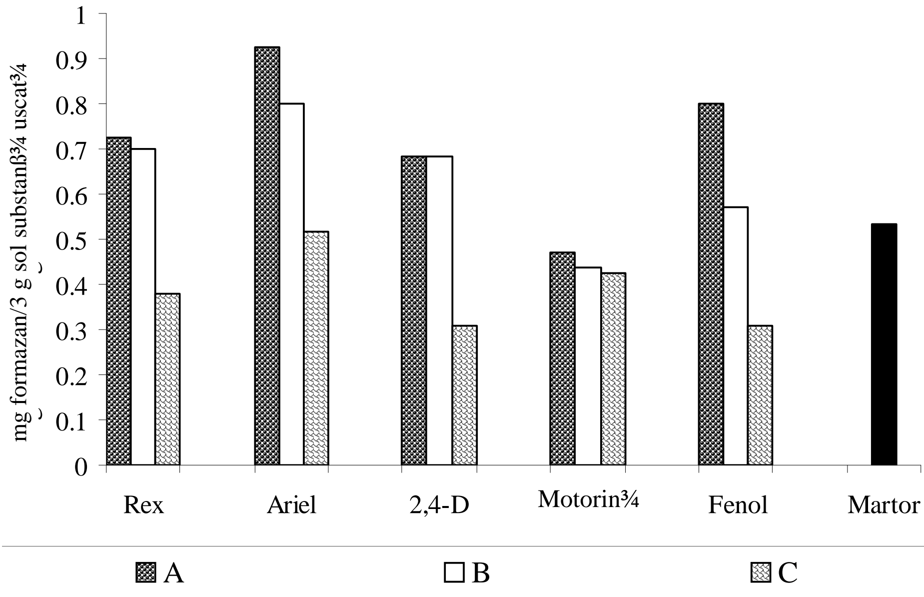

Fig. 3. *Activitatea dehidrogenazică actuală determinată prin metoda TTC - test ecotoxicologic pentru poluanţi organici într-un sol aluvial.*
Concentraţiile substanţelor organice: "Rex" şi "Ariel": A = 0,1%, B = 1%, C = 2%; 2,4-D şi Fenol: A = 0,001%, B = 0,01%, C = 0,1%; Motorină: A =0,1%, B = 1%, C = 5%.

Astfel, la concentrația de 0,1% a detergentului, activitatea dehidrogenazică actuală a crescut de 1,4 ori ("Rex"), respectiv de 1,73 ori ("Ariel"). Activitatea dehidrogenazică potențială a fost stimulată uşor, ea crescând de 1,13 ori ("Rex"), respectiv de 1,3 ori ("Ariel"), în toate cazurile creşterile fiind raportate la martorul netratat. Rezultatele analizelor noastre recomandă detergentul "Ariel" ca fiind un produs superior din punct de vedere ecologic.

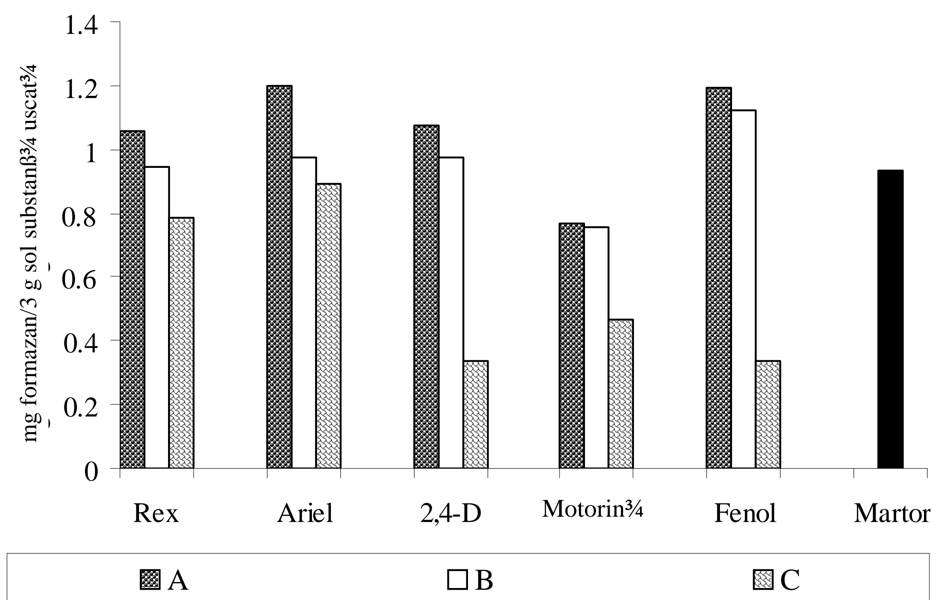

Fig. 4. *Activitatea dehidrogenazică potențială determinată prin metoda TTC - test ecotoxicologic pentru poluanți organici într-un sol aluvial.*
Concentrațiile substanțelor organice: "Rex" şi "Ariel": A = 0,1%, B = 1%, C = 2%; 2,4-D şi Fenol: A = 0,001%, B = 0,01%, C = 0,1%; Motorină: A =0,1%, B = 1%, C = 5%.

Ierbicidul hormonal 2,4-D şi fenolul se înscriu pe aceeaşi curbă a efectelor asupra activității dehidrogenazice în sol (stimulare la 0,001 şi 0,01%, inhibiție la 0,1%), iar valorile lor absolute sunt apropiate.

Al cincilea compus organic testat (motorina) inhibă activitatea dehidrogenazică, atât cea actuală cât şi cea potențială, la toate cele trei concentrații folosite. Această constatare subliniază odată în plus efectul deosebit de nociv asupra activității microbiene în sol, exercitat de poluanții derivați din petrol.

Între intensitatea activităţii dehidrogenazice şi concentraţia substanţelor organice testate s-a stabilit existenţa unor corelaţii negative. Valorile limită ale acestei corelaţii sunt $r = -0,398$ ($p > 0,05$) (activitatea dehidrogenazică actuală la varianta tratată cu detergentul "Ariel") şi $r = -0,980$ ($0,05 > p > 0,01$) (activitatea dehidrogenazică potenţială la varianta tratată cu ierbicidul hormonal 2,4-D).

Deci, intensitatea activităţii dehidrogenazice a solului scade odată cu creşterea concentraţiei substanţei organice. Totuşi, pentru concentraţiile testate de noi, efectul inhibitor, comparativ cu martorul netratat, este manifest numai la concentraţiile cele mai mari, cu excepţia amintită a motorinei. Existenţa corelaţiei negative între cei doi parametri (intensitatea activităţii dehidrogenazice şi concentraţia substanţei organice) arată fără dubiu că, pe măsura creşterii concentraţiei peste limitele experimentate de noi, toate substanţele testate se comportă ca veritabili poluanţi, care au drept efect scăderea intensităţii activităţii microbiene globale în sol. Deci, metoda poate fi recomandată ca test ecotoxicologic pentru poluanţi organici.

Concluzii. 1. Testul reducerii TTC, folosit ca test ecotoxicologic pentru poluanţi anorganici, demonstrează efectul inhibitor al ionilor metalici testaţi, mai ales la concentraţiile 0,01 şi 0,1% ale acestora asupra activităţii dehidrogenazice a solului. Ionul Hg^{2+} s-a dovedit a avea cel mai puternic efect toxic. La concentraţia de 0,1%, activitatea dehidrogenazică actuală este total inhibată.

2. Unicul caz în care nu numai că nu se constată vreun efect inhibitor, ci dimpotrivă, este stimulată activitatea dehidrogenazică actuală, este acela al ionului Zn^{2+} în concentraţie de 0,001%.

3. Folosind activitatea dehidrogenazică în sol ca test ecotoxicologic pentru poluanţi organici, am reuşit să facem o apreciere a calităţii ecologice a detergenţilor "Ariel" şi "Rex". Astfel, putem afirma că din punct de vedere ecologic, detergentul "Ariel" este superior detergentului "Rex", probabil datorită adaosului de enzime la conţinutul primului.

4. Cu excepţia motorinei, celelalte patru substanţe organice în concentraţiile mici şi mijlocii folosite stimulează activitatea dehidrogenazică actuală şi potenţială în solul studiat. Concentraţiile cele mai mari ale acestor patru substanţe organice inhibă în toate cazurile ambele tipuri de activitate dehidrogenazică.

5. Atât ierbicidul hormonal 2,4-D, cât şi fenolul, administrate în concentraţii de 0,001 şi 0,01%, stimulează activitatea dehidrogenazică, în schimb în concentraţie de 0,1% sunt foarte toxice, ambele substanţe determinând scăderea de 1,73 de ori a activităţii dehidrogenazice actuale, respectiv de 2,75 de ori a activităţii dehidrogenazice potenţiale, comparativ cu martorul netratat.

6. Motorina inhibă activitatea dehidrogenazică actuală şi potenţială la toate concentraţiile aplicate (0,1, 1 şi 5%), dar niciodată atât de puternic cum o inhibă concentraţiile maxime de 2,4-D şi fenol.

7. Între intensitatea activităţii dehidrogenazice şi concentraţiile substanţelor anorganice şi organice s-au stabilit corelaţii negative. Cele mai semnificative corelaţii s-au stabilit în cazul activităţii dehidrogenazice potenţiale la variantele tratate cu Co^{2+} ($r = -0,985$; $0,05 > p > 0,01$), respectiv la variantele tratate cu 2,4-D ($r = -0,980$; $0,05 > p > 0,01$).

BIBLIOGRAFIE

1. Anderson, J. R., *Pesticide effects on non-target soil microorganisms*, în Hill, I. R., Wright, S. J. (Eds.), *Pesticide Microbiology*, p. 313-533, Acad. Press, London, 1978.
2. Bååth, E., *Effects of heavy metals in soil on microbial processes and populations (a review)*, "Water, Air, Soil Pollut"., **47**, 1989, 335-379.
3. Casida, L. E. Jr., Klein, D. A., Santoro, T., *Soil dehydrogenase activity*, "Soil Sci.", **98**, 1964, 371-376.
4. Doelman, P., *Lead and terrestrial microbiota*, în Nriagu, J. O. (Ed.), *The Biogeochemistry of Lead in the Environment*, p. 343-353, Elsevier, Amsterdam, 1978.
5. Domsch, K. H., Jagnow, G., Anderson, T.-H., *An ecological concept for the assessment of side-effects of side-affects of agrochemicals on soil microorganisms*, "Residue Rev.", **86**, 1983, 65-105.
6. Francis, A. J., *Effects of acidic precipitation on soil microorganisms*, în Adriano, D. C., Johnson, A. H. (Eds.), *Acidic Precipitation, Vol. 2, Biological and Ecological Effects*, p. 305-326, Springer, New York, 1989.
7. Galiulin, R. V., *Indikaţiia zagriazneniia pociv tiajelîmi metallami putem opredeleniia aktivnosti pocivennîh fermentov*, "Agrohimiia", Nr. 11, 1989, 133-142.
8. Gianfreda, L., Bollag, J.-M., *Influence of natural and anthropogenic factors on enzyme activity in soil*, în Stotzky, G., Bollag, J.-M. (Eds.), *Soil Biochemistry, Vol.9,* p. 123-193, Dekker, New York, 1996.
9. Giménez Verdú, I., *Efecto inhibidor de algunos biocidas sobre la actividad enzimática del suelo*, "Bol. Serv. Plagas" (Madrid), **10**, 1984, 257-298.
10. Hicks, R. J., Stotzky, G., Van Voris, P., *Review and evaluation of the effects of xenobiotic chemicals on microorganisms in soil*, "Adv. Appl. Microbiol.", **35**, 1990, 195-253.

11. Ismailov, N. M., *Mikrobiologhiia i fermentativnaia aktivnost neftezagriaznennîh pociv*, în Glazovskaia, M. A. (Red.), *Vosstanovlenie neftezagriaznennîh ekosistem*, p. 42-56, Izd. Nauka, Moskva, 1988.
12. Kinzel, H., Baumgarten, A., Spadinger, K., Zechmeister-Boltenstern, S., *Influence of environmental pollution on nitrogen metabolism and other metabolic activities in forest soils*, în Szabolcs, I. (Ed.), *Ecological Impact of Acidification*, p. 69-77, Magy. Tud. Akad., Budapest, 1988.
13. Kireeva, N. A., *Mikrobiologhiceskie proțessî v neftezagriaznennîh pocivah*, p. 1-172, Başk. Gos. Univ., Ufa, 1995.
14. Kiss, S., Paşca, D., Drăgan-Bularda, M., *Enzymology of Disturbed Soils,* p. 1-336, Elsevier, Amsterdam, 1998.
15. Klein, D. A., Sorensen, D. L., Redente, E. F., *Soil enzymes: a predictor of reclamation potential and progress*, în Tate, R. L. III, Klein, D. A. (Eds.), *Soil Reclamation Processes. Microbiological Analyses and Applications*, p. 141-171, Dekker, New York, 1985.
16. Kraatz, M., Schumacher, B., Emmerling, C., Schröder, D., *Development of soil microbial activities, soil fauna and humic matter during remediation and recultivation of PAH-contaminated soil*, "II. Euro-Forum Altlasten (Saarbrücken, 1993)", **1**, 1993, 255-270.
17. Malkomes, H.-P., *Existing alternative tests to measure side-effects of pesticides on soil microorganisms: dehydrogenase activity*, "Toxicol. Environ. Chem.", **30**, 1991, 167-176.
18. Nannipieri, P., Badalucco, L., Landi, L., Pietramellara, G., *Measurement in assessing the risk of chemicals to the soil ecosystem*, în Zelikoff, J. T. (Ed.), *Ecotoxicology: Responses, Biomarkers and Risk Assessment*, Chapter 34, SOS Publ., Fair Haven, New Jersey, 1997.
19. Ohtonen, R., Markkola, A. M., Heinonen-Tanski, H., Fritze, H., *Soil biological parameters as indicators of changes in Scots pine forests* (Pinus sylvestris *L.) caused by air pollution*, în Kauppi, P., Kenttämies, K., Anttila, P. (Eds.), *Acidification in Finland*, p. 373-393, Springer, Berlin, 1990.
20. Schäffer, A., *Pesticide effects on enzyme activities in the soil ecosystem*, în Bollag, J.-M., Stotzky, G. (Eds.), *Soil Biochemistry, Vol. 8*, p. 273-340, Dekker, New York, 1993.
21. Simon-Sylvestre, G., Fournier, J.-C., *Effects of pesticides on the soil microflora*, "Adv. Agron.", **31**, 1979, 1-92.
22. Tyler, G., *Heavy metals in soil biology and biochemistry*, în Paul, E. A., Ladd, J. N. (Eds.), *Soil Biochemistry, Vol. 5*, p. 371-414, Dekker, New York, 1981.
23. Wang, X., Bartha, R., *Effects of bioremediation on toxicity, mutagenesis, and microbiota in hydrocarbon-polluted soils*, în Wise, D. L., Trantolo, D. J. (Eds.), *Remediation of Hazardous Waste Contaminated Soils*, p. 175-197, Dekker, New York, 1994.

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MICROORGANISME IZOLATE DIN FILOSFERA PLANTELOR DE OVĂZ CRESCUTE PE MATERIAL DE HALDĂ DE LA O MINĂ DE PLUMB ŞI ZINC

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SUMMARY. - Microorganisms Isolated from the Phyllosphere of Oat Plants Grown on Lead and Zinc Mine Spoils. Microflora of oat (*Avena* sp.) leaves was studied. Malt extract agar was used as nutrient medium, the inoculated media were incubated under aerobic conditions. It has been found that the colony-forming microorganisms belong to six different groups: rod-shaped Gram-negative bacteria, nonsporogenous Gram-positive bacteria, endospore-forming Gram-positive bacteria, Gram-positive cocci, yeasts and filamentous fungi. The nutrient medium used made it possible to evidence a more frequent occurrence of rod-shaped Gram-positive bacteria and Gram-positive cocci than that expectable based on literature data.

Frunzele plantelor superioare sunt colonizate de diferite microorganisme saprofite, care alcătuiesc o microfloră epifită foarte specifică, cunoscută sub denumirea de filosferă. Microorganismele de pe suprafaţa frunzelor sănătoase joacă un rol important în viaţa plantei gazdă, exercitând efecte favorabile sau acţionând în dezavantajul plantei. Unele microorganisme filosferice fixează N_2 atmosferic şi o parte din azotul fixat poate fi utilizată de plantă. Microorganismele filosferice intervin în protecţia plantei gazdă prin stimularea plantei de a produce fitoalexine şi prin capacitatea lor de a intra în competiţie cu agenţii patogeni. Unele microorganisme saprofite eliberează compuşi organici şi anorganici care sunt accesibili plantei, altele produc substanţe care reglează creşterea plantelor.

Microorganismele filosferice degradează stratul ceros extern al membranei cuticulare, determinând creşterea permeabilităţii şi astfel, probabil, îmbătrânirea timpurie a frunzelor [5, 9, 11-13]. Unele bacterii de pe suprafaţa frunzelor, în special cele din genurile *Pseudomonas* şi *Erwinia*, la temperaturi scăzute secretă pe suprafaţa foliară o proteină de nucleare (ice-nucleation protein) care serveşte ca nucleu în jurul căruia se depun cristale de gheaţă. La temperaturi cuprinse între -2 şi -5^{0}C, pot apărea deteriorări grave care duc la

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lezarea şi moartea frunzelor, respectiv a plantelor [10, 12]. Din punct de vedere ecologic, cea mai importantă funcţie a microflorei filosferice este legată de capacitatea lor de a utiliza unele substanţe poluante ajunse pe frunze din aerul atmosferic, neutralizând astfel acţiunea acestora [12]. Modificarea numărului bacteriilor şi, în general, schimbările apărute în compoziţia microflorei filosferice pot furniza informaţii valoroase referitoare la gradul de poluare al mediului [12]. Numeroase microorganisme din filosferă sunt rezistente la anumite metale grele, iar în cazul unor metale (de ex. Hg) au capacitatea de a transforma şi elimina din mediu aceste metale. Aceste microorganisme pot fi utilizate ca bioindicatori ai poluării mediului cu diferite metale [1-3].

Lucrarea de faţă prezintă grupele de microorganisme care s-au putut izola din filosfera unor plante de cultură crescute pe material de haldă provenit de la o mină de plumb şi zinc şi conţine date referitoare la diversitatea populaţiilor filosferice în funcţie de compoziţia variantelor experimentale.

Materiale şi metode. Pentru studierea microflorei filosferice s-au folosit plante de ovăz (*Avena* sp.) obţinute din seminţe semănate pe material de haldă provenit de la mina de plumb şi zinc din Rodna (jud.Bistriţa-Năsăud). Materialul de haldă utilizat conţine doar urme de substanţe organice, este lipsit de compuşi cu azot şi foarte sărac în compuşi cu fosfor (0,004 g P_2O_5/100 g substanţă uscată). Conţinutul în metale grele este relativ ridicat (0,09 g Pb, 0,15 g Zn, 0,26 g Mn, 1,02 Ti şi 3,41 g Fe/100 g substanţă uscată). Conţinutul în Ca, Mg şi K prezintă, de asemenea, valori mari (13,2 g CaO, 5,2 g MgO şi 1,07 g K_2O/100 g substanţă uscată), iar pH-ul este 8,5 [7]. La materialul de haldă s-au adăugat: sol nativ cu fertilitate scăzută de la baza haldei de steril, cernoziom, adsorbanţi, substanţe organice şi anorganice conform Tabelului 1.

Solul nativ conţine 4,43% humus, 0,183% N total, 1,51 mg P_2O_5, 10,46 mg K_2O şi 2,83 mg carbonaţi/100 g substanţă uscată; pH=7,45.

Seminţele au fost semănate în două loturi. Primul lot de seminţe a fost semănat la 27 februarie 1998, iar cel de-al doilea lot la 9 iunie 1998. Lotul al doilea a fost semănat pe acelaşi material de haldă pe care au crescut plantele primului lot, după îndepărtarea resturilor cu excepţia celor radiculare. În cazul fiecărui lot, s-au efectuat periodic studii asupra compoziţiei populaţiilor filosferice dezvoltate pe suprafaţa plantelor de ovăz.

Cultivarea microorganismelor filosferice s-a efectuat pe mediul de malţ agarizat (Merck, lot 3857020), care a fost preparat din 4,8 g malţ agarizat, 0,5 g agar-agar şi 100 ml apă distilată.

Mediul de cultură s-a sterilizat prin autoclavare la 121^{0}C, timp de 30 minute. Mediul de cultură steril s-a repartizat în cutii Petri sterile, iar după solidificarea mediului cutiile s-au însămânţat cu 0,1 ml de lichid obţinut prin spălarea unei frunze de ovăz în 2 ml de apă de robinet fiartă şi sterilizată la 121^{0}C, timp de 30 minute. În cazul fiecărei variante s-au inoculat câte două cutii Petri. Incubarea a avut loc la temperatura de 28^{0}C şi a durat 72 ore.

Compoziția variantelor experimentale

Nr.	Material de haldă (g)	Sol nativ (g)	Cernoziom(g)	Adsorbanți (g)					Fertilizare organică (g)		Fertilizare minerală NPK	Număr de semințe	Apă de robinet (ml)
				Cărbune activ	Caolin	Bentonit	Zeolit	Cărămidă	Gunoi de grajd	Pulbere de fân			
1	413,2	20,6	-	-	-	-	-	-	34,5	-	0,37 g	15	137,7
2	413,2	-	20,6	-	-	-	-	-	34,5	-	NH_4NO_3+	15	137,7
3	413,2	-	-	20,6	-	-	-	-	34,5	-	0,33 g	15	137,7
4	413,2	-	-	-	20,6	-	-	-	34,5	-	KH_2PO_4+	15	137,7
5	413,2	-	-	-	-	20,6	-	-	34,5	-	0,16 g	15	137,7
6	413,2	-	-	-	-	-	20,6	-	34,5	-	KCl	15	137,7
7	413,2	-	-	-	-	-	-	20,6	34,5	-		15	137,7
8	413,2	20,6	-	-	-	-	-	-	-	6,9		15	137,7
9	413,2	-	20,6	-	-	-	-	-	-	6,9	0,37 g	15	137,7
10	413,2	-	-	20,6	-	-	-	-	-	6,9	NH_4NO_3+	15	137,7
11	413,2	-	-	-	20,6	-	-	-	-	6,9	0,33 g	15	137,7
12	413,2	-	-	-	-	20,6	-	-	-	6,9	KH_2PO_4+	15	137,7
13	413,2	-	-	-	-	-	20,6	-	-	6,9	0,16 g	15	137,7
14	413,2	-	-	-	-	-	-	20,6	-	6,9	KCl	15	137,7
15	413,2	-	-	-	-	-	-	-	34,5	-		15	137,7
16	413,2	-	-	-	-	-	-	-	-	6,9		15	137,7
17	413,2	-	-	-	-	-	-	-	-	-	-	15	137,7

Tabel 2

Caracterizarea plantelor crescute pe material de haldă

Parametrii dezvoltării	Nr. lot	Varianta experimentală																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Semințe germinate (%)	1	93,33	93,33	93,33	93,33	80,0	100	93,33	100	100	100	93,3	93,33	93,33	86,66	93,33	80	93,33
	2	100	100	100	100	100	100	100	93,33	93,33	100	100	100	100	100	100	93,33	93,33
Lungimea frunzelor (cm)	1	12,63	10,30	13,13	11,70	9,93	10,30	13,20	12,63	11,00	13,50	11,40	11,53	12,36	9,56	12,53	12,16	9,43
	2	17,53	18,43	16,03	15,21	17,88	21,40	15,31	15,00	15,41	21,28	19,28	19,26	17,26	16,05	18,98	16,61	11,13

După incubare, s-a studiat aspectul macroscopic şi microscopic al coloniilor de microorganisme dez-voltate. Studiul macroscopic s-a efec-tuat luând în considerare anumite ca-ractere morfologice ale coloniilor (for-mă, margini, luciu etc.). Pentru studiul microscopic s-au preparat frotiuri care s-au colorat după metoda Gram şi s-au examinat la microscopul cu imersie.

Pe baza caracterelor morfolo-gice şi tinctoriale ale microorganis-melor filosferice izolate s-au putut delimita 6 grupe: bastonaşe Gram-negative, bastonaşe Gram-pozitive nesporogene, bastonaşe Gram-pozitive endosporogene, coci Gram-pozitivi, drojdii şi fungi filamentoşi.

Rezultate. Creşterea şi dez-voltarea plantelor este influențată în mare măsură de cantitatea şi calitatea substanțelor nutritive existente în sol şi de gradul de poluare al mediului înconjurător. Caracteristicile mediului afectează nu numai ciclul de viață al plantelor gazdă, dar şi compoziția populațiilor rizosferice şi filosferice ce se dezvoltă pe seama excrețiilor radi-culare, respectiv foliare ale plantelor.

Plantele de ovăz cultivate pe materialul de haldă provenit de la mi-na din Rodna au prezentat variații în dezvoltarea lor în funcție de compoziția variantelor experimentale (Tabel 2). În cazul primului lot, procentul de ger-minație a fost mai scăzut, iar plantele erau mai puțin dezvoltate şi nu au ajuns în faza de înflorire. La cel de-al doilea lot, procentul de germinație la majoritatea variantelor a

fost de 100%, plantele erau bine dezvoltate şi au ajuns în faza de înflorire, pe multe apărând chiar şi spicuri. Aceste rezultate s-ar putea datora faptului că în cazul primei serii experimentale variantele erau afectate de un grad ridicat de poluare cu metale grele şi condiţiile de cultivare erau nefavorabile, ceea ce a influenţat negativ creşterea normală a plantelor, acestea neputând supravieţui timp îndelungat. Plantele din lotul al doilea s-au dezvoltat pe acelaşi material de haldă ca şi plantele primului lot, dar cultivarea lor s-a efectuat în perioada de vară şi, în acelaşi timp, ele au beneficiat şi de substanţele anorganice şi organice eliberate de plantele din lotul precedent, compuşi care probabil au exercitat un efect favorabil asupra creşterii acestor plante.

Tabel 3

Microorganismele izolate din filosfera plantelor de ovăz

Tipuri de colonii	Grupa	Aspectul macroscopic al coloniilor	Aspectul microscopic al celulelor
1	Bacterii	Colonii galbene, lucioase, bombate, netede, cu margini regulate	Bastonaşe Gram-negative, cu lungimea de 1,7-2,5 μm
2	Bacterii	Colonii de culoare roz, lucioase, bombate, netede, cu margini regulate	Bastonaşe Gram-negative, cu lungimea cuprinsă între 1,5 şi 2,6 μm
3	Bacterii	Colonii albe, lucioase, bombate, netede, cu margini regulate	Coci Gram-pozitivi, cu dimensiuni de 0,8-1,3 μm
4	Bacterii	Colonii alb-cenuşii, mate, rugoase, plate, cu centrul adâncit, margini ondulate	Bastonaşe Gram-pozitive, având lungimi de 1,8-3,2 μm, se grupează în lanţuri, endosporogene
5	Bacterii	Colonii alb-gălbui, plate, cu luciu slab, margini neregulate	Bastonaşe Gram-pozitive, cu lungimea de 1,5-3,0 μm, nesporogene
6	Drojdii	Colonii de culoare portocalie, lucioase, netede, bombate, cu margini regulate	Celule elipsoidale, cu dimensiuni de 4-4,5 μm
7	Drojdii	Colonii albe, lucioase, netede, bombate, cu margini regulate	Celule ovale, cu dimensiuni de 2,5-3,3 μm
8	Drojdii	Colonii albe, lucioase, netede, bombate, cu margini regulate	Celule elipsoidale, cu dimensiuni de 4,5-6,5 μm
9	Micromicete	Colonii albe, pufoase, cu spori de culoare neagră	Hife segmentate, l=7,0-8,0 μm, ϕ=1,5-4,0 μm, spori alungiţi cu lungimea de 2,5-3,0 μm
10	Micromicete	Colonii verzi,cu margini albe	Hife nesegmentate, ϕ=3,0-4,0 μm, sporangii negre cu dimensiuni de 16-20 μm, spori sferici de 1,5-2,0 μm
11	Micromicete	Colonii negre	Hife nesegmentate, ϕ=1,5-2,0 μm, spori sferici cu ornamentaţii şi dimensiuni de 1,5-2,0 μm

Microorganismele izolate de pe suprafaţa foliară a plantelor de ovăz reprezintă numai o parte din microorganismele filosferice, deoarece nu există

mediu de cultură cu o compoziție favorabilă pentru dezvoltarea tuturor microorganismelor care alcătuiesc microflora frunzelor. Microorganismele izolate din filosfera plantelor de ovăz sunt prezentate în Tabelul 3.

Din datele tabelului se poate observa că pe mediul de malț agarizat s-au putut cultiva şi izola 11 tipuri de colonii de microorganisme filosferice. Aceste microorganisme pot fi grupate în 6 categorii: bastonaşe Gram-negative, bastonaşe Gram-pozitive nesporogene, bastonaşe Gram-pozitive endosporogene, coci Gram-pozitivi, drojdii şi fungi filamentoşi (micromicete). Majoritatea coloniilor (5 din 11) aparțin grupei bacteriilor, iar numărul coloniilor de drojdii este egal cu cel al micromicetelor. Aceste rezultate sunt în conformitate cu datele din literatura de specialitate, după care bacteriile sunt principalele componente ale populațiilor filosferice, urmate de drojdii şi fungi filamentoşi [5, 6, 9]. Este mai neobişnuită prezența în microflora filosferică a diferitelor grupe de bacterii Gram-pozitive. Aceste microorganisme sunt rar întâlnite în microflora frunzelor verzi sănătoase, deşi prezența lor a fost semnalată şi de alți cercetători în filosfera arborelui *Quercus ilex* [4].

Tabelul 4 prezintă observațiile referitoare la distribuția coloniilor de microorganisme în filosfera celor 17 variante studiate. Din datele tabelului reiese că bastonaşele Gram-negative care formează colonii galbene, lucioase au fost izolate în majoritatea cazurilor, excepție făcând variantele 2 şi 17. Bacteriile Gram-negative care se prezintă sub formă de colonii de culoare roz au fost depistate numai la varianta 2. Este surprinzătoare frecvența ridicată cu care s-au putut izola cocii Gram-pozitivi, aceştia fiind, împreună cu bacteriile Gram-negative, principalii componenți ai populațiilor filosferice. Aceste bacterii, asemănător bastonaşelor Gram-pozitive, se caracterizează prin rezistență crescută față de factorii nefavorabili ai mediului înconjurător [8].

Bacteriile Gram-pozitive endosporogene sunt întâlnite doar în unele cazuri, iar cele nesporogene au fost identificate numai la varianta 6. Nu s-au putut izola bacterii din filosfera plantelor aparținând variantei 17.

In cazul drojdiilor se poate observa frecvența mai mare în microflora frunzelor a drojdiilor albe, drojdiile care formează colonii portocalii fiind mult mai rar depistate. Fungii filamentoşi reprezintă, alături de bacterii, o grupă semnificativă în compoziția populațiilor filosferice. Majoritatea micromicetelor izolate formează colonii albe, pufoase, care în cursul sporogenezei prezintă spori de culoare neagră. Micromicetele care formează colonii verzi sunt mult mai rar observate, iar colonii negre de fungi filamentoşi s-au dezvoltat numai din filosfera variantelor 1 şi 16.

Tabel 4

Distribuția microorganismelor în microflora frunzelor de ovăz

Varianta experimentală	Tipuri de colonii											Nr. tipuri de colonii
	1	2	3	4	5	6	7	8	9	10	11	
1	+	-	+	-	-	+	-	+	+	+	+	7
2	-	+	+	-	-	-	-	+	+	-	-	4
3	+	-	-	+	-	-	-	+	+	-	-	4
4	+	-	-	-	-	-	-	-	+	-	-	2
5	+	-	+	+	-	-	-	-	-	-	-	3
6	+	-	+	-	+	+	-	-	+	+	-	6
7	+	-	+	-	-	-	-	+	-	+	-	4
8	+	-	-	-	-	-	-	-	+	-	-	2
9	+	-	+	-	-	-	-	-	+	-	-	3
10	+	-	+	+	-	-	-	+	+	-	-	5
11	+	-	-	-	-	+	-	-	-	-	-	2
12	+	-	+	+	-	-	-	+	-	-	-	4
13	+	-	-	+	-	-	-	-	+	+	-	4
14	+	-	-	-	-	-	-	-	+	+	-	3
15	+	-	+	-	-	+	-	-	+	-	-	4
16	+	-	+	+	-	-	-	-	+	+	+	6
17	-	-	-	-	-	-	+	-	-	+	-	2

La majoritatea variantelor, diversitatea populațiilor filosferice se rezumă la 4 tipuri de colonii diferite de microorganisme. Populațiile mai puțin diversificate, cu 3, respectiv 2 colonii microbiene, sunt, de asemenea, mai bine reprezentate, în timp ce populațiile filosferice cu o diversitate mai ridicată sunt destul de rar întâlnite. Astfel, comunitățile microbiene alcătuite din 5 tipuri de colonii au fost evidențiate în cazul variantei 10, iar cele cu 6, respectiv 7 colonii diferite au fost observate la variantele 6 şi 16, respectiv 1.

Concluzii. 1. Materialul de haldă provenit de la mina Rodna are un conținut ridicat de metale grele, ceea ce a avut un efect negativ asupra dezvoltării plantelor din primul lot, acestea nu au putut supraviețui timp îndelungat, chiar dacă la materialul de haldă s-au adăugat adsorbanți şi substanțe nutritive. În cursul creşterii lor, plantele din primul lot au eliberat în mediu substanțe care se pare că au influențat favorabil dezvoltarea plantelor care au apărut din semințele semănate după îndepărtarea resturilor plantelor din primul lot.

2. Microflora filosferică a plantelor de ovăz cuprinde 6 grupe de microorganisme care se pot cultiva pe mediul de malț agarizat: bastonaşe Gram-negative, bastonaşe Gram-pozitive nesporogene, bastonaşe Gram-pozitive endosporogene, coci Gram-pozitivi, drojdii şi micromicete. Cele mai frecvente microorganisme în filosfera plantelor sunt bacteriile Gram-negative, urmate de coci Gram-pozitivi şi fungi filamentoşi.

3. Pe mediul de cultură utilizat s-au putut evidenția bastonaşe şi coci Gram-pozitivi cu o frecvență mult mai ridicată decât s-ar putea aştepta pe baza datelor din literatura de specialitate.

4. Diversitatea populațiilor microflorei filosferice se rezumă în majoritatea cazurilor la 2, 3, respectiv 4 tipuri de colonii microbiene, populațiile cu un grad mai mare de diversitate fiind mai rar evidențiate.

BIBLIOGRAFIE

1. Andersen, G. L., Menkissoglou, O., Lindow, S. E., *Occurrence and properties of copper-tolerant strains of* Pseudomonas syringae *isolated from fruit trees in California*, "Phytopathology", **81**, 1991, 648-656.
2. Baldi, F., Bianco, M. A., Pepi, M., *Mercury, arsenic and boron resistant bacteria isolated from the phyllosphere of airborne pollution near geothermal plants*, "Sci. Total Environ.", **164**, 1995, 99-107.
3. Baldi, F., Parati, F., Semplici, F., Tandoi, V., *Biological removal of inorganic Hg(II) as gaseous elemental Hg(0) by continuous culture of a Hg-resistant* Pseudomonas putida *strain FB-1*, "World J. Microbiol. Biotechnol.", **9**, 1993, 275-279.
4. Brunel, B., Perissol, C., Fernandez, M., Boeufgras, J. M., Le Petit, J., *Occurrence of* Bacillus *species on evergreen oak leaves,* "FEMS Microbiol. Ecol.", **14**, 1994, 331-342.
5. Dommergues, Y., Mangenot, F., *Écologie microbienne du sol*, p. 538-547, Éd. Masson, Paris, 1970.
6. Jacques, M. A., Morris, C.E., *A review of issues related to the quantification of bacteria from the phyllosphere,* "FEMS Microbiol. Ecol.", **18**, 1995, 1-14.
7. Kiss, S., Paşca, D., Drăgan-Bularda, M., Cristea, V., Blaga, G., Crişan, R., Muntean, V., Zborovschi, E., Mitroescu, S., *Enzymological analysis of lead and zinc mine spoils submitted to biological recultivation*, "Stud. Univ. Babeş-Bolyai, Biol.", **35**(2), 1990, 70-79.

8. Kuliceva, N. N., Lîsak, L. V., Kojevin, P. A., Zviaghințev, D. G., *Bakterii v pocive, opade i filloplane gorodskoi ekosistemî,* "Mikrobiologhiia", **65**, 1996, 416-420.

9. Last, F.T., Warren, R.C., *Non-parasitic microbes colonizing green leaves: their form and functions,* "Endeavour", **31**(114), 1972, 143-150.

10. Lindow, S. E., *The role of bacterial ice nucleation in frost injury to plants,* "Annu. Rev. Phytopathol.", **21**, 1983, 363-384.

11. Pattantyus, J., Kiss, S., *Contribution to study of phyllosphere microflora*, "Stud. Univ. Babeş-Bolyai, Biol.", **36**(2), 1994, 97-103.

12. Szabó, I.M., *A bioszféra mikrobiológiája*, Vol. 2, p.1438; Vol. 3, p.2065-2080, Akad. Kiadó, Budapest, 1989.

13. Zarnea, G., *Tratat de microbiologie generală*, Vol. 5, p. 651-654, Ed. Acad. Rom., Bucureşti, 1994.

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THE EFFECTS OF SOIL MANAGEMENT PRACTICES ON THE ENZYMATIC ACTIVITIES IN A BROWN LUVIC SOIL

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SUMMARY.- Actual and potential dehydrogenase and catalase activities were determined in the 0-20-, 20-40- and 40-60-cm layers of a brown luvic soil submitted to a complex tillage, crop rotation and fertilisation experiment. It was found that no-till - in comparison with conventional tillage - resulted in significantly higher soil enzymatic activities in the 0-20-cm layer and in significantly lower activities in the deeper layers. The soil under maize or wheat was more enzyme-active in the 6- than in the 2-year rotation. In the 2-year rotation, higher enzymatic activities were registered under wheat than under maize. In the 6-year rotation, the enzymatic indicators of soil quality decreased, depending on the nature of crops and kind of fertilisers (mineral NP or farmyard manure), in the following order: minerally fertilized (m.f) wheat≈m.f. oats-clover mixture> farmyard-manured maize> m.f. soybean>m.f. clover>m.f. maize. It should be emphasised that farmyard-manuring of maize - in comparison with its mineral (NP) fertilisation - led to a significant increase in each of the three enzymatic activities determined. Each activity in both non-tilled and conventionally tilled soil under all crops of both rotations decreased with increasing sampling depth.

The effects of tillage, crop rotation and fertilisation on soil enzymatic activities were studied in many countries, including Romania (see the reviews published by [1, 2, 4, 6, 7, 9]). In order to obtain new data on the soil enzymological effects of soil management practices we have determined some enzymatic activities in a brown luvic soil submitted to a complex tillage, crop rotation and fertilisation experiment at the Agricultural and Animal Breeding Experiment Station in Oradea (Bihor county).

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The first enzymological data on this soil were published by Ştefanic and his collaborators [10-12]. They studied the soil enzymological effect of mineral (NP) fertilisation and liming and found that catalase activity was higher while dehydrogenase, invertase and phosphatase activities were lower in the NP-fertilised and limed soil samples than in the unfertilised limed ones. Ştefanic also dealt with the effect of compost application and irrigation on the enzyme activities in this soil, but he published no paper on these investigations (personal communication, 1999).

Materials and methods. The ploughed layer of the studied brown luvic soil is of mellow loam texture, it has a pH value of 5.5, medium humus (2.32%) and P (22 ppm) contents, but it is rich in K (83 ppm).

The experiment started in 1992. The experimental field occupying 3.84 ha was divided into plots and subplots for comparative study of no-till and conventional tillage, rotations of 2 and 6 years, and mineral (NP) fertilisation and farmyard-manuring.

The crops of the 2- and 6-year rotations are specified in Table 1. Each plot consisted of two subplots representing the no-till and conventional tillage variants. The plots were annually NP-fertilised at rates of 120 kg of N/ha and 90 kg of P/ha, excepting, in each year, a maize plot (in the 6-year rotation) which received farmyard manure (50 t/ha) instead of mineral fertilisers. The plots (and subplots) were installed in three repetitions.

Table 1

Crops of the rotations of 2 and 6 years

Year	Rotation of 2 years		Rotation of 6 years					
	Plots		Plots					
	1	2	1	2	3	4	5	6
1992	Wheat	Maize	Maize	Soybean	Clover	Wheat	Maize (FYM)*	Flax
1993	Maize	Wheat	Soybean	Maize	Maize (FYM)	Oats-clover	Hemp	Wheat
1994	Wheat	Maize	Oats-clover	Maize (FYM)	Flax	Maize	Wheat	Soybean
1995	Maize	Wheat	Maize (FYM)	Maize	Wheat	Soybean	Maize	Oats-clover
1996	Wheat	Maize	Flax	Wheat	Soybean	Maize	Oats-clover	Maize (FYM)
1997	Maize	Wheat	Wheat	Soybean	Maize	Maize (FYM)	Clover	Oats-clover

*(FYM) - (farmyard-manured)

In October 1997, soil was sampled from the 0-20-, 20-40- and 40-60-cm depths of the subplots. The soil samples were allowed to air-dry, then ground and passed through a 2-mm sieve and, finally, used for enzymological analyses. Three enzymatic activities (actual and potential dehydrogenase and catalase) were determined according to the methods described in [3]. Dehydrogenase activities are expressed in mg of triphenylformazan (TPF) produced (from 2,3,5 - triphenyltetrazolium chloride, TTC) by 10 g of soil in 24 hours, whereas catalase activity is recorded as mg of H_2O_2 decomposed by 1 g of soil in 1 hour. The enzymatic activity values were submitted to statistical evaluation by the two-way *t* - test [8].

Results and discussion. Results of the enzymological analyses are presented in Table 2, and those of the statistical evaluation are summarised in Table 3.

The effect of tillage practices on the enzymatic activities in soil. Each of the three enzymatic activities determined was significantly higher (at least at $p<0.02$) in the upper (0-20-cm) layer of the non-tilled subplots than in the same layer of the conventionally tilled subplots. The reverse was true in the deeper (20-40- and 40-60-cm) layers. These findings are valid for subplots under each crop of both rotations.

The effect of crop rotations on the enzymatic activities in soil. For evaluation of this effect, the results obtained in the three soil layers analysed in the two subplots of each plot were considered together.

- The soil enzymological effect of the same crop in the two rotations. As maize and wheat were crops in both rotations, it was possible to compare the soil enzymological effect of the 2- and 6-year rotations. The soil under both plants was more enzyme-active in the 6- than in the 2-year rotation. But in the soil under maize, the difference between the two rotations was significant ($p<0.05$) only in the case of potential dehydrogenase activity, whereas in the soil under wheat each activity was significantly higher ($p<0.02$) in the 6 - than in the 2-year rotation.

- The soil enzymological effect of different crops in the same rotation. The 2-year rotation. Actual and potential dehydrogenase activities were significantly higher ($p<0.05$ and $p<0.01$, respectively), while catalase activity was unsignificantly higher ($p>0.05$) in the wheat soil than in the soil under maize.

Table 2

The effects of soil management practices on enzymatic activities in a brown luvic soil

Soil enzymatic activity	Soil depth (cm)	Rotation of 2 years				Rotation of 6 years											
		Maize		Wheat		Wheat		Soybean		Maize		Maize (FYM)***		Clover		Oats-clover	
		N.t.*	C.t.**	N.t.	C.t.	N.t.	C.t.	N.t.	C.t.	N.t.	C.t.	N.t.	C.t	N.t.	C.t.	N.t.	C.t.
Actual dehydrogenase	0-20	4.68	4.50	7.36	6.02	7.76	6.80	7.56	6.72	5.76	4.88	5.82	5.12	6.16	5.32	8.68	6.16
(mg TPF/10 g soil/24	20-40	2.68	3.10	4.84	5.20	5.01	5.60	4.08	4.81	2.86	3.52	2.52	3.92	4.04	4.36	3.36	4.48
hours)	40-60	1.12	1.80	1.36	1.84	2.80	3.01	1.40	2.52	1.02	1.84	1.40	2.40	2.24	2.80	2.40	3.90
Potential dehydro-	0-20	23.52	22.36	22.96	21.20	25.20	23.20	26.60	20.90	24.12	22.96	27.16	25.48	24.92	20.72	29.68	23.24
genase (mg TPF/10 g	20-40	15.68	16.52	14.08	15.40	15.28	18.96	16.40	17.72	16.44	17.48	17.92	18.48	12.60	12.88	14.52	15.40
soil/24 hours)	40-60	2.52	3.36	5.32	5.72	5.60	6.76	7.00	7.56	5.64	7.00	6.01	6.72	4.88	5.60	5.20	6.36
Catalase	0-20	1.27	1.17	1.86	1.52	1.98	1.77	1.42	1.23	1.44	1.37	1.73	1.68	1.43	1.39	2.15	1.73
(mg H_2O_2/g soil/hour)	20-40	0.62	1.06	1.17	1.45	1.37	1.49	1.11	0.90	1.02	1.16	1.05	1.10	0.93	1.27	0.74	1.59
	40-60	0.25	0.53	0.59	0.62	0.62	0.34	0.22	0.57	0.27	0.55	0.49	0.65	0.56	0.46	0.53	1.34

* No-till.

** Conventional tillage.

*** (farmyard-manured).

Table 3

Significance of the differences between enzymatic activities in a brown luvic soil submitted to different management practices

Management practices	Soil enzymatic activity*	Soil depth (cm)	Mean activity values in management practices			Significance of the differences
			a	b	(a-b)	
1	2	3	4	5	6	7
No-till (a) versus conventional tillage (b)	ADA	0-20	6.72	5.69	1.03	0.01>p>0.002
		20-40	3.67	4.37	-0.70	0.002>p>0.001
		40-60	1.72	2.51	-0.79	0.001>p>0.0001
	PDA	0-20	25.52	22.51	3.01	0.02>p>0.01
		20-40	15.36	16.61	-1.25	0.02>p>0.01
		40-60	5.27	6.14	-0.87	0.001>p>0.0001
	CA	0-20	1.66	1.48	0.18	0.01>p>0.002
		20-40	1.00	1.25	-0.25	0.01>p>0.002
		40-60	0.44	0.63	-0.19	0.02>p>0.01
The same crop in the two rotations						
Maize in 2-year rotation (a) versus maize in 6-year rotation (b)	ADA	0-60	2.98	3.31	-0.33	0.10>p>0.05
	PDA		13.99	15.61	-1.62	0.05>p>0.02
	CA		0.82	0.97	-0.15	0.10>p>0.05
Wheat in 2-year rotation (a) versus wheat in 6-year rotation (b)	ADA	0-60	4.44	5.16	-0.72	0.02>p>0.01
	PDA		14.11	15.83	-1.72	0.02>p>0.01
	CA		1.20	1.26	-0.06	0.02>p>0.01
Different crops in the same rotation						
2-year rotation						
Maize (a) versus wheat (b)	ADA	0-60	2.98	4.44	-1.46	0.05>p>0.02
	PDA		13.98	14.11	-0.13	0.01>p>0.002
	CA		0.82	1.20	-0.38	0.10>p>0.05
6-year rotation						
Wheat (a) versus soybean (b)	ADA	0-60	5.16	4.52	0.64	0.05>p>0.02
	PDA		15.83	16.03	-0.20	0.002>p>0.001
	CA		1.26	0.91	0.35	0.002>p>0.001
Wheat (a) versus maize (b)	ADA	0-60	5.16	3.31	1.85	0.0002>p>0.0001
	PDA		15.83	15.61	0.22	0.05>p>0.02
	CA		1.26	0.97	0.29	0.001>p>0.0001
Wheat (a) versus maize (FYM)** (b)	ADA	0-60	5.16	3.53	1.63	0.002>p>0.001
	PDA		15.83	16.96	-1.13	0.05>p>0.02
	CA		1.26	1.17	0.09	0.01>p>0.002
Wheat (a) versus clover (b)	ADA	0-60	5.16	4.16	1.00	0.01>p>0.002
	PDA		15.83	13.60	2.23	0.05>p>0.02
	CA		1.26	1.01	0.25	0.02>p>0.01

Table 3 (continued)

1	2	3	4	5	6	7
Wheat (a) versus oats-clover (b)	ADA	0-60	5.16	4.83	0.33	0.01>p>0.002
	PDA		15.83	15.73	0.10	0.10>p>0.05
	CA		1.26	1.35	-0.09	0.10>p>0.05
Soybean (a) versus maize (b)	ADA	0-60	4.52	3.31	1.21	0.01>p>0.002
	PDA		16.03	15.61	0.42	0.05>p>0.02
	CA		0.91	0.97	-0.06	0.10>p>0.05
Soybean (a) versus maize (FYM) (b)	ADA	0-60	4.52	3.53	0.99	0.05>p>0.02
	PDA		16.03	16.96	-0.93	0.10>p>0.05
	CA		0.91	1.17	-0.26	0.05>p>0.02
Soybean (a) versus clover (b)	ADA	0-60	4.52	4.16	0.36	0.05>p>0.02
	PDA		16.03	13.60	2.43	0.02>p>0.01
	CA		0.91	1.01	-0.10	0.02>p>0.01
Soybean (a) versus oats-clover (b)	ADA	0-60	4.52	4.83	-0.31	0.01>p>0.002
	PDA		16.03	15.73	1.30	0.001>p>0.0001
	CA		0.91	1.35	-0.44	0.001>p>0.0001
Maize (a) versus maize (FYM) (b)	ADA	0-60	3.31	3.53	-0.22	0.01>p>0.002
	PDA		15.61	16.96	-1.35	0.002>p>0.001
	CA		0.97	1.17	-0.20	0.02>p>0.01
Maize (a) versus clover (b)	ADA	0-60	3.31	4.16	-0.85	0.01>p>0.002
	PDA		15.61	13.60	2.01	0.02>p>0.01
	CA		0.97	1.01	-0.04	0.10>p>0.05
Maize (a) versus oats-clover (b)	ADA	0-60	3.31	4.83	-1.52	0.01>p>0.002
	PDA		15.61	15.73	-0.12	0.10>p>0.05
	CA		0.97	1.35	-0.38	0.01>p>0.002
Maize (FYM) (a) versus clover (b)	ADA	0-60	3.53	4.16	-0.63	0.05>p>0.02
	PDA		16.96	13.60	3.36	0.02>p>0.01
	CA		1.17	1.01	0.16	0.01>p>0.002
Maize (FYM) (a) versus oats-clover (b)	ADA	0-60	3.53	4.83	-1.30	0.02>p>0.01
	PDA		16.96	15.73	1.23	0.01>p>0.002
	CA		1.17	1.35	-0.18	0.05>p>0.02
Clover (a) versus oats-clover (b)	ADA	0-60	4.16	4.83	-0.67	0.10>p>0.05
	PDA		13.60	15.73	-2.13	0.05>p>0.02
	CA		1.01	1.35	-0.34	0.05>p>0.02

* ADA - Actual dehydrogenase activity.
PDA - Potential dehydrogenase activity.
CA - Catalase activity.
** (FYM) - (farmyard-manured).

The 6-year rotation. Significant and unsignificant differences were registered in the soil enzymatic activities depending on the kind of enzymatic activity and the nature of crop. Based on these differences the following decreasing orders of the enzymatic activities could be established in the soil of the six plots:

actual dehydrogenase activity: wheat > oats-clover > soybean > clover > maize (FYM) > maize;

potential dehydrogenase activity: maize (FYM) ≈ soybean > wheat ≈ oats-clover ≈ maize >clover;

catalase activity: oats-clover≈wheat>maize (FYM)> clover≈ maize≈ soybean.

It is evident from these orders that each of the six plots presented either a maximum or a minimum value of the three soil enzymatic activities. Consequently, these orders do not make it possible to establish such an enzymatic hierarchy of the plots which takes into account each activity for each plot. For establishing such a hierarchy, we have applied the method suggested in [5]. Briefly, by taking the maximum mean value of each activity as 100%, we have calculated the relative (percentage) activities. The sum of the relative activities is the enzymatic indicator which is considered as an index of the biological quality of the soil in a given plot. The higher the enzymatic indicator of soil quality, the higher the position of plots is in the hierarchy. Table 4 shows that the first three positions are occupied by those plots in which actual dehydrogenase activity, potential dehydrogenase activity and catalase activity, respectively, were the highest. The soil under the minerally fertilised maize plot occupying the last position can be considered as the least enzyme-active soil.

Table 4

Enzymatic indicators of soil quality in plots of the 6-year rotation

Position	Plot	Enzymatic indicator of soil quality
1	Minerally fertilised (M.f.) wheat	286.67
2	M.f. oats-clover mixture	286.35
3	Farmyard-manured maize	255.08
4	M.f. soybean	249.53
5	M.f. clover	235.62
6	M.f. maize	228.04

The effect of fertilisation on the enzymatic activities in soil. The two maize plots in the 6-year rotation could serve for comparing the soil enzymological effect of mineral (NP) fertilisation and farmyard-manuring. One can see from Table 2 that the enzymatic activities were always higher in the 0-20-cm layer and were generally higher in the 20-40- and 40-60-cm layers of the farmyard-manured subplots in comparison with the subplots that had received mineral (NP) fertilisers. When the three soil layers were considered together (Table 3), each of the three enzymatic activities was found to be significantly higher (at least at $p<0.02$) in the farmyard-manured plot than in the minerally fertilised plot. In concordance with these findings, Table 4 shows that the farmyard-manured maize plot occupies position 3, whereas the other maize plot is placed on the last position in the hierarchy of plots in the 6-year rotation.

Variation of soil enzymatic activities in dependence of sampling depth. Each activity in both non-tilled and conventionally tilled subplots under all crops of both rotations decreased with increasing sampling depths.

Our results are in good agreement with the literature data reviewed by [1, 2, 4, 6, 7, 9] and constitute novelties for the enzymological characterisation of a brown luvic soil submitted to complex management practices.

Conclusions. 1. No-till - in comparison with conventional tillage - resulted in higher enzymatic activities in the 0-20-cm soil layer and in lower activities in the 20-40- and 40-60-cm soil layers under each crop of both rotations.

2. The 6-year rotation - as compared to the 2-year rotation - led, in general, to higher enzymatic activities in the soil layers under maize or wheat.

3. Farmyard-manuring - in comparison with mineral (NP) fertilisation - proved to be more efficient in increasing enzymatic activities in soil layers under maize in the 6-year rotation.

4. The soil enzymatic activities decreased with increasing sampling depth.

REFERENCES

1. Burns, R.G. (Ed.), *Soil Enzymes*, Acad. Press, London, 1978.
2. Dick, R.P., *A review: long-term effects of agricultural systems on soil biochemical and microbial parameters*, "Agric., Ecosyst. Environ.", **40**, 1992, 25-36.
3. Drăgan-Bularda, M., *Lucrări practice de microbiologie generală*, pp. 163-167, Univ. Babeş-Bolyai, Cluj-Napoca, 1983.

4. Kiss, I., *Talajenzimek*, in *Csapó*, M.J. (Ed.), *Talajtan*, pp. 491-622, Mezőgazd. Erd. Könyvkiadó, Bukarest, 1958.
5. Kiss, S., Paşca, D., Drăgan-Bularda, M., Cristea, V., Blaga, G., Crişan, R., Muntean, V., Zborovschi, E., Mitroescu, S., *Enzymological analysis of lead and zinc mine spoils submitted to biological recultivation*, "Stud. Univ. Babeş-Bolyai, Biol.", **35** (2), 1990, 70-79.
6. Kiss, S., Ştefanic, G., Drăgan-Bularda, M., *Soil enzymology in Romania (Part II)*, "Contrib. Bot." (Cluj), 1975, 197-207.
7. Lynch, J.M., *Soil Biotechnology: Microbiological Factors in Crop Productivity*, Blackwell, Oxford-London, 1983.
8. Sachs, L., *Statistische Auswertungsmethoden*, pp. 140, 309-310, Springer, Berlin, 1968.
9. Skujins, J.J., *Enzymes in soil*, in *McLaren, A.D., Peterson, G.H.* (Eds.), *Soil Biochemistry*, pp. 371-414, Dekker, New York, 1967.
10. Ştefanic, G., *Enzimologia solurilor cultivate*, in Kiss, S., Ştefanic, G., Paşca, D., Drăgan-Bularda, M., Zborovschi, E., Crişan, R., *Enzimologia mediului înconjurător, Vol. 1*, pp. 65, 71, 78, 84, Ceres, Bucureşti, 1991.
11. Ştefanic, G., Eliade, G., Chirnogeanu, I., *Influența aplicării îndelungate a îngrăşămintelor chimice asupra unor însuşiri biologice ale solului*, "Lucr. Conf. Naț. Ştiința Solului (Brăila, 1982)", Publ.Soc. Naț. Rom. Ştiința Solului, Bucureşti, No.21B, 1983, 87-93.
12. Ştefanic, G., Eliade, G., Chirnogeanu, I., *Researches concerning a biological index of soil fertility*, "Fifth Symp. on Soil Biology (Iaşi, 1981)", Rom. Natl. Soc. Soil Sci., Bucharest, 1984, 35-45.

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ENZYMOLOGICAL RESEARCH ON SEDIMENTS FROM THE URSU AND NEGRU SALT LAKES (SOVATA, MUREŞ COUNTY)

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SUMMARY. - Seasonal enzymological analyses were performed over a 5-year period on sediments from the Ursu and Negru lakes (Sovata, Mureş county). The following 7 enzymatic and nonenzymatic catalytic activities were measured: phosphatase, catalase, nonenzymatic H_2O_2-splitting, 2,3,5-triphenyltetrazolium chloride (TTC) reduction in nonautoclaved (dehydrogenase activity) and autoclaved samples, without or with glucose addition. The assessment of the enzymatic potential of the sediments was made by calculating an enzymatic indicator of their quality. The studied enzymatic and nonenzymatic catalytic activities were significantly high through all seasons, with irregular seasonal variations. The evolution of this potential, which tended to decrease during the 5 years of research, was followed.

The locality Sovata is situated in a central submountain area of Romania, at 46°35' North latitude and 25°4' East longitude. In terms of geology, the zone consists of sedimentary formations of Badenian, Sarmatian and Pannonian age, deposited at the bottom of the tertiary sea which covered the region many millions of years ago. Salt massifs, which are genetically related to salt lakes, have an important place in the stratigraphy and tectonics of the region. Badenian age salt deposited at the bottom of tertiary sea lagoons millions of years ago was subsequently covered by more recent Sarmatian and Pannonian deposits. The geographical and physico-chemical data concerning the two lakes are cited from Gâştescu [4].

The Ursu lake is one of the largest heliothermal salt lakes in Europe. It was formed in a sinking depression, as the consequence of an intense process of salt dissolution, at the end of the XIX century (1870-1880). The lake has a surface of approximately 40,000 m^2 and a maximum depth of 18.4 m. It has a several cm deep surface layer of very poorly mineralised water, resulting from

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rainfall and from the Toplița and Auriu rivulets which flow into the lake. The salt concentration increases linearly up to the depth of 3 m, where it reaches over 300 g/l and maintains this value up to the sediment surface. NaCl dominates, but SO_4^{2-}, HCO_3^-, Ca^{2+}, Mg^{2+}, Fe^{2+}, Fe^{3+} ions are also found. The Ursu lake shows an inverse thermal stratification up to the depth of 3 m and, then, a direct thermal stratification up to the maximum depth.

The Negru lake has an anthroposaline origin, being formed in an abandoned mine dating from the time of the Romans. It has a surface of about 3 800 m^2 and a maximum depth of 6.8 m. Salinity from the depth of 3 m to the bottom is over 120 g/l.

The finely granulated and colloidal iron sulphide-rich sapropel type sediment, which is found in considerable amounts especially in the Ursu lake, has been used ever since the beginning of the century for its curative qualities. Today Sovata is a modern balneary resort, known in all Europe, especially due to the use of therapeutic mud from the Ursu lake in the treatment of various rheumatic, posttraumatic diseases of the locomotor system, gynecologic, neurologic disorders etc.

The interest in understanding the properties of the sediments from the two lakes is therefore justified. Our research continues the enzymological studies on Sovata salt lake sediments, initiated several years ago [1, 2, 6, 7, 9]. This paper presents the results of seasonal enzymological research performed during the period 1990-1994 on the sediments of the two salt lakes.

Materials and methods. Sediments of the Ursu and Negru salt lakes from the balneary resort Sovata (Mureş county, Romania) were studied enzymologically over a 5-year (1990-1994) period. In the Ursu lake sediment samples were taken and analysed every year in spring, summer and autumn from 7 places: raft, balneary sector, Northern gulf, entrance of the Northern gulf, spillway of the rivulet Auriu, zone to Aluniş and the basket of natural regeneration of mud utilised in balneotherapy. Samples taken from all places were analysed in winter 1992 as well. In the Negru lake sediment samples were gathered from three places: zone of the spillway of mud used in balneary treatment, central zone and tail of the lake. Analyses were carried out in spring, summer and autumn. In 1991 only spring and summer samples of sediment from the same places were analysed and in winter 1992 a sediment sample from the central zone of the lake.

The following 7 enzymatic and nonenzymatic catalytic activities have been measured: phosphatase, catalase, nonenzymatic H_2O_2-splitting capacity in autoclaved samples, TTC (2,3,5-triphenyltetrazolium chloride) reduction in nonautoclaved (dehydrogenase) and autoclaved samples, without or with glucose

addition. The sediment samples were centrifuged at 4000 r.p.m./30 minutes. After the removal of the supernatant, the dry matter and the 7 enzymatic and nonenzymatic catalytic activities specified were measured in the sediments. In order to determine the nonenzymatic catalytic activities, parts of the samples were autoclaved at 120°C for 1 hour in three consecutive days.

Phosphatase activity was assayed according to K r á m e r and E r d e i [8], in reaction mixtures consisting of 2.5 g sediment + 2 ml toluene (antiseptic) + 10 ml 0.5% disodium phenylphosphate solution or 10 ml distilled water. A technique based on K a p p e n's method [5] was applied for determination of catalase activity, in reaction mixtures composed of 1.5 g sediment + 10 ml distilled water + 2 ml 3% H_2O_2 solution or 2 ml distilled water. A similar technique was used for determination of nonenzymatic H_2O_2-splitting capacity, but the sediment was previously inactivated by autoclaving. TTC reduction in nonautoclaved (dehydrogenase) and autoclaved samples were assayed according to the method of C a s i d a *et al.* [3]. Composition of the reaction mixtures was: 0.5 g sediment + 1 ml distilled water or 1 ml 3% glucose solution + 0.5 ml 3% 2,3,5-triphenyltetrazolium chloride (TTC) solution or 0.5 ml distilled water.

The activities were expressed as follows: phosphatase activity in mg phenol/2.5 g dry sediment/24 hours at 37°C; catalase activity and nonenzymatic H_2O_2-splitting capacity in mg H_2O_2/1.5 g dry sediment/1 hour at 20°C; TTC reduction activities in mg triphenylformazan/0.5 g dry sediment/24 hours at 37°C. For each enzymatic and nonenzymatic catalytic activity the coefficient of variation was calculated [14].

Results and discussion. In all the analysed samples, the presence of all 7 enzymatic and nonenzymatic catalytic activities was noted. On the basis of absolute values of enzymatic and nonenzymatic catalytic activities, the mean seasonal and annual values were calculated. The results show a higher phosphatase activity in the sediments of the Negru lake as compared to that of the Ursu lake. This is obvious in all seasons, every year. The greatest difference is found in 1990, when the annual mean phosphatase activity in the sediment of the Ursu lake (1.954 mg phenol/2.5 g dry sediment) is only 27.58% of that from the sediment of the Negru lake (7.084 mg phenol/2.5 g dry sediment).

In the sediment of the Ursu lake, the annual evolution of this activity is regular, increasing until 1992, then decreasing until 1994, and its variation is low (minimum 1.954 mg phenol/2.5 g dry sediment in 1990 and maximum 2.555 mg phenol/2.5 g dry sediment in 1992). In the sediment of the Negru lake, these variations are larger (minimum 3.634 mg phenol/2.5 g dry sediment in 1991 and maximum 8.409 mg phenol/2.5 g dry sediment in 1993) and the evolution is less uniform.

The intensity of nonenzymatic H_2O_2-splitting is generally lower, but close to that of catalase activity, sometimes even exceeding it. This is true for all the 5 years of research. Both activities are higher in the sediment of the Negru lake than in that of the Ursu lake between 1991-1992. In the case of catalase activity, the greatest difference is noted in 1991, when the annual mean value of this activity in the sediment of the Ursu lake (48.077 mg H_2O_2/1.5 g dry sediment) represents only 75% of the annual mean value found in the sediment of the Negru lake (64.118 mg H_2O_2/1.5 g dry sediment). In 1990 and 1994, the catalase activity from the sediment of the Ursu lake is by 1.5% and 4.3%, respectively, higher than in the sediment of the Negru lake.

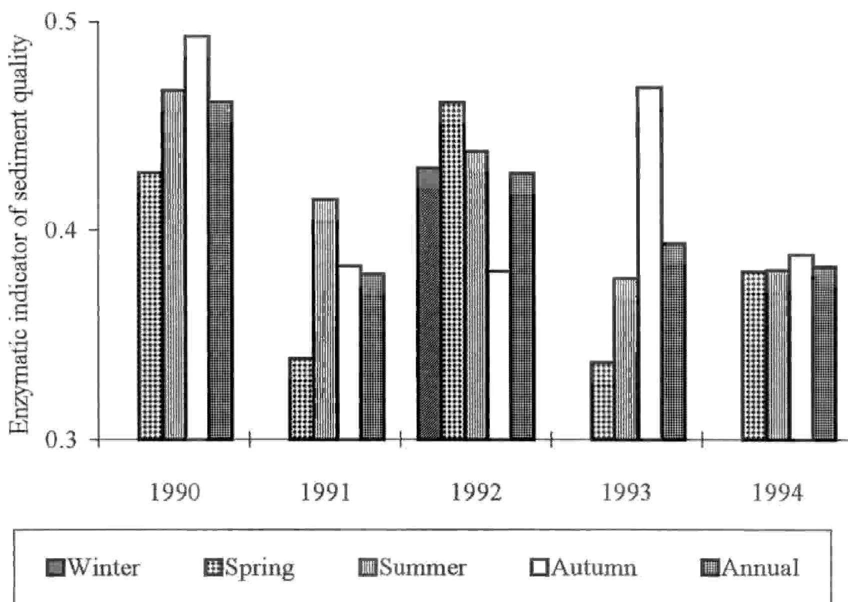
The intensity of the nonenzymatic TTC reduction is, with no exceptions, lower than that of dehydrogenase activity, but its level is however high, generally over 1 mg formazan/0.5 g dry sediment. The highest values of nonenzymatic activity were registered in 1990, when the annual mean exceeds 3.5 mg formazan/0.5 g dry sediment (Ursu lake) and 2 mg formazan/0.5 g dry sediment (Negru lake), respectively.

The relatively high values of the nonenzymatic H_2O_2-splitting activity and TTC reduction activities, respectively, could be explained by the presence in the sediments of highly reduced substances (*e.g.* sulphides) and humic acids, which can act as electron donors-acceptors and can reduce tetrazolium salts [11, 12, 15, 16].

Glucose addition has a constant but weak stimulating effect on dehydrogenase activity in the sediments of both lakes. A possible explanation could be the presence in these sediments of sufficiently high amounts of organic substances, which provide a good development of microorganisms, their activity being characterised by the maintenance of a relatively high level of the actual dehydrogenase activity.

The greatest difference between the actual and potential dehydrogenase activity is found in both lakes in 1993. In this year, the potential dehydrogenase activity in the Ursu lake (6.666 mg formazan/0.5 g dry sediment) is by 36.9% higher than the actual dehydrogenase activity (4.205 mg formazan/0.5 g dry sediment). In the Negru lake, the potential dehydrogenase activity (5.633 mg formazan/0.5 g dry sediment) is by 42.6% higher than the actual dehydrogenase activity (3.233 mg formazan/0.5 dry sediment).

On the basis of the mean values of the enzymatic and nonenzymatic catalytic activities studied, the enzymatic indicators of sediment quality (EISQ) were calculated, according to the formula presented in a previous paper [10]. Figs. 1 and 2 show the seasonal and annual evolution of the sediment enzymatic potential in both lakes, as it is reflected by the EISQ values.

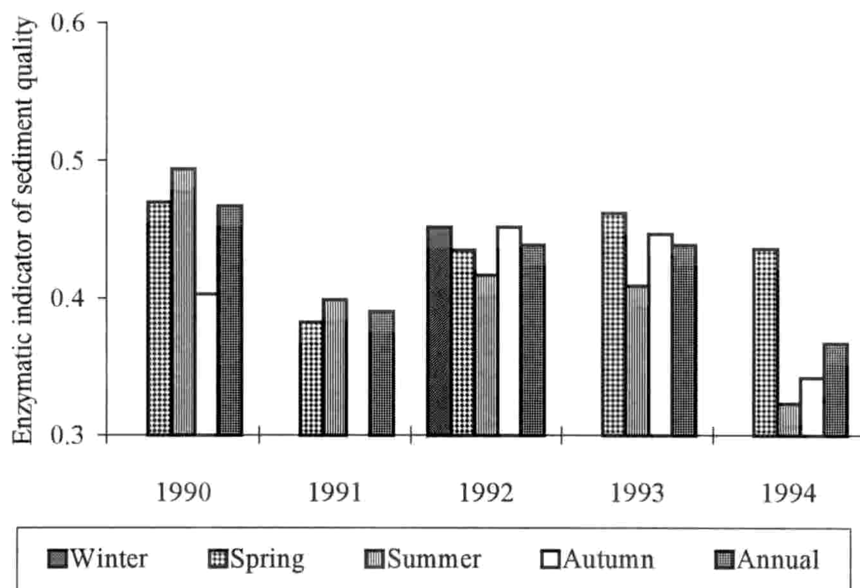


F i g. 1. *Seasonal and annual enzymatic indicators of sediment in Ursu lake.*

Only in the case of the Ursu lake and only for the years 1990, 1993 and 1994, an increase in the enzymatic and nonenzymatic catalytic activities may be noted in the order spring < summer < autumn. For the rest, seasonal variation is aleatory. The relatively high values of EISQ in winter (1992) in both lakes (0.430 in the Ursu lake and 0.452 in the Negru lake) might be surprising. The explanation for this could consist of the higher inertia of these ecosystems, due to which the organic matter sedimented at the end of the warm season is decomposed at a lower rate, providing the support for the maintenance of a high microbial and, consequently, enzymatic activity, inclusively in the cold season. Also, due to the particular thermal conditions (heliothermia in summer, inverse thermal stratification, but with somewhat higher bottom temperatures in winter and in transitional seasons), the lakes from salt massifs, like those studied by us, show a thermal stability of the depth water horizon all through the year, with temperature differences between seasons not exceeding 5°C [4].

It is noteworthy that EISQ never exceeds the value of 0.500 (half of the maximum theoretical value). So, globally, the sediment of neither lake can be enzymologically evaluated as very good. In the classification system of salt

lakes from Romania, based on the enzymatic indicator values of sediment quality, taking as a calculation basis the mean values of enzymatic and catalytic nonenzymatic activities during the period 1990-1994, the Negru lake is ranged at the position 21 (EISQ = 0.421) and the Ursu lake at the position 24 (EISQ = 0.409) [10]. As we shall show in the following part, the term of good, enzymatically active sediment, may be given to the sediment from certain areas of the Ursu lake.

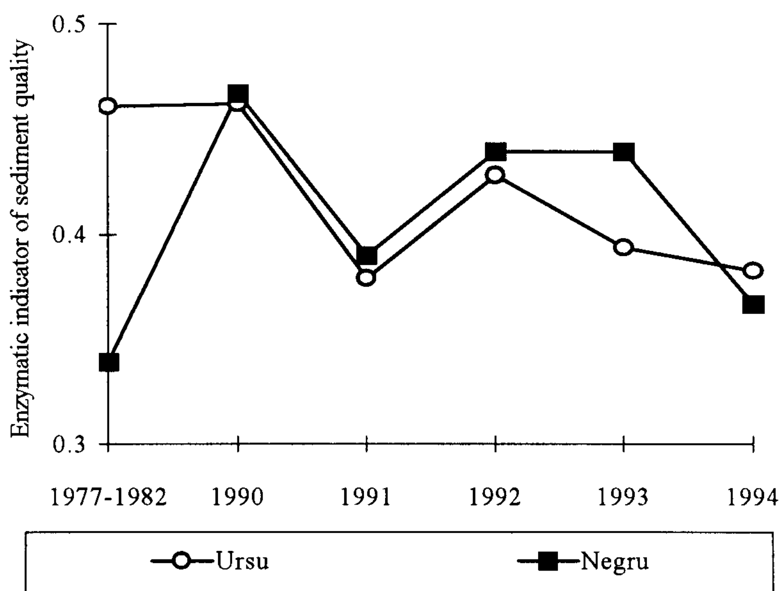


F i g. 2. *Seasonal and annual enzymatic indicators of sediment in Negru lake.*

The mean annual values of enzymatic and nonenzymatic catalytic activities in the sediment from each sampling site indicate some particularities. Important differences are found in the intensity of enzymatic activities between the sampling sites from the same lake. Three areas are distinguished in the Ursu lake (raft, balneary sector and spillway of the Auriu rivulet), whose sediments have much more intense activities than those from other areas. In the first three areas, the sediment is black and unctuous, being used in balneotherapy. In the case of the Negru lake, the differences between the three sites are not so great, due to the smaller size of the lake and, consequently, to the higher homogeneity of the sediment, which is black, unctuous, like in the case of the three sites of the Ursu lake.

The relatively high values of the variability coefficients for each activity, at the level of the whole lake or of each sampling site, values which frequently exceed 40%, illustrate a marked instability of the studied activities. Catalase activity and nonenzymatic H_2O_2-splitting activity are more stable. The annual variability coefficients for each sampling site generally have lower values than those established seasonally for all the sites of a lake. The phenomenon can be explained by the higher homogeneity of the samples taken from the same site and is more obvious in the case of the Negru lake and of the three sites with a higher enzymatic potential (raft, balneary sector and spillway of the Auriu rivulet) from the Ursu lake.

Enzymological studies on the sediments from both lakes were also carried out during the period 1977-1982 [6]. The same 7 enzymatic and nonenzymatic catalytic activities were investigated. Using the published data of the cited authors, we calculated EISQ for the period in question. Fig. 3 shows the evolution of the enzymatic potential of the sediments from the two lakes studied over the whole period.



F i g. 3. *Evolution of the enzymatic potential in sediments of the Ursu and Negru lakes.*

The sediment of the Ursu lake has a decreasing tendency of EISQ values. The value of 0.461 of EISQ in 1977-1982 decreases to 0.383 in 1994. The evolution is not linear. The enzymatic potential is practically maintained at

the same level until 1990 (EISQ = 0.462), but a sudden decline occurs in 1991, when EISQ reaches the minimum value recorded for the whole period (0.379). A slight increase follows in the next two years, without reaching the initial potential, then this decreases again in the last two years during which research was carried out. The need to know exactly the overall evolution of the sediment from the Ursu lake is illustrated by the observation that, although contradictory, literature data confirm the existence of a clogging process whose amplitude is differently estimated [13].

The sediment of the Negru lake shows a spectacular increase in the intensity of enzymatic and nonenzymatic catalytic activities between 1982 and 1990, the EISQ value increasing from 0.339 to 0.467 (the maximum for the whole period). Then, the evolution of the enzymatic potential of the sediment from the Negru lake parallels that of the sediment potential of the Ursu lake, during the last year of research the EISQ value in the first lake being even lower (0.367 compared to 0.383). During the first research period (1977-1982), the therapeutic mud used for balneotherapy at the Sovata sanatorium was spilt in the Negru lake, which might explain the very low enzymatic potential of this lake's sediment. The spill stopped in 1986. As the marked increase in the enzymatic potential is noted in the period during which the spill stopped, we may conclude that the natural regeneration of the sediment used in balneotherapy was effective. In fact, even if the evolutional tendency was decreasing in the last years, due to the very low initial EISQ value, unlike the Ursu lake, the enzymatic potential of the sediment from the Negru lake had an increasing tendency over the whole period of research.

REFERENCES

1. B u l g ă r e a n u, V.A.C., I o n e s c u, V., I o a n i ţ e s c u, E., *The contribution of wood vegetation leaf material to the peloidogenesis in salt, meromic and pelogenous lake Ursu-Sovata (Transylvania, Romania)*, "Rev. Roum. Biol., Biol. Vég.", **34**, 1989, 149-155.
2. B u l g ă r e a n u, V.A., K i s s, S., R ă d u l e s c u, D., D r ă g a n - B u l a r d a, M., H a n n i c h, D., *Influence of limnogeological conditions on the enzymatic and nonenzymatic catalytic activities in the sediments of salt lakes in Sovata*, "Stud. Univ. Babeş-Bolyai, Biol.", **26** (1), 1981, 68-72.
3. C a s i d a, L.E.jr., K l e i n, D.A., S a n t o r o, T., *Soil dehydrogenase activity*, "Soil Sci.", **98**, 1964, 371-376.
4. G â ş t e s c u, P., *Lacurile din România. Limnologie regională*, Ed. Acad. Rom., Bucureşti, 1971.

5. K a p p e n, H., *Die katalytische Kraft des Ackerbodens*, "Fühlings Landw. Ztg.", **62**, 1913, 377-392.

6. K i s s, S., D r ă g a n - B u l a r d a, M., P a ş c a, D., *Environmental enzymology*, "Stud. Univ. Babeş-Bolyai, Biol.", **31** (1), 1986, 3-13.

7. K i s s, S., R ă d u l e s c u, D., D r ă g a n - B u l a r d a, M., B u l g ă r e a n u, V.A., N i c u l a, G., *Contributions to the enzymological study of therapeutic muds*, "Stud. Univ. Babeş-Bolyai, Biol.", **24** (2), 1979, 54-63.

8. K r á m e r, M., E r d e i, G., *Primenenie metoda opredeleniya aktivnosti fosfatazy v agrokhimicheskikh issledovaniyakh*, "Pochvovedenie", No.9, 1959, 99-102.

9. M u n t e a n, V., *Enzymological study of muds from the Ursu and Negru Lakes (Sovata, Mureş county)*, "Evol. Adapt." (Cluj-Napoca), **5**, 1995, 97-105.

10. M u n t e a n, V., C r i ş a n, R., P a ş c a, D., K i s s, S., D r ă g a n - B u l a r d a, M., *Enzymological classification of salt lakes in Romania*, "Int. J. Salt Lake Res.", **5**, 1996, 35-44.

11. O k a z a k i, M., H i r a t a, E., T e n s h o, K., *TTC reduction in submerged soils*, "Soil Sci. Plant Nutr.", **29**, 1984, 489-497.

12. P a m a t m a t, M.M., *In situ metabolism of benthic communities*, "Cah. Biol. Mar.", **16**, 1975, 613-633.

13. P i ş o t ă, I., *Noi date hidrologice asupra lacurilor din depresiunea Sovatei*, "Probl. Geogr.", **7**, 1960, 179-192.

14. S a c h s, L., *Statistische Auswertungsmethoden*, p. 485, Springer, Berlin, 1968.

15. S c h i n d l e r, J.E., W i l l i a m s, D.J., Z i m m e r m a n, A.P., *Investigation of extracellular electron transport by humic acids*, in N r i a g u, J.O. (Ed.), *Environmental Biogeochemistry*, Vol. 1, pp.109-115, Ann Arbor Sci., Ann Arbor, Michigan, 1976.

16. V o s j a n, J.H., *Respiratory electron transport system activities in marine environments*, "Hydrobiol. Bull.", **16**, 1982, 61-68.

RECENZII

Ioan Petrescu, **Biochimie, Vol. 1. Constituenţii chimici ai celulei, Vol. 2. Reacţii chimice în celula vie** (*Biochemistry, Vol. 1, Chemical Constituents of the Cell, Vol. 2, Chemical Reactions in the Living Cell*), Presa Universitară Clujeană, Cluj, 1998, 203+327 pages with 92 + 167 figures in the text.

Volume 1 comprises Part I of the book (*Chemical Constituents of the Cell*), including 7 chapters (Saccharides; Lipids; Amino acids and proteins; Nucleotides and nucleic acids; Vitamins; Hormones; Tetrapyrrolic compounds). Volume 2 contains Part II (*Enzymatic Catalysis*) with two chapters (Enzymes; Coenzymes) and Part III (*Metabolism*) with 8 chapters (Metabolism of saccharides; Metabolism of lipids; Metabolism of amino acids and proteins; Metabolism of nucleotides and nucleic acids; Metabolism of porphyrins; Interrelations within the intermediary metabolism; Cellular bioenergetics; Cellular regulation of the metabolism).

A drawing presenting the structural formulae of the most important cyclic compounds, a list of abbreviations and a list of selective bibliography are enclosed to both volumes. Additionally, an original (40 by 40 cm) drawing entitled "Principal metabolic pathways - Interrelations within the intermediary metabolism" is enclosed to Volume 2.

Professor Ioan Petrescu's *Biochimie* is addressed to a broad circle of readers, especially students in biology, chemistry, human and veterinary medicine, pharmacy, agronomy.

This work can be characterised by a series of qualities: up-to-date information; comprehensive presentation of the fundamental topics in modern biochemistry; logical grouping of these topics into chapters divided into subchapters and many sections and subsections; clarity of the descriptions; attractive style; richness of illustrations. All these qualities facilitate the learning process, the easy assimilation of new knowledge and, undoubtedly, stimulate the sentimental attachment of the reader (student) for studying biochemistry.

Being convinced that Professor Petrescu's excellent book would be a bestseller in other countries, too, I warmly recommend its translation not only into world-wide spoken languages, but also into smaller ones.

ŞTEFAN KISS

Nicolae Dragoş, Leontin Ştefan Péterfi, Laura Momeu, Cristina Popescu, **An Introduction to the Algae and the Culture Collection of Algae at the Institute of Biological Research Cluj-Napoca** *(Einführung in die Algenkunde und die Sammlung der Algenkulturen am Institut für Biologische Forschungen in Klausenburg)*, Cluj University Press, 1997, 267 Seiten mit 11 Tabellen und 84 Abbildungen in dem Text.

Die Autoren des vorliegenden Bandes veröffentlichen hiermit erstmals einen vollständigen Katalog der am Institut für Biologische Forschungen Cluj-Napoca aufbewahrten Sammlung von Algenkulturen

mit insgesamt 631 registrierten Algenstämmen, die seit 1970 nach und nach aus der Algenflora Rumäniens isoliert und weiterkultiviert wurden.

Sammlungen von Algenkulturen spielen heute eine immer bedeutendere wissenschaftliche Rolle nicht nur im Hinblick auf die Erweiterung unserer Kenntnisse über diese überaus artenreiche und mannigfaltige Gruppe Niederer Pflanzen, die, wie im vorliegenden Buch, wenigstens in 16 systematische Abteilungen gegliedert wird, sondern sie liefern auch ein leicht zugängliches und wertvolles Untersuchungsmaterial zur Durchführung genetischer und stoffwechselphysiologischer Analysen, zur Klärung phylogenetischer Fragen über die Abstammung der Eukaryonten, aber auch für weltweit unternommene Bemühungen zur besseren wirtschaftlichen Nutzung der Algen. Demgemäβ besteht ein wachsendes wissenschaftliches Interesse für Sammlungen von Algenkulturen, und es ist ein besonderes Verdienst der Autoren, die Liste der in der Kollektion kultivierten Algenstämme in einem Katalog zu veröffentlichen.

Das Buch gliedert sich in zwei Teile, wobei im ersten, allgemein theoretischen Teil, die Grundkenntnisse über die allgemeinen Merkmale der Algen hinsichtlich Morphologie der Thalli, Feinstruktur der Zelle, Ernährung, Fortpflanzungstyp und Habitat durch kurzgefaβte und mit originellen Abbildungen illustrierte Beschreibungen der verschiedenen systematischen Abteilungen vermittelt werden, der als Leitfaden insbesondere für interessierte Studenten und sonstige Nutznieβer der Kollektion gedacht ist. Dazu werden in einem Wörterverzeichnis die wichtigsten Fachbegriffe der Phytologie erläutert. Ein Sachverzeichnis und ein Artenindex erleichtern die Benutzung des Katalogs. Die angeführte generelle phykologische Fachliteratur und die für jede einzelne systematische Abteilung angegebenen speziellen Literaturlisten sind für die weitere Information und die Orientierung auf dem Gebiet der Algenkunde besonders wertvoll.

Der eigentliche Katalog der Algensammlung bildet den 2. Teil des Buches. Die im Katalog angeführten 631 kultivierten Algenstämme sind nach den 16 systematischen Abteilungen der Algen angeordnet. Für jeden einzelnen Stamm werden Autor, Jahr und Standort der Isolierung, das jeweils verwendete Kulturmedium und die für die taxonomische Identifizierung der Art verantwortlichen Autoren angegeben. In einer kurzen Einführung über Status und Funktion der Sammlung, Erhalt der Kulturen und verwendete Kulturmedien wird darauf hin-gewiesen, daβ die allen Interessenten ohne Einschränkung zugängliche Sammlung keine nutznieβerischen Zwecke verfolgt. Die Algensammlung steht für Aus-bildung, wissenschaftliche und sonstige, nicht profitbringende Zwecke unentgelt-lich zur Verfügung, wobei die Nutznieβer nur die Transportspesen und teilweise die Kosten für den Erhalt der Kulturen (Glaswaren, Chemikalien u.s.w.) zu tragen haben.

Der vorliegende Katalog der Algensammlung des Instituts für Biologische Forschungen ist zweifellos ein verdienstvoller Beitrag der Autoren zur Erfassung der in Rumänien isolierten Algen und eine wertvolle Informationsquelle für Studenten, Algologen und sonstige Interessenten auf dem Gebiet der Algologie.

MARTIN KEUL

Nagy-Tóth Francisc, Adriana Barna, **Alge verzi unicelulare** (Chlorococcales). **Determinator** *(Unicellular Green Algae* (Chlorococcales). *Determinator),* Presa Universitară Clujeană, Cluj, 1998, 200 pages with 7 tables and 310 figures in the text.

The book is a remarkable contribution of the Cluj algological school, its authors being senior scientists of the Biological Research Institute in Cluj-Napoca.

The book is written in the first place for specialists, being the first determinator of algae published in Romania, but it can also be used by teaching staff or students interested in this field.

The authors establish first the taxonomic position of the *Chlorococcales* within the class *Chlorophyceae* (*Euchlorophyceae*), division *Chlorophyta,* based on morphological and structural characters. At the same time, it is mentioned that there are new electron microscopical and biochemical criteria which are used in the taxonomy of the order.

On the basis of diacritical characteristics of the *Chlorococcales* order, suborders to subfamilies are briefly presented. Emphasising the importance of morpho-structural criteria, including electron microscopic ones and also referring to the tendency towards the involvement of multiplication processes, the authors present the determination key of the 15 families of the *Chlorococcales*, including a diagram with the possible evolutionary lines within the order, based on recent literature.

The main part of the book (pp. 18-180) consists of the determination keys of infrageneric taxa within the 15 families, hierarchically organised according to the degree of evolution of the thallus, a criterion according to which some families (*Hormontilaceae, Ankistrodesmaceae*), that are included in recent monographs in other taxonomic units, have been retained. For each of the 310 species (within 135 genera) there are given brief descriptions based on their morpho-structural features, being accompanied by elements of ecology and chorology (especially for Europe), as well as by their principal synonyms. All the species included in the determinator are accompanied by drawings in black ink, which illustrate both the phenetic characters of the thalli and their wide variability, and provide sequences of their life cycles when this is necessary and significant for their identification. The graphic part is completed by a number of diagrams illustrating either the main characters of some genera from some families or subfamilies (*Chlorella, Raphidaelis, Kirchneriella, Crucigenia, Tetrastrum, Hofmania, Tetrachlorella, Westella*) or their life cycles (*Pediastrum*).

The book is accompanied by a selective bibliography including a series of works from the international literature and by an index of taxa.

The importance of the book is also due to the fact that the 310 species presented are the most common ones, wide-spread in pools, lakes, rivers, ponds and in soils, many of them occurring in the Romanian flora. They represent approximately 1/3 (1/5) of all *Chlorococcales* species, estimated to be known at present in the world (1000-1500 species), the group having a large ecological tolerance, its members being found from glaciers and long-lasting snow to thermal waters.

LAURA MOMEU and ANA RASIGA

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, XLIV, 1-2, 1999

CRONICĂ

Centenary of Soil Enzymology

The first paper on soil enzymes appeared one hundred years ago, more precisely on 15th of November 1899, in the Centralblatt für Bakteriologie, Parasitenkunde u. Infektionskrankheiten, Zweite Abteilung: Allgemeine, landwirtschaftlich - technologische Bakteriologie, Gärungsphysiologie, Pflanzenpathologie und Pflanzenschutz (Band V, No. 22, Seiten 745-754).

The paper entitled "The Destruction of Chlorophyll by Oxidizing Enzymes" was elaborated by Albert F. Woods (co-worker of the Division of Vegetable Physiology and Pathology, U.S. Department of Agriculture). Most of his experiments were carried out for studying oxidase and peroxidase enzymes in leaves of a series of plants. But as he wrote *"I have also determined by experiment that the oxidizing enzymes, especially the peroxidase may occur in the soil and, as a rule, are not destroyed by the ordinary bacteria of decay. These enzymes enter the soil through the decay of roots and other parts of plants which contain them. The peroxidase also endures drying for a long time without injury."* He reiterated in the Synopsis of Conclusions that *"The oxidase and peroxidase may remain in the soil uninjured for several months"*.

In the light of present-day soil enzymology, Woods' results on occurrence, persistence and resistance of soil enzymes are valid findings. His statement on plant origin of soil enzymes is only partly true, as soil enzymes may also originate from microorganisms and animal residues.

Woods' paper, due to its historical importance and fundamental value, deserves, I think, these commemorative lines.

ŞTEFAN KISS