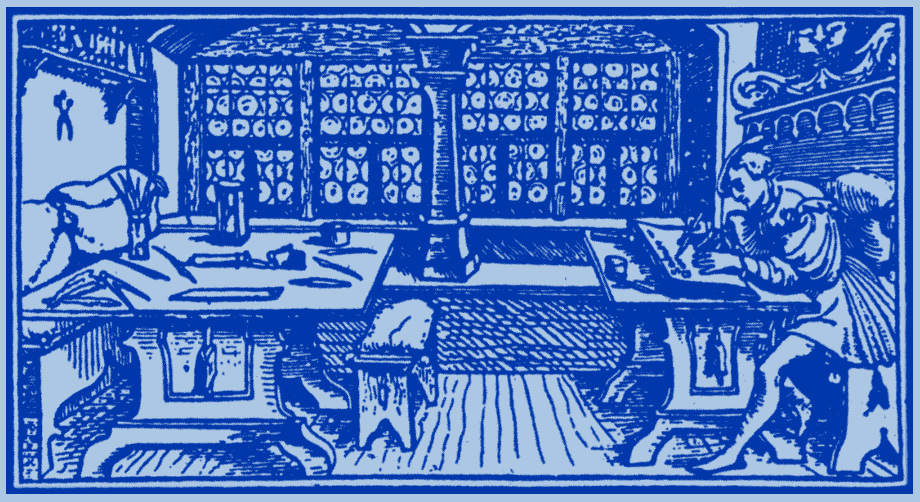
STUDIA

UNIVERSITATIS BABEŞ-BOLYAI

Biologia

CLUJ-NAPOCA 2000



1

ANUL XLV **2000**

S T U D I A
UNIVERSITATIS BABEŞ-BOLYAI

BIOLOGIA

1

Editorial Office: 3400 CLUJ-NAPOCA, Gh. Bilaşcu no. 24 • Phone: 405300 ex. 167

SUMAR - CONTENTS - SOMMAIRE - INHALT

Recenzii - Book Reviews - Comptes Rendus - Buchbesprechungen

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, XLV, 1, 2000

SOIL ENZYME ACTIVITIES AS INFLUENCED BY EARTHWORMS

ŞTEFAN KISS[*]

SUMMARY. - This article is a review of the earthworm-related soil enzymological investigations described in the western and eastern literature mostly during the last 40 years. In the review, the literature data are grouped into 13 Sections entitled: 1. Comparison of enzyme activities in earthworm casts and underlying soil; 2. Comparison of enzyme activities in drilosphere and matrix soil; 3. Comparison of enzyme activities in soils containing and lacking earthworms, respectively; 4. Comparison of enzyme activities in soils with and without addition of earthworms; 5. Origin of earthworm cast enzymes; 6. Earthworm-related enzyme activities in cycling of plant nutrients in soils; 7. Enzyme activities and earthworms in soils as influenced by management practices; 8. Enzyme activities and earthworms as related to pesticide degradation in soils; 9. Enzyme activities and earthworms in pasture soils submitted to restoration after removal of their top layer used for landscape improvement; 10. Enzyme activities and earthworms in urban soils; 11. Enzyme activities and earthworms in mine spoils submitted to recultivation; 12. Enzyme activities in earthworm-worked dungs, composts, toxic crop residues and organic industrial wastes; and 13. Enzyme activities in soils treated with earthworm-worked manures.

Introduction. The first study showing that the earthworm casts are more enzyme-active than the underlying soil was published in 1957 [22]. The investigations along this line and in new directions have been amplified by many research groups and led to a rich literature on the relations between soil enzyme activities and earthworms. Some of these investigations were referred to in excellent review works on earthworms (*e.g.*[2, 30, 39, 45, 62]), but an updated review, considering the western and eastern literature with an equal emphasis, is lacking. This is why this review article was elaborated. The literature data reviewed are grouped into 13 Sections. Within Sections 6 and 12, subsections (3 and 4, respectively) are delineated.

[*] *Babeş-Bolyai University, Department of Plant Physiology, Laboratory for Environmental Enzymology and Microbiology, 3400 Cluj, Romania*

1. Comparison of enzyme activities in earthworm casts and underlying soil

In 1957, Kiss [22] reported that invertase activity was higher in the casts of *Lumbricus terrestris* than in the most enzyme-active underlying 0-2-cm soil layer and much higher than in the 5-10-cm layer. Two neighbouring areas, covered by dense and sparse vegetation, under a meadow and an arable land, on a neutral, clayey brown forest soil (located in the vicinity of the town of Dej, Transylvania, Romania) were studied (Table 1).

Table 1

Invertase activity in earthworm casts and underlying soil [22]

Studied area	Analysed material	Invertase activity*	
		$\Delta\alpha^0$	%
Meadow	Earthworm casts	2.70	177.6
	Soil from the 0-2-cm depth	1.52	100.0
	Soil from the 5-10-cm depth	0.70	46.0
Arable land	Earthworm casts	1.73	180.2
	Soil from the 0-2-cm depth	0.96	100.2
	Soil from the 5-10-cm depth	0.69	71.8

* Invertase activity, determined polarimetrically, is expressed as difference between optical rotations in reaction mixtures (prepared from 20 g air-dried casts or soil + 2.5 ml toluene as antiseptic + 10 ml 20% (weight/volume) sucrose solution + 50 ml distilled water) before and after 24 hours of incubation at 37^0C: $\alpha_0^0 - \alpha_{24}^0 = \Delta\alpha^0$. The percentage invertase activity was calculated by taking as 100% the activity measured in the 0-2-cm soil layer.

Hoffmann [19] sampled three layers (0-10-, 10-20- and 20-30-cm depths) from a sandy loam soil under meadow vegetation in the Weihenstephan area (Bavaria, Germany). The 0-10-cm layer consisted of earthworm casts and casts + soil mixture; cast-free soil could not be found in this layer. In the other two layers, three materials: casts, casts+soil mixture and cast-free soil could be separated. The results of the enzymological analyses are reproduced in Table 2.

Table 2 shows that the absolute activity of each enzyme was highest in the casts, lower in the casts+soil mixture and lowest in the cast-free soil. The absolute activities in each material decreased with decreasing organic matter content and with increasing soil depth. Based on these dependences of the absolute activities and also on the finding that the relative activities, excepting amylase activity, were not highest in casts at all soil depths, Hoffmann [19] has drawn the conclusion that the increased activities in the casts are due to microbial enzymes and, thus, the earthworms themselves do not contribute to a marked increase of the enzyme content in soil.

Table 2

Enzyme activities in earthworm casts and underlying sandy loam soil under meadow vegetation [19]

Depth of soil layer (cm)	Analysed material	Organic substance (%)	Enzyme activities*							
			Invertase		β-Glucosidase		Amylase		Urease	
			A.a.	R.a.	A.a.	R.a.	A.a.	R.a.	A.a.	R.a.
0-10	Casts	3.95	10.1	127.8	5.7	72.2	4.8	60.8	12.1	153.2
	Casts+ soil mixture	3.87	9.4	121.4	5.3	68.5	4.0	51.7	11.1	143.4
10-20	Casts	2.40	3.5	72.9	1.7	35.4	1.8	37.5	5.2	108.3
	Casts+ soil mixture	1.50	2.5	83.3	0.9	30.0	1.0	33.3	2.5	83.3
	Cast-free soil	0.98	1.8	91.8	0.4	20.4	0.7	35.7	1.7	86.7
20-30	Casts	1.77	2.7	76.3	1.5	42.3	1.4	39.5	1.3	36.7
	Casts+ soil mixture	0.67	1.1	82.0	1.3	97.0	0.5	37.3	0.9	67.2
	Cast-free soil	0.51	0.8	78.4	0.1	-	0.3	-	0.5	49.0

* Invertase, β-glucosidase and amylase activities are expressed in ml 0.1 N $Na_2S_2O_3$ equivalent to the amounts of reducing sugars and urease activity is expressed in ml 0.1 N H_2SO_4 equivalent to the amount of NH_3, produced from the enzyme substrates during incubation of reaction mixtures and reported to 2 g of analysed material (Absolute activity = A.a.) or to 1 g of organic substance (Relative activity = R.a.).

According to a report published in the Communications of the Agricultural University and Experiment Station in Gent (Belgium), Coucke [7] introduced *ca*. 12 earthworms (the species name is not given) into 2.5-kg samples of a garden soil of sandy loam texture placed in glass containers. Other samples of the same soil, at the same humidity, but without earthworm addition were the controls. The casts collected on the surface of soil treated with earthworms and the control soil were submitted to enzymological and microbiological analyses.

The results have shown that invertase activity was a little higher and amylase and cellulase activities were much higher in the casts than in the control soil, whereas urease activity was practically the same in the casts and control soil. Contrarily to the increased amylase and cellulase activities in the casts, the numbers of amylolytic, aerobic and anaerobic cellulolytic microorganisms were lower in the casts than in the control soil. This is why Coucke [7] considered that the increased amylase and cellulase activities in the casts resulted from the amylase and cellulase produced by the earthworms themselves.

Kozlovskaya and her collaborators [26-34] have determined enzyme activities in casts of *Lumbricus rubellus* and *Octalasium lacteum* and in the underlying peat soils and in casts of *Eisenia rosea* and the underlying soddy-podzolic soils. Investigations on earthworms and other soil invertebrates were carried out in 85 biogeocoenoses located in different European and Asian areas of the former USSR [27].

Casts of *L. rubellus* were more enzyme-active than the underlying peat soil. Thus, invertase and urease activities (expressed in mg of glucose and NH_3, respectively/g dry casts or soil/24 hours at 37^0C) were 80.00 and 3.66, and 8.50 and 1.05 in the casts and soil, respectively [29, 31, 32]. Mean values of cellulase activity (expressed in mg of glucose/g dry casts or soil/10 days at 37^0C) were also higher in *L. rubellus* casts (2.025) than in the peat soil (1.057) and in *O. lacteum* casts (1.909) than in the peat soil (1.080) [26-28, 31, 32, 34].

The enzyme activities underwent changes during "aging" of casts. Table 3 shows that protease activity was higher in the casts of each earthworm species than in the underlying soil. At the same time, protease activity was higher in the 1-month-old than in the 6-day-old casts of both *L. rubellus* and *O. lacteum*, but further aging of the *L. rubellus* casts led to a decrease in protease activity and, thus, in the 3-month-old casts, protease activity declined nearly to the activity of the underlying peat soil.

Table 3

Protease activity in earthworm casts and underlying soils [27, 33, 34]

Analysed material	Age of casts	Protease activity*
Lumbricus rubellus casts	6 days	9.797±0.155
	1 month	10.560±0.046
	2 months	9.594±0.077
	3 months	6.692±0.109
Peat soil	-	6.462±0.049
Octalasium lacteum casts	6 days	6.924±0.136
	1 month	12.900±0.120
Peat soil	-	5.266±0.127
Eisenia rosea casts	6 days	2.066±0.104
Soddy-podzolic soil	-	1.666±0.081

* Expressed in mg NH_2-N/g dry casts or soil/24 hours at 37^0C.

As the age-dependent increase then decrease of enzyme activity in casts are the results of microbial enzyme synthesis then destruction, the conclusions has been drawn that the earthworm casts (and excrements of other soil invertebrates) should be considered as centres of the microbial activity, of the biochemical synthesis and decomposition of organic substances [26-28, 30, 31, 34].

Sharpley and Syers [68] have determined phosphatase activity (by using *p*-nitrophenyl phosphate as substrate) in fresh earthworm casts and the underlying surface (0-5 cm) soil of silty loam texture. The casts of earthworms (dominantly *Allolobophora caliginosa*[*]) were collected from the soil surface at several sites close to undrained surface run-off plots in an experimental watershed (under permanent pasture) located adjacent to Massey University (Palmerston North, New Zealand).

The casts contained appreciably more total, inorganic and organic phosphorus than the underlying soil. Phosphatase activity was much higher in the freshly deposited (< 14 hours) casts than in the surface soil. Thus, this activity (expressed in μmoles of *p*-nitrophenol/g casts or soil/hour) was, after 1 and 5 days of incubation, 190 and 60 (after 1 day), and 60 and 40 (after 5 days) in casts and soil, respectively. It was also found that release of total P from fresh *casts* to 0.1 M NaCl at a solution: solid ratio of 50:1 did not change during the 5-day incubation at 16^0C. However, the amount of inorganic P released increased significantly and in parallel with the decrease in the amount of organic P released during the first 3 days of incubation, and these changes were related to and preceded by increased phosphatase activity. At the same time, little changes occurred not only in the amount of total P but also in the amounts of inorganic and organic P released from the *soil* under identical conditions of incubation.

Studying the seasonal variation of casting activity in the experimental watershed area specified above, Sharpley and Syers [69] have established that the amount of inorganic P released from freshly deposited (< 14 hours) casts to 0.1 M NaCl at a solution:solid ratio of 400:1 at 16 and 4^0C decreased progressively from a maximum in May to a minimum in August. In parallel, the amount of organic P released showed a reverse trend over the same May-August period. Consequently, the decrease in the inorganic P was attributed to a reduction in the conversion of organic to inorganic P because of the lower phosphatase activity as a result of declining soil temperature (the decrease being a little greater at 4 than at 16^0C). In contrast, seasonal changes in the release of inorganic and organic P to solution from the underlying soil (0-10 cm depth) were small at both 16 and 4^0C.

These investigations were referred to also in [73, 74].

Loquet *et al.* [42] and Loquet [41] have described investigations carried out on a *Lolio-cynosuretum* meadow permanent since at least 1840. It is situated in Cîteaux Monastery, at 30 km south of Dijon (France), on a nearly neutral, leached silty soil. The earthworms living here belong to 12 species, of which

[*] Syers *et al.* [72] note that the dominant earthworm species was *Lumbricus rubellus* and not *Allolobophora caliginosa* as suggested by Sharpley and Syers [68, 69].

Nicodrilus longus longus and *N. nocturnus cistercianus* are dominant. Earthworm casts and the 0-6-, 6-20-, 20-40-, 40-60- and 60-100-cm layers of the underlying soil were sampled several times in the March 1974-November 1976 period, for different analyses, including determination of invertase, urease, amylase and cellulase activities.

Invertase activity was higher in the casts than in the underlying soil, in which the activity progressively decreased with increasing soil depth. A single exception was recorded: in March 1974, the 0-6-cm soil layer was more invertase-active than were the casts.

Urease activity was also higher in casts than in soil, but amylase activity was very low and appreciable cellulase activity was lacking in both casts and soil, including the 0-6-cm soil layer.

Mulongoy and Bedoret [52] have determined enzyme activities in turret-shaped and granular casts presumably produced by the most numerous earthworm species in southern Nigeria, namely *Hyperiodrilus africanus* and *Eudrilus eugeniae (Eudrilidae)*, respectively, and in the underlying surface soils (0-15-cm layer).

Turret-shaped casts and underlying surface soils were sampled from four sites under different plant covers. Three sampling sites (designated A, B and C) are located at the International Institute of Tropical Agriculture in Ibidan (south-western Nigeria) and the fourth site (D) is located at the high rainfall substation of this Institute at Onne, near Port Hartcourt (south-eastern Nigeria). Site A is under *Leucaena leucocephala (Mimosae)*, site B - under non-leguminous vegetation in a secondary forest, site C - under *Treculia africana (Moraceae)* and site D - under *Pueraria phaseoloides (Papilionaceae)*.

Granular casts and underlying surface soil were sampled from a plot of *Treculia africana* (site C).

All samplings were done during the rainy season of 1985.

The results obtained (Table 4) clearly show that at each sampling site each enzyme activity was much higher in the earthworm casts than in the underlying surface soil. In general, no differences were found among turret-shaped casts or surface soils from different sites, but the turret-shaped casts and surface soil from site C were relatively low in acid phosphatase activity. β-Glucosidase, urease and acid phosphatase activities were not significantly different in the turret-shaped and granular casts sampled from the same site C, but the granular casts - in comparison with the turret-shaped casts - were more dehydrogenase - and alkaline phosphatase-active. Acid phosphatase activity exceeded the alkaline one in both casts and underlying soils.

Table 4

Enzyme activities in surface soil and earthworm casts from sampling sites A, B, C and D [52]

Analysed material	Sampling site	Enzyme activities*				
		Dehydro-genase	β-Gluco-sidase	Urease	Acid phosphatase	Alkaline phosphatase
Surface soil	A	192	38	6	99	27
	B	193	19	5	144	20
	C	145	11	4	78	27
	D	215	20	7	192	65
Turret-shaped casts	A	584	309	34	498	360
	B	404	134	16	540	124
	C	484	119	23	263	127
	D	265	69	23	528	137
Granular casts	C	614	129	21	335	202
LSD (5%)**		22	34	2	89	44

* Expressed in μg reaction product/g soil or casts/hour. Reaction products: triphenylformazan (dehydrogenase); ammonium (urease); *p*-nitrophenol (β-glucosidase, acid and alkaline phosphatases).

** LSD - Least significant difference. Means for granular casts are excluded.

Tiwari *et al.* [77] have determined dehydrogenase, urease and acid phosphatase activities in earthworm casts and underlying red sandy soil of laterite origin, at the Pineapple Research Station Nayabunglow (about 30 km north of Shillong, India). Samplings were done monthly, in the 15 April 1986-15 March 1987 period, during which five earthworm species (*Amynthas alexandri, Drawida assamensis, Megascolides antrophyes, Metaphire houlleti* and *Nelloscolex strigosus*) were recorded, *Drawida assamensis* being the dominant species.

Each activity was found at each sampling date to be higher in casts than in underlying soil. The trend of temporal variation in enzyme activities was similar in casts and soil. The maximum values were registered in July (dehydrogenase and acid phosphatase) and in May (urease), whereas each activity was lowest in January.

Tiunov [76] described enzymological studies related to the earthworms living in a spruce, a lime and a mixed forest (spruce, poplar, oak, lime) located on podzolic soils in the Moscow region (Russia). *Aporrectodea caliginosa* was found to be the dominant species in each of the three forests. *Lumbricus terrestris* was found in the lime and mixed forests.

Urease activity was determined in the spruce forest and mixed forest soils and in the casts of *A. caliginosa* cultured in these soils. The spruce forest soil was less urease-active than the mixed forest soil. Urease activity in fresh, 1- and 24-day-old casts exceeded the urease activity of the soil in which *A. caliginosa* was cultured. The cast urease activity in the mixed forest soil was higher than the cast urease activity in the spruce forest soil.

Matsumoto and Taniguchi [47] compared enzyme (phosphomono- and phosphodiesterase) activities in earthworm (*Pheretima communissima*) casts with those in the earthworm gut. Both phosphatase activities were much higher in the gut than in the casts. Thus, the activities measured at pHs 4.9, 6.6 and 8.2 and expressed in μmoles of *p*-nitrophenol/g material/hour gave the following values: 160.5, 92.28 and 120.3 in gut and 5.86, 7.23 and 6.40 in casts (phosphomonoesterase), and 5.74, 13.81 and 14.88 in gut and 1.07, 1.85 and 1.70 in casts (phosphodiesterase). The activities were also much higher in the gut than in the surrounding soil.

In another experiment, Matsumoto *et al.* [46] have found that phosphomonoesterase activity (expressed again in μmoles of *p*-nitrophenol/g material/hour) decreased in the order: 184.19 (foregut) > 58.33 (midgut) > 18.84 (hindgut) > 2.86 (casts). These investigators have also studied the effect of aging of casts on their phosphomonoesterase activity. Taking the activity in fresh casts as 100%, it decreased to 66, 56 and 43% after 1, 3 and 5 days of aging, respectively, and showed a slight increase (48%) after 7 days.

2. Comparison of enzyme activities in drilosphere and matrix soil

Drilosphere is a thin soil layer around the walls of the burrows of earthworms. It separates the burrows from the neighbouring, matrix soil, devoid of burrows.

The investigations carried out by Loquet *et al.* [42] and Loquet [41] for studying enzyme activities in earthworm casts and in the underlying soil were referred to in Section 1. On the same permanent meadow and in the same March 1974-November 1976 period, Loquet [41] has studied enzymologically the drilosphere, too.

A soil pit (1.4 m deep and 1.2 m wide) was examined and it was found that the earthworm (*Nicodrilus longus longus* and *N. nocturnus cistercianus*) burrows did not exceed 1 m in depth. For enzymological analyses, Loquet [41 collected the 2-mm wide drilosphere soil layer round the burrows at depths of 0-6, 6-20, 20-40, 40-60 and 60-100 cm; samples from the same depths of the matrix soil were also taken. Invertase, urease and dehydrogenase activities were determined.

The drilosphere enzyme activities changed rather irregularly in dependence of sampling depth and time and in comparison with activities in the matrix soil. For example, the ratio invertase activity in drilosphere/invertase activity in matrix soil was highest at the 60-100-cm depth in June 1975 and February 1976 and at the 6-20-cm depth in November 1975, April and November 1976. This activity was higher in the drilosphere than in the matrix soil at all depths in June 1975, but the reverse was true at depths of 20-100 cm in April 1976. In dependence of depth, invertase activity fluctuated in the drilosphere and progressively decreased in the matrix soil in February 1976.

Urease activity in drilosphere largely differed from that in the matrix soil at the 40-60-cm depth in June 1975, but the differences were small in June and November 1976.

The ratio between dehydrogenase activity in drilosphere and matrix soil at the four depths studied were 1.0, 2.5, 8.3 and 3.0, respectively, in June 1976.

As specified in a short report, Stehouwer *et al.* [70] have studied drilosphere and matrix soil at 7 depth intervals from 0 to 50 cm. Alkaline phosphatase activity, organic C and water-soluble organic C contents decreased with depth in both drilosphere and matrix soil. Concentrations in drilosphere were 2-3 times higher than in the matrix soil at the profile surface and up to 80 times higher at the depth of 50 cm.

Tiunov [75, 76] has determined enzyme activities in drilosphere of *Lumbricus terrestris* and *Aporrectodea caliginosa* and in matrix soil. The investigations were carried out in a lime and a mixed forest on podzolic soils in the Moscow region (see also Section 1). In the lime forest, *L. terrestris* drilosphere and matrix soils were collected from the 30-cm depth, whereas in the mixed forest, both *L. terrestris* and *A. caliginosa* drilosphere and matrix soils were taken from the 15-cm and 30-cm depths (*L. terrestris*) and the 10-20-cm depth (*A. caliginosa*).

Urease activity was 2-12 times higher in the drilosphere than in the matrix soil. It should be added that this activity was even lacking in the matrix soil of lime forest. The *A. caliginosa* drilosphere was more urease-active than the *L. terrestris* drilosphere. Protease activity was also higher (2-4 times) in the drilosphere than in the matrix soil. Contrarily, cellulase activity was often lower in the drilosphere as compared to the matrix soil.

3. Comparison of enzyme activities in soils containing and lacking earthworms, respectively

Atlavinyte *et al.* [1] conducted field experiments on a soddy-podzolic soil in the Širvintu district (northern Lithuania), in the 1975-1977 period. They used biometers, *i. e.* 2-m^2 microplots, in which the soil to depth of 50 cm was isolated from the surrounding soil by means of corrosion-resistant metal walls. The earthworms (*Allolobophora caliginosa* f. *typica*) were removed from a part of

biometers (biometers I) which remained free of earthworms during the 3-year experiments. In the other biometers (biometers II), the number of earthworms was maintained at an average of 150/m^2 by introduction of earthworms after sowing of winter rye in autumn 1975, barley in spring 1976 and red clover in spring 1977. Biometers I were sown with the same plants and at the same time as biometers II. Both biometers I and II were fertilised with ammonium nitrate, superphosphate and potash salt at rates (per ha) of $N_{60}P_{90}K_{90}$ (in 1975), $N_{110}P_{90}K_{90}$ (in 1976) and $P_{90}K_{90}$ (in 1977).

In August 1977 (3 years after the beginning of the experiments), soil samples were taken from the 0-13- and 13-26-cm depths for different analyses, including determination of invertase and protease activities.

Invertase activity (expressed in mg glucose/g dry soil) in the 0-13- and 13-26-cm soil layers was 32.7 and 26.7 in biometers I (no earthworms) and 32.4 and 25.0 in biometers II (with earthworms); the corresponding values for protease activity (expressed in mg NH_2-N/g dry soil) were 0.34 and 0.37, and 0.46 and 0.45, respectively. In other words, the earthworms did not affect invertase activity of soil, but led to increased soil protease activity. It should be added that under the influence of earthworms the crop yields increased (winter rye with 15%, barley with 27% and red clover with 45%).

Studying the earthworm *Pheretima communissima* in the soil of a reclaimed land near the seashore of the Osaka Bay (Japan), Matsumoto *et al.*[48] have found that invertase and urease activities were higher in the earthworm-worked soil and in the casts than in the surrounding soil lacking earthworms.

In another study, Matsumoto and Taniguchi [47] have found higher phosphomono- and phosphodiesterase activities in the upper layer of a soil rich in *Pheretima* earthworms than in a forest soil and a paddy soil in which no earthworms lived.

Tiunov [76] has collected soil samples from the 5-10-cm layer and earthworms (*Aporrectodea caliginosa*) in spruce, mixed and lime forests on podzolic soils in the Moscow region (see also Sections 1 and 2). The field-moist soils were passed through a 3-mm sieve, then placed into pots (1 kg/pot) and left without earthworms or inoculated with 3 earthworms/pot. For inoculation of each soil, the earthworms, collected from the respective soil, were used. The incubation, at 25-28% (weight/weight) soil moisture content, lasted 12 days. Before inoculation and incubation and after incubation, the soils were analysed for determination of their urease activity. In soil of each forest, urease activity was higher in the earthworm-containing variant than in that lacking earthworms. But the level of soil urease activity was dependent on forest type: the activity increased - in soils before inoculation and incubation as in those left non-inoculated or inoculated and incubated - in the order: spruce forest < mixed forest < lime forest.

4. Comparison of enzyme activities in soils with and without addition of earthworms

Subler *et al.* [71] conducted field experiments in two agroecosystems established in 1991 at the Ohio Management Systems Evaluation Area in Pike county (Ohio). The agroecosystems, each replicated (n=3) on 0.4-ha plots, were a corn-soybean rotation (CS) and a corn-soybean-wheat-hairy vetch cover-crop rotation (CSW) on predominantly silt loam and sandy loam soils. The corn in both systems was N-fertilised at the following rates (kg N/ha): 135 (as NH_3) + 44 (as liquid fertiliser, l.f.) in 1992 and 157 (as l.f.) in 1994 in the CS system; 56 (as NH_3) + 12 (as l.f.) + 28 (as manure) in 1991 and 105 (as l.f.) + ≈ 28 (as vetch) in 1994 in the CSW system. The liquid fertiliser contained 40% NH_4NO_3 and 30% urea, by weight.

In November 1993, earthworms were added (100/m^2) to enclosures (6.1 x 6.1 m) within plots of both agroecosystems. Other enclosures, to which no earthworms were added, served as controls. The enclosure walls, made of translucent corrugated plastic, were inserted *ca*. 5 cm into the soil and extended 25 cm above the soil. The added earthworms were collected from another area, namely from a no-till corn field in Columbus (Ohio) and were predominantly immature and adult *Lumbricus terrestris*.

In April 1994 (*i.e.* 5 months after addition of earthworms), some earthworm and control enclosures were used for counting earthworms. It was found that, in both CS and CSW systems, addition of earthworms led to less abundant surface-dwelling earthworms (collected above 15 cm) and most abundant deep-dwelling earthworms (collected below 15 cm) than in the control enclosures (to which, as mentioned above, no earthworms were added). Other earthworm and control enclosures were used for soil sampling at four depths: 0-5, 5-15, 15-30 and 30-45 cm. Besides a series of soil parameters, dehydrogenase activity was also determined.

In the CS system, dehydrogenase activity was insignificantly lower in the 0-5-cm soil layer and significantly ($P<0.05$) higher in deeper soil layers of the earthworm enclosure as compared to the control enclosure. Contrarily, in the CSW system, earthworm addition led to significant decrease in dehydrogenase activity at the soil surface (0-5 cm) and to insignificant changes in the deeper soil layers. These findings were interpreted as being the consequence of decreased abundance and activity of surface-dwelling earthworms in the earthworm enclosures.

The experiment performed by Ross and Cairns [61] and briefly described in Section 9 should also be mentioned here.

5. Origin of earthworm cast enzymes

It is a general opinion that the enzymes in earthworm casts originate from two sources: the microorganisms living in gut and casts and the earthworm tissues themselves. Table 5 lists literature data, according to which there are enzymes produced at least partly or not produced at all by tissues of the earthworm species studied.

Two other sources of cast enzymes can also be envisaged. If the enzymes present in the ingested food (*e.g.* plant residues) and in the ingested soil resist - as free proteins or as protein-humic complexes - to decomposition in the digestive tract, they will be ejected with the faeces. The experimental verification of these possibilities received no attention up to now, which seems to be attributed to the difficulties in elaboration of methodologies for such a verification.

Table 5

Production of enzymes in earthworms

Abbreviation for some earthworm genera: *A. - Allolobophora. D. - Dendrobaena. E. - Eisenia. L. - Lumbricus. O. - Octalasium (Octalasion)*

Enzyme	Earthworm	Examined material	Reference
1	2	3	4
Enzymes in earthworms produced, at least partly, by earthworm tissues			
Hydrolases			
Hydrolases participating in C-cycle			
Cellulase	17 species*	Extract from entire worms	78
Chitinase	12 species*	The same as above	78
Cellulase, chitinase	*L. terrestris*	Extract of foregut and hindgut wall	78
Invertase, maltase, cellobiase, melibiase, lactase, amylase, cellulase, chitinase	*D. octaedra*	Extract from homogenate of gut and gut content	54
Maltase, cellobiase, melibiase, lactase, amylase	*A. caliginosa*	The same as above	54
Laminarinase	*L. terrestris*, *L. rubellus*	The same as above	55
Amylase, cellulase, lichenase, chitinase, lipase	*L. terrestris*, *Allobophora* sp., *Pheretima* sp.	Extract from tissue of alimentary tract	37
Glycosidases	*L. terrestris*	Intestinal extract	40

Table 5 (continued)

1	2	3	4
Carbohydrases, lipases	*A. caliginosa*, *A. smaragdina*, *Allolobophora* sp., *L. rubellus*, *D. veneta*	Extract from digestive tract	44
Cellulase	*E. foetida*, *L. terrestris*, *O. tyrtaeum*	Cell-free extract from homogenate of entire worms	18
Cellulase	*E. fetida andrei*	Homogenate from gut wall and from gut wall+gut content	43
Cellulase	*D. vejdovskyi*, *D. octaedra*, *Dendrodrilus rubidus*, *L. castaneus*, *L. rubellus*, *A. caliginosa*, *A. rosea*, *O. lacteum*	Extract from homogenised gut wall (tissue)	79
α-Amylase, glucoamylase, laminarinase, xylanase, lichemase, C_x-cellulase, cellulase complex (exo- and endo-β-1,4-glucanase)	*D. octaedra*, *L. castaneus*, *L. rubellus*, *A. caliginosa*, *O. lacteum*	The same as above	80
Invertase, amylase, cellulase	*Lampito mauritii*, *Octochaetona surensis*, *Drawida willsi*	Gut-cleaned worms	8
Maltase, β-N-acetylglucosaminidase, amylase, carboxymethylcellulase, xylanase, laminarinase, galactomannanase	*Pontoscolex corethrurus*	*In vitro* tissue culture of gut wall	81
Glycolytic enzymes	*Millsonia anomala*	The same as above	35

Table 5 (continued)

1	2	3	4
Carbohydrases hydrolysing oligosaccharides (sucrose, maltose, cellobiose, laminaribiose, gentiobiose), heterosides (α- and β-glucoside, β-N-acetyl-glucosamine, β-mannoside, β-xyloside, β-galactoside) and polysaccharides (starch, cellulose, laminarin, mannan, galactomannan, pullulan, lichenin)	*Polypheretima elongata*	The same as above	36
Xylanase, acetylesterase, β-glucuronidase, α-arabinosidase, β-xylosidase	*E. andrei*	Extract from homogenate of entire, gut-cleaned worms	50
Hydrolases participating in N-cycle			
Urease	*Lampito mauritii*, *Drawida willsi*	Gut-cleaned worms	8
Protease	*L. terrestris*, *Allolobophora* sp., *Pheretima* sp.	Extract from tissue of alimentary tract	37
Protease	*A. caliginosa*, *A. smaragdina*, *Allobophora* sp., *L. rubellus*, *D. veneta*	Extract from digestive tract	44
Protease	*D. octaedra*, *L. castaneus*, *L. rubellus*, *A. caliginosa*, *O. lacteum*	Extract from homogenised gut wall (tissue)	80
Hydrolases participating in P-cycle			
Alkaline phosphatase	*Barogaster annandalei*	Different components of alimentary canal examined histoenzymologically	9

Table 5 (continued)

1	2	3	4
Alkaline phosphatase	*E. fetida*, *D. veneta*, *L. rubellus*, *A. caliginosa*	Fresh faecal material, containing acid phosphatase of microbial origin and alkaline phosphatase produced, at least partly, by the earthworms	62
Acid and alkaline phosphomono- and phosphodiesterase	*Pheretima communissima*	Foregut, midgut and hindgut	46, 47
Alkaline phosphodiesterase, acid and alkaline phosphomonoesterase	*L. terrestris*	Extract of homogenate from entire worms	58-60
Alkaline phosphomonoesterase	*E. andrei*	Extract of homogenates prepared during embryonic and postnatal stages of worms	57
Alkaline phosphomonoesterase	*A. andrei*	Extract of homogenate from midgut	56
Oxidoreductases			
Dehydrogenase, catalase, polyphenol oxidase, peroxidase	*E. nordenskioldi*	Extract from entire worms	25
Peroxidase	*O. tyrtaeum*, *L. terrestris*, *E. foetida*, *Pheretima hupiensis*, *A. chlorotica*	Cell-free extract from homogenate of entire worms	53
Peroxidase, catalase	*E. foetida*, *L. terrestris*, *O. tyrtaeum*	The same as above	18
Transferases			
Glutathione-S-transferase	*Pheretima posthuma*	Extract of homogenate from entire worms	17
Enzymes in earthworms not produced by earthworm tissues			
Hydrolases			
Hydrolases participating in C-cycle			
Trehalase, cellulase, pectinase, xylanase, chitinase	*E. rosea*	Extract from homogenate of gut and gut content	54
Trehalase, pectinase, xylanase, galactanase	*D. octaedra*	The same as above	54
Trehalase, pectinase	*A. caliginosa*	The same as above	54

Table 5 (continued)

1	2	3	4
β-N-acetylglucosa-minidase	*L. terrestris*	Intestinal extract	40
Cellulase, mannanase	*Pontoscolex corethrurus*	*In vitro* tissue culture of gut wall	81
Cellulase, mannanase	*Millsonia anomala*	The same as above	35
Hydrolases participating in N-cycle			
Urease	*Octochaetona surensis*	Gut-cleaned worms	8
Oxidoreductases			
Polyphenol oxidase, aldehyde oxidase	*E. foetida, L. terrestris, O. tyrtaeum*	Cell-free extract from homogenate of entire worms	18

* *A. caliginosa, A. chlorotica, A. icterica, A. longa, A. nocturna, (Bimastus eiseni), (D. mammalis), (D. rubica), D. subrubicunda, E. foetida, E. rosea, (Eiseniella tetraedra), (L. castaneus), L. rubellus, L. terrestris, O. cyaneum, O. lacteum.*
Five species, in which lack of material prevented the detection of chitinase, are placed in brackets.

Besides producing some own enzymes contributing to the enzyme content in casts, the earthworms stimulate the production of microbial enzymes, as demonstrated by Satchell *et al.* [64].

For culturing *Eisenia foetida* and *Dendrobaena subrubicunda* a medium was prepared from cellulose board shredded and mixed with distilled water in proportion of 1 to 9 and the resulting pulp was amended with phytin (calcium inositol hexaphosphate) in proportion of 1 to 40. Pulp (300 g) amended with phytin was placed over 10 g of fine acid-washed sand in plastic containers, to which 30 worms were then added. The cultures were kept at room temperature for 4 weeks and analysed weekly for determination of acid (microbial) phosphatase activity at pH 4 and of the amount of ATP considered as indicator of microbial biomass.

The results have shown that, in cultures of both earthworms, there was a significant correlation (r=0.93) between acid phosphatase activity and ATP content. Based on this finding the conclusion was drawn that the acid phosphatase activity observed reflects the effect of earthworm activity in increasing microbial biomass.

6. Earthworm-related enzymes activities in cycling of plant nutrients in soils

The investigations proving that the earthworm enzymes contribute to the cycling of plant nutrients in soils will be reviewed grouped according to the nature of the nutrient elements.

6.1. Nitrogen cycling. Syers *et al.* [72] have carried out investigations on 900-cm^2 plots in an experimental watershed under permanent pasture in New Zealand (see Section 1) and on an adjacent area. Casts of predominantly *Lumbricus rubellus* and underlying soil samples (from depths of 0-5 and 18-22 cm) were collected weekly in the April-October 1977 period and analysed for determination *e.g.* of exchangeable $NH_4^+ - N$ and $NO_3^- - N$ contents and urease activity. These three parameters were found to be appreciably higher in casts than in underlying soil. In casts, the $NH_4^+ - N$ content was always greater than the $NO_3^- - N$ content, but in casts incubated for 6 days at 4 and 16°C, the $NH_4^+ - N$ content continuously decreased during the incubation, while the $NO_3^- - N$ content decreased only during the first 4 days of incubation. Urease activity was highest at day 1, then continuously decreased in parallel with the decreasing $NH_4^+ - N$ content. All these changes were a little more intense at 16 than at 4°C. In the underlying soil, the duration and temperature of incubation had very little effect on the $NH_4^+ - N$ and $NO_3^- - N$ contents and urease activity.

These investigations were also referred to in [73, 74].

6.2. Phosphorus cycling. Sharpley and Syers' [68, 69] investigations, referred to in Section 1, have convincingly proved the role of earthworm cast phosphatase in conversion of organic P into plant-available, inorganic P. This role also results from the investigations described by Matsumoto and co-workers [46,47] and referred to in Sections 1 and 3.

Based on an experiment, in which 1.5-kg samples of a sandy soil, collected at Zwijnaarde (Belgium), were amended with fresh lettuce leaves without or with addition of two *Lumbricus terrestris* and maintained at 15-20°C for 5 weeks, Devliegher and Verstraete [10] have stated - commenting the results of the experiment - that "P-availability is increased in the presence of *L. terrestris* due to the production of phosphatases and the activation of microbial phosphatase production".

A similar conclusion was drawn by Ross and Cairns [61](see Section 9).

Ganeshamurthy *et al.* [14] studied a red and a black tropical soil (India) and the earthworm *Drawida assamensis*. No or ten worms per pot were added to 500-g soil samples, then the pots were kept, at constant soil humidity,

at 28^0C for 4 weeks. The soils were analysed weekly. The analytical results have shown that under the influence of earthworms the exchangeable P and SO_4-S contents increased significantly in both soils. The increases were explained by admitting that some of the ester-bound P and S were enzymatically hydrolysed to inorganic P and S thereby contributing to their availability.

6.3. Sulphur cycling. See the preceding paragraph.

7. Enzyme activities and earthworms in soils as influenced by management practices

Gehlen [15] determined dehydrogenase, catalase and alkaline phosphatase activities and number of earthworms in 16 soils, namely in four arable (cereals), four vegetable (cabbage, carrot, lettuce, potato etc.), four orchard (apple) and four vineyard soils, at seven localities in Nordrhein-Westfalen and Rheinland-Pfalz (Germany). On each soil, two neighbouring plots were selected for studies. One plot was under conventional management and the other plot was under organic-biological or biological-dynamic management. Soil samplings were done four times: in springs and autumns of 1984 and 1985.

All enzyme activities in soils of all cultures, at all sampling dates, were significantly ($P < 0.05$ or $P < 0.01$) higher under biological management than under the conventional one.

The number of earthworms in all arable soils (at all sampling dates), in all soils under vegetables (in autumn 1984, and in spring and autumn 1985), in all vineyard soils (in autumn 1985), as well as in some soils under vegetables (in spring 1984) and in some orchard soils (at all sampling dates) was higher under the biological than under the conventional management. In general, the enzyme activities were more intense and the earthworms were more numerous in the orchard soils than in the other soils.

Taking into consideration the results obtained in all soils at all sampling dates, it was established that the correlations among the three enzymes activities were positive and more pronounced (r=0.72-0.83) than those between each enzyme activity and number of earthworms (r=0.47-0.48).

Fraser *et al.* [12] determined protease, phosphatase and arylsulphatase activities as well as number and biomass of earthworms in samples of a stony silt loam soil collected from three experimental sites on the Winchmore Irrigation Research Station in the mid-Canterbury region (New Zealand).

The first site is a flood-irrigated, grazed pasture fertilised with super-phosphate at rates of 0 (control), 188 and 376 kg/ha annually for 37 consecutive years. The second site had been intensely cultivated with arable crops for the last 11 consecutive years. The third site was a wilderness nearby which had not been used for agriculture and was covered with native grasses, herbs and introduced species.

For enzymological analysis, the soil was sampled from depths of 0-5, 5-10 and 10-20 cm. The earthworm population (*Aporrectodea caliginosa, A. rosea, Lumbricus rubellus, Octalasion cyaneum*) were taken for analysis from the top soil layer (to a depth of approximately 30 cm).

Protease and arylsulphatase activities decreased and phosphatase activity increased with increasing soil depth in the soil of pasture and wilderness. The activities showed a nearly even distribution in the 0-20-cm layer of the arable soil.

The enzyme activities and the number and biomass of earthworms varied in dependence of sites (Table 6).

Table 6

Site-dependent increasing order of soil enzyme activities and earthworms
(compiled based on data from [12])

Soil enzyme activity and earthworms	Soil depth (cm)	Site*-dependent increasing order
Protease	0-5, 5-10 and 10-20	A<W<P(0)<P(188)=P(376)
Phosphatase	0-5	A<W<P(376)<P(188)<P(0)
	5-10 and 10-20	A<W<P(0)=P(376)=P(188)
Arylsulphatase	0-5 and 5-10	A<W<P(0)<P(188)=P(376)
	10-20	A=W<P(0)=P(188)=P(376)
Number of earthworms	0-30	A<W<P(0)<P(188)<P(376)
Biomass of earthworms	0-30	A<W=P(0)<P(188)<P(376)

* A - Arable. W - Wilderness, P (0) - Pasture (control), not fertilised. P (188) and P(376) - Pasture fertilised with 188 and 376 kg superphosphate/ha, respectively.

It is evident from this table that the site dependence of soil enzyme activities, especially protease activity and that of earthworms are similar.

The experiments conducted by Subler *et al.* [71] for studying soil dehydrogenase activity and earthworms in a corn-soybean and a corn-soybean-winter rotation were already dealt with in Section 4.

8. Enzyme activities and earthworms as related to pesticide degradation in soils

Park *et al.* [58-60] have studied *Lumbricus terrestris* obtained from stock cultures at the Indiana State University in Terre Haute (USA). For removal of gut content with its microbiota, the worms were purged by incubation for 24 hours on moist filter paper. Then, approximately 20 g of live earthworm biomass (3.5-5 g dry weight) was homogenised in 1:4 (weight/volume) 0.1 or 0.05 M Tris-HCl buffer (pH 8.8) with addition of no or 0.1% Triton X-100 and centrifuged at 13,000 *g* (6^0C) for 10 minutes. The supernatant (*i.e.* the extract from the homogenate of entire worms) was used as source of phosphodi- and phosphomonoesterase. Bis-(*p*-nitrophenyl) phosphate (BNPP) and *p*-nitrophenyl phosphate (NPP) served as enzyme substrates. These compounds are not insecticidal organophosphates, but their hydrolytic product, *p*-nitrophenol, is toxic to plants and animals.

It was found that the earthworm extract exhibited both phosphodi- and monoesterase activities, producing *p*-nitrophenol from both BNPP and NPP, which means that *L. terrestris* is able of toxic bioactivation of nitrophenyl phospho-ester xenobiotics.

Contrarily, another enzyme, found in earthworms, too, can participate not in toxic bioactivation of xenobiotics, but in their detoxification. This enzyme, glutathione-S-transferase (GST), the activation of which in the earthworm *Pheretima posthuma* was studied by Hans *et al.* [17], catalyses dealkylation, dearylation and dehalogenation of insecticides by their conjugation with glutathione, these reactions being the primary steps in detoxification.

Hans *et al*. [17] collected mature earthworms from the campus soil of the Industrial Toxicology Research Centre in Lucknow (India). The earthworms were introduced in potted soil, to which no insecticide (control) or aldrin, endosulphan or lindane was added in a concentration of 1 μg/g. The pots were kept at 25^0C at 70% relative humidity for 4 weeks. After 1, 2 and 4 weeks, GST was extracted from the earthworms, from the guts of which the soil was previously removed. Then, the worms were homogenised in 0.05 M saline phosphate buffer (pH 6.6). The homogenate was centrifuged at 1000 *g* for 5 minutes. The supernatant was the GST-containing extract. GST activity was determined using 1-chloro-2,4-dinitrobenzene as substrate.

The residual insecticide concentrations in earthworms and soil were also determined.

The extract from earthworms kept in the control soil exhibited GST activity, but this activity was significantly lower than that registered in extracts obtained from earthworms of the insecticide-treated soil. This means that each of the three insecticides tested has induced synthesis of GST in the earthworms. The insecticide-induced increase in GST activity was, generally, at a maximum after 1 week, then the activity declined. Thus, after 4 weeks of exposure to anyone of the three insecticides, GST activity declined to near the control values.

The increased GST activity was accompanied by glutathione conjugation-caused increased accumulation of each insecticide in earthworms and by a simultaneous decrease of the insecticide concentration in soil.

9. Enzyme activities and earthworms in pasture soils submitted to restoration after removal of their top layer used for landscape improvement

Ross and Cairns [61] have dealt with the restoration of a pasture soil (silt loam) at Judgeford, Wellington area (New Zealand). The top (0-15-cm) layer of this soil had been removed to be used for landscape improvement in urban areas. The remaining soil was rotary hoed to 15-cm depth to mix the AB and B_1 horizons. The resulting mixed subsoil was collected and packed into pots with each containing 24.9 kg oven-dry weight of soil.

The restoration experiment comprised addition of earthworms, *Allolobophora (Aporrectodea) caliginosa* (100 per pot) and sowing of ryegrass, *Lolium perenne* (100 seeds per pot). Lime and fertilisers (P, K, S, trace elements, and N as urea) were added at sowing and during the experiment. Four treatments were applied: 1. no earthworms and ryegrass (subsoil alone); 2. no earthworms, with ryegrass; 3. with earthworms, no ryegrass; 4. with earthworms and ryegrass. The pots were maintained in a glasshouse and watered regularly.

The experiment started in October 1978 and lasted some 13 months. The soil was analysed at approximately 4-monthly intervals. The results obtained in enzymological analyses are reproduced in Table 7.

One can deduce from Table 7 that in subsoil alone (treatment 1), xylanase, urease, phosphatase and sulphatase activities declined, but invertase activity increased during the experiment. The presence of earthworms resulted in increased cellulase and sulphatase activities. Generally, the presence of ryegrass enhanced all enzyme activities. The additional presence of earthworms further stimulated invertase, amylase, urease and phosphatase activities.

The conclusion was drawn that the earthworms - due to their stimulating effect on biochemical activities - contribute to the restoration of pasture productivity after topsoil removal.

Table 7

Influence of earthworms and ryegrass on soil enzyme activities [61]

Enzyme activity*	Duration (months)	Treatment**			
		Earthworms absent		Earthworms present	
		Ryegrass absent	Ryegrass present	Ryegrass absent	Ryegrass present
1	2	3	4	5	6
Invertase	4	170 aA	600 bA	140 aA	760 cA
	8	280 aB	810 bA	290 aB	1250 cB
	13	290 aB	1360 bB	380 aB	1570 bC
Amylase	4	36 aA	43 abA	61 abA	65 bA
	8	60 aA	78 aB	96 abB	130 bB
	13	43 aA	110 bC	55 aA	150 cB
Cellulase	4	7 aA	24 bA	23 bA	26 bA
	8	11 aB	27 bA	18 abA	31 bA
	13	6 aA	43 bB	8 aA	42 bB
Xylanase	4	15 aC	90 bA	17 aA	87 bA
	8	9 aB	110 bA	11 aA	140 bA
	13	5 aA	120 bA	10 aA	110 bA

Table 7 (continued)

1	2	3	4	5	6
Protease	4	19 aA	32 bA	21 abB	26 abA
	8	19 aA	28 abA	25 abB	32 bA
	13	14 aA	31 bA	12 aA	37 bA
Urease	4	190 aB	340 bB	180 aA	300 bA
	8	180 aB	240 bA	170 aA	290 cA
	13	160 aA	340 bB	150 aA	420 cB
Phosphatase	4	620 aB	800 bB	640 aB	840 bB
	8	540 bA	580 bA	460 aA	690 cA
	13	570 aA	910 bB	580 aB	910 bB
Sulphatase	4	84 aB	100 aB	95 aA	100 aA
	8	56 aA	74 bA	95 cA	80 bcA
	13	68 aA	79 abA	79 abA	87 bA

* Expressed in pmoles of reaction product/g dry soil/second. Products: "glucose" (invertase, amylase, cellulase); "xylose" (xylanase); "tyrosine" (protease); ammonium-N (urease); *p* - nitrophenol (phosphatase, sulphatase).

** a, b, c (effects of treatments) : any two means within a row not marked with the same letter are significantly different ($P < 0.05$).
A, B, C (effects of the duration of experiment): any two means within a column not marked with the same letter are significantly different ($P < 0.05$).

10. Enzyme activities and earthworms in urban soils

Based on the values of catalase, xylanase and urease activities in soils of 12 places and on the abundance of earthworms in soils of 42 places in Bonn-Bad Godesberg (Germany), Fründ *et al.* [13] found that none of the soil enzyme activities correlated significantly ($P > 0.05$) with the number and fresh biomass of earthworms. The most frequently found earthworms were *Lumbricus terrestris* and *Allolobophora caliginosa*. See also page 131 in [23].

Studying soils in the city of Dorsten, located at the northern rim of the Ruhr industrial area (Germany), Keplin and Broll [20] have determined dehydrogenase activity of the 0-8-cm soil layer and the number and dry biomass of earthworms (the earthworms with their gut content were dried at 65^0C for 24 hours). Seven lumbricid species were identified. Formerly, the studied soils were used as gardens, grassland and arable land. Soil dehydrogenase activity, like the number and dry biomass of earthworms, decreased from ancient garden land to grassland and arable land. Thus, in the most dehydrogenase-active soil (97 mg triphenylformazan/g soil) of the ancient garden, the number and dry biomass of earthworms were 224/m^2 and 19.4 g/m^2, respectively. The corresponding values in the least active formerly arable land (at present shrub woodland) were: 54 mg/g soil, 57/m^2 and 2 g/m^2, respectively. See also page 131 in [23].

11. Enzyme activities and earthworms in mine spoils submitted to recultivation

Müller *et al.* [51] studied 12 technogenic soils located in the Köln-Bergheim zone, within the surface-mined brown coal area in the Rhine region (Germany). These soils were recultivated after redeposition of mine spoils (loess) as dry materials 20 years previously. Since that time, four of them were used as grasslands, four as maple and hornbeam forests and four as arable lands.

Dehydrogenase activity was highest in the grassland soils, intermediary in the forest soils and lowest in the arable soils. Numerous earthworm burrows were observed in the grassland and forest soils, but only a few occurred in the arable soils. See also page 190 in [23].

In a similar study in the same surface-mined brown coal zone of the Rhine region, Schneider *et al.* [65] compared about 10- and 25-year-old recultivation spoil (loess) plots used as arable lands or forests. Dehydrogenase activity (expressed in μg triphenylformazan/5 g dry soil) in the 5-10-cm layer was ~ 100 in both 10- and 25-year-old arable spoil plots, and ~ 200 and ~ 320 in the 10- and 25-year-old forest spoil plots, respectively.

Total number of lumbricid earthworms (belonging to seven species) in the 0-10-cm soil layer of the 10-year-old arable and forest spoil plots was 60 and 141 /m^2, respectively, whereas in the same layer of the 25-year-old arable and forest spoil plots 223 and 339 earthworms/m^2, respectively, were recorded.

On the basis of these and other data, it was recommended that agricultural recultivation of brown coal mine spoils in the studied region should begin with forest recultivation; under such conditions, the arable soils will reach a "maturity stage" more rapidly. See also page 191 in [23].

By using coal mine spoils from the Nazarovo Basin (Siberia), Bezkorovainaya [5] carried out a pot experiment to study the role of earthworms in decomposition of litters produced by six tree species: aspen (*Populus tremula*), birch (*Betula fruticosa*), Siberian larch (*Larix sibirica*), Siberian pine (*Pinus sibirica*), Scotch pine (*Pinus sylvestris*) and Siberian spruce (*Picea obovata*). Each pot contained 2.5 kg of spoils. Litter (50 g dry weight) and five earthworms (*Eisenia* sp.) were placed on the surface of spoils. Pots with spoils and litter, but without earthworms were the controls. After moistening, the pots were exposed to hydrothermal conditions similar to the natural ones. The experiment lasted 3 years (1985-1987). The litters and spoils were submitted yearly to different analyses.

It was found that decomposition of each litter was stimulated in the presence of earthworms and, as expected, the decomposition degree was more advanced in deciduous than in coniferous litters. In the presence of earthworms, protease and urease activities of litters increased. The increase was highest in the urease activity of birch litter: it was 1.5-3-fold in comparison with the other litters and 2.5-fold compared with the control birch litter. See also pages 183-184 in [23].

12. Enzyme activities in earthworm-worked dungs, composts, toxic crop residues and organic industrial wastes

12.1. Dungs. Businelli *et al.* [6] have determined five hydrolase (amylase, acid and alkaline phosphatase, phosphodiesterase, arylsulphatase) activities as well as dehydrogenase activity in casts produced by *Lumbricus rubellus* from: cow and horse dungs (Sample 1), cattle and horse dungs (Sample 2), horse dung (Sample 3), cow and sheep dungs (Sample 4) and municipal waste compost (Sample 5).

Hydrolase activities were high and virtually the same in all samples. But dehydrogenase activity was low in Sample 5, which was attributed to the particularly high concentration of heavy metals, especially lead in the municipal waste.

The investigations of Benedetti *et al.* [3] indicate that during bioconversion of pig dung into a manure under the action of *Eisenia foetida*, the high protease and urease activities in the fresh dung gradually decrease.

12.2. Composts. In contrast to findings of Benedetti *et al.* [3], Seregina and Lysak [66] have found that urease activity, like invertase and dehydrogenase activities, exhibited some increases during composting of cattle dung, sewage sludge and dung+sludge mixture in the presence of added earthworms. It should be emphasised that such increases did not occur in the absence of earthworms. Catalase activity was low and remained practically unchanged during composting in both absence and presence of earthworms.

The wet vermicompost prepared from cattle dung, peat and straw (Lazarchik *et al.* [38]) exhibited high dehydrogenase and catalase activities.

Korotkova [24] carried out experiments for modelling vermicomposting of dung-peat mixture under climatic conditions of Siberia. Vermicomposting was conducted at 6-8^{0}C or at 20-25^{0}C. The vermicompost obtained at 6-8^{0}C was more protease- and catalase-active than that prepared at 20-25^{0}C.

For estimation of compost maturity, Forster *et al.* [11], soil scientists of the Bayreuth University (Germany), have measured dehydrogenase activity, arginine ammonification, respiration (CO_2 evolution) rate and several chemical parameters of six composts obtained from different parent materials, the composting technology and time being also different. Designation, parent materials and age of composts are specified below: S - barley and wheat straw, ~ 5 months; B - spruce and pine bark, 4 weeks; HB - hope rape from brewery waste and spruce bark, 1 year; Z - household and garden waste in the presence of *Eisenia foetida*, 6-9 months; O - household and garden waste (no earthworms added), ~ 18 months; and PS - paper dust, minicipal sewage sludge and willow shavings, 12 weeks.

It was found that for estimation of the maturity of these composts, the chemical data were contradictory. But a combination of the values of dehydrogenase activity and arginine ammonification was considered sufficient to estimate compost maturity and, implicitly, the consequences of applying a compost to soil. A high dehydrogenase activity and negative arginine ammonification indicate immaturity of composts, while low dehydrogenase activity and positive, but low arginine ammonification are characteristics of mature composts.

The values in Table 8 allow to establish the following increasing order of maturity of the six composts studied: B < PS < HB ≈ Z < S < O. It results from this order that compost Z, in which the household and garden waste had been worked by *E. foetida* during 6-9 months, was less mature than compost O, obtained from household and garden waste after composting without earthworms but during 18 months.

Table 8 also shows that respiration rate would give a mostly other order of compost maturity. It was pointed out that respiration rate did not contribute to the assessment of compost maturity.

Table 8

Dehydrogenase activity, arginine ammonification and respiration of composts [11]

Compost	Dehydrogenase activity*	Arginine ammonification**	Respiration***
S	2.06 b	13.5 cd	2.16 d
B	2.95 c	-18.5 a	2.19 d
HB	5.64 e	35.3 d	2.35 d
Z	4.63 d	78.4 e	1.49 b
O	0.83 a	5.3 bc	0.95 a
PS	5.89 e	-12.9 ab	1.81 c

* Expressed in mg triphenylformazan/g dry compost/24 hours.

** Expressed in μg $NH_4^+ - N$ / g dry compost/hour.

*** Expressed in mg CO_2/g dry compost/24 hours.

Means within columns followed by the same letter are not significantly different at the 1% level.

The three composts studied by Serra-Wittling *et al.* [67] were prepared from the organic fraction of the municipal solid household wastes in the town of Bapaume (France). The C1 compost was obtained by 6-week fermentation, followed by 7 months of maturation. For the C2 compost, the fermentation period lasted 10 weeks, followed by 3-month maturation. The LC2 compost was prepared as follows: after fermentation, a part of the C2 compost was lumbricomposted with *Eisenia andrei* for 2 months, then was subjected to 1-month maturation without earthworms.

Activities of seven hydrolases participating in the C- and N-cycles (cellulase, β-glucosidase, β-galactosidase, β-N-acetylglucosaminidase, protease, urease, amidase) and two oxidoreductases (dehydrogenase, peroxidase) were determined in the composts.

Protease activity was higher in the older C1 compost than in the younger C2. Urease and peroxidase activities did no differ significantly between these two composts. The other six activities gave higher values in C2 than in C1, up to 10 times higher for cellulase and amidase.

Little differences were registered in enzyme activities of C2 and LC2. Thus, cellulase, β-glucosidase, β-galactosidase, protease, urease and dehydrogenase activities were a little higher, while β-N-acetylglucosaminidase, amidase and peroxidase activities were a little lower in C2 than in LC2, but all differences were statistically insignificant. As C2 and LC2 were prepared from the same municipal wastes and had the same age (10 weeks + 3 months), one can deduce that the effect of *E. andrei* on enzyme activities in compost was not long-lasting.

Based on enzymological and chemical analyses of sewage sludge during its vermicomposting, Benítez *et al.* [4] have established that the ratio between dehydrogenase activity and water-soluble organic carbon content may be considered as an index which makes it possible to distinguish between hydrolytic and maturation phases in the sewage sludge composting process.

For vermicomposting a 1:1 mixture of two sewage sludges were used, namely an anaerobically digested sewage sludge from the wastewater treatment plant of a paper mill and an aerobically digested municipal sewage sludge. The mixture (1 kg fresh weight) was placed in 1-l cylindrical plastic container. The surface of the mixture was covered with 100 g (fresh weight) of vermicomposted sewage sludge to act as a microbial inoculum and to provide suitable conditions for earthworms. Then, ten *Eisenia foetida* were added to the vermicomposted material.

During the vermicomposting period (10 weeks), the moisture content of the mixture was maintained at 75-80% and the containers were kept in darkness at room temperature. Enzyme activities and chemical parameters of sludge and biomass of earthworms were determined weekly. After the vermicomposting period, the containers with compost and worms were left at room temperature for further 8 weeks to complete the stabilisation of organic matter. At the end of the 18th week, the enzymological and chemical analyses were repeated.

Four hydrolytic enzyme (β-glucosidase, urease, protease, phosphatase) activities and dehydrogenase (DHase) activity were determined. Of the chemical parameters analysed, the content of water-soluble organic carbon (WSC) should be emphasised.

The activities showed a decreasing tendency during the first 4-7 weeks of composting; then, they remained practically stable (constant) up to the end of the composting period and even at week 18.

Each enzyme activity correlated significantly ($P < 0.01$) with WSC. The correlations among enzyme activities were also significant ($P < 0.01$), excepting a single pair (urease-phosphatase) with insignificant correlation.

As DHase activity reflects the overall microbial activity and as it has been stable (constant) after 5 days of vermicomposting, Benítez *et al.* [4] used this activity, more precisely the ratio between this activity and WSC as an index which allows to distinguish two phases in the advancement of the vermicomposting process. The time - during which this index exhibits, after an initial increase, a decreasing tendency and the earthworm biomass shows an increasing trend - corresponds to the hydrolytic phase. During the next, maturation phase, the time-dependent changes in this index are not significant. Benítez *et al.* [4] have found that the hydrolytic phase lasted 7 weeks and the maturation phase comprised weeks 8-18. During the last 3 weeks of the composting period (weeks 8-10), the earthworm biomass did not increase and began to decrease.

12.3. Toxic crop residues. For disposal of cassava peel, a toxic crop residue, M b a [49] utilised the earthworm *Eudrilus eugeniae*. The bitter cassava (*Manihot utilissima)* is widely grown in Nigeria and other tropical countries for its large tuberous roots rich in food carbohydrate. The rind of the roots has a high cyanide content and, as cassava peel, forms a toxic waste.

Air-dried cassava peel samples (200 g each) were introduced to plastic pots with perforated bottoms and watered to field capacity. Eight sub-adult *E. eugeniae* were added to each pot. The developing worm cultures were watered every other day and amended with 100 g of air-dried cassava peel at monthly intervals. After 4 months, the worms and cocoons were separated by hand and the worm culture residue was submitted to different analyses, including measurement of dehydrogenase and acid phosphatase activities. For comparison, two controls were used: air-dried peel and decomposed peel (peel allowed to decompose without earthworms for 8 months).

The earthworm biomass, determined several times during the 4-month culturing, increased continuously with time.

The cyanide content in the worm culture residue was about 50% of that measured in the air-dried peel and was similar to that in the decomposed peel.

Dehydrogenase activity (expressed in mg 2,3,5-triphenyltetrazolium chloride reduced/100 g dry weight) and acid phosphatase activity (expressed in mg phenol/g dry weight) showed the order: 14.0 (air-dried peel) < 59.3 (decomposed peel) < 127.3 (culture residue) and 4.9 (air-dried peel) < 24.6 (decomposed peel) < 182.0 (culture residue), respectively. Thus, the earthworm-worked cassava peel became less toxic and more enzyme-active.

12.4. Organic industrial wastes. Satchell [62] and Satchell and Martin [63] used paper mill waste, rich in cellulose and poor in phosphate, for culturing *Eisenia foetida, Dendrobaena veneta, Lumbricus rubellus* and *Allolobophora caliginosa.*

The culture medium was prepared from 40 g of sterilised paper waste (pH 6.8; water content ≈ 85%) amended with 1 g phytin (calcium inositol hexaphosphate) serving as P source. Identical amounts of the medium (*i. e.* 40 g paper waste + 1 g phytin) were placed in small polythene tubs, then 20 earthworms were introduced in separate tubs for each worm species. No worm was added to the control medium.

The cultures were left for 24 days for the worms to work through the medium, and samples of the fresh faecal material and of the control medium were then collected for determination of phosphatase activity. It was found that phosphatase activity was higher in the faecal material of each worm species than in the control medium. When phosphatase activity was assayed in reaction mixtures buffered to provide a pH range of 2-10, two peaks could be registered in the faecal phosphatase activity, namely at about pH 3-5 and about pH 9-10, respectively. These findings were interpreted by the suggestion that the acid phosphatase in earthworm faeces is of microbial origin, whereas the alkaline phosphatase was produced, at least partly, by the earthworms themselves.

13. Enzyme activities in soils treated with earthworm-worked manures

A vermicompost prepared from cattle dung, peat and straw was used by Lazarchik *et al.* [38] for manuring soddy-podzolic soils. Pot and field experiments were carried out. In the manured soil, the green biomass of the test plants (*e. g.* lettuce) showed a 35-40% increase. Enzyme activities also increased in the manured soils.

Serra-Wittling *et al.* [67], who determined nine enzyme activities in the C1, C2 and LC2 composts (see Subsection 12.2), also determined the effect of these composts on enzyme activities in a loamy soil sampled from the 0-20-cm layer of a bare experimental plot at Grignon (France).

Fresh soil samples (50 g each) were amended with 10 or 30% of compost (weight/weight). Soil samples without compost and compost samples without soil (prepared from 25 g compost and 25 g inert sand) served for comparison. Then, all samples were moistened to 95% water-holding capacity and incubated in the dark at 28^0C for 189 days. During the incubation, the same nine enzyme activities were determined as those measured in the composts (see Subsection 12.2).

The results obtained may be summarised as follows.

The enzyme activities were higher in the composts than in the soil, except for urease which was similar in the composts and soil.

In the soil-compost mixtures, the enzyme activities were not additive.

The C-cyle enzyme (cellulase, β-glucosidase, β-galactosidase and β-N-acetylglucosaminidase) activities were not affected by addition of C1, but C2 and LC2, at 30% additions, increased these activities.

The N-cycle enzyme (protease, urease and amidase) activities in soil were stimulated by the 30% compost addition, but the increase was only transient for urease and amidase.

Dehydrogenase activity was the only activity which increased significantly in soil-compost mixtures as the rate of compost addition increased.

Peroxidase activity in soil increased at both compost addition rates, the increase being lower with C1 than with C2 and LC2.

No significant differences were observed in enzyme activities of the soil-C2 and soil-LC2 (lumbricompost) mixtures. In the absence of soil, a single important difference was found between C2 and LC2: after 189 days of incubation, urease activity was greater in C2 than in LC2.

Govedarica *et al.* [16] conducted a field experiment on a calcareous chernozem soil in Yugoslavia. Three 1-ha plots were used. The first plot was treated with 5 t of earthworm manure; the second received 15 t of green manure - rape (*Brassica napus oleifera*), and the third, serving as control, was not manured. The test plant was wheat. At the beginning, middle and end of the growing period, soil samples were taken from the 0-30- and 30-60-cm depths for enzymological and microbiological analyses.

Earthworm manuring increased dehydrogenase activity to a large extent and protease and urease activities to a lesser extent in the 0-30-cm layer at all sampling dates and in the 30-60-cm layer at two sampling dates. Green manuring increased dehydrogenase activity, as did earthworm manuring, but it had a negligible effect on protease activity and a strong decreasing effect on urease activity at both soil depths and at all sampling dates. In other words, the 5 t of earthworm manure enhanced more positively the enzymatic potential of soil than did the 15 t of green manure. Total number of microorganisms was also higher in the earthworm-manured than in the green-manured soil.

For studing remediation of oil-contaminated soils, Kireeva [21] carried out a field experiment. Microplots on a grey forest soil in Bashkiria (Russian Federation) were contaminated with 0, 8, 16 and 25 l of crude oil/m^2, then treated or not treated with Biohumus. Biohumus is a compost which was prepared from cattle, pig and horse dungs mixed with sawdust and chopped straw and inoculated with *Eisenia foetida*, then subjected to maturation for 6 months. Biohumus was applied several times at rates of 4-8 t/ha. The test plants were oats and barley. Enzyme activities were determined in the 0-20-cm soil layer 1 and 12 months after the Biohumus treatment.

Enzymological analysis of soil samples showed that invertase, phosphatase, dehydrogenase, catalase, peroxidase and polyphenol oxidase activities decreased and urease activity increased in parallel with the rate of crude oil contamination. Biohumus attenuated the negative effects of crude oil, increasing each enzyme activity in each plot. The only exception was urease activity as in the contaminated plots Biohumus diminished the oil-induced high urease activity.

Biohumus enhanced oil degradation. Grain yields of oats and barley, in both the second and third years, were significantly higher in the Biohumus-treated contaminated plots than in the untreated contaminated plots. The yields in the third year in plots contaminated with 8 and 16 1 of crude oil/m^2 and treated with Biohumus attained the yield obtained in the uncontaminated plots. But in the plots contaminated with 25 l of crude oil/m^2 and treated with Biohumus, the yields were about 60% of those of the uncontaminated plots. See also pages 32-33 in [23].

Concluding remarks. It is considered since Charles Darwin that the earthworms play an important role in soil fertility. The mechanisms by which the earthworms play this role is multiple as comprehensively reviewed by Syers and Springett [74].

Our present article dealt with only a single aspect related to contribution of earthworms to soil fertility. This, enzymological aspect was the objective of investigations performed by many research groups in a series of countries under different climatic conditions. The conclusion which can be drawn from the results of these investigations is that the activity of earthworms leads to increased enzymatic (catalytic) potential of soils, this potential having a major biological significance as the biochemical reactions in the living systems, including the soils, are catalysed by enzymes.

REFERENCES

1. Atlavinyte, O., Stanislavičiute, I., Šiuliauskiene, N., *Vliyanie dozhdevykh chervei na agrokhimicheskie svoistva, mikrofloru i fermentativnuyu aktivnost' dernovo-podzolistoi pochvy*, "Liet. TSR Mokslu Akad. Darb., Ser.C", **3(75)**, 1981, 75-82.
2. Bachelier, G., *La Vie Animale dans les Sols*, ORSTOM, Paris, 1963; *La Faune des Sols - Son Écologie et Son Action*, ORSTOM, Paris, 1978.

3. Benedetti, A., Ceccanti, B., Calcinai, M., *Caratterististiche chimico-strutturali e biochimiche dei casting da letame suino*, in Senesi, N., Miano, T.M. (Eds.), *Riciclo di Biomasse di Rifiuto e di Scarto e Fertilizzazione Organica del Suolo. Realtà e Prospettive, Vantaggi e Rischi per l'Agricoltura e l'Ambiente*, pp. 65-68, Pàtron Editore, Bologna, 1991.
4. Benítez, E., Nogales, R., Elvira, C., Masciandaro, C., Ceccanti, B., *Enzyme activities as indicators of the stabilization of sewage sludges composting with* Eisenia foetida, "Bioresource Technol.", **67**, 1999, 297-303.
5. Bezkorovainova, I.N., *Eksperimental'noe izuchenie roli dozhdevykh chervei v mineralizatsii podstilki*, "Ekologiya", No. 5-6, 1994, 67-73.
6. Businelli, M., Perucci, P., Patumi, M., Giusquiani, P. L., *Chemical composition and enzyme activity of some worm casts*, "Plant Soil", **80**, 1984, 417-422.
7. Coucke, P., *Invloed van de regenwormen op de mikrobiologische eigenschappen van de grond*, "Meded. Landbouwhogesch. Opzoekingssta. Staat, Gent", **29**, 1964, 1277-1285.
8. Dash, M.C., Patnaik, H.K., *Effect of fenitrothion on gut enzyme activity of tropical earthworms*, "Progr. Abstr., 11th Int. Colloq. Soil Zool. 'Soil Organisms and Soil Health' (Jyväskylä, Finland, 1992)", 1992, 139.
9. Dev, B., Vyas, I., *Histoenzymatic pattern of alkaline phosphatase in the different components of the alimentary canal of the common earthworm*, Barogaster annandalei (*Stephenson*), *and its possible physiological significance*, "Acta Morphol. Neerl.-Scand.", **9**, 1972, 365-369.
10. Devliegher, W., Verstraete, W., *The effect of* Lumbricus terrestris *on soil in relation to plant growth: Effects of nutrient-enrichment processes (NEP) and gut-associated processes (GAP)*, "Soil Biol. Biochem.", **29**, 1997, 341-346.
11. Forster, J.C., Zech, W., Würdinger, E., *Comparison of chemical and microbiological methods for the characterization of the maturity of composts from contrasting sources*, "Biol. Fert. Soils", **16**, 1993, 93-99.
12. Fraser, P.M., Haynes, R.J., Williams, P.H., *Effects of pasture improvement and intensive cultivation on microbial biomass, enzyme activities, and composition and size of earthworm populations*, "Biol. Fert. Soils", **17**, 1994, 185-190.
13. Fründ, H.-C., Ruszkowski, B., Söntgen, M., Graefe, U., *Besiedlung städtischer Böden durch Regenwürmer, Enchytraeiden und bodenlebende Gehäuseschnecken*, "Mitt. Dtsch. Bodenk. Ges.", **56**, 1988, 351-356.
14. Ganeshamurthy, A.N., Manjaiah, K.M., Subba Rao, A., *Mobilization of nutrients in tropical soils through worm casting: Availability of macronutrients*, "Soil Biol. Biochem.", **30**, 1998, 1671-1672.
15. Gehlen, P., *Bodenchemische, bodenbiologische und bodenphysikalische Untersuchungen konventionell und biologisch bewirtschafteter Acker-, Gemüse-, Obst- und Weinbauflächen*, Dr. Diss., Friedrich-Wilhelms-Univ., Bonn, 1987.

16. Govedarica, M., Milošević, N., Jarak, M., Ubavić, M., Radanović, Z., *The effect of earthworm and green manure on the microbiological activity in wheat soil*, "Zemljište Biljka" (Belgrade), **45**, 1996, 121-126.
17. Hans, R.K., Khan, M.A., Farooq, M., Beg, M.U., *Glutathione-S-transferase activity in an earthworm* (Pheretima posthuma) *exposed to three insecticides*, "Soil Biol. Biochem.", **25**, 1993, 509-511.
18. Hartenstein, R., *Soil macroinvertebrates, aldehyde oxidase, catalase, cellulase and peroxidase*, "Soil Biol. Biochem.", **14**, 1982, 387-391.
19. Hoffmann, G., *Verteilung und Herkunft einiger Enzyme im Boden*, "Z. Pflanzenernähr., Düng., Bodenk.", **85**, 1959, 97-104.
20. Keplin, B., Broll, G., *Earthworms and dehydrogenase activity of urban biotopes*, "Soil Biol. Biochem.", **29**, 1997, 533-536.
21. Kireeva, N.A., *Ispol'zovanie biogumusa dlya uskoreniya destruktsii nefti v pochve*, "Biotekhnologiya", No. 5-6, 1995, 32-35.
22. Kiss, S., *A gilisztaürülék és hangyabolyföld invertázaktivitása*, "Agrokém. Talajtan", **6**, 1957, 65-68.
23. Kiss, S., Paşca, D., Drăgan-Bularda, M., *Enzymology of Disturbed Soils*, Elsevier, Amsterdam, 1998.
24. Korotkova, E.V., *Ekologicheskoe znachenie zoogumusa kaliforniiskogo chervya*, "Sb. Tez. Dokl. 3 Novosibir. Mezhvuz. Nauch. Stud. Konf. 'Intellektual'nyi Potentsial Sibiri' (Novosibirsk, 1996)", **3**, 1996, 71.
25. Kozlov, K.A., *Über die Rolle der Bodenfauna bei der Anreicherung von Fermenten im Boden*, "Pedobiologia", **5**, 1965, 140-145.
26. Kozlovskaya, L.S., *Der Einfluβ der Wirbellosen auf die Tätigkeit der Mikroorganismen in Torfböden*, "C. R. 4ème Colloq. Int. Zool. Sol (Dijon, 1970)", 1971, 173-180.
27. Kozlovskaya, L.S., *Rol' Bespozvonochnykh v Transformatsii Organicheskogo Veshchestva Bolotnykh Pochv*, Nauka, Leningrad, 1976.
28. Kozlovskaya, L.S., *Rol' bespozvonochnykh v pochvoobrazovanii v taezhnoi zone*, in Kozlovskaya, L.S., Medvedeva, V.M. (Red.), *Priroda Bolotno-lesnykh Sistem Karelii i Puti Ikh Osvoeniya*, pp. 4-18, Inst. Lesa, Petrozavodsk, 1982.
29. Kozlovskaya, L.S., *Vliyanie melioratsii na zhiznedeyatel'nost' pochvennykh bespozvonochnykh*, in P'yavchenko, N.I. (Red.), *Izmenenie Biologicheskoi Aktivnosti Torfyanykh Pochv pod Deistviem Melioratsii*, pp. 58-96, Nauka, Leningrad, 1982.
30. Kozlovskaya, L.S., Archegova, I.B., Rakova, N.N., *Biokhimicheskoe vozdeistvie pochvennykh bespozvonochnykh na rastitel'nye ostatki*, in P'yavchenko, N.I. (Red.), *Bolotnye Biogeotsenozy i Ikh Izmenenie v Rezul'tate Antropogennogo Vozdeistviya*, pp. 24-46, Nauka, Leningrad, 1983.

31. Kozlovskaya, L.S., Rakova, N.N., *Fermentativnaya aktivnost' ekskrementov bespozvonochnykh*, in P'yavchenko, N.I. (Red.), *Issledovaniya po Lesnomy Bolotovedeniyu i Melioratsii*, pp. 64-80, Inst. Lesa, Petrozavodsk, 1978.

32. Kozlovskaya, L.S., Solov'eva, G.I., *Pochvennye bespozvonochnye bolot yuzhnoi Karelii i ikh rol' v pochvoobrazovanii*, in *Statsionarnoe Izuchenie Bolot i Zabolochennykh Lesov v Svyazi s Melioratsiei*, pp. 67-88, Inst. Lesa, Petrozavodsk, 1977.

33. Kozlovskaya, L.S., Zagural'skaya, L.M., *Vliyanie dozhdevykh chervei na proteoliticheskuyu aktivnost' torfyanykh pochv*, in *Problemy Pochvennoi Zoologii*, pp. 90-91, Nauka, Moskva, 1969.

34. Kozlovskaya, L.S., Zagural'skaya, L.M., *Znachenie bespozvonochnykh v sozdanii fermentativnoi aktivnosti pochv*, in P'yavchenko, N.I. (Red.), *Osnovnye Printsipy Izucheniya Bolotnykh Biogeotsenozov*, pp. 89-97, Nauka, Leningrad, 1972.

35. Lattaud, C., Locati, S., Mora, P., Rouland, C., *Origin and activities of glycolytic enzymes in the gut of the tropical geophagous earthworm* Millsonia anomala *from Lamto (Côte d'Ivoire)*, "Pedobiologia", **41**, 1997, 242-251.

36. Lattaud, C., Zhang, B.G., Locati, S., Rouland, C., Lavelle, P., *Activities of the digestive enzymes in the gut and in tissue culture of a tropical geophagous earthworm*, Polypheretima elongata (Megascolecidae), "Soil Biol. Biochem.", **29**, 1997, 335-339.

37. Laverack, M.S., *The Physiology of Earthworms*, pp. 18-23, Pergamon Press, Oxford, 1963, as cited in [39].

38. Lazarchik, V.E., Orlov, D.S., Ovchinnikova, M.F., Kapinos, V.A., Ras'kova, N.V., Solov'ev, G.A., Lazarchik, V.M., Smirnov, G.V., Shirskaya, G.M., *Biologicheskaya tsennost' vermikomposta i perspektivy ego ispol'zovaniya na dernovo-podzolistykh pochvakh*, "Khim. Sel'sk. Khoz.", No. 4, 1994, 12-13.

39. Lee, K.E., *Earthworms. Their Ecology and Relationships with Soils and Land Use*, Acad. Press, Sydney, 1985.

40. Li, Y.-T., Shetlar, M.R., *Glycosidases in the earthworm*, Lumbricus terrestris, "Comp. Biochem. Physiol.", **14**, 1965, 275-279, as cited in [79, 81].

41. Loquet, M., *The study of respiratory and enzymatic activities of earthworms-made pedological structures in a grassland soil at Cîteaux, France*, "Sci. Proc., Royal Dublin Soc., Ser. A", **6**, 1978, 207-214.

42. Loquet, M., Bhatnagar, T., Bouché, M.B., Rouelle, J., *Essai d'estimation de l'influence écologique des lombriciens sur les microorganismes*, "Pedobiologia", **17**, 1977, 400-417.

43. Loquet, M., Vinceslas, M., *Cellulolyse et ligninolyse liées au tube digestif d'*Eisenia fetida andrei *Bouché*, "Rev. Écol. Biol. Sol", **24**, 1987, 559-571.

44. Marcuzzi, G., Torchetto Lafisca, M., *First observations on the digestive enzymes of some Italian earthworms* (Lumbricidae, Oligochaeta), "Pedobiologia", **20**, 1980, 144.

45. Martin, A., Marinissen, J.C.Y., *Biological and physico-chemical processes in excrements of soil animals*, "Geoderma", **56**, 1993, 331-347.

46. Matsumoto, S., Nishihara, H., Kataoka, I., *Earthworms contribution to soil fertilization. II. Relationship between earthworm action and phosphatase activity in soils*, "Edaphologia", No. 54, 1995, 25-31 (in Japanese with English abstract).

47. Matsumoto, S., Taniguchi, T., *Earthworms contribution to soil fertilization. I. A mutual relationship between the available phosphate and phosphatase activity in earthworm casts*, "Edaphologia", No. 53, 1995, 19-24 (in Japanese with English abstract).

48. Matsumoto, S., Tumura, A., Koh, T., Kasai, Z., *The role of earthworms on soil fertility of reclaimed land*, "Edaphologia", No. 43, 1990, 41-49 (in Japanese with English abstract).

49. Mba, C.C., *Utilization of* Eudrilus eugeniae *for disposal of cassava peel*, in Satchell, J.E. (Ed.), *Earthworm Ecology from Darwin to Vermiculture*, pp. 315-321, Chapman & Hall, London, 1983.

50. Merino-Trigo, A., Sampedro, L., Rodríguez-Berrocal, F.J., Mato, S., Páez de la Cadena, M., *Activity and partial characterisation of xylanolytic enzymes in the earthworm* Eisenia andrei *fed on organic wastes*, "Soil Biol. Biochem.", **31**, 1999, 1735-1740.

51. Müller, R., Schneider, R., Schröder, D., *Physikalische, chemische und biologische Eigenschaften trocken rekultivierter Löβböden unter Wald-, Grünland- und Ackernutzung*, "Mitt. Dtsch. Bodenk. Ges.", **56**, 1988, 387-392.

52. Mulongoy, K., Bedoret, A., *Properties of worm casts and surface soils under various plant covers in the humid tropics*, "Soil Biol. Biochem.", **21**, 1989, 197-203.

53. Neuhauser, E.F., Hartenstein, R., *Reactivity of soil macroinvertebrate peroxidases with lignins and lignin model compounds*, "Soil Biol. Biochem.", **10**, 1978, 341-342.

54. Nilsen, C.O., *Carbohydrases in soil and litter invertebrates*, "Oikos", **13**, 1962, 200-215

55. Nielsen, C.O., *Laminarinases in soil and litter invertebrates*, "Nature", **199**, 1963, 1001.

56. Park, H.W., Koh, K.S., Park, S.C., *Molecular weights and inhibitor sensitivities of alkaline phosphatase isoenzymes from the midgut of the earthworm*, Eisenia andrei, "Soil Biol. Biochem.", **30**, 1998, 831-832.

57. Park, S.C., Park, H.W., Smith, T.J., *Expression of alkaline phosphatases during embryonic development and immature stages of the earthworm*, Eisenia andrei, "Soil Biol. Biochem.", **28**, 1996, 579-582.

58. Park, S.C., Smith, T.J., Bisesi, M.S., *Hydrolysis of bis(4-nitrophenyl) phosphate by the earthworm* Lumbricus terrestris, "Soil Biol. Biochem.", **22**, 1990, 729-730.

59. Park, S.C., Smith, T.J., Bisesi, M.S., *Activities of phosphomonoesterase and phosphodiesterase from* Lumbricus terrestris, "Soil Biol. Biochem.", **24**, 1992, 873-876.

60. Park, S.C., Smith, T.J., Bisesi, M.S., *Bioactivation of bis(p-nitrophenyl) phosphate by phosphoesterases of the earthworm,* Lumbricus terrestris, "Drug Chem. Toxicol.", **16**, 1993, 111-116.

61. Ross, D.J., Cairns, A., *Effcts of earthworms and ryegrass on respiratory and enzyme activities of soil*, "Soil Biol. Biochem.", **14**, 1982, 583-587.

62. Satchell, J.E., *Earthworm microbiology*, in Satchell, J.E. (Ed.), *Earthworm Ecology from Darwin to Vermiculture*, pp. 351-364, Chapman & Hall, London, 1983.

63. Satchell, J.E., Martin, K., *Phosphatase activity in earthworm faeces*, "Soil Biol. Biochem.", **16**, 1984, 191-194.

64. Satchell, J.E., Martin, K., Krishnamoorthy, R.V., *Stimulation of microbial phosphatase production by earthworm activity*, "Soil Biol. Biochem.", **16**, 1984, 195.

65. Schneider, R., Schneider, H., Schröder, D., *Beziehung zwischen Bodenorganismen und Gefügeentwicklung auf Lö*β*-Neuland unter Acker- und Waldnutzung*, "Z. Pflanzenernähr. Bodenk.", **158**, 1995, 197-204.

66. Seregina, E.V., Lysak, L.V., *Izmenenie mikrobiologicheskoi aktivnosti substratov pri vermikompostirovanii*, "Tez. Dokl. 4 Vses. Nauch. Konf. 'Mikroorganizmy v Sel'skom Khozyaistve' (Pushchino, 1992)", 1992, 185.

67. Serra-Wittling, C., Houot, S., Barriuso, E., *Soil enzymatic response to addition of municipal soild-waste compost*, "Biol. Fert. Soils", **20**, 1995, 226-236.

68. Sharpley, A.N., Syers, J.K., *Potential role of earthworm casts for the phosphorus enrichment of run-off waters*, "Soil Biol. Biochem.", **8**, 1976, 341-346.

69. Sharpley, A.N., Syers, J.K., *Seasonal variation in casting activity and in the amounts and release to solution of phosphorus forms in earthworm casts*, "Soil Biol. Biochem.", **9**, 1977, 227-231.

70. Stehouwer, R.C., Dick, W.A., Traina, S.J., *Characterization of earthworm burrow lining material*, "Agron. Abstr.", 1991, 53.

71. Subler, S., Baranski, C.M., Edwards, C.A., *Earthworm additions increased short-term nitrogen availability and leaching in two grain-crop agroecosystems*, "Soil Biol. Biochem.", **29**, 1997, 413-421.

72. Syers, J.K., Sharpley, A.N., Keeney, D.R., *Cycling of nitrogen by surface-casting earthworms in a pasture ecosystem*, "Soil Biol. Biochem.", **11**, 1979, 181-185.

73. Syers, J.K., Springett, J.A., *Earthworm ecology in grassland soils*, in Satchell, J.E. (Ed.), *Earthworm Ecology from Darwin to Vermiculture*, pp. 67-83, Chapman & Hall, London, 1983.

74. Syers, J.K., Springett, J.A., *Earthworms and soil fertility*, "Plant Soil", **76**, 1984, 93-104.

75. Tiunov, A.V., *Biological activity in drilosphere*, "Progr. Abstr., 11th Int. Colloq. Soil Zool. 'Soil Organisms and Soil Health' (Jyväskylä, Finland, 1992)", 1992, 90.

76. Tiunov, A.V., *Sravnitel'noe issledovanie ureaznoi aktivnosti v pochve i v ekskrementakh dozhdevykh chervei*, "Izv. Akad. Nauk, Ser. Biol.", No. 3, 1993, 472-475.

77. Tiwari, S.C., Tiwari, B.K., Mishra, R.R., *Microbial populations, enzyme activities and nitrogen-phosphorus-potassium enrichment in earthworm casts and in the surrounding soil of a pineapple plantation*, "Biol. Fert. Soils", **8**, 1989, 178-182.

78. Tracey, M.V., *Cellulase and chitinase of earthworms*, "Nature", **167**, 1951, 776-777.

79. Urbášek, F., *Cellulase activity in the gut of some earthworms*, "Rev. Écol. Biol. Sol", **27**, 1990, 21-28.

80. Urbášek, F., Pižl, V., *Activity of digestive enzymes in the gut of five earthworm species* (Oligochaeta; Lumbricidae), "Rev. Écol. Biol. Sol", **28**, 1991, 461-468.

81. Zhang, B.G., Rouland, C., Lattaud, C., Lavelle, P., *Activity and origin of digestive enzymes in gut of the tropical earthworm* Pontoscolex corethrurus, "Eur. J. Soil Biol.", **29**, 1993, 7-11.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, XLV, 1, 2000

NEW MORPHOMETRIC DATA AND GEOGRAPHICAL DISTRIBUTION OF CRICONEMATID SPECIES (*NEMATODA*: *CRICONEMATIDAE*) IN ROMANIA

IULIANA POPOVICI[*] and MARCEL CIOBANU[*]

SUMMARY. - Twenty nematode species (*Nematoda*: *Criconematidae*) from several habitats in Romania are studied under the light microscope. Details of the main taxonomic characters for seven species (*Criconemoides annulatus, C. informis, C. morgensis, C. parvus, Mesocriconema kirjanovae, M. rusticum* and *Criconema demani*) are discussed and their morphometric data are given in tables. Distribution maps for all recorded species in Romania are provided and their preferences for certain habitats are underlined.

Ecological surveys of soil nematodes throughout Romania provided a rich variety of criconematids from over one hundred different study sites. So far, several species belonging to the *Criconematidae* have been reported from Romania [17, 19-21]. More recently, seven newly recorded species belonging to the genera *Mesocriconema* and *Ogma* were described and illustrated [22].

The present paper refers to new morphometric data, details on taxonomic characters of seven species and the geographical distribution of all known criconematid species in Romania.

Material and methods. Sampled sites containing criconematid species are briefly described in Table 1. Nematodes were extracted using the centrifugal method of de Grisse [8], killed and preserved in a 4% formaldehyde solution, mounted in anhydrous glycerin [24] and examined under the light microscope.

All measurements in Tables 2 and 3 are in μm; dimensions and ratios of the species are presented as mean and limit values.

The main taxonomic characters used are those underlined by de Grisse [6,7], Andrássy [1, 2], Raski and Luc [23], Loof [15] and Loof and de Grisse [16].

Results and discussion. Twenty nematode species belonging to the *Criconematidae* family were identified in the study sites. The main taxonomic characters of some species with large ranges of variations and the geographical distribution of all recorded species in Romania are presented below.

[*] *Institute of Biological Research, 3400 Cluj-Napoca, Romania*

Criconemoides annulatus Cobb in Taylor, 1936
(Fig. 1A; Table 2)

This species is characterised by numerous, finely crenate body annuli with 6-14 anastomoses present, large bodies and long spear. Submedian lobes present as submedian pseudolips (lip-like); vulva simple, closed, anterior vulva lip without lobes, spermathecae round to oval, filled with sperm; tail conical-rounded with three-lobed terminus. Morphometric data are presented in Table 2. Details on its intraspecific variation have already been discussed [18].

Distribution and habitat. The first report on this species in Europe, except for the Island of Spitzbergen [14], was from Romania [17]. It has a very restricted distribution in Romania (site no. 4) (Table 1, Fig. 1A) and was found in soil of a spruce forest at depths of 10 – 60 cm.

Criconemoides informis (Micoletzky, 1922) Taylor, 1936
(Fig. 1B; Table 2)

The present measurements (Table 2) are identical to the range of variability given in the literature [7, 11].

Submedian lobes lip-like; body annuli smooth to very finely crenate with few anastomoses; vulva simple and closed; tail conical. Almost all specimens from grasslands in Romania have large spermathecae filled with sperm, while specimens found in forest soils had either empty spermathecae or with few sperm or the spermathacae were not observed. Grassland soils seem to be more favourable for the development of fertile populations of this species than forest soils, possibly due to the high diversity of nutrients. This phenomenon was also observed and discussed by de Grisse and Loof [9].

Distribution and habitat. This species was found at the following sites: 1-3, 18, 22, 29, 30, 58, 60, 62, 85 and 106 (Table 1) in varying habitats from the sand dunes of the Danube Delta to the subalpine grasslands, also in mine spoil dumps under bioremediation (Fig. 1B). It was previously reported in some faunistic and ecological studies [17, 20, 21] and our data confirm its wide distribution in the Northern Hemisphere [10].

Criconemoides morgensis (Hofmänner and Menzel, 1914) Taylor, 1936
Syn.: *C. pseudohercyniensis* apud Popovici,1992
(Fig. 1A; Table 2)

Body cylindrical, robust, slightly curved ventrally; body annuli smooth to crenate with 1-14 anastomoses, two or three annuli successively connected; submedian lobes lip-like; vulva closed, anterior lip with two lobes; vagina straight; spermatheca filled with sperm; tail conical- rounded, with the last 2-3 folded annuli. Details on morphometric data are given in Table 2.

Distribution and habitat. This species was found at only one site, no. 101 (Table 1, Fig. 1A) and was reported from Romania [19] under the name *C. pseudohercyniensis*.

Criconemoides parvus Raski, 1952
(Fig. 1A; Table 2)

The presence of this species in Romania was first reported by Popovici [19]. More information on the only specimen (a female) found is given in Table 2.

Anterior end truncate, submedian lobes absent; body annuli without anastomoses, margins of annuli very finely crenate (more visible on tail). First annulus 7 μm in diameter, mid-body 24 μm width; esophagus 76 μm long; vulva closed; spermatheca filled with sperm; body wall bend at the level of vulva; tail rounded, 9 μm long.

Distribution and habitat. This species was found in sandy soil with high moisture content (site no. 101) (Table 1, Fig. 1A).

Mesocriconema crenatum (Loof, 1964) Andrássy, 1965
(Fig. 1C)

Morphometric and taxonomic details are described in [22].

Distribution and habitat. The species was found at site 73 (Table 1, Fig. 1C), in subalpine area.

Mesocriconema curvatum (Raski, 1952) Loof & de Grisse, 1989
(Fig. 1D)

Morphometric and taxonomic details are presented in [22].

Distribution and habitat. The species was found at sites no. 38, 45, 54 and 63 (Table 1, Fig. 1D).

Mesocriconema kirjanovae (Andrássy, 1962) Loof & de Grisse, 1989
(Fig. 1D; Table 3)

Head with small and rounded submedian lobes; lateral plates small but visible; stylet robust; vulva open, anterior vulva lip with two acute projections; vagina straight; spermathecae oval filled with sperm; tail conical pointed.

Morphometrics (Table 3) fall within the range given by Brzeski [4].

Distribution and habitat. The species was found only at site no. 102 (Table 1, Fig. 1D). It was previously reported from Romania [19].

Mesocriconema rotundicauda (Loof, 1964) Loof, 1989
(Fig. 1C)

Details concerning morphometrics and taxonomic characters are described in [22].

Distribution and habitat. The species was only found at site no. 73 in natural subalpine habitat (Table 1, Fig. 1C).

Mesocriconema rusticum (Micoletzky, 1915) Loof & de Grisse, 1989
(Fig. 2A; Table 3)

Submedian lobes large truncate. Annuli smooth; vulva open; vagina straight, tail rounded. Morphometric data are given in Table 3.

Distribution and habitat. M. rusticum is wide spread and was found in 30% of the study sites (no. 1-3, 5, 10, 12, 18-20, 22, 27-29, 39-44, 47, 48, 55, 64, 67, 74, 76, 79, 85, 92, 95, 101 and 102) (Table 1). It was found in both natural and disturbed habitats (Fig. 2A).

Mesocriconema solivagum (Andrássy, 1962) Loof & de Grisse, 1989
Syn. *Criconema dubium* apud Popovici, 1987
(Fig. 1C)

Details concerning morphometric data and taxonomic characters are given in [22].

Distribution and habitat. This species was collected at sites no. 1, 4, 64, 78 and 80 (Table 1, Fig. 1C). Two specimens (from sites no. 1 and 4), previously identified as *Criconema dubium* (de Grisse, 1967) by Popovici [17], belong to this species.

Mesocriconema xenoplax (Raski, 1952) Loof, 1989
(Fig. 2B)

Details concerning morphometric data and taxonomic characters are presented in [22].

Distribution and habitat. This species was found in sandy soil only in the Danube Delta (sites no. 101,103-105, 107 and 108) (Table 1, Fig.2B). These habitats experience very dry conditions during the summer and early autumn.

Xenocriconemella macrodora (Taylor, 1936) de Grisse & Loof, 1965
(Fig. 2B)

Morphometric data and taxonomic characters of specimens from Romania [17] fall within the range of the species variability given by de Grisse [7].

Distribution and habitat. This is the most common criconematid species in Romania (Fig. 2B), found in over 50% of the listed sites (no. 1, 5, 8, 10, 18, 19, 22-25, 27, 29-31, 33, 34, 36, 41-44, 46, 49, 50-53, 59, 60, 61, 64, 66, 69, 75-77, 81-

84, 87-95, 97-99) (Table 1). It was also previously reported from Romania [17, 20, 21]. The wide distribution of this species in Romania is in contrast with the statement by Escuer and Bello [10] that it prefers a Mediterranean climate.

Criconema annuliferum (de Man, 1921) Micoletzky, 1925
(Fig. 1A)

Measurements of specimens from Romania were previously reported [17] and fall within the range of variability given by de Grisse [7].

Distribution and habitat. This species is the second most common criconematid species in Romania (identified in 35% of the listed sites). This species shows preferences for natural habitats (forests and grasslands), from sites no. 1, 3, 6, 7, 9, 11, 12, 14, 15, 17, 21, 29, 30, 32-35, 41-43, 44, 48, 52, 53, 55, 61, 64, 69, 72, 76, 77, 80, 83, 84, 97-100) (Table 1, Fig. 1A).

Criconema demani Micoletzky, 1925
(Fig. 1B; Table 3)

The measurements (Table 3) fall within the range of the species variability already published [7].

Distribution and habitat. C. demani was mostly found in soil from forest ecosystems (sites no. 10, 56 and 71) (Table 1, Fig. 1B). Its presence in Romania was formerly reported [17, 20].

Criconema longulum Gunhold, 1953
(Fig. 1D)

Popovici [17, 20] already reported morphometric data and first notes on its distribution in Romania.

Distribution and habitat. C. longulum was identified only from sites no. 56-59 and 71 (Fig. 1D), representing timberline spruce forests and dwarf scrub in the subalpine area of the Carpathian Mountains (Table 1).

Criconema princeps (Andrássy, 1962) Raski & Luc, 1985
(Fig. 1C)

Morphometric data of this species from Romania and data on its relative abundance in different habitats were previously reported [17, 20, 21].

Distribution and habitat. C. princeps was found in small numbers, in 15% of the listed sites (no. 3, 5, 29, 32, 33, 53, 55, 58, 59, 61, 64, 69, 70, 72 and 83) (Table 1, Fig. 1C).

Ogma danubiale Andrássy, 1985
(Fig. 2C)

The characters and morphometrics of the specimens found in Romania correspond well with those of the original description [3]. Details on the specimens from Romania are presented in [22].

Distribution and habitat. *O. danubiale* from Romania was found in sand dunes in the Danube Delta Biosphere Reserve, at sites no. 103 and 106 (Table 1, Fig. 2C).

Ogma menzeli (Stefanski, 1924) Schuurmans-Stekhoven & Teunissen, 1938
(Fig. 2C)

Morphometrics and data on relative abundance of this species in Romania were previously reported [17, 20, 21].

Distribution and habitat. The species was found in 34% of the study sites viz. no. 8, 10, 12, 16, 22, 26-31,33, 35-37, 55,56, 74-77, 80, 82, 83, 86-88, 92, 95-99 (Table 1, Fig. 2C). It was found only in natural ecosystems from the Carpathians.

O. menzeli is widely distributed in Europe [2] and not restricted to the Atlantic area only, as reported by Escuer and Bello [10].

Ogma murrayi Southern, 1914
(Fig. 2C)

Morphometric data, the presence and relative abundance of this species from Romania were reported [17, 20, 21].

Distribution and habitat. This species was infrequently found in natural habitats such as beech, oak, spruce forests and grasslands (sites no. 6, 8, 13, 16, 55, 77 and 105) (Table 1, Fig. 2C).

Ogma zernovi Kirjanova, 1948
(Fig. 2C)

Ogma zernovi was described from Russia [13] and subsequently from Spain [12].

The specimens from Romania differ slightly from those previously reported and details are given in [22].

Distribution and habitat. The species was recorded only from habitats in the subalpine areas of the Retezat Mountains, at sites no. 60, 62, 64 and 65 (Table 1, Fig. 2C).

Table 1

Site description

Site No.	Region, Place	Altitude (m)	Geographical position	Plant association*
1	Bihor Mountains, Padiș	1250	46°34`N-22°43`E	*Festuco rubrae-Agrostetum*
2	Bihor Mountains, Poiana Ponor	1000	46°34`N-22° 43`E	*Lolio-Trifolietum repentis*
3	Bihor Mountains, Poiana Ponor	1050	46°34`N-22°43`E	*Hieracio rotundati-Piceetum*
4	Bihor Mountains, Someșul Cald	1100	46°35`N-22°50`E	*Hieracio rotundati-Piceetum*
5	Bihor Mountains, Padiș	1300	46°40`N - 22° 45`E	*Symphyto cordati-Fagetum*
6	Bihor Mountains, Arieșeni	800	47°28`N-22°43`E	*Festuco rubrae- Agrostetum*
7	Bihor Mountains, Smida	1000	46°39`N-23°01`E	*Violo declinatae-Nardetum*
8	Bihor Mountains, Călineasa	1200	46°30`N-22°55`E	*Hieracio rotundati- Piceetum*
9	Vlădeasa Mountain, Poiana Frânturii	1400	46°46`N-22°48`E	*Scorsonero-Festucetum nigricantis*
10	Vlădeasa Mountains, Stâna de Vale, Poiana Priscop	1400	46°46`N-22°45`E	*Scorsonero-Festucetum nigricantis*
11	Vlădeasa Mountains, Stâna de Vale	1000	46° 46`N-22°45`E	*Symphyto cordati-Fagetum*
12	Vlădeasa Mountains, Dealul cu trei Poieni	1640	46° 46`N-22°47`E	*Scorsonero-Festucetum nigricantis*
13	Vlădeasa Mountains, Zârna Valley	1250	46°45`N - 22°46`E	*Festuco rubrae- Agrostetum*
14	Vlădeasa Mountains, Drăgan Valley	1100	46°47`N- 22°43`E	*Scorsonero-Festucetum nigricantis*
15	Vlădeasa Mountains, Drăgan Valley,	1130	46°55`N-22°53`E	*Violo declinatae-Nardetum*
16	Vlădeasa Mountains, near chalet	1400	46°55`N-22°53`E	*Hieracio rotundati-Piceetum*
17	Vlădeasa Mountains, Preluca Rabului	1250	46°55`N-22°53`E	*Leucanthemo waldsteinii-Fagetum*
18	Trascău Mountains, Tureni Gorges	400	46°30`N-23°41`E	*Melico-Phleetum montani*
19	Trascău Mountains, Vălișoara Gorges	400	46°26`N-23°29`E	*Agrostio-Festucetum sulcatae*
20	Trascău Mountains, Intregalde Gorges	430	46°14`N-23°23`E	*Festuco rubrae - Agrostetum*
21	Trascău Mountains, Poșaga	675	46°26`N-23°27`E	*Asperulo capitatae-Seslerietum rigidae*
22	Trascău Mountains, Râmeț Gorges	440	46°19`N-23 °31`E	*Symphyto cordati-Fagetum*

Table 1 (continued)

Site No.	Region, Place	Altitude (m)	Geographical position	Plant association*
23	Trascău Mountains, Sălciua	1000	46°23′N-23°26′E	*Symphyto cordati-Fagetum*
24	Trascău Mountains, Poșaga, Scărița Belioara	700	46°26′N-23°27′E	*Symphyto cordati-Fagetum*
25	Trascău Mountains, Intregalde Gorges	435	46°14′N-23°23′E	*Carpino-Fagetum*
26	Trascău Mountains, Turzii Gorges	400	46°34′N-23°36′E	*Pinetum sylvestris-Seslerietosum*
27	Trascău Mountains, Turzii Gorges	410	46°34′N-23°36′E	*Carpino-Quercetum petraeae*
28	Metaliferi Mountains, Bucești-Vulcan	500	46° 11′N- 22° 50′E	*Festuco rubrae- Agrostetum*
29	Metaliferi Mountains, Bucești-Vulcan	550	46° 11′N- 22° 50′E	*Carpino-Fagetum*
30	Metaliferi Mountains, Roșioara Valley	800	46° 09′N - 23° 4′E	*Symphyto cordati-Fagetum*
31	Metaliferi Mountains, Dealul Mare	800	46° 16′N - 23° 04′E	*Carpino-Fagetum*
32	Metaliferi Mountains, Ghețar-Scărișoara	1050	46°26′N-23°17′E	*Telekio- Petasitetum*
33	Metaliferi Mountains, Muncel	700	46° 23′N - 23° 17′E	*Symphyto cordati-Fagetum*
34	Metaliferi Mountains, Zlatna	700	46° 07′N - 23° 13′E	*Symphyto cordati-Fagetum*
35	Gilău Mountains, Huza Valley	700	46°30′N-23°14′E	*Symphyto cordati-Fagetum*
36	Gilău Mountains, Iara Valley	800	46°30′N-23°14′E	*Leucanthemo waldsteinii-Fagetum*
37	Gilău Mountains, Muntele Mare, Capu Dealului	1300	46°30′N-23°14′E	*Hieracio rotundati-Piceetum*
38	Turda, Dealul Durgău, Cluj county	350	46°34′N-23°48′E	*Salicornietum europaeae*
39	Cluj, Fânețele Clujului Botanical Reserve	350	46°45′N-23°35′E	*Jurineo transsilvanicae - Stipetum pulcherimae*
40	Suatu Botanical Reserve, Cluj county	370	46°46′N-23°58′E	*Salvio nutantis-Festucetum rupicolae*
41	Baciu, Cluj county	590	46°48′N-23°31′E	*Carpino-Quercetum petraeae*
42	Câțcău, Secătura Valley, Cluj county	450	47°12′N-23°47′E	*Carpino-Fagetum*
43	Câțcău, Secătura Valley, Cluj county	450	47°12′N-23°47′E	*Carpino-Quercetum petraeae*
44	Aluniș, Cluj county	400	47°02′N-23°45′E	*Carpino-Quercetum petraeae*

Table 1 (continued)

Site No.	Region, Place	Altitude (m)	Geographical position	Plant association*
45	Ocna Sibiului, Sibiu county	400	46°52′N-24°03′E	*Artemisio-Festucetum pseudovinae*
46	Noroieni, Satu Mare county	150	47°46′N-22°52′E	*Quercetum robori-cerris*
47	Tg. Mureș, Mureș county	350	46°32′N-24°34′E	Apple orchard
48	Copșa Mică, Sibiu county	350	46°06′N-24°16′E	Arable land
49	Semenic Mountains, Canton Borloveni	1380	45°14′N-22°41′E	*Festuco rubrae- Agrostetum*
50	Mehedinți Mountains, Piatra Cloșani	330	45°07′N-22°43′E	*Festuco rubrae- Agrostetum*
51	Cernei Mountains, confluence of Roșu and Roșeț brooks	240	44°55′N-22°27′E	*Deschampsio flexuosae-Fagetum*
52	Cernei Mountains, Jelerău brook	500	44°55′N-22°27′E	*Phyllitidi-Fagetum*
53	Retezat Mountains, Gura Zlata, Poiana Lăncița	1050	45°33′N-22°52′E	*Festuco rubrae- Agrostetum*
54	Retezat Mountains, Dobrun Valley	1500	45°29′N-22°52′E	*Hieracio rotundati-Piceetum*
55	Retezat Mountains, Fața Retezatului	1830	45°24′N-22°55′E	*Campanulo-Juniperetum bruckenthalietosum*
56	Retezat Mountains, Fața Retezatului	1810	45°28′N-22°55′E	*Bruckenthalio-Piceetum*
57	Retezat Mountains, Fața Retezatului	1950	45°28′N-22°55′E	*Rhododendro myrtifolii-Pinetum mugi*
58	Retezat Mountains, Drăgșan,	1765	45°20′N-22°57′E	*Poetum mediae*
59	Retezat Mountains, Șcaua Scorota	1850	45°19′N-22°57′E	*Potentillo-Festucetum airoidis*
60	Retezat Mountains, Fața Iarului	1700	45°18′N-22°60′E	*Festucetum xanthinae*
61	Retezat Mountains, Scocul Iarului	1200	45°18′N-23°03′E	*Leucanthemo waldsteinii-Fagetum*
62	Retezat Mountains, Piatra Iorgovanului	1750	45°18′N-23°03′E	*Rhododendro myrtifolii-Pinetum mugi*
63	Retezat Mountains, Scorota cu Apă	1350	45°18′N-23°03′E	*Leucanthemo waldsteinii- Fagetum*
64	Retezat Mountains, Piule	1850	45°19′N-22°57′E	*Festucetum xanthinae*
65	Retezat Mountains, Piule	1900	45°18′N-23°03′E	*Rhododendro myrtifolii-Pinetum mugi*

Table 1 (continued)

Site No.	Region, Place	Altitude (m)	Geographical position	Plant association*
66	Retezat Mountains, Câmpușel	1150	45°18′N-22°60′E	*Phyllitidi-Fagetum*
67	Retezat Mountains, Câmpușel	1100	45°18′N-22°60′E	*Festuco rubrae-Agrostetum*
68	Retezat Mountains, Șeaua Scorota	1850	45°18′N-23°03′E	*Rhododendro myrtifolii-Pinetum mugi*
69	Parâng Mountains, Păpușa	2050	45°28′N-23°29′E	*Violo declinatae-Nardetum*
70	Parâng Mountains, near Câlcescu Lake	1850	45°21′N-23°33′E	*Violo declinatae-Nardetum*
71	Parâng Mountains, Coasta lui Rus	1800	45°25′N-23°22′E	*Hieracio rotundati-Piceetum*
72	Ciucaș Mountains, Roșu Mountain	1500	45°26′N-25°52′E	*Violo declinatae-Nardetum*
73	Harghita-Mădăraș Mountains	1750	46°35′N-24°23′E	*Campanulo- Juniperetum*
74	Harghita-Mădăraș Mountains	1650	46°35′N-24°23′E	*Hieracio rotundati-Piceetum*
75	Harghita-Mădăraș Mountains	1500	46°35′N-24°23′E	*Hieracio rotundati-Piceetum*
76	Harghita-Mădăraș Mountains, Filiou Valley	800	46°35′N-24°23′E	*Symphyto cordati-Fagetum*
77	Gurghiu Mountains, Gurghiu Valley	830	46°45′N-25°01′E	*Symphyto cordati-Fagetum*
78	Gurghiu Mountains, Sălard Valley	870	46°57′N-25°06′E	*Hieracio rotundati-Piceetum*
79	Căliman Mountains, Negoiul Românesc	1780	47°14′N-25°20′E	*Rhododendro myrtifolii-Pinetum mugi*
80	Căliman Mountains, Ilișoara Valley	1150	46°59′N-25°02′E	*Leucanthemo waldsteinii-Fagetum*
81	Bârgău Mountains, Măgura	950	47°22′N-24°54′E	*Festuco rubrae-Agrostetum*
82	Rodna Mountains, Pietrosul Rodnei	1560	47°25′N-24°54′E	*Hieracio rotundati-Piceetum*
83	Năsăud Plateau, Largă Valley	550	47°17′N-24°27′E	*Symphyto cordati-Fagetum*
84	Năsăud Plateau, Izvorul Negru Valley	1200	47°17′N-24°27′E	*Leucanthemo waldsteinii-Fagetum*
85	Rodna Veche, Bistrița- Năsăud county	700	47°25′N-24°46′E	Mine spoil dumps
86	Maramureș Mountains, Bârjaba Valley	1300	47°43′N-24°26′E	*Hieracio rotundati-Piceetum*
87	Igniș Mountain, Ferneziu, Firiza Valley	490	47°41′N-23°36′E	*Symphyto cordati-Fagetum*
88	Gutâi Mountain, Bolduț Valley	850	47°42′N-23°54′E	*Symphyto cordati-Fagetum*

Table 1 (continued)

Site No.	Region, Place	Altitude (m)	Geographical position	Plant association*
89	Gutâi Mountain, Roșia Valley	550	47°39′N-23°34′E	*Castaneo-Quercetum*
90	Lăpușel, Maramureș county	205	47°37′N-23°29′E	*Carpino-Quercetum petraeae*
91	Țibleș Mountains, Bradu Valley	680	47°29′N-24°04′E	*Symphyto cordati-Fagetum*
92	Țibleș Mountains, Suciu Valley	850	47°29′N-24°04′E	*Pulmonario rubrae-Fagetum*
93	Lăpuș Mountains, Leorda	800	47°30′N-24°01′E	*Symphyto cordati-Fagetum*
94	Lăpuș Mountains, Strâmbu-Băiuț	850	47°40′N-23°52′E	*Hieracio rotundati-Abietetum*
95	Oaș Mountains, Călinești-Oaș	350	47°54′N-23°18′E	*Carpino-Quercetum petraeae*
96	Giumalău Mountains, Putna Valley	940	47°29′N-25°24′E	*Hieracio rotundati-Piceetum*
97	Rarău Mountains, Codrul Secular Slătioara	1060	47°27′N-25°34′E	*Hieracio rotundati-Piceetum*
98	Rarău Mountains, Toanca Valley	1350	47°27′N-25°34′E	*Hieracio rotundati-Piceetum*
99	Rarău Mountains, Colbu Valley	1280	47°27′N-25°34′E	*Festuco rubrae-Agrostetum*
100	Ceahlău Mountains, Dochia Valley	850	46°47′N-25°41′E	*Symphyto cordati-Fagetum*
101	Danube Delta, Maliuc	50	45°13′N-29°04′E	Poplar plantation
102	Danube Delta, Caraorman	80	45°04′N-29°22′E	*Elymetum gigantei*
103	Danube Delta, Caraorman	80	45°04′N-29°22′E	*Quercetum pedunculiflorae*
104	Danube Delta, Enisala	250	42°52′N-28°50′E	*Quercetum pedunculiflorae*
105	Danube Delta, Letea	80	45°19′N-29°33′E	*Quercetum pedunculiflorae*
106	Danube Delta, Letea	80	45°19′N-29°33′E	*Elymetum gigantei*
107	Danube Delta, Sf. Gheorghe	35	44°52′N-29°36′E	*Elymetum gigantei*
108	Danube Delta, Gura Portiței	10	44°46′N-28°52′E	*Elymetum gigantei*

*C o l d e a [5].

Table 2

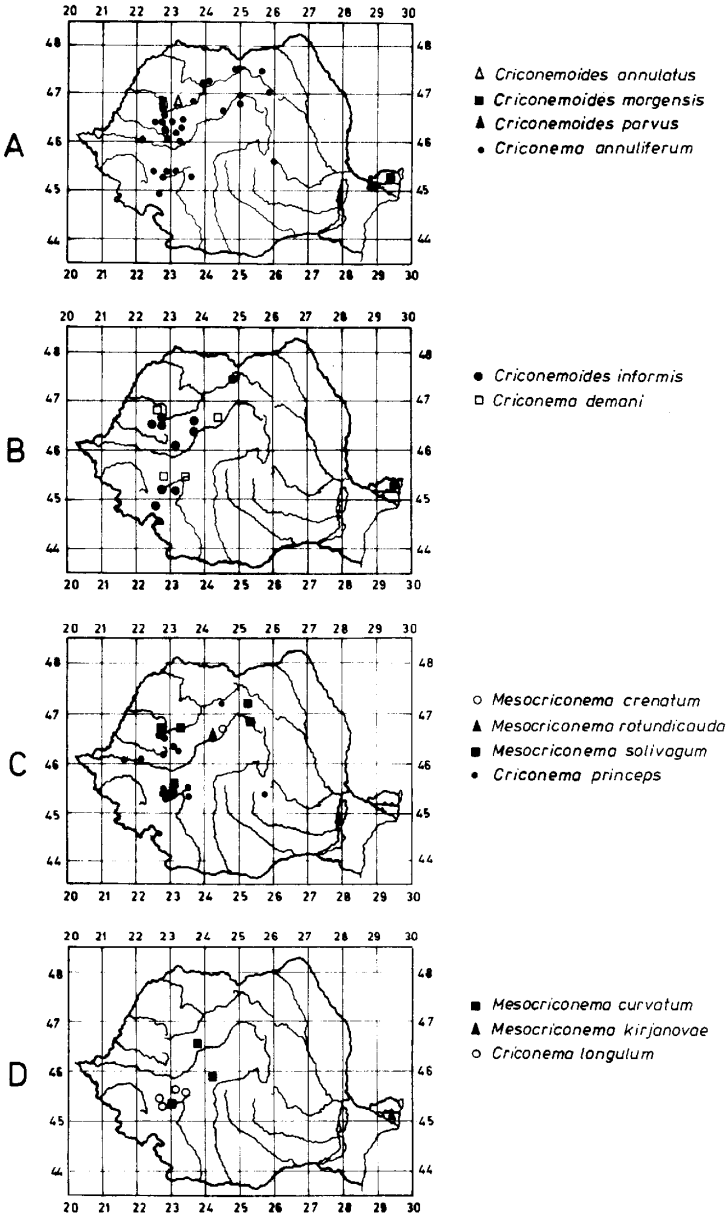
Measurements of Criconemoides annulatus, C. informis, C. morgensis and C. parvus

Characters	*C. annulatus*	*C. informis*			*C. morgensis*	*C. parvus*
	Bihor Mountains	Bihor Mountains	Metaliferi Mountains	Rodna	Danube Delta	Danube Delta
N	9 ♀♀	3 ♀♀	12 ♀♀	8 ♀♀	2 ♀♀	1 ♀
L	737 (683-902)	411 (356-482.5)	456 (401-492)	430 (372-530)	468-608	299
A	12.6 (11-15)	8.3 (7.7-8.9)	9.3 (8.2-10.6)	9.4 (7.4-12.6)	12.6-13.2	12.4
B	4.4 (3.5-5.2)	3.6 (3.3-3.8)	3.8 (3.3-4.2)	3.7 (3.2-4.5)	4.6-5.1	3.9
C	35.9 (26-45)	17.9 (17.5-18.3)	20.4 (13-29.5)	16.9 (10-20.2)	16.7-20.9	33.2
V	95 (94.3-95.8)	89 (88.6-90)	91(89.4-92.5)	90 (88.4-92.1)	92.9-93.2	95
St	107 (100-112)	67.5 (60-75)	70 (64-74)	67.5 (67-71)	54-66	35
R	162 (158-172)	57 (55-59)	60 (55-64)	59 (54-72)	122-124	173
RSt	29 (24-36)	10 (9-12)	11(10-12)	11 (9-12)	17-22	23
ROes	45 (39-55)	16 (14-17)	17 (15-19)	17 (15-19)	30-33	49
Rex	45 (44-48)	18 (17-19)	19 (17-20)	19 (17-20)	33-36	50
RV	9 (8-10)	7 (7-8)	7 (6-8)	8 (7-9)	10-11	12
Ran	6 (4-7)	5 (4-5)	4 (4-6)	5 (4-6)	8-9	9
Rvan	1 (0-3)	2 (1-2)	1 (0-2)	1 (1-2)	1	2
VL/VB	0.9 (0.8-1.1)	1.2 (1.1-1.2)	1.1 (0.9-1.2)	1.1 (0.8-1.4)	1.1-1.2	0.7
VL/St	0.3 (0.2-0.4)	0.6	0.6 (0.5-0.7)	0.6 (0.4-0.7)	0.6	0.3
St%L	15 (12.5-18)	16 (15.5-17)	15.3 (13.4-17.2)	16 (13-18)	10.8-11.5	12
St%Oes	62 (56-69)	59.5 (56-63)	58 (54-65)	59 (48-79)	50-57.5	46
CP%St	77 (72-82)	76 (75-78)	77 (75-81)	79 (74-81)	68.5-71	80

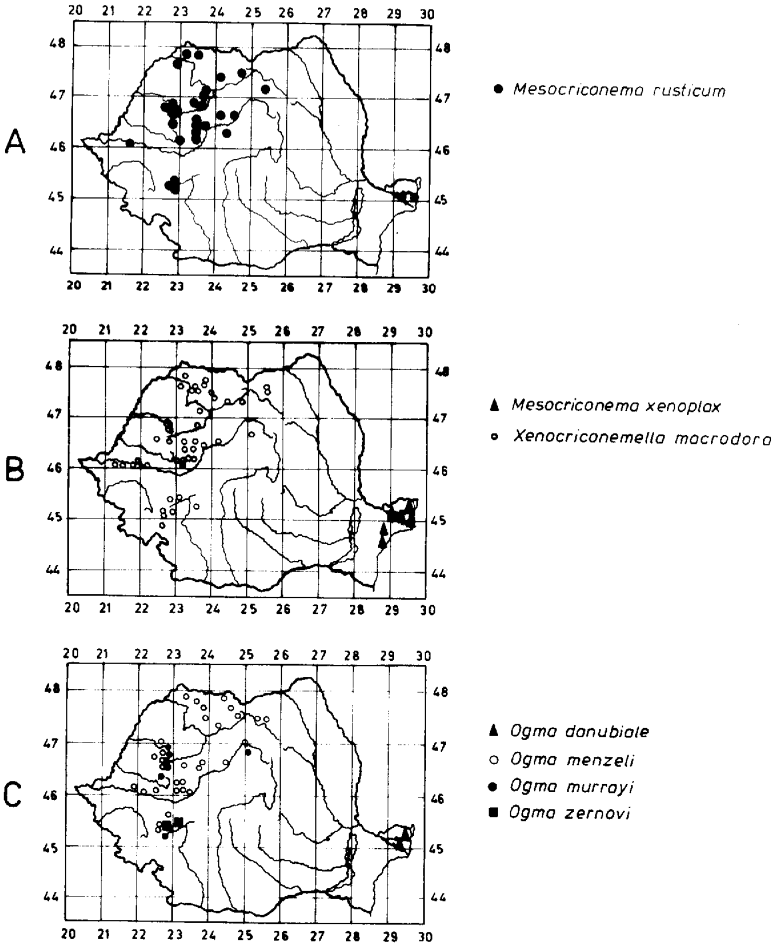
Table 3

Measurements of Mesocriconema kirjanovae, M. rusticum and Criconema demani

Characters	*M. kirjanovae*	*M. rusticum*				*C. demani*	
	Danube Delta	Rodna Veche	Tureni Gorges	Fânețele Clujului	Danube Delta	Harghita Mountains	Parâng Mountains
n	4 ♀♀	2 ♀♀	5 ♀♀	2 ♀♀	11 ♀♀	1 ♀	7 ♀♀
L	392 (372-420)	442-400	417 (371-490)	436-536	401 (350-440)	394	401 (377-428)
a	9.5 (7.8-10.8)	9.8-8	10.7 (9.3-11.6)	10.9-12.1	9.2 (7.4-11.9)	10.8	9.8 (9.1-10.4)
b	4 (3.6-4.4)	4	4.2 (3.8-4.9)	3.6-4.1	3.9 (3.8-4.3)	4	3.9 (3.5-4.4)
c	14.5(12.9-16.8)	25.2-26.9	20 (15.3-22.8)	29.7-33.5	26.4 (20.1-33.6)	10	12.4 (10.1-15)
V	90 (88.6-90.4)	93.2-94.4	93 (92-93.4)	93.8	93 (92-94)	86	87 (85-90)
St	57 (53-61)	55-62.5	53 (50-55)	63-84	55 (50-59.5)	63	61(58-63)
R	83 (81-84)	96-104	101 (94-109)	101-82	91(87-97)	75	74 (69-76)
RSt	15 (12-17)	14	17 (15-18)	15	15 (13-16)	14	12 (11-14)
ROes	24 (23-25)	26-27	26 (25-29)	22-28	26 (23-28)	19	19 (18-20)
Rex	25	30-31	29 (27-31)	23-29	26 (26-27)	20	15 (13-19)
RV	11 (10-11)	7-8	8 (8-9)	7	7 (6-9)	14	14 (11-15)
Ran	8 (7-9)	5-6	7 (6-7)	4	5 (3-6)	10	10 (9-11)
RVan	2 (1-2)	1	1 (0-1)	2	2 (1-3)	3	4 (3-5)
VL/VB	1.3 (1.2-1.4)	1-1.1	0.9 (0.8-1)	1.1	1.1- (1.0-1.2)	1.9	0.6 (0.5-0.7)
VL/St	0.6 (0.6-0.7)	0.5	0.5 (0.5-0.6)	0.4	0.6 (0.5-0.7)	0.9	0.4 (0.4-0.5)
St%L	14 (13-16)	12.4	13 (11-14)	14-16	14 (13-15)	16	15 (14-17)
St%Oes	57 (50-68)	50	53 (51-55)	52-66	55(51-58)	64	61 (57-64)
CP%St	76 (77-79)	75-77	76 (74-78)	74-75	77 (75-79)	84	83 (81-85)



F i g. 1 A - D. *Distribution maps of* Criconemoides, Criconema and Mesocriconema *species in Romania (geographical degrees).*



F i g. 2 A - C. *Distribution maps of* Mesoriconema, Xenocriconemella *and* Ogma *species in Romania (geographical degrees).*

General remarks on criconematid distribution in Romania. The *Criconematidae* species from Romania were the most prevalent in grasslands (32 % of sites), followed by deciduous forests (28 %) and coniferous forests (17.5 %).

The most frequently found species were *X. macrodora* (51.5 %), *C. annuliferum* (35 %), *O. menzeli* (34 %) and *M. rusticum* (31 %) (see also Figs.1 and 2) and C. *informis* and *X. macrodora* were the most widely distributed species in Romania.

Mesocriconema crenatum, M. rotundicauda and *Ogma zernovi* were found only in the subalpine areas of the Carpathians, while *M. kirjanovae, M. xenoplax, C. morgensis, C. parvus* and *O. danubiale* were found only in the sandy soils from the Danube Delta Biosphere Reserve.

Conclusions. 1. Twenty criconematid species from several habitats in Romania are identified in 108 study sites.

2. Details on the main taxonomic characters for seven species (*Criconemoides annulatus, C. informis, C. morgensis, C. parvus, Mesocriconema kirjanovae, M. rusticum* and *Criconema demani*) are discussed and their morphometric data are given in tables. The ranges of their variability are important for taxonomic studies of the *Criconematidae* family.

3. Distribution maps for all recorded species in Romania are provided. Their prevalence in grasslands and deciduous forests is noted. The most frequently found species are *Xenocriconemella macrodora*, *Criconema annuliferum*, *Ogma menzeli* and *Mesocriconema rusticum*. *Criconemoides informis* and *X. macrodora* are the most widely distributed species in Romania.

REFERENCES

1. A n d r á s s y, I., *Verzeichnis und Bestimmungsschlüssel der Arten der Nematoden-gattungen* Criconemoides *Taylor, 1936* und Mesocriconema *n. gen.*, "Opusc. Zool. Budapest.", **5,** 1965, 153-171.
2. A n d r á s s y, I., *Revision of the subfamily* Criconematinae *Taylor, 1936 (*Nematoda*)*, "Opusc. Zool. Budapest.", **16**, 1979, 11-57.
3. A n d r á s s y, I., *A dozen new nematode species from Hungary*, "Opusc. Zool. Budapest.", **19-20**, 1985, 3-39.
4. B r z e s k i, M.W., *Morphological observations on three species of* Mesocriconema *Andrássy, 1962 and nomenclatorial note on* M. goodeyi *(Jairajpuri, 1963) Loof & de Grisse, 1989 (*Nematoda: Criconematidae*)*, "J. Nem. Morphol. Syst.", **1**, 1998, 47-56.
5. C o l d e a, G., *Prodrome des associations végétales des Carpates du Sud-Est (Carpates Roumaines)*, "Doc. Phytosociol." (Camerino), **13**, 1991, 1-539.
6. D e G r i s s e, A., *Description of fourteen new species of* Criconematidae *with remarks on different species of this family*, "Biol. Jaarb. Dodonea", **35**, 1967, 66-125.
7. D e G r i s s e, A., *Contribution to the morphology and the systematics of the* Criconematidae *(Taylor, 1936) Thorne, 1949*, Dr. Thesis, Univ. Gent, 1969.

8. De Grisse, A., *Redescription ou modification de quelques techniques utilisées dans l´étude des nématodes phytoparasitaires*, "Meded. Rijksfac. Landbouww. Gent", **34**, 1969, 351-370.
9. De Grisse, A., Loof, P. A. A., *Intraspecific variation in some* Criconematidae (Nematoda), "Meded. Rijksfac. Landbouww. Gent", **35**, 1970, 41-63.
10. Escuer, M., Bello, A., *Nematodos del suelo de la familia* Criconematidae *en Cataluña*, "Orsis", **9**, 1994, 59-75.
11. Escuer, M., Bello, A., *Nematodos de la subfamilia* Macroposthoniinae *(*Nematoda*:* Criconematidae*) en la España peninsular*, "Orsis", **11**, 1996, 59-92.
12. Escuer, M., Palomo, A., Bello, A., *The genus* Ogma *Southern*, 1914 (Nematoda: Criconematidae) *in the Iberian Peninsula*, "Nematol. Medit.", **18**, 1990, 9-13.
13. Kiryanova, E. S., *Desyat` novykh vidov nematod iz semeistva* Ogmidae *Southern, 1914*, in *Sbornik Pamyati Akademika S.A. Zernova*, pp. 346-358, Izd. Akad. Nauk SSSR, Moskva, 1948.
14. Loof, P. A. A., *Free-living and plant parasitic nematodes from Spitzbergen, collected by Mr. H. van Rossen*, "Meded. Landbouwhogeschool Wageningen", **71**, 1971, 1-86.
15. Loof, P. A. A.,. *Identification of criconematids*, in Fortuner, R. (Ed.), *Nematode Identification and Expert System Technology*, pp. 139-152, Plenum Press, New York and London, 1989.
16. Loof, P. A. A., De Grisse, A., *Taxonomic and nomenclatorial observations on the genus* Criconemella *De Grisse & Loof, 1965 sensu Luc & Raski, 1981* (Criconematidae*)*, "Meded. Fac. Landbouww. Rijksuniv. Gent", **54**, 1989, 53-74.
17. Popovici, I., *Specii de nematode* (Nematoda: Criconematidae*) în fauna României*, "Stud. Cerc. Biol., Ser. Biol. Anim.", **39**, 1987, 95-102.
18. Popovici, I., *Intraspecific variations in* Criconemoides annulatus *Taylor, 1936* (Nematoda: Criconematidae) *from Romania*, "Rev. Roum. Biol., Sér. Biol. Anim.", **33**, 1988, 11-14.
19. Popovici, I., *Diversitatea faunei de nematode* (Nematoda) *din Delta Dunării*, "An. Şti. Inst. Delta-Dunării", 1992, 57-62.
20. Popovici, I., *Structura şi dinamica comunităţilor de nematode* (Nematoda), in Popovici, I. (Ed.coord.), *Parcul Naţional Retezat-Studii Ecologice*, pp. 200-214, West Side, Braşov, 1993.
21. Popovici, I., *Structure of nematode communities in mountain grasslands from Romania*, in De Goede, R. G. M., Bongers, T. (Eds.), *Nematode Communities of Northern Temperate Grassland Ecosystems*, pp. 221-240, Focus, Giessen, 1998.
22. Popovici, I., Ciobanu, M., Criconematidae (Nematoda) *from Romania*, "J. Nem. Morphol. Syst.", (in press).
23. Raski, D. J., Luc, M., *A reappraisal of the genus* Criconema *Hofmänner and Menzel, 1914* (Nematoda: Criconematidae), "Rev. Nématol.", **7**, 1984, 323-334.
24. Seinhorst, J. W., *A rapid method for the transfer of nematodes from fixative to anhydrous glycerin*, "Nematologica", **4**, 1959, 67-69.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, XLV, 1, 2000

ECOLOGY OF TERRESTRIAL ISOPODS IN THE NATURE RESERVE SCĂRIŢA-BELIOARA, ROMANIA

NICOLAE TOMESCU[*], GAVRIL ARDELEAN[**], DANIELA MUREŞAN[*] and VALENTIN POPA[*]

SUMMARY. - In the Scărița-Belioara Nature Reserve, located in the Gilău – Muntele Mare Massif, we have collected terrestrial isopods using pitfall traps, in four ecosystems: meadow, rocky area (open ecosystems), spruce and beech forests. Six species of terrestrial isopods live in these ecosystems, of which four species are sylvan (*Protracheoniscus politus*, *Trachelipus wächtleri*, *T. arcuatus* and *Armadillidium carniolense*) and two are praticolous species (*T. nodulosus* and *A. versicolor quinqueseriatum*).

In the open ecosystems of the Nature Reserve, the prohibition of grazing has favoured the expansion of the herbaceous layer, especially in the meadow, which caused, at the soil surface, the formation of a microclimate resembling that in the litter layer of the forests. Under these conditions, the species of sylvan isopods have extended their spread outside the forests, presenting large populations in the meadow and the rocky areas where they are the dominant species. In the meadow there live three sylvan species (*P. politus*, *T. wächtleri* and *A. carniolense*) and a praticolous one (*T. nodulosus*). In the rocky area there live four sylvan species (*P. politus*, *T. wächtleri*, *T. arcuatus* and *A. carniolense*) and two praticolous ones (*T. nodulosus* and *A. versicolor quinqueseriatum*). In the meadow the dominant species is *T. wächtleri* (84.3%), and in the rocky area *A. carniolense* (73.9%). In the spruce and the beech forests there live only sylvan species (*P. politus*, *T. wächtleri* and *A. carniolense*). The presence of sylvan species in open mountain ecosystems where grazing is prohibited shows that these species are not strictly sylvan. Their distribution in hilly and plane zones only in forests is determined by the microclimate that is formed under the litter layer, a microclimate that presents small variations of humidity and temperature.

The sex ratio in the isopod populations of the Nature Reserve varies between 20-80% and 40-60% males: females. The value of the specific density and equitability is low in the meadow, rocky area and the spruce forest, due to the great differences that exist among the sizes of the populations of isopod communities from the three ecosystems.

**Babeş-Bolyai University, Department of Zoology, 3400 Cluj-Napoca, Romania*
***University of Baia Mare, Department of Biology, 4800 Baia Mare, Romania*

The research on the ecology of terrestrial isopods was focussed mainly on the populations of terrestrial isopods that live in grassland, meadow ecosystems and in the forests from hilly and plane zones [1-8,10-12, 14, 15]. We have studied the isopod communities and the distribution of species in relation to the ecological factors that exist in these types of ecosystems. The research concerning the terrestrial isopod fauna from mountain ecosystems is less extended. In Romania there are mountains on a large surface of the country, thus this type of research will contribute to a better analysis of the ecology and biology of isopod species.

The research has been carried out in 1996 in the Scărița-Belioara Nature Reserve. The Reserve is located in the Gilău-Muntele Mare Massif, in the Apuseni Mountains at an altitude over 1200 m. The annual average temperature is 4.2-5^{o}C, and the rainfall varies between 850-900 mm. The geological substratum is made of limestone covered by brown and rendzinous soil.

The terrestrial isopod samples have been collected in four types of ecosystems: meadow, rocky area, spruce and beech forests.

The **meadow** is located on a plateau with an inclination of 5 degrees. The herbaceous plants are tall (40-60 cm) and cover 90-100% of the soil surface, grazing being prohibited in the Nature Reserve. A relatively thick layer of detritus, composed of decaying herbaceous plants, covers the surface of the soil. The detritus layer maintains the water in the soil and prevents excessive warming of the soil surface during summer. A microclimate is formed at the surface of the soil, resembling that located under the litter layer of the deciduous forests from hilly and plane areas.

The **rocky area** is located on slopes with an inclination of 35-40 degrees. The soil layer is thinner and discontinuous, being interrupted by outcrops of rock. The herbaceous vegetation has a lower density compared to the plateau meadow. In this area there also are isolated trees. There exists a greater ecological diversity, with many microhabitats, due to the diversity of the microrelief, the thickness and the discontinuity of the soil, the inclination and the aspect of the slopes, the presence of isolated trees etc.

Spruce forest mixed with juniper shrubs. In the forest there are many clearings with abundant herbaceous vegetation. The reduced density of the trees has favoured the formation of a herbaceous layer also in the forest. The litter layer is composed of fallen spruce leaves and decaying herbaceous plants.

The **beech forest** is made up of trees that are 70-80 years old. The density of the trees is high, and the herbaceous layer is very scarce. A thick and uniform layer of litter, made almost exclusively of fallen beech leaves covers the surface of the soil.

Materials and methods. The isopods were collected using pitfall traps. In each ecosystems we placed 7 pitfalls. The pitfalls have been placed at the end of May, and emptied three times, once every month. The captured animals were conserved in 70% alcohol and studied in the laboratory. We have captured a total number of 1785 individuals. We have studied the distribution of species in the Nature Reserve, the numerical abundance and relative abundance, sex ratio, diversity and equitability.

The ecological indices of quantity have been statistically calculated.

The relative abundance has been calculated using the formula $A_r=n_i/N\cdot100$, where n_i = number of individuals of species i, N = total number of individuals.

To calculate the diversity we have used the Shannon-Weaver index: $H'= -\Sigma pi\cdot\log pi$, where pi = the proportion of the individuals of species i, and log is the decimal logarithm. The equitability has been calculated using the following formula: $e=H'/H_{max}$; $H_{max}=\log S$, where S represents the total number of species in a biocoenosis.

Results and discussion. The isopods that we have collected belong to six species (Table 1), of which four species are sylvan (*Protracheoniscus politus*, *Trachelipus wächtleri*, *T. arcuatus* and *Armadillidium carniolense*) and two species are praticolous (*T. nodulosus* and *A. versicolor quinqueseriatum*).

The distribution of species in the four ecosystems is varied. The size of the populations in a biocoenosis also varies greatly (Table 1).

In the meadow, which is an open ecosystem (without trees), three sylvan species (*P. politus*, *T. wächtleri* and *A. carniolense*) and only one praticolous species (*T. nodulosus*) represent the isopod fauna. From the numerical point of view the dominant species is *T. wächtleri* (a sylvan species), the population of wich represents 86.4% of the isopod fauna of the studied zone. On the contrary, the other two sylvan species (*P. politus* and *A. carniolense*) have a very low numerical representation, which suggests the fact that the values of the ecological optimum of these species are different from the ecological optimum of *T. wächtleri*, although in many coniferous and deciduous forests the three species coexist and are represented by large populations [9, 13].

In the rocky area all the six species are present, but in different proportions. The ecological diversity found here is also reflected in the structure of the isopod communities. The sylvan species *T. wächtleri* and *A. carniolense* are again numerically dominant, in spite of the fact that this also is an open ecosystem. The two praticolous species are also present here in a small number, proving that there are few microhabitats that provide them with optimum life conditions.

Table 1

Distribution, numerical abundance, relative abundance and sex ratio for the species of terrestrial isopods in the studied Nature Reserve

Station	Parameters*	Species						Total amount of izopods/station		X / trap / station
		P.politus	*T.nodulosus*	*T. wächtleri*	*T. arcuatus*	*A. carniolense*	*A. versicolor quinqueseriatum*	Number of individuals	%	
Meadow	n	7	98	605	0	9	0	719	40.3	102.7
	a	1.0	14.0	86.4	0	1.28	0			
	b	0.9	13.6	84.3	0	1.2	0			
Rocky area	n	49	4	88	4	451	14	610	34.2	87.2
	a	7.0	0.57	12.57	0.57	64.42	2.0			
	b	8.1	0.65	14.4	0.65	73.9	2.3			
Spruce forest	n	40	0	361	0	33	0	434	24.3	62
	a	5.7	0	51.57	0	4.71	0			
	b	9.22	0	83.18	0	7.6	0			
Beech forest	n	5	0	10	0	7	0	22	1.2	3.2
	a	0.71	0	1.4	0	1.0	0			
	b	22.72	0	45.47	0	31.81	0			
Total number of individuals		101	102	1064	4	500	14	1785		
% / species		5.6	5.6	59.8	0.2	28.0	0.8			
Sex ratio M/F (%)		20/80	40/60	40/60		40/60				

* n - Number of individuals/species;
a - Average (X) of individuals/ trap;
b - Relative abundance/species/ station.

In the two types of forests we found the same communities of sylvan species (*P. politus, T. wächtleri* and *A. carniolense*), but with much more numerous populations in the spruce forest, due to the more abundant herbaceous layer and a great number of glades. Analysing the distribution of the six species, we observe that the sylvan species *P. politus, T. wächtleri* and *A. carniolense* are present in all four ecosystems, which suggests that the boundaries of their ecological valences are broader compared to those of the other species. *T. nodulosus* is present in both open ecosystems (meadow and the rocky area), and *T. arcuatus* and *A. versicolor quinqueseriatum* live only in the rocky area.

The presence of sylvan species in the open ecosystems at the Scărița-Belioara Nature Reserve, which is located in a mountainous area, suggests that these species are not strictly sylvan. Their distribution, limited to forest ecosystems from hilly and plane zones, is determined mainly by the constant and moderate temperature values and the lack of excessive decrease of humidity during summer, conditions which exist only under the litter layer of the forests, and not in the open ecosystems encountered here.

The values of the numerical abundance (Table 1) are related to the sizes of the populations existent in an ecosystem. In the Scărița-Belioara Nature Reserve, *P. politus* and *A. carniolense* have more numerous populations in the rocky area and the spruce forest, *T. wächtleri* in the meadow and the spruce forest, *T. nodulosus* in the meadow. *T. arcuatus* and *A. versicolor* have a low numerical abundance, which suggests that in the examined area the values of the environmental factors are at the boundary of the ecological optimum of the two species.

The relative abundance, calculated for the communities of isopod species from each ecosystem, indicates the numerical dominance of *T. wächtleri* in the meadow and the spruce forest (84.3 and 83.18%, respectively), and of *A. carniolense* in the rocky areas (73.9%). We consider that for the beech forest the values of the relative abundance are not relevant, due to the small number of isopods collected here (22 individuals). From the total number of individuals which we have captured, 59.8% belong to *T. wächtleri* (1064 individuals), 28.0% - *A. carniolense* (500 individuals), 5.6% - *P. politus* (101 individuals), 5.6% - *T. nodulosus* (102 individuals), 0.2% belong to *T. arcuatus* (4 individuals) and 0.8% to *A. versicolor quinqueseriatum* (14 individuals). For the species with populations which are numerically reduced, the general ecological factors in the studied ecosystems are less favourable, and their presence in small populations is probably due to the small number of microhabitats in which the ecological factors are maintained within the optimum range.

In order to evaluate the abundance of the terrestrial isopod fauna in the four ecosystems, we have also calculated the average number of isopods captured with each trap, taking into consideration the total number of isopods

sampled in each ecosystem. The most numerous isopod populations were found in the meadow (X=102.7), then in the rocky areas (X=87.2) and the spruce forest (X=62.2). We are able to conclude that in the open mountain ecosystems, where grazing is prohibited, there are optimum ecological conditions for the isopod populations, including the sylvan species, and these populations are much more numerous compared to the isopod populations that live in forests located in mountainous areas.

The sex ratio has been calculated for four species, from which we have collected over 100 individuals (Table 1). For all the populations of the species that we have studied, we found that the females were predominant: 60-80%. The great percentage of females in the populations of the ecosystems from the Nature Reserve constitutes an advantage for the species by increasing their reproductive potential. In the mountainous areas, the low temperatures and the late spring frost increase the mortality rate of the isopods at young ages.

For the isopod communities that live in the meadow and the rocky area the values of the diversity and equitability index are lower (Table 2). Although in these ecosystems there live more species compared to the spruce or beech forest, with a number of three species of terrestrial isopods living in each ones (Table 1). These low values indicate the numerical dominance of only one species the population of which has a major contribution to the biological activities that take place in the biocoenosis.

Table 2

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

Index	Type of ecosystem			
	Meadow	Rocky area	Spruce forest	Beech forest
H'	0.22292	0.37172	0.24374	0.46005
e	0.37026	0.47767	0.51090	0.96422

Conclusions. 1. In the Scărița-Belioara Nature Reserve, located in the Gilău-Muntele Mare Massif, at an altitude over 1200 m, there live six species of terrestrial isopods: four sylvan species (*P. politus, T. wächtleri, T. arcuatus* and *A. carniolense*) and two praticolous species (*T. nodulosus* and *A. versicolor quinqueseriatum*).

2. In open ecosystems (meadow and rocky areas) the predominant species are the sylvan ones. Their presence here suggests that in mountainous open ecosystems the climate conditions are similar to those existent in the litter layer of the forests from hilly and plane zones, where these species are frequent.

3. The presence in mountainous open ecosystems of species that are generally considered sylvan indicates the fact that these species are not strictly sylvan, and their distribution only in the forests of hilly and plane zones is determined by the temperature and humidity that here are within the optimal range.

4. The highest value of the numerical and relative abundance has been recorded for *T. wächtleri* in the meadow and the spruce forest, and for *A. carniolense* in the rocky areas. From the total number of captured individuals (1785), 59.8% belong to *T. wächtleri*, 28.0% - *A. carniolense*, 5.6% - *P. politus*, 5.6% - *T. nodulosus*, 0.8% - *A. versicolor quinqueseriatum*, 0.2% - *T. arcuatus*.

5. The sylvan species are numerically predominant in mountainous open ecosystems where grazing is prohibited. It is necessary to extend the prohibition of grazing in mountain areas, in order to protect the epigeous fauna.

6. Females (60-80%) mainly represent the populations of terrestrial isopods from the Scărița-Belioara Nature Reserve.

7. The values of the diversity and equitability express the existent differences regarding the size of the populations of the terrestrial isopod communities, differences which are emphasized in the meadow, rocky areas and in the spruce forest.

REFERENCES

1. D a v i s, R. C., S u t t o n, S. L., *Spatial distribution and niche separation of woodlice and millipedes in a dune grassland ecosystem*, "Ecol. Bull." (Stockholm), **25**, 1977, 45-55.
2. H a s s a l, M., D a v i s, R. C., P r o c t e r, D., *Lateral movements of isopods in a dune grassland*, "Proc. 4th Eur. Congr. Entomol. (Gödöllő, 1991)", 1991, 733-740.
3. H o r n u n g, E., *Characteristics of the population of an isopoda species* (Trachelipus nodulosus *C. L. Koch*) *at sandy soil grassland*, "Acta Biol. Szeged.", **30**, 1984, 153-158.
4. H o r n u n g, E., *Preliminary data to the clutch-size of* Trachelipus nodulosus *C.L. Koch in different habitats*, "Acta Biol. Szeged.", **34**, 1988, 169-171.
5. H o r n u n g, E., *Population dynamics and spatial distribution of* Trachelipus nodulosus (*C.L. Koch, 1838*) (Crustacea, Isopoda) *in a sandy grassland*, "Monit. Zool. Ital. (N.S.) Monogr.", **4**, 1989, 399-409.
6. H o r n u n g, E., *Comparison of different grassland types based on isopod communities*, " Proc. 4th Eur. Congr. Entomol. (Gödöllő, 1991)", 1991, 741-746.

7. H o r n u n g, E., W a r b u r g, M. R., S z l á v e c z, K., *Trends and methods in terrestrial isopod ecology: a round-table discussion*, "Proc. 4th Eur. Congr. Entomol. (Gödöllő, 1991)", 1991, 747-750.
8. K h e i r a l l a h, A. M., *Aspects of the distribution and community structure of isopods in the Mediterranean coastal desert of Egypt*, "J. Arid Environ.", **3**, 1980, 69-74.
9. L i u b o m i r a, O. D., T o m e s c u, N., *Aspects écologiques de la population d'isopodes terrestres de la zone des Dornas*, "An. Mus. Natl. Bucovina", 1997, 81-92.
10. R a d u, V., T o m e s c u, N., *Quantitativ-ökologische Untersuchungen an Landisopoden*, "Pedobiologia", **16**, 1976, 36-43.
11. S l á v e c z, K., L o s k a, I., *Diversity of soil Arthropods in the Bátorliget nature reserve, Hungary*, "Proc. 4th Eur. Congr. Entomol. (Gödöllő, 1991)", 1991, 801-807.
12. S u t t o n, S. L., H a r d i n g, P. T., *Interpretation of the distribution of terrestrial isopods in the British Isles*, "Monit. Zool. Ital. (N.S.) Monogr.", **4**, 1989, 43-61.
13. T o m e s c u, N., A c c o l a, S., P a ş c a, C., *Ecology of the terrestrial isopods* (Crustacea: Isopoda) *in Cheile Turzii*, "Stud. Univ. Babeş-Bolyai, Biol.", **40** (1-2), 1995, 78-94.
14. W a r b u r g, M. R., L i n s e n m a i r, K. E., B e r c o v i t z, K., *The effect of climate on the distribution and abundance of isopods*, "Symp. Zool. Soc. Lond.", **53**, 1984, 339-367.
15. W a r b u r g, M. R., R a n c h e v i c h, D., C h a s a n m u s, K., *Isopod species diversity and community structure in mesic and xeric habitats of the Mediterranean region*, "J. Arid Environ.", **1**, 1978, 157-163.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, XLV, 1, 2000

INFLUENȚA VÂRSTEI ASUPRA COMPORTAMENTULUI ŞI CAPACITĂȚII DE REPRODUCERE LA *MAMESTRA BRASSICAE* L. (*LEPIDOPTERA: NOCTUIDAE*) ÎN CONDIȚII DE LABORATOR

GHEORGHE STAN[*], SERGIU MIHUȚ[*], VIORICA CHIŞ[**], IOAN COROIU[***] şi ALEXANDRU CRIŞAN[***]

SUMMARY. - Influence of Age on Mating Behaviour and Reproductive Capacity of Mamestra brassicae L. (Lepidoptera: Noctuidae) under Laboratory Conditions. Under laboratory conditions, at 22°C, an optimum calling behaviour of *Mamestra brassicae* L. was recorded in the females of 2-5 days and the percentage of female calling gradually decreased with age increase. On the other hand, an age increase ascertained an increase of the calling duration, the number of calling bouts and an early initiation of calling. At 24°C, in the females of one day, 53.6% of them showed a clear calling behaviour. An optimum of successful matings was obtained for adult moths of 2-5 days and the number significantly decreased at the age greater than 10 days. In the mating tests with adult moths of the same age or of different age, the data obtained showed that the presence of young sexually mature males was necessary for achievement of successful matings and great fecundity. The oviposition pattern dependend on female reproductive status. In mated females the length of oviposition period gradually decreased with age increase; the percentage of eggs laid in the first day after copulation increased, too. In the virgin females a typical egg retention behaviour with adaptative value was recorded. Fecundity, as total number of eggs laid and eggs from ovaries, was influenced by adult age. The optimum fecundity was recorded in females of 3-6 days and the presence of young males was necessary. The fertility of eggs was greatest in the females of 2-5 days and decreased with age increase.

Vârsta este unul din factorii interni (fiziologici) cu semnificație deosebită în modelarea comportamentului de reproducere la insecte, dar alături de ea şi alți factori din această categorie (ritmul circadian, hrana, numărul de împerecheri, expunerea la mediatori chimici, densitatea populației, hormonii) influențează

[*] *Institutul de Cercetări Biologice, 3400 Cluj-Napoca, România*
[**] *Institutul de Chimie "Raluca Ripan", 3400 Cluj-Napoca, România*
[***] *Universitatea Babeş-Bolyai, Catedra de Zoologie, 3400 Cluj-Napoca, România*

comportamentul general. Fiecare secvență în parte este controlată neuro-endocrin, dar o serie de factori ecologici modifică modelul specific asigurând valoare adaptativă pentru specie. La nivelul reproducerii, comportamentul de chemare (eliberarea feromonului sexual) este o secvență declanşatoare, dar între ea şi cele care se succed (curtarea, acuplarea, ovipozitarea) există o strânsă dependență.

Cercetările referitoare la influența vârstei adulților asupra reproducerii prezintă atât o importanță teoretică cât mai ales una practică, în relație cu introducerea şi apoi extinderea metodelor şi procedeelor biotehnice şi biologice în monitoring, management sau combatere la specii de insecte cu importanță economică sau științifică. Studii aprofundate au fost făcute în special la lepidoptere [1, 6-9, 11, 12, 21].

Mamestra brassicae L. este o specie importantă în agricultură, dar în ceea ce priveşte influența factorilor ecologici asupra comportamentului s-au studiat în special factorii externi. Influența vârstei a fost analizată doar la nivelul comportamentului de chemare [20] şi asupra împerecherii în relație cu suşa crescută în laborator şi generația din care a provenit materialul biologic [18].

Lucrarea prezintă rezultatele obținute în perioada 1980-1994 cu privire la influența vârstei adulților de *M. brassicae* asupra chemării, împerecherii, ovipozitării, fecundității şi fertilității. Studiile au fost făcute la diferite linii de creştere în laborator şi număr mare de generații.

Material şi metode. *Creşterea speciei în laborator*. Adulții folosiți în experimentări au provenit din populații de larve crescute în laborator (23 ± 1^{o}C; 16:8 ore regim fotoperiodic; UR > 70%) pe diete artificiale [16, 19], în condiții specifice şi după "metoda cutiilor Petri şi a sistemului celular" [17]. Menținerea heterogenității la suşele din laborator a fost asigurată prin control dirijat al creşterii larvelor şi al selecției adulților pentru reproducere, cu material biologic provenit din 5 linii de creştere (LF, LG, C, LV, V) [17]. In fiecare an au fost aclimatizate şi introduse în laborator alte linii noi. Pupele, separate pe sexe, s-au menținut la 22^{o}C şi întuneric continuu, emergența adulților s-a înregistrat zilnic (în unele situații de două ori/zi), iar adulții au fost puşi în vase de sticlă sau cuşti speciale, în funcție de varianta experimentală. In interior s-a introdus hârtie de filtru ca suport şi soluție de glucoză 20%, ca sursă de hrană.

Condițiile de experimentare. Observațiile directe asupra secvențelor comportamentale sau vasele de împerechere s-au făcut în scotofază, în intervalul care coincide cu perioada circadiană a activității de reproducere la această specie [14, 15], valorile unor factori climatici esențiali fiind: temperatură constantă - 22 ± 1^{o}C; fotoperioada de 16:8 ore lumină:întuneric; umiditatea relativă >70%. Pentru femelele de 0-2 zile, comportamentul de chemare s-a studiat şi la 24^{o}C. Montarea experiențelor a fost făcută în fotofază.

Influența vârstei asupra comportamentului de reproducere. Comportamentul de chemare a fost studiat prin metoda observării directe cu stenografierea elementelor comportamentale şi înregistrare pe casetofon [14, 15]. Studiul s-a făcut pe femele de 0, 1, 2....15 zile, observate individual sau în grup. Chemarea în relație cu vârsta femelelor a fost caracterizată prin periodicitate, % chemare, durata chemării, ora medie a perioadei de chemare, ora medie a perioadei de inițiere a chemării, modelul de chemare şi numărul de reprize de chemare/femelă. Datele finale reprezintă valori medii pentru un număr mare de femele (Fig. 1). Acest număr a depăşit frecvent valoarea optimului de informație necesar în prelucrarea matematică a datelor, iar femelele au provenit din diferite suşe crescute în condiții similare. In aceleaşi condiții s-a studiat şi comportamentul de curtare. S-a înregistrat periodicitatea, durata, ora medie a perioadei de activitate. S-au studiat împerecherile multiple dependent de vârsta adulților şi capacitatea de supraviețuire. In acest caz s-au folosit perechi de adulți, iar numărul mediu cumulat de spermatofori a fost evaluat după 1, 2, 3....15 zile din momentul în care s-au format perechile cu adulți de 0 zile. Evaluarea numărului de spermatofori s-a făcut după metoda descrisă pentru acest tip de cercetări [17]. In studiul împerecherii, inițial s-a folosit câte o singură pereche de adulți/vas, dar ulterior s-au folosit două perechi, constatând că procentajul de acuplări reuşite şi fecunditatea au fost mai mari (chiar semnificativ pentru unele linii de creştere în laborator). In acest studiu datele reprezintă valori medii ale testelor de acuplare, diferențele fiind analizate într-un alt studiu. In cadrul comportamentului de ovipozitare în relație cu vârsta adulților s-a analizat modelul de depunere al ouălor, perioada de ovipozitare, durata şi eclozarea.

Pentru primele două secvențe, chemare şi împerechere, s-a înregistrat ora medie a perioadei de inițiere a chemării (respectiv împerecherii), reprezentând ora medie (în exprimare zecimală) a intervalului de timp dintre prima şi ultima femelă care au inițiat chemarea la nivelul unei populații observate în aceleaşi condiții (respectiv, intervalul dintre prima şi ultima acuplare). Ora medie a perioadei de chemare (respectiv de împerechere), în exprimare zecimală [22], s-a estimat după relația:

$$x_H = \frac{[\Sigma(t_i \cdot \frac{n}{t_i})]}{N}$$

(t_i = ora observării, la nivelul unei scotofaze de 8 ore, notată de la 0 la 8; n/t_i = numărul de femele în chemare, respectiv acuplate, în momentul observării; N = numărul total de femele în chemare, respectiv de perechi, la nivelul variantei experimentale).

Durata medie a chemării, fără a lua în considerare reprizele de chemare (intervalul de timp în care a existat o postură tipică de chemare, separată de o altă postură printr-o activitate locomotoare în care chemarea nu a fost evidentă), s-a estimat după relația:

$$D_C = \frac{[\sum(D_i \pm 15)]}{N}$$

(D_i = durata individuală de chemare (min.); ± 15 = se adaugă sau se scad 15 minute în funcție de postura femelei respective în momentul observării la intervale de 15 minute; N = numărul total de femele în chemare). Similar s-a evaluat şi durata împerecherii.

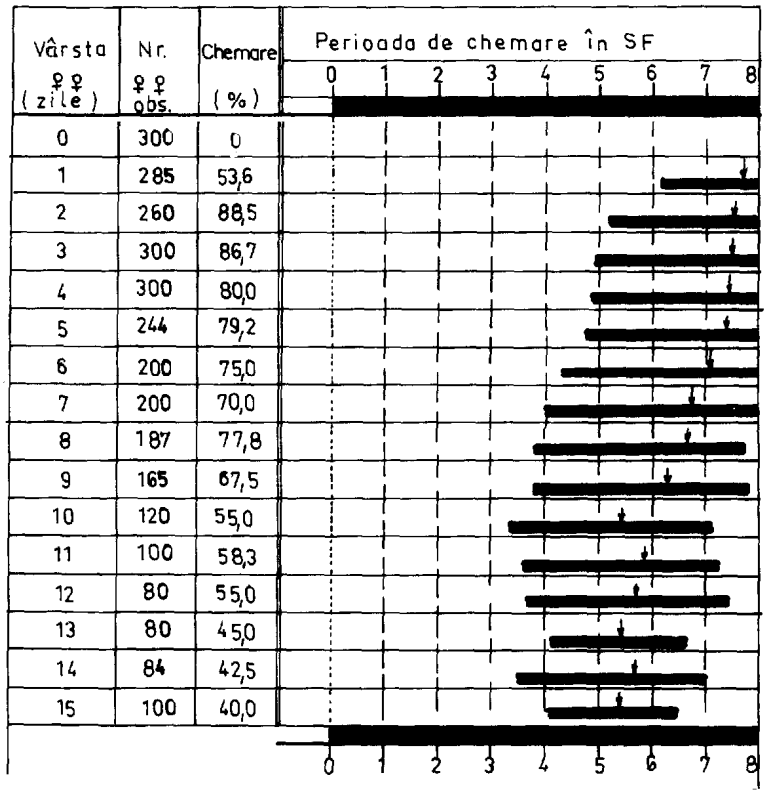
Influența vârstei asupra fecundității şi fertilității. Fecunditatea a fost acceptată ca număr total de ouă/femelă (ouă depuse + ouă existente în ovare la moartea femelei), iar fertilitatea se referă la ouăle viabile din care au eclozat larvele. De asemenea, s-a înregistrat şi % de pontă sterilă. Numărul de ouă din ovare a fost înregistrat la moartea femelelor prin disecții, iar clasificarea s-a făcut după metoda descrisă [17].

Prelucrarea datelor. Datele obținute au fost prelucrate statistic. Spre deosebire de teste experimentale similare unde informația este furnizată de număr relativ mic de variante sau repetiții, datele din acest studiu reprezintă valori medii pentru un număr foarte mare de adulți pentru a atenua variația individuală a răspunsului şi a modificării comportamentului în condițiile creşterii insectelor în laborator. Pentru analiza variației s-a folosit Duncan's New Multiple Range Test (D'sNMRT; P=0,05), cu transformarea inițială a şirului de date în log (x+1).

Rezultate şi discuții. *Vârsta femelelor şi comportamentul de chemare*. Rezultatele obținute sunt prezentate în Fig. 1. Intervalul optim pentru eliberarea feromunului sexual s-a înregistrat pentru femelele de 2-4 zile, nivelul menținându-se ridicat şi la femelele de 5-8 zile. S-a observat comportament de chemare şi la femelele de o zi. Datele confirmă studiile citologice efectuate asupra structurii şi dezvoltării glandei feromonale la această specie [10].

Pentru *M. brassicae* nu s-a observat un comportament de chemare la femelele proaspăt emerse (0 zile), iar în alte investigații nici pentru femelele de o zi. Procentul a crescut şi curba a fost similară pentru femelele de 3-5 zile [20]. Referitor la *M. brassicae*, studii citologice şi fiziologice ale glandei feromonale [10] au arătat că la femelele de o zi celulele glandei feromonale sunt formate, eliberare de feromon sexual are lor şi în prima zi, dar maximul s-a inregistrat în zilele 2-3. Datele noastre sugerează existența la *M. brassicae* a unui comportament de chemare (cu predominarea chemării slabe) şi la femelele de o zi. De altfel modele similare au mai fost evidențiate şi pentru alte specii (*Lacanobia suasa, Autographa gamma, Agrotis ipsilon*), autorii sugerând că la femelele

proaspăt emerse există frecvent un comportament de "chemare slabă" [21, 22] şi chemarea este evidentă la femelele de 2-3 zile. Factorul care induce cele mai pronunţate modificări la nivelul localizării perioadei de chemare este temperatura [15]. De aici rezultă şi diferenţele obţinute de diferiţi autori. Modelul comportamental de sinteză şi eliberare a feromonului sexual este dependent de specie şi este determinat genetic fiind sub control neuro-endocrin. Există însă o serie de factori interni şi externi care modifică acest comportament. La specia *Choristoneura rosaceana* modelul de chemare a fost corelat cu vârsta şi modificat de influenţa temperaturii constante sau fluctuante [4].



F i g. 1. *Influenţa vârstei asupra nivelului şi periodicităţii comportamentului de chemare al femelelor de* Mamestra brassicae.

Banda neagră reprezintă scotofaza de 8 ore, iar benzile mai subţiri reprezintă locali-zarea perioadei de chemare (săgeţile indică ora medie a perioadei de chemare).

Perioada de chemare la această specie este localizată la sfârşitul scotofazei [14]. Odată cu creşterea vârstei chemarea s-a inițiat mai timpuriu, iar ora medie a perioadei de chemare la nivel populațional s-a deplasat spre începutul scotofazei. Acesta este un comportament cu valoare adaptativă pentru specie, oferind şanse mai mari, pentru acuplare, femelelor mai în vârstă în competiția lor cu cele tinere. O semnificație similară, în relație cu creşterea vârstei, o are creşterea duratei chemării şi a numărului de reprize de chemare / femelă (Tabel 1).

Tabel 1

Influența vârstei adulților de Mamestra brassicae asupra comportamentului de chemare în condiții de laborator

Datele reprezintă valori medii pentru testele efectuate la diferite linii de creştere şi generații, în perioada 1983-1993

Vârsta ♀♀ (zile)	N	Ora medie a chemării*		D_{CH}**	NR_{CH}***
		I_{CH}	P_{CH}		
1	820	6,28	7,68	86,4 a	3,6 a
3	760	5,27	7,46	92,8 b	4,2 b
5	565	5,04	7,29	99,7 b	4,6 bc
7	540	4,21	6,78	115,6 c	5,1 cd
9	420	4,08	6,24	121,3 cd	5,7 de
11	384	4,01	5,92	128,9 d	6,1 ef
13	280	4,37	5,41	116,6 c	6,4 f

* **I_{CH}** - Ora medie pentru perioada de inițiere a chemării la nivel populațional. P_{CH} - Ora medie a perioadei de chemare. Faza de întuneric (scotofaza) de 8 ore este notată de la 0 la 8, cu exprimare zecimală a orei [22].

** **D_{CH}** - Durata chemării. Aceeaşi literă indică diferențe nesemnificative în funcție de vârstă, pentru acelaşi parametru (D'sNMRT; P=0,05).

*** **NR_{CH}** - Numărul de reprize de chemare/femelă în chemare.

Cercetările arată că există două grupe de specii: a) cele la care modelul de chemare a variat dependent de variația vârstei [5, 6, 20, 21]; b) specii la care modelul de chemare nu a variat corelat cu vârsta [1, 2].

In cazul primei categorii, la specia *Dioryctria abietella* cca. 20% dintre femelele observate au inițiat chemarea încă din ziua 1. Pentru vârsta de 2-10 zile nu au existat variații mari (pentru vârsta de 9-10 zile chemarea a fost chiar mai mare ca în rest) [5]. Pentru specia *Agrotis ipsilon* pe intervalul 1-4 zile, odată cu creşterea vârstei, au crescut procentul de femele în chemare, durata chemării, numărul de reprize de chemare (calling bouts)/femelă în chemare, iar chemarea s-a inițiat mai timpuriu, fapt care arată că femelele mature sunt mai competitive decât cele tinere [21]. Modelul de chemare al speciei *Chilo suppressalis* a variat de asemenea cu

vârsta [6]. Numărul de femele în chemare a fost mare şi aproximativ egal pentru vârsta de 1-5 zile, după care a scăzut semnificativ. Numărul de reprize de chemare şi lungimea acestora a crescut uşor la femelele mai bătrâne (pe intervalul 1-5 zile), dar pentru intervalul de peste 6-8 zile a scăzut din nou.

Caracteristic pentru grupa a doua este specia *Laspeyresia pomonella*, la care nu au existat diferenţe privind %chemare, ora medie a perioadei de chemare şi durata chemării pe intervalul vârstei de 0-6 zile [2]. Ca şi la alte specii, în paralel cu creşterea vârstei chemarea s-a iniţiat mai timpuriu, iar durata chemării a crescut. Trebuie precizat că observaţiile au fost făcute pe grupe de femele de 0-1; 1-2; 2-3; 3-4; 4-5; 5-6 zile (totuşi la cei 3 parametri amintiţi au existat diferenţe semnificative între femelele de 0-1 zile şi celelalte grupe). La *Grapholitha molesta*, femelele proaspăt emerse (1-9 ore) nu au avut un comportament de chemare, dar pe intervalul 1-6 zile modelul a fost similar [1].

Relaţia dintre împerechere (acuplare) şi vârsta adulţilor. Datele sunt prezentate sintetic în Fig. 2. Un model comportamental similar cu cel observat pentru chemare la *M. brassicae*, s-a observat şi în cazul împerecherii. Eliberarea feromonului sexual (chemarea) este secvenţa care declanşează răspunsul masculilor. In urma derulării a două faze caracteristice (răspunsul la distanţă şi curtarea) are loc împerecherea. Există un sincronism în timp al modelelor comportamentale, acuplarea fiind caracterizată şi ea de o iniţiere mai timpurie odată cu creşterea vârstei, în paralel cu creşterea duratei (Tabel 2).

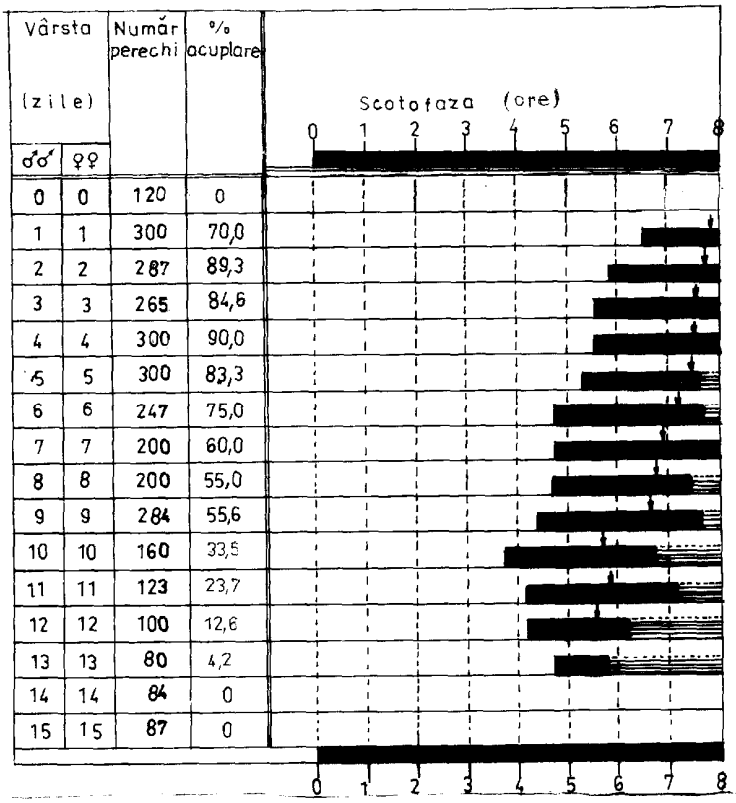
Tabel 2

Influenţa vârstei adulţilor de Mamestra brassicae asupra comportamentului de împerechere în condiţii de laborator

Datele reprezintă valori medii pentru rezultatele obţinute în toate experimentele efectuate la diferite linii de creştere şi generaţii (1983-1993)

P*	Vârsta adulţilor (zile) (♂:♀)														
	1:1	**1:3**	**3:1**	**3:2**	**3:3**	**5:1**	**5:3**	**7:1**	**7:2**	**1:5**	**3:5**	**1:7**	**2:7**	**1:9**	**3:9**
I_{AC}	6,61a	6,38a	6,44a	6,18b	5,77bc	5,32cd	5,18c	5,02cd	4,92d	6,32a	6,28b	6,08b	6,16b	5,92b	4,98d
P_{AC}	7,62a	7,64a	7,78a	7,52a	7,24b	7,22b	6,39c	6,18c	5,38d	7,65a	7,12b	7,18b	7,28b	6,32c	5,98e
D_{AC}	68,2a	76,1b	68,4a	56,3c	68,5a	64,8a	76,8b	82,2b	70,6a	108,5d	94,8e	84,2b	78,5b	101,8d	118,34f

* P - Parametrii analizaţi. **I_{AC}** - Ora medie pentru perioada de iniţiere a acuplării la nivel populaţional. **P_{AC}** - Ora medie a perioadei de acuplare. **D_{AC}** - Durata acuplării (în minute). Faza de întuneric (scotofaza) de 8 ore este notată de la 0 la 8, cu exprimare zecimală a orei [22]. Aceeaşi literă indică diferenţe nesemnificative între variante, în cadrul aceluiaşi parametru (D'sNMRT; P=0,05).



F i g. 2. *Influența vârstei adulților de* Mamestra brassicae *asupra împerecherii în condiții de laborator*.

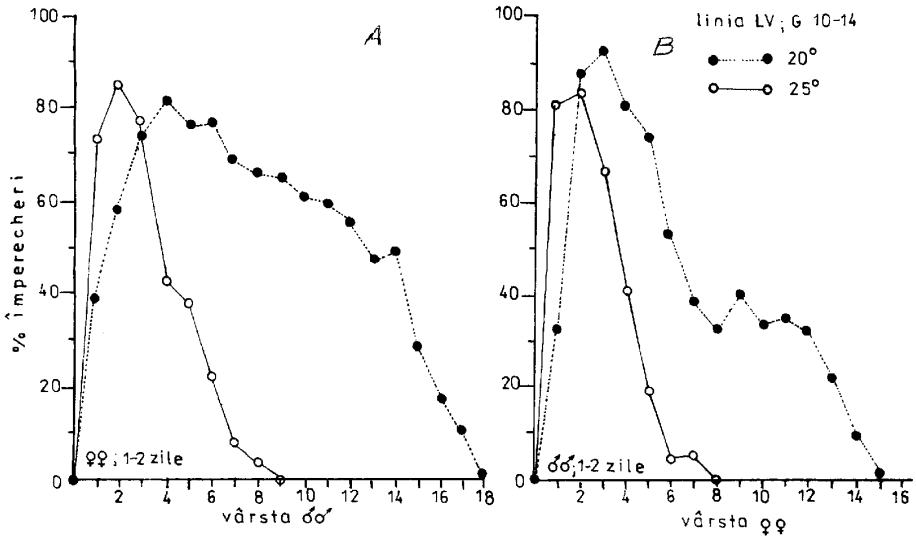
Banda neagră reprezintă scotofaza de 8 ore, iar benzile mai subțiri reprezintă localizarea perioadei de împerechere (săgețile indică ora medie a perioadei de împerechere).

Pe lângă sincronismul evidențiat între secvențele de chemare - curtare - acuplare, cercetările efectuate la speciile de lepidoptere, unde există o comunicare evidentă prin feromoni sexuali, s-a dovedit clar că masculii sunt deja pregătiți pentru acuplare şi chiar manifestă un comportament caracteristic chiar înainte de eliberarea feromonului de către femelă (în acest caz perioada lor de activitate este mai largă) [15]. Aceasta are o valoare adaptativă deosebită pentru specie. Ca şi în cazul chemării, împerecherea este influențată de vârsta adulților.

La *M. brassicae* cercetările noastre referitoare la comportamentul de reproducere al diferitelor linii de creştere în relaţie cu generaţia au arătat că împerecherea poate fi influenţată doar de generaţie, între linii nefiind diferenţe semnificative pentru o aceeaşi generaţie şi vârstă a adulţilor [18]. In schimb, temperatura este un factor extern deosebit de important care poate modifica puternic un model obţinut în relaţie cu vârsta (Fig. 3). Trendul curbelor referitoare la nivelul împerecherii în relaţie cu vârsta s-a modificat semnificativ datorită scurtării duratei de viaţă sub influenţa temperaturii ridicate. Din Fig. 3 se desprinde şi un alt aspect interesant. Astfel, se pot observa modificările în evoluţia trendului curbelor în funcţie de sex, mai pronunţat pentru intervalul vârstei de 6-12 (14) zile. Se poate constată că o împerechere este reuşită în condiţiile în care cuplul este format în special cu femele tinere, mature sexual.

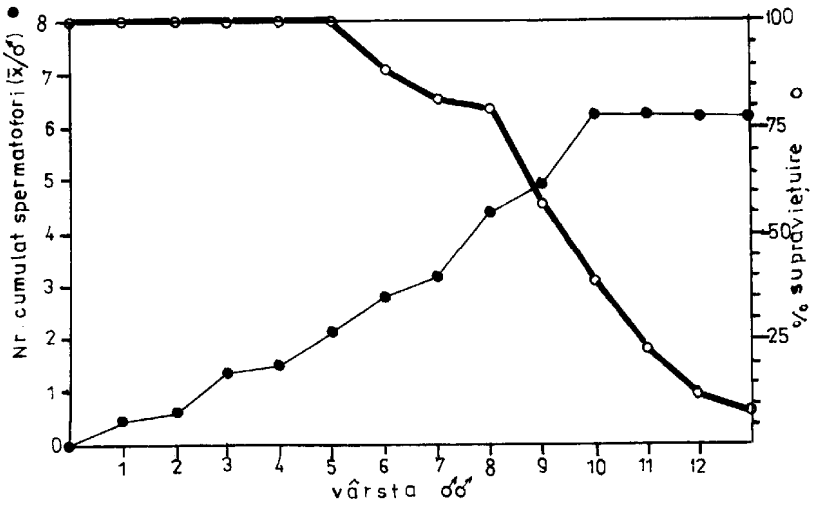
Odată cu creşterea vârstei a crescut numărul de spermatofori transmişi în secvenţa de împerechere, corelat cu scăderea procentajului de supravieţuire (Fig. 4).

Un model comportamental similar cu cel de faţă obţinut la *M. brassicae*, pentru toate secvenţele reproducerii a fost evidenţiat la diferite nivele de aprofundare şi la alte specii de lepidoptere cu activitate nocturnă.



F i g. 3. *Influenţa vârstei masculilor (A) şi a femelelor (B) în funcţie de temperatură, asupra împerecherii la* Mamestra brassicae, *în condiţii de laborator*.

In testele experimentale, masculii sau femelele de diferite vârste s-au pus la împerecheat cu femele, respectiv masculi, de 1-2 zile. N = minim 200 perechi/vârstă. Linia de creştere (suşa) LV, generaţiile 10-14.

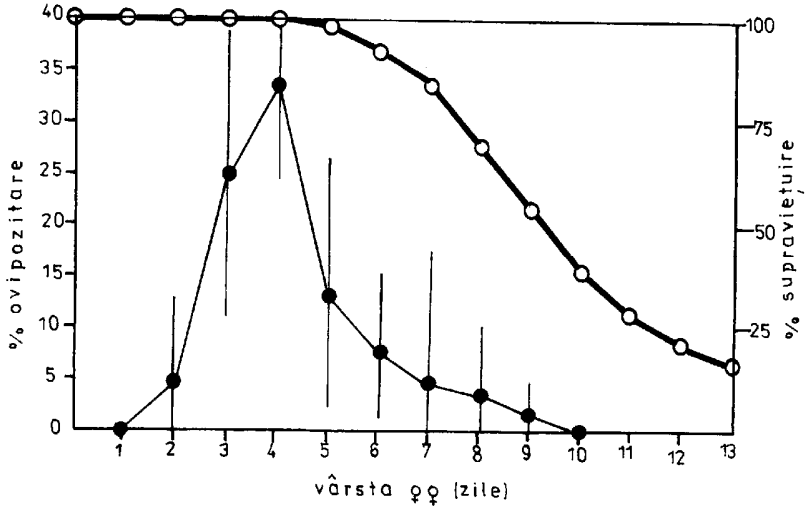


F i g. 4. *Numărul cumulat de spermatofori transmişi de masculii de* Mamestra brassicae *pe toată durata de viață şi relația cu evoluția nivelului de supraviețuire*.
Perechi de adulți puşi la împerecheat la vârsta de 0 zile.

In cadrul acuplării în relație cu vârsta adulților semnalăm cazul speciei *Spodoptera littoralis* [7] unde există diferențe în ceea ce priveşte vârsta la care cele două sexe ating maturitatea sexuală. In ambele cazuri femelele au fost atractive şi la vârsta de o zi, dar masculii numai la vârsta de 2-3 zile. O valoare maximă a fost atinsă pentru vârsta de 3 zile a ambelor sexe. La *Earias insulana* acuplarea a fost absentă la vârsta de o zi a celor două sexe, indiferent de combinațiile făcute, iar valoarea cea mai mare s-a obținut pentru adulții de 4 zile [8].

In ceea ce priveşte împerecherile multiple, numărul acestora în general creşte odată cu creşterea vârstei. Modelul este însă foarte diferit de la o specie la alta, dependent de durata de viață a adulților şi factorii interni şi externi implicați în experiment. La *S. littoralis* masculii care au trăit maxim 26 zile au transferat în medie 5,2 spermatofori, iar numărul mare al acestora (4, 5 şi 6) s-a înregistrat pe intervalul vârstei de 14-26 zile [7]. Un model similar a fost obținut şi la specia *E. insulana* [8].

Ovipozitarea şi vârsta adulților. La femelele de *M. brassicae*, acuplate şi care au depus pontă fertilă, ovipozitarea s-a inițiat chiar din a doua zi după împerechere. In condițiile noastre de experimentare, numărul mediu cel mai mare de ouă depuse în zilele succesive de ovipozitare s-a înregistrat la femelele de 4 zile, cu un procentaj destul de mare şi pentru femelele de 3 zile (Fig. 5). Odată cu înaintarea în vârstă, nivelul de ovipozitare s-a diminuat progresiv.



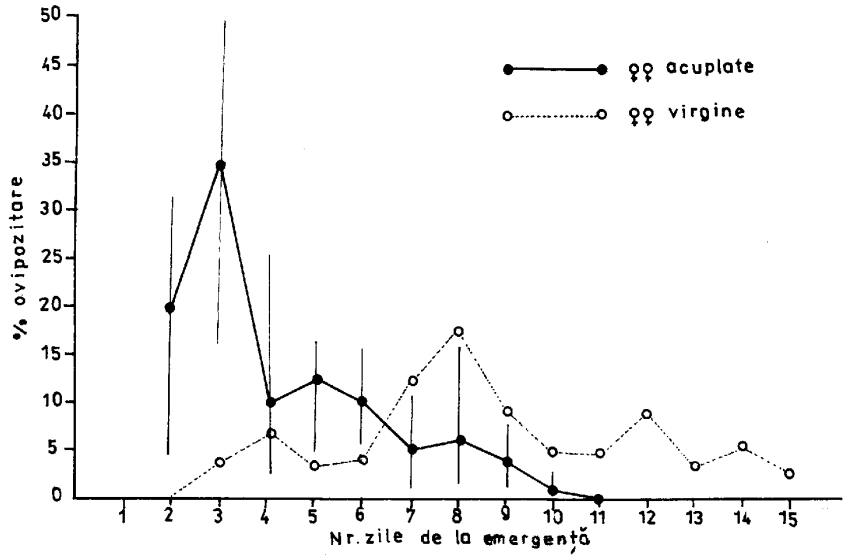
F i g. 5. *Dinamica ovipozitării şi supravieţuirea la femelele acuplate de* Mamestra brassicae.

Adulţi puşi la împerechere la vârsta de 0 zile. Datele reprezintă valori medii, iar liniile verticale marchează intervalul de variaţie pentru 36-64 serii experimentale din diferite linii de creştere şi generaţii (1983-1993).

Există diferenţe semnificative între modelul de ovipozitare al femelelor acuplate care depun pontă fertilă şi femelele virgine (Fig. 6). Conform datelor prezentate în figură, decalajele sunt pronunţate la nivelul de maxim şi terminarea ovipozitării. Fenomenul a fost atenuat în ceea ce priveşte iniţierea ovipozitării, deşi dominant femelele virgine au iniţiat mult mai târziu ovipozitarea, rata zilnică a fost redusă şi a durat mai multe zile corelat şi cu o durată de viaţă mai mare a acestora. Fenomenul de reţinere al ouălor are valoare adaptativă deosebită şi caracterizează şi alte specii.

Ca durată de timp în care a avut loc ovipozitarea, modelul şi procentajul de femele care au ovipozitat au fost corelate cu vârsta femelelor (Fig. 7). Femelele tinere acuplate (2-3 zile în momentul iniţierii ovipozitării) au depus numărul cel mai mare de ouă în ziua a doua după acuplare şi ovipozitarea a durat mai multe zile. Pentru femelele cu vârsta de la 3 zile în sus, procentul maxim de ouă depuse s-a înregistrat în ziua imediat următoare acuplării, iar durata ovipozitării s-a redus treptat, asociat cu creşterea vârstei. Şi la alte lepidoptere nocturne a existat un model asemănător, dar variabil în funcţie de specie şi dependent de o serie de factori interni şi externi. La toate speciile împerecherea stimulează ovipozitarea

care creşte progresiv pe perioada maturității sexuale [3], după care descreşte treptat. Femelele virgine depun ouăle la o rată mică, după o perioadă de retenție, dar frecvent rata creşte odată cu creşterea vârstei [11]. In medie o femelă împerecheată depune de cel puțin două ori mai multe ouă decât una virgină. La alte specii femelele neîmperecheate ovipozitează zilnic, dar tot la o rată semnificativ mai mică decât cele acuplate şi toate ouăle sunt sterile [9].



F i g. 6. *Reprezentarea comparativă a dinamicii ovipozitării femelelor virgine şi acuplate de* Mamestra brassicae, *în condiții de laborator*.
Alte explicații - ca în Fig. 5.

Fecunditatea, fertilitatea şi vârsta adulților. In acest gen de cercetări efectuate la insecte, fecunditatea este acceptată ca numărul total de ouă/femelă (ouă depuse pe toată durata de viață + numărul de ouă existente în ovare la moartea femelelor). Dominant în lucrări se ține cont cel mai mult de numărul de ouă depuse, bazat pe faptul că între cele două componente există o relație strânsă [17]. Astfel, fecunditatea femelelor acuplate a fost semnificativ ridicată în perioada optimă a maturității reproductive. Diminuarea procentajului de supraviețuire se corelează atât cu diminuarea numărului de ouă depuse cât şi cu golirea ovarelor. Femelele cu durată de viață lungă, care au depus un număr mare de ouă au avut, la moartea acestora, ovarele goale (sau cu un număr foarte redus de ouă) (Tabel 3).

Tabel 3

Influenţa vârstei adulţilor de Mamestra brassicae asupra numărului de spermatofori transmişi, fecundităţii şi fertilităţii, în condiţii de laborator

Datele reprezintă valori medii pentru testele efectuate în perioada 1983-1993

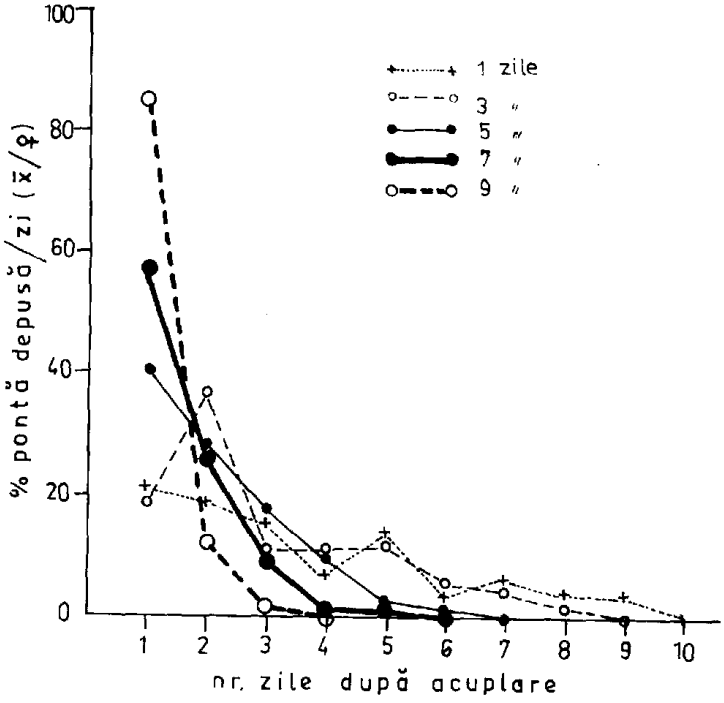
Vârsta*		Fecunditatea ($\bar{x}$ ouă/♀)			Număr spermatofori***	%f****
♂♂	♀♀	Număr de ouă depuse (± AS)	Ouă/ovare**			
			Imature	Corionate		
1	1	870,9 ± 28,4	+; o	0; I	4,8	86,4
3	3	1680,5 ± 118,2	o; +	0	3,1	92,5
5	5	1412,8 ± 168,8	+	I	2,4	96,4
7	7	1108,4 ± 174,6	+	I; II	1,6	88,3
9	9	1005,5 ± 214,4	++	II; III	0,7	72,8
11	11	982,3 ± 112,2	+++	III	0,5	74,5
13	13	706,8 ± 164,2	+++	IV	0,7	62,3
1	1	964,8 ± 38,4	o; +	0	5,8	82,7
1	2	1612,5 ± 116,2	o	0	4,1	96,3
1	3	1512,7 ± 84,5	o; +	0; I	5,1	95,2
3	1	1528,4 ± 138,9	o	0	3,2	93,4
3	2	1584,2 ± 176,2	o	0	2,1	92,5
3	3	1418,7 ± 212,8	o; +	0; I	2,4	96,9
5	1	1226,5 ± 174,1	o; +	I	2,3	94,1
5	2	1104,6 ± 132,8	+	0; I	1,5	86,2
5	3	1074,8 ± 224,5	+	I	1,1	91,4
7	1	1229,5 ± 158,3	+; ++	I; II	0,6	88,2
7	2	1176,5 ± 170,0	++	I	0,8	84,1
7	3	986,4 ± 175,5	+++	I	0,4	86,4
1	5	1312,1 ± 324,2	o; +	I	3,1	90,4
2	5	1176,3 ± 286,5	+	0; I	2,6	91,2
3	5	1342,2 ± 324,8	o; +	I	1,4	88,3
1	7	1014,2 ± 346,7	o; +	I; II	1,6	86,5
2	7	987,5 ± 322,2	+	II	1,8	82,4
3	7	1078,4 ± 284,9	++	II	1,4	79,8
1	9	879,6 ± 322,1	+	II; III	1,4	74,7
2	9	912,5 ± 294,8	+	III	1,2	78,6
3	9	848,4 ± 345,4	+++	III; IV	0,9	76,2

* Cifrele reprezintă vârsta la care adulţii s-au pus la împerecheat.

** Evaluare la moartea femelelor (notarea reprezintă minim 50% din femele, pentru ouă imature şi mature [18]). Ouă imature: **o** - absent; **+** - foarte puţine, izolate (până la 20); **++** - multe; **+++** - foarte multe. Ouă mature (corionate): **0** - ouă absente; **I** - ouă puţine (1-50); **II** - nr. mic de ouă (51-100); **III** - nr. mare de ouă (101-150); **IV** - ouă multe (>150 - frecvent abdomen plin).

*** Număr de spermatofori transmişi pe durata de viaţă.

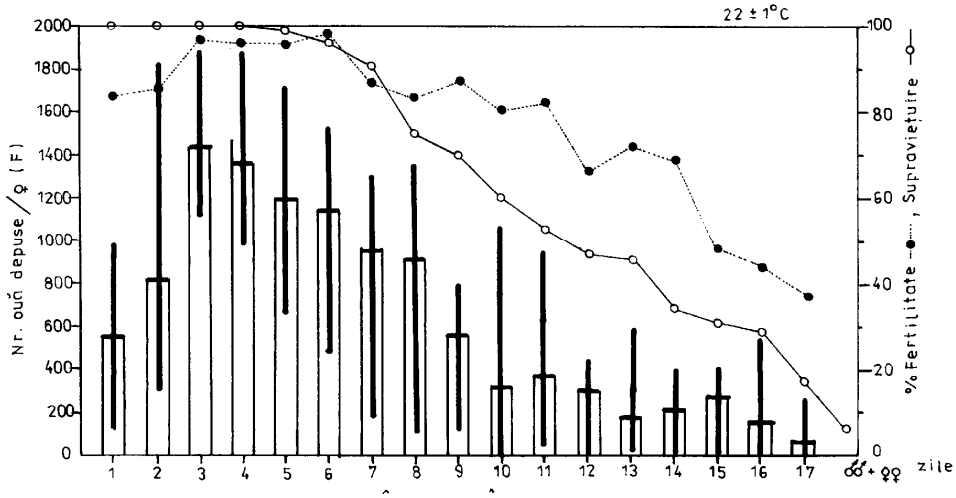
**** %f - Procentul de fertilitate a ouălor depuse.



F i g. 7. *Modelul de ovipozitare la* Mamestra brassicae, *pentru femelele acuplate, în funcție de vârsta la care au fost puse la împerecheat.*

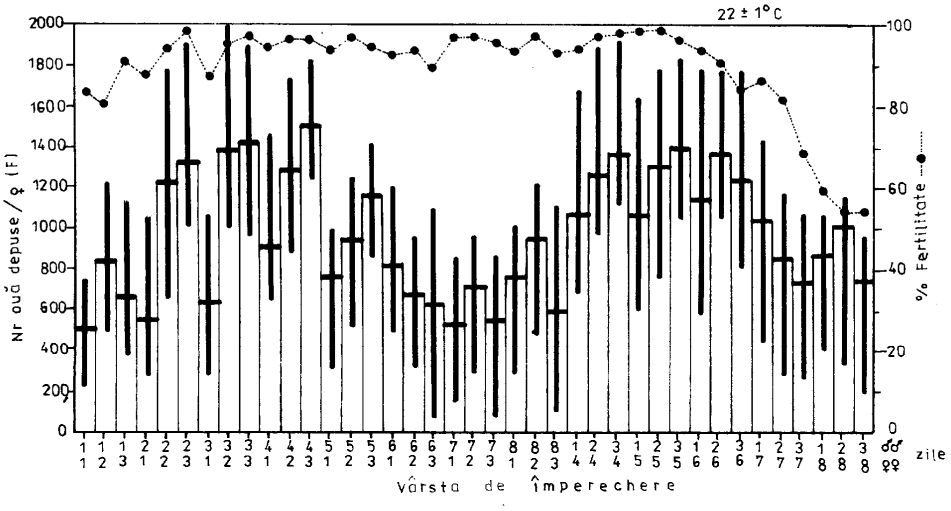
Datele reprezintă valori medii, iar liniile verticale marchează intervalul de variație pentru 12-18 serii experimentale din diferite linii de creştere şi generații (1990-1993).

In condițiile în care s-au pus la împerechere adulți de aceeaşi vârstă, numărul cel mai mare de ouă depuse s-a înregistrat pentru vârsta de 3-6 zile (Fig. 8). Şi femelele cu vârsta de împerechere de 2 zile au depus un număr mare de ouă, dar s-a constatat un interval de variație foarte larg. Incepând cu vârsta de acuplare de 7 zile, fecunditatea s-a redus progresiv odată cu scăderea procentajului de supraviețuire. Un trend similar a avut şi curba fertilității. Referitor la ponta sterilă se impun unele observații. Astfel, un număr variabil de ouă sterile (depuse) au marcat fecunditatea practic pe toată perioada de ovipozitare. Numărul lor a crescut odată cu creşterea vârstei, dar ouă sterile au fost prezente şi la femelele tinere (1-2 zile). In procentaj de 0,1-3,6% au fost evidențiate şi la femelele de 3-6 zile.



F i g. 8. *Influența vârstei asupra fecundității și fertilității la* Mamestra brassicae *în condițiile variantelor experimentale de împerechere cu adulți de aceeași vârstă*. Datele reprezintă valori medii, iar liniile verticale marchează intervalul de variație pentru 74-96 serii experimentale din diferite linii de creștere și generații (1987-1993).

Modelele fecundității şi fertilității au avut o evoluție interesantă în condițiile în care s-au pus la împerechere adulți de vârste diferite (Fig. 9). Numărul cel mai mare de ouă depuse a fost înregistrat în condițiile în care s-au împerecheat masculi de 2-4 zile cu femele de 2-3 zile. Nivelul s-a menținut ridicat şi pentru masculii de o zi împerecheați cu femele de 2-3 şi chiar de 8 zile. În schimb, creşterea vârstei masculilor, pe intervalul 5-8 zile, a indus o diminuare a fecundității chiar în condițiile împerecherii cu femele de 2-3 zile. Un alt aspect interesant se referă la fertilitate. Pentru un nivel al fecundității relativ acelaşi, fertilitatea s-a menținut ridicată când împerecherile au fost făcute între masculi bătrâni şi femele tinere, dar a scăzut semnificativ în cazul acuplărilor dintre adulți tineri şi femele bătrâne. Rezultatele obținute confirmă faptul că fertilitatea este strict dependentă de vârsta femelelor. Pentru adulții tineri creşterea procentajului de pontă sterilă este asociată cu acuplări nereuşite sau cu o capacitate mai redusă de fertilizare a spermei. Reducerea capacității de fertilizare se diminuează semnificativ odată cu creşterea vârstei, astfel că şi la femelele mature sexual, care au ovipozitat numai ouă fertile şi în număr mare, la bătrânețe a crescut numărul de ouă sterile.

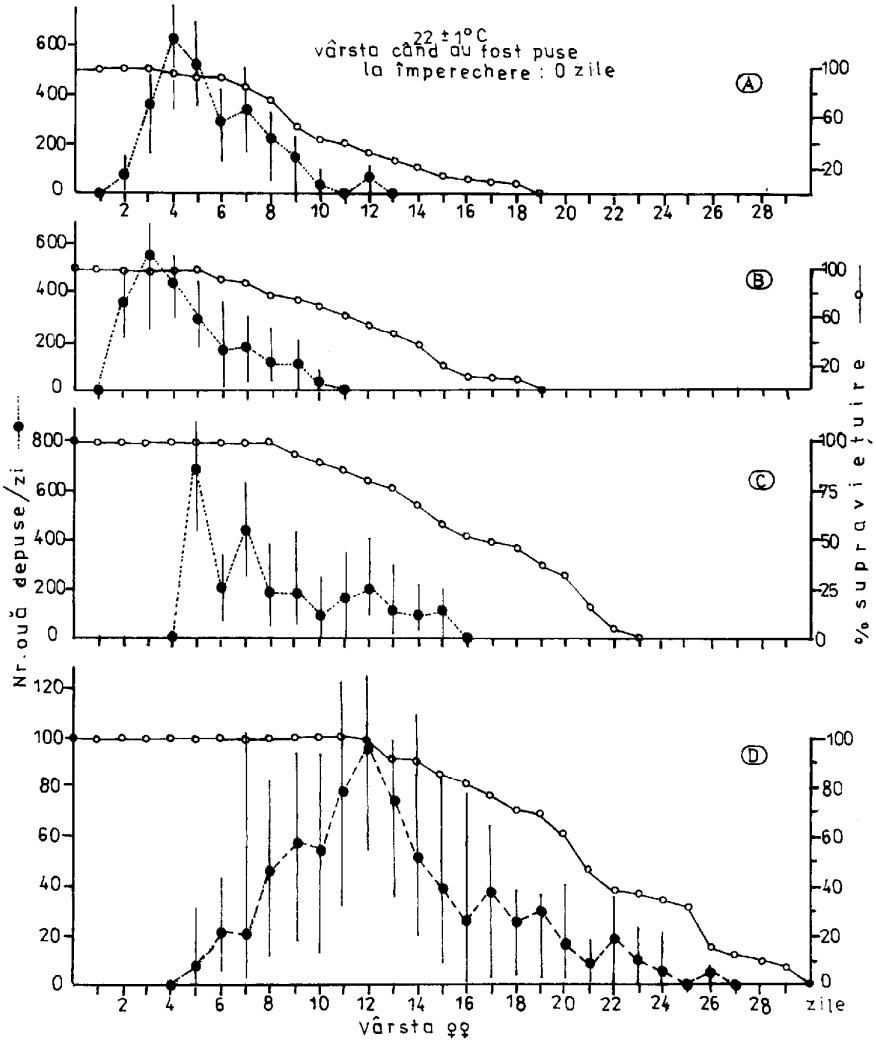


F i g. 9. *Influența vârstei asupra fecundității şi fertilității la* Mamestra brassicae *în condițiile variantelor experimentale de împerechere cu adulți de vârste diferite.*
Alte explicații - ca în Fig. 8.

Dacă nivelul fertilității este asociat cu calitatea femelelor, fecunditatea este influențată şi de calitatea masculilor. Astfel, un nivel ridicat al fecundității a implicat neapărat prezența adulților tineri şi maturi sexual, chiar în condițiile împerecherii cu femele mai bătrâne.

Modelul de ovipozitare şi nivelul fecundității femelelor de *M. brassicae* a fost parțial influențat şi de comportamentul de împerechere (Fig. 10). Nu au fost diferențe între femelele cu o singură împerechere şi cele cu 2-3 acuplări, pe toată durata de viață. In schimb, dacă prima acuplare a avut loc după 4-5 nopți, s-a modificat trendul curbei de ovipozitare odată cu creşterea duratei de supraviețuire la nivel populațional. Pentru femelele neacuplate modelul se aseamănă cu cel din Fig. 6.

Un comportament asemănător cu cel prezentat pentru *M. brassicae* a fost descris şi la alte lepidoptere, dar există particularități specifice chiar dacă el se aseamănă mult la noctuide [12]. La *S. littoralis* evoluțiile numărului de ouă depuse şi supraviețuirea au fost corelate atât cu vârsta cât şi cu rata de împerechere [7]. La *Mythimna separata* numărul de ouă/femelă a fost mic (10-250) pentru vârsta de 1-5 zile şi mare (820-900) la femelele de 20 zile [13]. In schimb, la *Bombyx mori* a existat o corelație semnificativ negativă (r = -0,928) între fertilitate şi vârstă [11].



F i g. 10. *Modelul de ovipozitare şi evoluţia numărului de ouă depuse de femelele de* Mamestra brassicae *pe toată durata de viaţă, în funcţie de statutul reproductiv.*

Adulţi puşi la împerecheat la vârsta de 0 zile. **A** - O singură acuplare/durata de viaţă (N=420). **B** - 2-3 acuplări/durata de viaţă (N=380). **C** - 2 împerecheri, prima acuplare după 3-4 nopţi (N=240). **D** - Femele virgine (N=464). Valori medii din testele experimentale făcute pe material biologic din diferite linii de creştere şi generaţii (1987-1993). Liniile verticale indică intervalul de variaţie.

Cunoaşterea particularităților comportamentale ale unor specii importante din punct de vedere economic, asociat cu prolificitatea şi longevitatea lor şi în funcție de valorile factorilor ecologici prezintă valoare practică deosebită în vederea elaborării unor modele predictive în cadrul acțiunilor de monitoring şi management.

Concluzii. 1. Un comportament optim de chemare (eliberarea feromonului sexual) la *M. brassicae* a fost evidențiat pentru femelele de 2-5 zile. La un regim termic de 24^{o}C, 53,6% din femele au prezentat o postură de chemare şi la vârsta de o zi. La 22^{o}C, peste vârsta de 5 zile procentajul de femele în chemare s-a redus progresiv odată cu creştere vârstei (aceasta a indus şi o creştere a duratei chemării, a numărului de reprize de chemare şi o inițiere mai timpurie a chemării).

2. In ceea ce priveşte periodicitatea a existat o similaritate între chemare şi acuplare. Procentul cel mai mare de acuplări reuşite s-a obținut pentru femelele de 2-5 zile, numărul s-a redus semnificativ peste vârsta de 10 zile şi au fost absente peste 13 zile, vârstă la care 45% din femele erau totuşi în chemare. Rezultatele obținute în testele de împerechere cu adulți de aceeasi vârstă şi de vârste diferite au evidențiat că vârsta masculilor este esențială în realizarea acuplărilor reuşite.

3. Modelul de ovipozitare a fost diferit la femelele împerecheate care au depus pontă fertilă şi cele virgine. In primul caz, odată cu creşterea vârstei a crescut şi procentajul de pontă depusă în prima zi după acuplare, iar durata de ovipozitare s-a redus progresiv. Pentru femelele virgine s-a observat un comportament de reținere al pontei (cu valoare adaptativă pentru specie), ovipozitarea s-a întins pe durată de timp aproape dublă şi la o rată zilnică mică.

4. Fecunditatea nu a fost influențată de numărul de acuplări, dar vârsta a fost un factor intern cu o semnificație mare. In condițiile în care împerecherea a fost analizată pe perechi de adulți de aceeaşi vârstă, fecunditatea cea mai mare s-a obținut pentru femelele de 3-6 zile. In varianta cu adulți de vârstă diferită, nivelul cel mai mare al fecundității s-a înregistrat pentru combinația dintre masculi de 1-4 zile cu femele de 1-8 zile. Datele sugerează că realizarea acuplărilor reuşite este dependentă de prezență masculilor tineri.

5. Fertilitatea pontei a fost mare pentru adulții de 2-5 zile şi a scăzut mult odată cu creşterea vârstei. Prezența unui procentaj mai mare de pontă sterilă s-a obținut şi în cazul adulților de 1-2 zile, când ovipozitarea a avut loc în ziua imediat următoare după acuplare.

B I B L I O G R A F I E

1. B a k e r, T. C., C a r d e, R. T., *Endogenous and exogenous factors affecting periodicities of female calling and male pheromone response in* Grapholitha molesta *Busck*. "J. Insect Physiol.", **25**, 1979, 943-950.
2. C a s t r o v i l l o, P. J., C a r d e, R. T., *Environmental regulation of female calling and male pheromone response periodicities in the codling moth* (Laspeyresia pomonella), "J. Insect Physiol.", **25**, 1979, 659-667.
3. D a n t h a n a r a y a n a, W., G u, H., *Multiple mating and its effect on the reproductive success of female* Epiphyes postvittana (Lep. Tortricidae), "Ecol. Entomol.", **16**, 1991, 169-176.
4. D e l i s l e, J., *Age-related changes in the calling behaviour and the attractiveness of obliquebended leafroller virgin females*, Choristoneura rosaceana *under different constant and fluctuating temperature conditions*, "Entomol. Exp. Appl.", **63**, 1992, 55-62.
5. F a t z i n g e r, C. W., A s h e r, W. C., *Mating evidence for a sex pheromone of* Dioryctria abietella (Lepidoptera: Pyralidae, Phycitinae), "Ann. Entomol. Soc. Amer.", **64**, 1971, 612-620.
6. K a n n o, H., *Effects of age on calling behaviour of the rice stem borer*, Chilo suppressalis (*Walker*) (Lepidoptera: Pyralidae), "Bull. Entomol. Res.", **69**, 1979, 331-335.
7. K e h a t, M., G o r d o n, D., *Mating, longevity, fertility and fecundity of the cotton leafworm*, Spodoptera littoralis (*Boisd*.) (Lepidoptera: Noctuidae), "Phytoparasitica", **3**, 1975, 87-102.
8. K e h a t, M., G o r d o n, D., *Mating ability, longevity and fecundity of the spiny bollworm*, Earias insulana (Lepidoptera: Noctuidae), "Entomol. Exp. Appl.", **22**, 1977, 267-273.
9. L a n d o l t, P., C u r t i s, C. E., *Mating frequency of female navel orangeworm moths* (Lepidoptera: Pyralidae) *and patterns of oviposition with and without mating*, "J. Kansas Entomol. Soc.", **64**, 1991,414-420.
10. M o l n a r, B., R o m a n, M. C., *Glanda feromonală la* Mamestra (Barathra) brassicae *L*. (Lepidoptera: Noctuidae) *şi modificările ei structurale în raport cu vârsta*, "Stud. Cercet. Biol., Ser. Biol. Anim.", **35** (1), 1983, 28-31.
11. P a u l, D. C., K i s h o r - K u m a r, C. M., S e n, S. K., *Effect of age on mating, oviposition, fertility and longevity of female silkmoth* Bombyx mori *L*. (Lepidoptera: Bombycidae), "Indian J. Sericic.", **32** (1), 1993, 21-25.
12. S i m m o n s, A. M., R o g e r s, C. E., *Fall armyworm* (Lepidoptera: Noctuidae) *mating effects of age and scotophase on pre-mating time, mating incidence, and fertility*, "J. Entomol. Sci.", **29**, 1994, 201-208.

13. S i n g h, R., C h a u d h a r y, J. P., *Influence of female and male age and association periods of the adults of oriental armyworm* (Lepidoptera: Noctuidae) *on oviposition, egg hatch and longevity,* "Beitr. Entomol.", **41**, 1991, 271-275.
14. S t a n, G., *Influența regimului fotoperiodic, temperaturii şi vârstei asupra comportamentului de chemare la femelele de* Mamestra brassicae *L.* (Lepidoptera: Noctuidae), *în condiții de laborator,* "Lucr. a III-a Conf. Entomol., Iaşi, 1983", 1986, 241-248.
15. S t a n, G., *Biologia reproducerii, comportamentul de reproducere şi feromonii sexuali la specii de lepidoptere dăunătoare. Studiul comportamentului feromonal în condiții de laborator şi câmp la* Mamestra brassicae *L. şi* Xestia c-nigrum *L.* (Lepidoptera: Noctuidae), "Bul. Inf. Soc. Lepidopterol. Rom.", Suppl. **1**, 1991, 87-133.
16. S t a n, G., *Creşterea pe diete artificiale a insectelor fitofage utilizate ca suport de parazitare pentru entomofagi*, în C i o c h i a, V., I s a c, G., S t a n, G., *Tehnologii de creştere industrială a câtorva specii de insecte auxiliare folosite în combaterea biologică a dăunătorilor*, p. 193-237, Ceres, Bucureşti, 1993.
17. S t a n, G., C h i ş, V., *Studiul capacității reproductive la* Mamestra brassicae *L.* (Lepidoptera: Noctuidae): *Evaluarea numărului de spermatofori şi ouă la diferite generații şi linii de creştere în laborator şi la femelele capturate la capcana luminoasă (1986-1991)*, "Bul. Inf. Soc. Lepidopterol. Rom.", **6** (3-4), 1995, 247-266.
18. S t a n, G., C h i ş, V., M o r a r, M., *Studiul capacității reproductive la* Mamestra brassicae *L.* (Lepidoptera: Noctuidae): *Influența vârstei asupra împerecherii în relație cu generația, la două linii de creştere în laborator,* "Bul. Inf. Soc. Lepidopterol. Rom.", **7** (1-2), 1996, 61-79.
19. S t a n, G., C h i ş, V., N i c o l e s c u, M. A., *Growth, development and food consumption by larvae of* Mamestra brassicae *L.* (Lepidoptera: Noctuidae) *reared on artificial diets with and without agar-agar,* "Entomol. Rom.", **3**, 1998 (in press).
20. S u b c h e v, M. A., *Effect of age, temperature, photoperiod and light on the calling of* Mamestra brassicae *L. females*, "Ecology" (Bulgaria), **12**, 1983, 65-75.
21. S w i e r, S. R., R i n g s, R. W., M u s i c k, S. W., *Age-related calling behavior of the black cutworm,* Agrotis ipsilon, "Ann. Entomol. Soc. Amer.", **70**, 1977, 919-924.
22. T e a l, P. E. A., B y e r s, J. R., P h i l o g e n e, B. J. R., *Differences in female calling behavior of three interfertile sibling species of* Euxoa (Lepidoptera: Noctuidae), "Ann. Entomol. Soc. Amer.", **71**, 1978, 630-634.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, XLV, 1, 2000

USING OF ALLELOMORPHIC FEATURES IN IDENTIFYING THE TWO SPECIES BELONGING TO THE GENUS *BOMBINA* (*ANURA: DISCOGLOSSIDAE*) FROM TRANSYLVANIA

IOAN GHIRA[*] and GYÖNGYVÉR MARA[*]

SUMMARY. - The paper analyses the allelomorphic features of seven *Bombina* populations, living in Transylvania (Romania) and in Hungary between 150 and 1700 m altitude, in order to establish which of them are the most important for the determination of belonging to *bombina* or *variegata* species, or for the determination of *bombina*-like or *variegata*-like hybrids. The analysed material consists of 259 toads (122 adult males, 110 adult females and 27 juveniles) belonging to the genus *Bombina*. The populations found in the plain areas belong to *bombina* species: Marghita with 98.18 % of the features and Parassapuszta with 94.57 % of the features. The most hybrid populations were found in the hilly regions: Cluj with 71.15% and Ciuc with 69.65% of the features belonging to *B. variegata*. The most *variegata* population was not found at the highest altitude as was expected (Soarbele – 1400-1550 m above sea level), but in Câmpuşel at 1000 m altitude, with 93.42% of the features belonging to *variegata*. Two features are the most important for species determining: the length of the leg (the tibio-tarsal articulations are/are not in connection) and the presence/absence of the dorsal spots.

M é h e l y [9] was the first to report in 1905 the natural hybridisation between two European species of fire-bellied toads, *Bombina bombina* (L.) and *Bombina variegata* (L.).

Artificial hybrids were obtained in laboratory and heterospecific pairs in amplexus were observed in field [10], but fertility of hybrids was unknown and the morphology of F1 hybrids did not resemble most intermediate forms found in nature [13]. Several authors, among them M i c h a l o w s k i [10,11], S t u g r e n [16], L á c [4] and M a d e j [7] conducted studies of morphological variation in the contact zone of their ranges. Furthermore, the lowland and the mountain populations of *Bombina* have different mating calls [5], which traditionally have been regarded as effective barriers to gene exchange in many amphibian species.

[*] *Babeş-Bolyai University, Department of Zoology, 3400 Cluj-Napoca, Romania*

It has been suggested that the mixture of species characters is due to interspecific crosses and backcrosses [10,12]. S t u g r e n and V a n c e a [17] sustain that interspecific crosses explain the mixture of species-specific characters when both species occur together (sympatrics), or are at least ineffectively isolated reproductively, when living in different neighbouring habitats. Moreover, the phenotypical heterogeneity with toad populations, the admixture of characters of the other species can not be entirely explained by the assumption of interspecific crosses.

The newly developed molecular techniques could answer the question of hybridisation. The application of protein electrophoresis demonstrated that the fire-bellied toads and yellow-bellied toads do interbreed west of Cracow [18].

Molecular evidence suggests that these taxa have been diverging for 3-5 million years [8]. During this period, their behaviour, life history and morphology diverged extensively, and many of these differences are thought to be adaptations to their respective habitats. Briefly, *B. variegata* lays larger eggs that develop more quickly [15], and also has thicker skin and longer legs [7]. The more quiescent behaviour of *B. bombina* tadpoles is likely to give them a survival advantage over *B. variegata* in the presence of invertebrate predators found in ponds [3].

S z y m u r a and B a r t o n [19] examined the pattern of genotype frequencies - in particular, cline shape and linkage disequilibra among marker loci - to estimate the strength and nature of selection which maintains the hybrid zone in southern Poland. They cited the hybrid zone in *Bombina* as a typical example of a tension zone. At the same time, they suggested two lines of evidence for the occurrence of endogenous selection against hybrids: embryonic mortality and morphological aberrations. However, their evidence was limited to a single study with small samples, and the conjecture of lowered fitness from such phenotypic aberrations is doubtful [2].

The structure of narrow hybrid zones depends above all on the balance between selection and recombination. Selection and recombination typically maintain the distinct adaptations and coadaptations of the pure taxa. Those are typically maintained by selection favouring different genotypes in different environments, by selection against hybrids or by combination of both. Yet, the dispersal in hybrid zones creates mixed populations in which recombinations can act. If the selection is not too strong, a wide range of recombinants is produced. The break-up of parental gene combinations then frees individual genes from the effects of the selected loci with which they were originally associated: the effective selection acting on each gene becomes weaker, and so the hybrid zone becomes wider. With sufficient hybridisation, particularly fit recombinant genotypes can establish themselves, further broadening the hybrid zone, or even allowing the creation of hybrid taxa. However, if selection is sufficiently strong, then the integrity

of the two gene pools can be maintained despite the continuous production of hybrid genotypes. A strong barrier to gene flow will be maintained because individual alleles will be trapped in a largely unrecombined genetic background. Barriers to gene exchange can be strengthened by genetic variation for habitat preference [6]. These authors demonstrate the effect of habitat heterogeneity and a habitat preference on the genetic structure of the hybrid zones between fire-bellied and yellow-bellied toads.

The problem of hybridisation and the characteristics of this hybrid zone between *B. bombina* and *B. variegata* are not yet clarified. Therefore, we have analysed the allelomorphic features of seven populations of *Bombina* genus, between 150 to 1700 m altitude in order to establish which of them are the most important for the determination of belonging to *bombina* or *variegata* species, or for the determination of *bombina*-like or *variegata*-like hybrids.

Material and methods. The analysed material consists of 259 toads (122 adult males, 110 adult females and 27 juveniles) belonging to the genus *Bombina*. The specimens were collected in 10 different geographical populations as follows (see also: Fig. 1):

I. **Săcărâmb**: 19 individuals (16 males, 3 females); the population is from Hunedoara county, 20 km north-east from Deva in the Metaliferi Mountains, at 950 m altitude;

II. **Câmpuşel**: 45 individuals (18 males, 27 females), from the Retezatul Calcaros Mountains (Hunedoara county), at 1000 m altitude;

III. **Soarbele Valley,** at 1400 m altitude, and **Tăul Soarbele,** at 1550 m altitude, in the Retezatul Calcaros Mountains (Hunedoara county): 32 specimens (22 males, 10 females);

IV. **Ciuc region**: 48 individuals (27 males, 21 females), in Harghita county: **Şoimeni** - 12 km north-east to Miercurea-Ciuc at 750 m altitude, and **Armăşeni,** at 20 km south-east from Miercurea-Ciuc at 750 m altitude;

V. **Cluj region**: 44 individuals (22 males, 14 females, 8 juveniles) resulted from **Baci Valley**, 7-8 km west from Cluj-Napoca (Cluj county), at 350 m altitude, and **Fânațele Clujului,** 10 km north from Cluj, 450 m altitude;

VI. **Petreu**: 48 individuals (8 males, 21 females, 19 juveniles), 3 km south-west from Marghita (Bihor county), at 150 m altitude;

VII. **Parassapuszta**: 23 individuals (9 males, 14 females) from the Ipoly Valley, the Börzsöny Mountains, Hungary, at 200 m altitude.

The *Bombina* specimens were studied morphologically according to the method of S t u g r e n [16]. This method consists of analysing 9 allelomorphic characters, but we considered only 5 of these and further 3 features were added (Table 1). The proportions of the characters were expressed as percentages: 0% for pure *Bombina bombina* and 100% for pure *Bombina variegata*. The results were compared to the theoretical features of pure *Bombina bombina* (0 %) and *B. variegata* (100%) populations (Table 2).

Table 1

The allelomorphic features of the two species of genus *Bombina* living in Romania (after[16] modified)

No.	Allelomorphic feature	*B. bombina*	*B. variegata*
1.	Colour of the light spots on the lower surface of the body	Red, orange, yellowish	Yellow
2.	Colour of the upper part of the thumb and of the tips of the toes (right and left)	Black	Light
3.	Relation between the light tarsal and sole spots	Separated	United
4.	The proportion between head length (HL) and head width (HW)	HL>HW	HL<HW
5.	Patterns of the lateral and lower surface of the body	White spots around the lateral and ventral warts	Lateral and ventral warts without white spots around
6.	Patterns of the upper surface of the body	Regularly disposed dark tubercles	Scattered dark tubercles
7.	Dorsal warts	Flat, sleek, lenticular	Cone-shaped, rugged
8.	Tibio-tarsal articulations when the femur and the tibia are perpendicular	Not touching	Touching

The results were statistically analysed: before using the tree diagram analysis, the differences between *Bombina* populations were checked by the analysis of variance (ANOVA and Duncan tests).

Results. The variation of characters is summarised in Table 2. The population of Săcărâmb is the purest *Bombina variegata*, with 93.42% of the characters belonging to "*variegata*". The head length is larger than the head width, this being a typical feature for the yellow-bellied toad. In the majority of specimens, the dorsal tegument has visible tubercles and the dorsal warts are cone-shaped. The specimens do not show any white spots around the lateral and ventral warts. In 78.91% of them the yellow spots reach the tip of the toes. In *Bombina variegata*, the posterior leg is longer than in *B. bombina* [12,14] because of its more terrestrial life. In the toads of Săcărâmb the tibio-tarsal articulations are connected in 84.21%, showing a larger leg.

Table 2

The percentage of the eight allelomorphic features in the seven studied populations of *Bombina*, in comparison to the theoretical pure *Bombina bombina* and *B. variegata* populations

Char.1 – Char.8: the number of the respective allelomorphic feature as in *Table 1*

Populations	Char.1 (%)	Char.2 (%)	Char.3 (%)	Char.4 (%)	Char.5 (%)	Char.6 (%)	Char.7 (%)	Char.8 (%)	Total (%)
Pure *B.v.*	100	100	100	100	100	100	100	100	100
Săcărâmb	100	84.21	100	100	78.94	84.21	100	100	93.42
Câmpuşel	100	75.09	93.75	95.53	97.41	73.8	100	100	92.18
Soarbele	100	21.87	81.25	100	87.5	37.5	100	100	78.51
Ciuc region	91.66	68.75	100	0	100	91.66	83.33	100	71.15
Cluj region	100	34.09	100	0	100	29.54	93.18	100	69.65
Marghita	0	10.41	0	0	0	4.16	0	0	1.82
Hungary	0	34.78	0	0	0	8.96	0	0	5.43
Pure *B. b.*	0	0	0	0	0	0	0	0	0

The population of Câmpuşel shows 92.11 % *Bombina variegata* characters. The head is wider than longer and the dorsal spots are weakly formed in 75.89 %. The corn-shaped dorsal warts are pointed at 93.75% of specimens and lenticular in three females. F u h n [1] shows that *Bombina variegata* females' spins can be underdeveloped. There are no white spots on the belly and the yellow spots of the tarsal and sole regions are united. The yellow spots reach the tip of the toes in 97.41 % of the specimens and in 73.8 % of them the tibio-tarsal articulations are connected.

The populations of Soarbele Valley and Tăul Soarbele have *Bombina variegata* characters in 78.51 % of individuals. The head is typically "*variegata*" being more wide than long. The dorsal tegument has pronounced spots even kidney-like in most specimens (*B. bombina* character), only in 21.87 % the dorsal spots are totally absent (*B. variegata* character). The corn-shaped dorsal warts are pointed in 81.25 % of the specimens.

In Ciuc region the populations have *B.variegata* characters in 71.15 %. The heads are typical *B. variegata*. In 68.75 % the dorsal spots are missing, the remainder having kidney-like spots and small ones; the dorsal warts are pointed. These populations have lateral and ventral warts surrounded by white spots. The yellow ventral spots are united (tarsal and sole) in 83.33 % of specimens. The tibio-tarsal articulations are connected in most specimens (91.67 %), which prove the adaptations to a more terrestrial habitat.

The populations of Cluj region have in 69.65 % "*variegata*" characters. The analysed specimens have clearly visible and kidney-like dorsal spots in most of the specimens. The tibio-tarsal articulations are connected only in 29.54 %, the remainder having shorter legs. There are white spots in the ventral and lateral tegument and the yellow-orange spots between tarsal and sole are united.

The *B. bombina* population close to Petreu (Marghita, Bihor county) has in 1.82 % *B. variegata* characters. The specimens' heads (*B. bombina* character) are longer than wide. The dorsal tubercles are obvious, almost all specimens have two kidney-like spots. The lateral and ventral warts are flat and surrounded by white spots. The leg is shorter and the tibio-tarsal articulations are connected in only 4.16% of the toads. The colour of the ventral spots is dark orange; the tarsal and sole spots are not united. The ventral tegument is predominantly black.

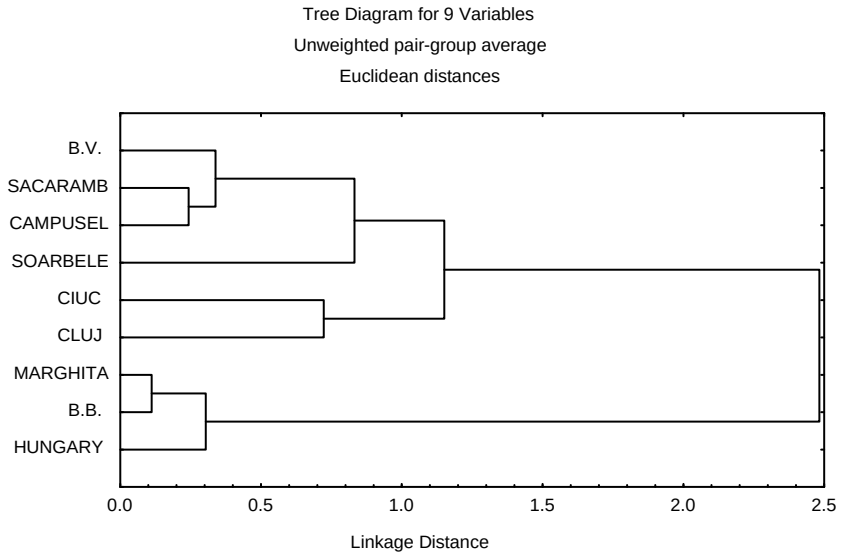
The population of Parassapuszta (Hungary) has 5.43 % characters of *B. variegata*. The dorsal tubercles are *B. variegata*-like in 34.78% and the tibio-tarsal articulations are connected only in a few specimens (8.69%). The colour of the ventral spots is orange, the tarsal and sole spots are separated. The lateral and ventral tegument is predominantly black and has warts surrounded by white spots.

For the comparison of the *Bombina* populations, we used the tree diagram analysis (Table 3 and Fig. 1).

Table 3

Values of the Duncan test following the ANOVA test (F=31.001; df=63: p< 0.0001), showing the significant statistical differences among the *Bombina* populations

	Pure *B.v.*	Săcărâmb	Câmpuşel	Soarbele	Ciuc	Cluj	Marghita	Hungary	Pure *B.b.*
Pure *B.v.*									
Săcărâmb	0.2361								
Câmpuşel	0.1462	0.7183							
Soarbele	**0.0277**	0.2483	0.3842						
Ciuc	**0.0257**	0.2346	0.3707	0.9328					
Cluj	**0.0100**	0.1238	0.2120	0.6332	0.6701				
Marghita	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0001**	**0.0001**			
Hungary	**0.0000**	**0.0000**	**0.0000**	**0.0001**	**0.0001**	**0.0001**	0.8432		
Pure *B.b.*	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0001**	0.9232	0.7834	



F i g. 1. *Tree diagram of the seven* Bombina *populations for the eight analysed features.*

Conclusions. 1.Two of the seven studied populations belong to *Bombina bombina*: the population of Marghita (98.18 % of the features) and the population of Parassapuszta - Hungary (94.57 % of the features).

2.The populations of Săcărâmb and Câmpuşel belong to *B. variegata* and have 93.42 % and, respectively, 89.58% characters of *B. variegata*.

3.The population of Soarbele though living near Câmpuşel at a higher altitude exhibits fewer characters of *B. variegata* (76.16 %) than that of Câmpuşel (93.42 %), a fact yet unexplained.

4.The populations of Ciuc and Cluj regions are natural hybrid populations between *Bombina bombina* and *Bombina variegata.* In this populations the "*variegata*" characters are predominant (71.15% and 69.65%, respectively).

5.The belonging to *Bombina bombina* or *B. variegata* can be established on the basis of two characters: the length of the leg (the tibio-tarsal articulations are/are not in connection) and the presence/absence of the dorsal spots. The dendrogram analysis of the two features, confirming this statement, proves to be similar to the dendrogram of all the features.

REFERENCES

1. F u h n, I., *Fauna R.P.R.,* Amphibia, **14**(1), Ed. Acad. Rom., Bucureşti, 1960.
2. G o l l m a n n, G., *Die Hybridzone des europäischen Unken. Ein natürliches Laboratorium der Evolutionsbiologie,* "Stapfia", **47,** 1991, 103-108.
3. K r u u k, L. E. B., G i l c h r i s t, J., *Mechanism maintaining species differentiation: predator-mediated selection in a* Bombina *hybrid zone,* "Proc. Roy. Soc. London", **264**, 1997, 105-110.
4. L á c, J., *Verbreitung der Unken (Tiefland-Unke* Bombina bombina *(L.) und Berg-Unke* Bombina variegata *(L.)) in der Slowakei und Problematik deren gegenseitigen Kreuzung,* "Biol. Prace Slov. Akad. Vied", **7**(3), 1961, 5-32.
5. L ö r c h e r, K., *Vergleichende bio-akustische Untersuchung an der Rotund Gelbbauch-unke,* Bombina bombina *und* Bombina variegata, "Oecologia", **3**, 1969, 84-124.
6. M a c C a l l u m, C. J., N ü r n b e r g e r, B., B a r t o n, N. H., S z y m u r a, J. M., *Habitat preference in the* Bombina *hybrid zone in Croatia,* "Evolution", **52**, 1998, 227-239.
7. M a d e j, Z., *Studies on the fire-bellied toad (*Bombina bombina*) and yellow-bellied toad* (Bombina variegata*) of Upper Silesia and Moravian Gate,* "Acta Zool. Cracov.", **9**(3**)**, 1964, 291-334.
8. M a x o n, L. E. R., S z y m u r a, J. M., *Quantitative immunological studies of the albumins of several species of fire-bellied toad, genus* Bombina, "Comp. Biochem. Physiol.", **63**, 1979, 517-519.
9. M é h e l y, L., *Die herpetologishen Verhältnisse des Mecsek Gebirges und der Kapela,* "Ann. Hist. Mus. Hung.", **3**, 1905, 256-316.
10. M i c h a l o w s k i, J., *Rozmieszczenie geograficzne kumaków (*Bombina *Oken) miedzy Wisla, Skawa i Raba (Województwo Krakówskie),* "Acta Zool. Cracov.", **3**(3), 1958, 247-283.
11. M i c h a l o w s k i, J., *Studies on the species characters in* Bombina variegata *(L.) and* Bombina bombina *(L.),* "Acta Zool. Cracov.", **6**(3), 1961, 51-59.
12. M i c h a l o w s k i, J., *Isolationsmechanismen und Bastardungsmöglichkeiten bei den Amphibien,* "Biol. Zentralbl.", **83**, 1964, 561-585.
13. M i c h a l o w s k i, J., M a d e j, Z., *Studies on the relationship of* Bombina variegata *(Linneaus) and* Bombina bombina*. III. Taxonomic characters of both species from laboratory and in interspecific hybrids,* "Acta Zool. Cracov.", **14**(9), 1969, 173-200.
14. N ü r n b e r g e r, B., B a r t o n, N. H., M a c C a l l u m, C. J., G i l c h r i s t, J., A p p l e b y, M., *Natural selection on quantitative trait in the* Bombina *hybrid zone,* "Evolution", **49**, 1995, 1224-1238.
15. R a f i n s k a, A., *Reproductive biology of the fire-bellied toad,* Bombina bombina *and* Bombina variegata (Anura: Discoglossidae): *egg size, clutch size and larval period length differences,* "Biol. J. Linn. Soc.", **43**, 1991, 624-630.
16. S t u g r e n, B., *Eidonomische Untersuchungen an* Bombina *Oken* (Amph., Discoglossidae) *aus dem Gurghiu-Tale (Siebenbürgen),* "Zool. Jahrb. Syst.", **86**(4/5), 1959, 382-394.

17. S t u g r e n, B., V a n c e a. Ş., *Geographic variation of the yellow-bellied toad* (Bombina variegata) *(L.) from the Carpathian Mountains of Romania and the USSR*, "J. Herpetol.", **2**(3-4), 1968, 97-105.
18. S z y m u r a, J. M., *Hybridization between discoglossid toads* Bombina bombina *and* B. variegata *in southern Poland as revealed by the electrophoretic technique*, "Z. Zool. Syst. Evol. Forsch.", **14**, 1976, 227-236.
19. S z y m u r a, J. M., B a r t o n N. H., *The genetic structure of the hybrid zone between the fire-bellied toads* Bombina bombina *and* B. variegata: *comparisons between transects and between loci*, "Evolution", **45,** 1991, 237-261.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, XLV, 1, 2000

BIOMETRIC STUDY OF THE SHREWS (*SORICIDAE, INSECTIVORA*) IN TWO HILLY ZONES OF THE SOMEŞUL MIC BASIN (ROMANIA)

VICTORIA NISTREANU[*]

SUMMARY. - Researches were carried out in the Gilău and Bonțida hilly zones of the Someşul Mic basin. In both zones, six shrew species were recorded: *Sorex araneus, S. minutus, Neomys fodiens, N. anomalus, Crocidura leucodon* and *C. suaveolens.* Ten body and skull measurements, important from biometric and taxonomic points of view, were made on each individual. The differences between the adult and subadult age groups were found to be significant ($P < 0.05$) in most of the ten biometric traits determined, but the differences between the males and females were significant only in some biometric traits, those of the males being greater. Exceptionally, none of the ten biometric traits of males and females was significantly different in the *Crocidura suaveolens* population from the Bonțida zone.

In the two studied zones, the populations of *Sorex araneus* and *S. minutus* are morphologically similar to the populations from the northern part of Romania and Central Europe, while the *Crocidura leucodon* and *C. suaveolens* populations are similar to those from different regions of Romania and Central Europe.

There are only few data published on the insectivore mammals living in the north-western part of Romania [4, 9, 13]. This is why we have initiated researches, especially biometric ones, concerning the shrew populations from this part of Romania.

Materials and methods. The researches were carried out in the hilly zones around the localities Gilău and Bonțida in the Someşul Mic basin. Gilău is situated at $46^0$44' North latitude and $23^0$22' East longitude at an altitude of 420 m above sea level, and this zone represents the north-eastern gate of the Apuseni Mountains. Bonțida is situated at $46^0$54' North latitude and $23^0$51' East longitude at an altitude of 350 m above sea level, on the western bottom of the Transylvanian Plain.

[*] *Academy of Sciences, Institute of Zoology, 2028 Chişinău, R. Moldova*

The shrews were caught with live traps in several types of ecosystems with different plant associations: *Salicetum albae, Carpino-Fagetum sylvaticae, Quercetum robori-petreae*, pasture with brush vegetation (*Crataegus monogyna, Cornus mas, Rosa canina, Pyrus pyraster, Malus sylvestris, Viburnum lantana*) and cultivated lands. All the caught individuals were measured and weighed. Ten body and skull measurements, important from biometric and taxonomic points of view, were made: body length (BL), tail length (TL), hindfoot length (HFL), body weight (BW), condylobasal length (CBL), breadth of brain case (BBC), interorbital constriction (IOC), height of brain case (HBC), mandible length (ML) and mandible height (MH). The biometric traits were registered in mm, excepting body weight which was recorded in g. For each biometric trait the following statistical parameters were considered: mean ($\bar{x}$), standard deviation (SD), minimum and maximum values (Min-Max). Significance of the differences between the biometric values of the adult and subadult age groups and of those of males and females was calculated by using Student's *t* test.

Results and discussion. In both hilly zones, six shrew species were caught [1]. The species and the number of the caught individuals were the following: *Sorex araneus* 34, *S. minutus* 11, *Neomys fodiens* 4, *N. anomalus* 3, *Crocidura leucodon* 47 and *C. suaveolens* 33.

In Tables 1-4, referring to *Sorex araneus, S. minutus, Crocidura leucodon* and *C. suaveolens*, NS and S indicate nonsignificant and significant ($P<0.05$) differences, respectively, between the age groups (adults vs. subadults; A/SA) and between the sexes (males vs. females; M/F). The results obtained for *Neomys fodiens* and *N. anomalus* were not taken into consideration because of the small number of the caught individuals belonging to these two species.

Table 1 shows that, in both Gilău and Bonțida populations of *Sorex araneus*, significant differences were found between the adult and subadult individuals concerning three biometric traits: body length, hindfoot length and interorbital constriction. The differences between the biometric traits of males and females of both *S. araneus* populations were significant only in the height of brain case and mandible length.

The values of biometric traits, especially those of body and condylobasal lengths and body weight of the *S. araneus* individuals from the Gilău and Bonțida zones are greater than those indicated for *S. araneus* living in other regions of the country [4, 6-8]. The biometric values of the *S. araneus* individuals caught by us are close to those recorded for the Central European *S. araneus* populations, but the maximum value of the condylobasal length is greater [10, 11]. The body and hindfoot length values are lower and the maximum value of condylobasal length is greater as compared to values registered in the East European *S. araneus* populations [2].

Table 1

Statistical parameters of the biometric traits of Sorex araneus

Biometric trait	Zone and number of the caught individuals							
	Gilău (26)				Bonțida (8)			Gilău+ Bonțida (34)
	$\bar{x}$ ±SD	Min-Max	A/SA	M/F	$\bar{x}$ ±SD	Min-Max	A/SA	$\bar{x}$ ±SD
BL	67.635 ±2.379	63.5-70.9	S	NS	62.738 ±7.965	52.1-77.9	S	66.482 ±4.711
TL	43.923 ±2.453	39.7-48.6	NS	NS	42.000 ±3.179	36.6-46.6	NS	43.471 ±2.718
HFL	12.250 ±0.432	11.4-13.2	S	NS	11.863 ±1.087	10.3-13.3	S	12.159 ±0.648
BW	7.196 ±1.014	6.0-9.5	NS	NS	5.675 ±1.322	4.4-7.7	S	4.838 ±1.257
CBL	19.831 ±0.682	18.2-21.3	NS	NS	19.025 ±0.688	18.3-20.0	S	19.641 ±0.757
BBC	8.577 ±0.657	7.5-9.9	NS	NS	8.775 ±0.900	7.4-9.8	NS	8.623 ±0.507
IOC	4.158 ±0.495	3.3-5.0	S	NS	3.988 ±0.464	3.4-4.6	S	4.188 ±0.486
HBC	5.692 ±0.373	5.0-6.2	NS	S	5.5 ±0.2	5.2-5.9	NS	5.647 ±0.347
ML	9.792 ±0.682	8.2-10.9	NS	S	9.825 ±0.557	9.0-10.5	NS	9.800 ±0.647
MH	4.515 ±0.211	4.1-4.9	S	NS	4.613 ±0.280	4.3-5.1	NS	4.538 ±0.228

The males of *Sorex minutus* have significantly greater body length than the females, whereas the differences between the other biometric traits are nonsignificant (Table 2).

The values of biometric traits of the Gilău and Bonțida populations of *S. minutus* are close to those recorded for the Suceava region [3] and are lower than those mentioned for other regions of Romania [4, 8]. Our results are similar to those described for the Central European *S. minutus* populations, excepting the condylobasal length, the minimum value of which is lower, and the mandible height, the minimum value of which is greater in the *S. minutus* individuals caught by us [10, 11]. In comparison with the *S. minutus* individuals from East Europe, those captured by us have greater limit values of the body length and much greater limit values of the condylobasal length [2, 5, 12].

Table 2

Statistical parameters of the biometric traits of Sorox minutus

Biometric trait	Zone and number of the caught individuals					
	Gilău (9)			Bonțida (2)		Gilău+ Bonțida (11)
	$\bar{x} \pm SD$	Min-Max	M/F	$\bar{x} \pm SD$	Min-Max	$\bar{x} \pm SD$
BL	52.044 ±3.661	47.5-58.1	S	53.450 ±1.768	52.2-54.7	52.300 ±3.317
TL	39.178 ±2.427	33.2-41.2	NS	39.400 ±1.273	38.5-40.3	39.218 ±2.209
HFL	10.556 ±0.456	9.9-11.2	NS	10.050 ±0.212	9.9-10.2	10.464 ±0.461
BW	3.511 ±0.744	2.7-4.5	NS	2.950 ±0.071	2.9-3.0	3.409 ±0.703
CBL	11.867 ±2.528	9.5-15.3	NS	16.650 ±0.212	16.5-16.8	12.736 ±2.977
BBC	7.278 ±0.222	7.0-7.7	NS	7.550 ±0.212	7.4-7.7	7.327 ±0.237
IOC	3.256 ±0.151	3.0-3.5	NS	3.150 ±0.071	3.1-3.2	3.236 ±0.143
HBC	4.267 ±0.180	4.0-4.5	NS	4.750 ±0.071	4.7-4.8	4.354 ±0.254
ML	5.611 ±1.358	4.4-7.8	NS	7.950 ±0.212	7.8-8.1	6.036 ±1.541
MH	3.2 ±0.1	3.0-3.3	NS	3.150 ±0.071	3.1-3.2	3.191 ±0.094

One can see from Table 3 that the differences between the adult and subadult individuals of *Crocidura leucodon* are significant in seven biometric traits (Gilău population) and in four traits (Bonțida population). In the Gilău population, the males have significantly greater body length, hindfoot length and breadth of brain case than the females, but in the Bonțida population, only two biometric traits (tail length and height of brain case) are significantly greater in the males than in the females.

Table 3

Statistical parameters of the biometric traits of Crocidura leucodon

Biometric trait	Zone and number of the caught individuals								
	Gilău (24)				Bonțida (23)				Gilău+ Bonțida (47)
	$\bar{x} \pm SD$	Min-Max	A/SA	M/F	$\bar{x} \pm SD$	Min-Max	A/SA	M/F	$\bar{x} \pm SD$
BL	64.821 ±8.467	53.8-77.8	S	S	68.609 ±7.240	56.3-81.3	S	NS	66.674 ±8.040
TL	34.754 ±3.089	28.7-39.4	NS	NS	32.922 ±3.369	27.4-40.0	NS	S	33.857 ±3.325
HFL	11.333 ±0.653	10.5-12.8	S	S	11.296 ±0.861	9.6-12.6	NS	NS	11.315 ±0.754
BW	6.229 ±2.459	2.5-11.0	S	NS	7.643 ±2.971	3.0-13.5	NS	NS	6.921 ±2.801
CBL	19.179 ±0.412	18.4-20.0	NS	NS	19.043 ±0.614	18.1-20.0	S	NS	19.113 ±0.519
BBC	9.200 ±0.389	8.7-9.9	S	S	9.078 ±0.288	8.7-9.8	NS	NS	9.140 ±0.345
IOC	4.608 ±0.259	4.2-5.0	S	NS	4.596 ±0.146	4.3-4.9	NS	NS	4.602 ±0.209
HBC	5.292 ±0.376	4.8-5.8	S	NS	5.191 ±0.241	4.8-5.7	NS	S	5.243 ±0.318
ML	9.823 ±0.324	9.3-10.6	S	NS	9.930 ±0.370	9.2-10.9	S	NS	9.878 ±0.348
MH	4.575 ±0.227	4.3-5.2	NS	NS	4.517 ±0.308	3.9-5.1	S	NS	4.547 ±0.269

The variability limits of the biometric trait values in the studied two *C. leucodon* populations are wider than those recorded for other *C. leucodon* populations in the country [4, 5, 8]. The values of body and condylobasal lengths of the *C. leucodon* individuals caught by us are greater and the variability limits of body weight are wider than those described for Central European *C. leucodon* populations [10, 11]. In the Gilău and Bonțida populations of *C. leucodon*, the body and hindfoot are shorter and the condylobasal length is greater in comparison with the East European *C. leucodon* populations [2, 12].

Significant differences were found between the adult and subadult *Crocidura suaveolens* individuals in eight biometric traits (Gilău population) and five traits (Bonțida population). The differences between the biometric traits of males and females were significant in two traits (body weight and mandible length), while no significant difference was found between the biometric traits of males and females of the Bonțida population (Table 4).

Table 4

Statistical parameters of the biometric traits of Crocidura suaveolens

Biometric trait	Zone and number of the caught individuals								
	Gilău (16)				Bonțida (17)				Gilău+ Bonțida (33)
	$\bar{x} \pm SD$	Min-Max	A/SA	M/F	$\bar{x} \pm SD$	Min-Max	A/SA	M/F	$\bar{x} \pm SD$
BL	55.844 ±3.442	50.7-64.2	S	NS	58.982 ±4.299	52.5-66.1	S	NS	57.461 ±4.163
TL	34.538 ±1.602	32.8-38.4	S	NS	32.624 ±1.591	29.6-34.5	NS	NS	33.552 ±1.847
HFL	10.569 ±0.665	10.0-11.6	S	NS	10.453 ±0.501	9.7-11.1	NS	NS	10.509 ±0.580
BW	4.188 ±0.609	3.2-4.9	S	S	3.594 ±0.766	2.7-5.1	S	NS	3.882 ±0.747
CBL	16.525 ±0.686	15.9-17.9	S	NS	16.712 ±0.598	15.2-17.1	S	NS	16.621 ±0.639
BBC	8.156 ±0.405	7.6-8.8	S	NS	7.706 ±0.590	6.3-8.2	NS	NS	7.924 ±0.551
IOC	3.813 ±0.280	3.2-4.1	NS	NS	4.076 ±0.315	3.8-4.8	S	NS	3.948 ±0.323
HBC	4.556 ±0.432	4.0-5.4	S	NS	4.871 ±0.400	4.2-5.7	NS	NS	4.718 ±0.439
ML	8.456 ±0.429	7.6-9.2	S	S	8.418 ±0.488	7.6-9.5	S	NS	8.436 ±0.453
MH	3.775 ±0.259	3.4-4.1	NS	NS	3.935 ±0.226	3.6-4.2	NS	NS	3.858 ±0.252

The biometric trait values, especially the minimum limits, of the *C. suaveolens* individuals captured by us are lower than those of the *C. suaveolens* individuals from other regions of the country [4, 6]. The *C. suaveolens* individuals from the Gilău and Bonțida zones have the minimum value of body length smaller and the maximum value of condylobasal length greater than those from Central Europe [10, 11]; they have the minimum value of hindfoot length lower and the maximum values of tail and condylobasal lengths greater in comparison with the East European *C. suaveolens* populations [2, 12].

Conclusions. 1. In the studied two hilly zones (Gilău and Bonțida) in the Someşul Mic basin, six insectivore mammal species were recorded: *Sorex araneus, S. minutus, Neomys fodiens, N. anomalus, Crocidura leucodon* and *C. suaveolens*.

2. The differences between the adult and subadult age groups were found to be significant in most of the ten biometric traits determined, but the differences between the males and females were significant only in some traits, those of the males being greater. Exceptionally, none of the biometric traits of males and females was significantly different in the *Crocidura suaveolens* population from the Bonțida zone.

3. In the Gilău and Bonțida zones, the populations of *Sorex araneus* and *S. minutus* are morphologically similar to the populations from the northern part of Romania and Central Europe, while the *Crocidura leucodon* and *C. suaveolens* populations are similar to those from different regions of Romania and Central Europe.

REFERENCES

1. Banaru, V., Coroiu, I., *Preliminary studies on micromammal fauna from the Someşul Mic basin (Cluj district) - Romania*, "Trav. Mus. Hist. Nat. Gr. Antipa", **39**, 1997, 241-255.
2. Gureev, A. A. (Red.), *Fauna SSSR. Mlekopitayushchie, Nasekomoyadnie* (Mammalia, Insectivora), *Tom IV (2)*, Nauka, Leningrad, 1972.
3. Hellwing, S., *Contribuții la cunoaşterea unor mamifere mici din regiunea Suceava (raioanele Vatra-Dornei, Câmpulung şi Rădăuți)*, "Trav. Mus. Hist. Nat. Gr. Antipa", **2**, 1960, 337-378.
4. Ionescu, V., *Vertebratele din România*, pp. 402-408, Ed. Acad. Rom., București, 1968.
5. Lozan, M., *Mlekopitayushchie. Zhivotnyi Mir Moldavii*, pp. 32-40, Ştiința, Chişinău, 1979.
6. Murariu, D., *Contribuții la cunoaşterea răspândirii şi ecologiei mamiferelor din zona Deltei Dunării şi a lacului Razelm*, "Trav. Mus. Hist. Nat. Gr. Antipa", **23**, 1981, 283-286.
7. Murariu, D., *Mamiferele mici* (Mammalia, Insectivora *şi* Rodentia) *din nordul Moldovei (România)*, "Trav. Mus. Hist. Nat. Gr. Antipa", **33**, 1993, 411-419.
8. Murariu, D., Andreescu, I., *Consideraţii ecologice asupra ordinelor* Insectivora *şi* Rodentia (Mammalia) *din județele Vrancea, Buzău, Prahova şi Argeş (România)*, "Trav. Mus. Hist. Nat. Gr. Antipa", **20**, 1979, 487-500.
9. Popescu, A., Barbu, P., *Data privind răspândirea şi frecvența soricidelor* (Soricidae - Insectivora) *în România*, "Ocrot. Nat.", **23** (2), 1979, 163-168.
10. Pucek, R. (Ed.), *Keys to Vertebrates of Poland. Mammals*, Pol. Sci. Publ., Warszawa, 1981.

11. Ruprecht, J., *Taxonomic value of mandible measurement in* Soricidae (Insectivora), "Acta Theriol.", **16** (21), 1971, 341-357.
12. Sokolov, I. I. (Red.), *Mlekopitayushchie. Fauna SSSR, Tom II*, Izd. Akad. Nauk SSSR, Moskva-Leningrad, 1963.
13. Szabó, I., *Contribuţii la cunoaşterea faunei de mamifere mici din partea nord-vestică a R. P. România*, "Stud. Univ. Babeş-Bolyai, Biol.", **5** (2), 1960, 119-126.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, XLV, 1, 2000

AN ORIGINAL DATA ACQUISITION SYSTEM FOR MONITORING THE BIOELECTRIC ACTIVITY OF FROG HEART

CRISTIAN SEVCENCU[*] and ANDREI PATRICIU[**]

SUMMARY. - An electrical signal generated by the frog heart was for the first time photographically recorded in 1883. Since then, the recording devices and methods have been continuously improved. One of the most powerful signal recording and processing technique is the computer-based data acquisition. During the last years, part of our work was focused on designing and improving a data acquisition system (DAQS) to be used for the study of the signals generated by the self-pacing myocardium. An original four-modular DAQS was built and our recordings show that its capacities in signal acquisition and processing are comparable to those of other similar tools.

The first step towards the study of certain electrical signals generated by the heart became possible in the 1880s, after the construction of a device (*i.e.* the Lippmann capillary electrometer) capable to acquire voltage fluctuations from living structures. To record the traces for further analysis, photographic methods were used [15].

In the years that followed, parallel to the evolution of the acquiring tools, the signal storage methods have also been improved.

The transposing of the traces reflecting a bioelectric process on film or photographic paper directly from the screen of the oscilloscope [7] was for many years the single recording method and it is still used [1, 11]. The construction of memory oscilloscopes made possible the storage of the traces and their reactivation for ulterior measurements [4-6].

In the last decade, due to the explosion of the computational techniques, a new generation of data acquisition methods has been developed and they are widely used today [2, 3, 9, 14, 16].

[*] *Babeş-Bolyai University, Department of Animal Physiology, 3400 Cluj-Napoca, Romania*
[**] *Aalborg University, Center for Sensory-Motor Interaction, 9220 Aalborg, Denmark*

Combining an astonishing data transferring speed with practically unlimited storage capacities of the harddisks, the computer-based data acquisition systems (DAQS) became powerful tools for the study of biological signals all over the world.

Beside these characteristics, one of the most important facilities offered by the PC is that the information, once stored on the harddisk, can be actually used any time. Moreover, many programs may also process the data by means of sophisticated analyzing software including, for example, harmonic analysis and statistical methods.

Our first attempt to acquire electrical signals generated by isolated frog hearts was performed by means of an ECG recorder. Two active electrodes and an indifferent one were properly placed into a self made perfusion chamber filled with Ringer solution. An ECG trace, similar to the human one, appeared on the screen of the apparatus.

The next step we made was to adapt the perfusion techniques to the requirements of a direct extracellular recording. To solve the problems of the electrode attachment to a moving organ, as well as to ensure a proper tensioning of the heart during the recordings, we had to design a novel perfusion device. Firstly, the signals were recorded with the ECG apparatus. We connected then its amplifier module to a self made data acquisition board, which was able to communicate with the PC by means of a proper program. The results of that work were previously reported [12, 13]. New improvements were made, both regarding the perfusion device and the acquiring software package, thus resulting our present DAQS.

We describe here the main technical characteristics of this system and the methods we used to monitor electrical signals generated by the frog heart.

Material and methods. *Animals.* The hearts can be prepared from any frog species. The size of the animals is not important because the fitting devices from the perfusion chamber are adaptable to the heart length.

The data acquisition system. Our system consists of four main modules. These are: the perfusion module - which ensures the proper conditions for the heart activity, the signal amplifier - which increases the signal amplitude to the requested value, the data acquisition (DAQ) board - which pre-processes and transmits the data to an IBM compatible PC - the fourth component of the DAQS (see, for illustration, Fig. 1).

The perfusion module consists of a perfusion tank (1) and a perfusion device. Each reservoir (R1,R2 and R3) of the perfusion tank can contain any requested solution, one of them always being the Ringer one for the reference cardiac activity. The Ringer solution can be instantly replaced by one from the other reservoirs by manipulating their taps (2).

Three plastic tubes (3) connect the reservoirs at the perfusing head (4), where the cardiac cannula (6) will also be fitted. A displacement device (5) adapts the position of the perfusing head to the heart size, thus ensuring the organ's proper tensioning. Made from steel, this cannula also plays the role of the reference electrode and it is connected at the signal amplifier by means of an electric cable.

The active electrode (7) consists of a steel spring, with an end in form of a hook and the other one straight. It has this special design because it must accomplish three tasks. Thus, the hook end stabs the tip of the ventricle and acquires the electrical activity of the tissue. The spring middle ensures a counter-force which opposes to the ventricular contraction and brings the heart to its initial length in the relaxing period. The straight end of the electrode serves for fixing it into the perfusion chamber by means of a pincers (8) connected to the amplifier.

A transparent Plexiglas lid covers the perfusion chamber (9), thus ensuring a damp atmosphere around the heart.

To amplify the electrical signals acquired by means of the devices described above, we used the amplifier of a Cardior KTD2 apparatus. To do that, both electrodes were connected to the original cables of its ECG module. The exit of the amplifier was further linked to the entrance of the DAQ board. This connection between the ECG apparatus and the DAQ board allows not only the transmission of the amplified signals to the PC, but also a visual control of the electrical activity of the heart on the ECG screen. According to the stability of the signals watched on this screen, one could establish the right moment to start the PC monitoring.

The DAQ board is an electronic device which plays two crucial roles in the acquisition process: those of a digital voltmeter and a transmission element.

The IBM compatible PC must have the minimum characteristics to be able to rule Windows 95, which is necessary for the acquiring LabView 4.1-based software package.

The heart preparation begins with the isolation procedures.

The frog is killed by pithing and the heart is discovered. The hook end of the active electrode is implanted into the ventricular mass. The tip of the cannula is introduced into the base of the venous sinus, which is then tightly bound around the metallic pipe.

After the removal, the heart is placed into the perfusion chamber. To avoid the formation of air bubbles into the heart, the flowing of the Ringer solution must be started before fitting the cannula at the perfusion head. The straight end of the electrode is then fixed into its pincers from the perfusion device.

By manipulating its displacement device, the perfusion head is positioned according to the length of the heart. Thus, at the end of these operations, the organ must stay in horizontal position, gently tensioned by the spring zone of the active electrode (see Fig.1).

The two electrodes are then connected to the cables of the amplifier module. Starting from this moment, the self-pacing bioelectrical activity of the ventricular tissue may be watched on the screen of the ECG.

After the stabilization of heart beating, the PC monitoring may start by triggering the acquisition program.

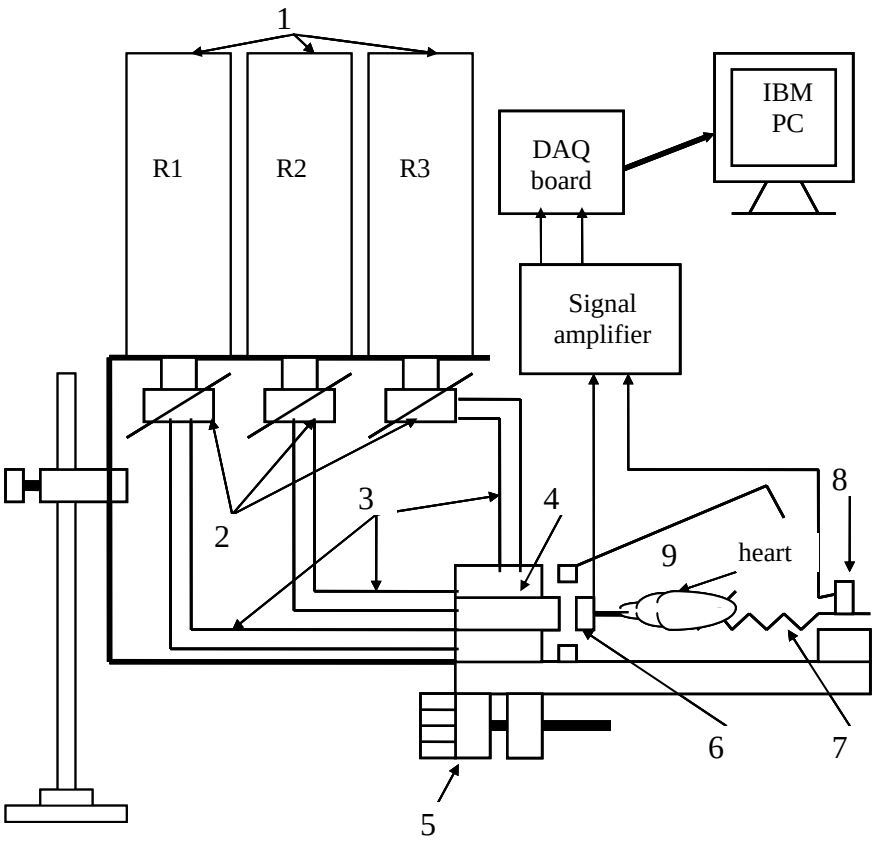


Fig. 1. *Schematic diagram of the data acquisition system.*
1 - Perfusing reservoirs. 2 – Taps. 3 - Plastic tubes. 4 - Perfusing head.
5 - Displacement device. 6 - Perfusion cannula/reference electrode.
7 - Active electrode. 8 – Pincers. 9 - Perfusion chamber.

Results and discussion. *The perfusion device.* In chronological order, the first result of our work was the realization of an original perfusion device.

In comparison to the classical Fühner model, which was the only one used in our laboratories, the new one has important improvements.

Thus, the perfusion can be successively performed from three different reservoirs which can be filled with three different solutions. As we have already mentioned, one of them must always contain the Ringer solution under normal conditions of temperature and pressure, for monitoring the reference bioelectrical cardiac functions. If the effects of any disturbing substance have to be tested, this can be solved in the Ringer solution from one of the other two reservoirs. By simply stopping the initial Ringer current and starting at the same time that containing the tested factor, the second solution will pass through the perfusing head, instantly replacing into the cannula the normal Ringer one.

This perfusing method has two main advantages.

First, the concentration of the disturbing substance is a strictly controlled factor, whereas by adding drops in the cardiac cannula the final concentration of the solution which enters the heart remains unknown.

Second, the technical characteristics of the perfusion device allow an almost instant replacement of a solution with another one from a second reservoir, with a very short transition period (see Fig. 4).

Moreover, we have thought of a third reservoir to be used for serial perfusions, such as a pretreatment with a blocker followed by a perfusion with its specific antagonist. For example, one could test the effect of epinephrine after the heart was previously perfused with its β-blocker, propranolol. Thus, the reservoir R1 (see Fig. 1) would contain normal Ringer solution, R2 - Ringer with propranolol and R3 - Ringer with epinephrine, the perfusing order being R1, R2, R3.

Another new element of our system is the mobile perfusing head. Its main role is to fit the cardiac cannula into the perfusion chamber, at the same time supplying it with the solutions which flow from the reservoirs. Taking into consideration that the hearts may have different sizes, we had to think of a technical formula in order to ensure the adaptability of this attachment device to the heart length. Thus, instead of fixing the perfusing head into the perfusion chamber, we have connected it to a thread based displacement device which ensures its foreword and backward movements, as the heart length would require.

When one has to record signals generated by a moving cell, tissue or organ, a major problem is the attachment of the electrodes so that the motion artifacts should be reduced or eliminated. Different methods have been established to solve this demand and we mention here the using of suction [8-10] or the calcium removal in order to inhibit the contractions [8].

Part of our solution to this problem is based on using a metallic cannula instead of the classical glass one. Anyhow, this must be strongly bound to the sinus wall, so that it can also serve as a reference electrode.

The other half of the problem, that of a proper and stable contact between the ventricular tissue and the active electrode, was solved by the particular design of this electrode (see the description above and Fig. 1). As we have previously presented, during the isolation procedures, the hook end of this electrode is implanted into the ventricular mass. After the isolation, the heart with the electrode hanging on its tip is attached to the perfusing head by means of the cardiac cannula. The second attachment point is represented by the pincers, where the straight end of the electrode is fixed. The next operation is to move backward the perfusing head till the spring middle of the electrode remains gentle stretched even during the relaxing period. This state of permanent tension ensures the indispensable tissue-electrode contact along the whole cardiac cycle. The elastic force accumulated by the spring during the contraction also serves for bringing the heart back to its initial length.

The DAQ board. We have mentioned before that this electronic device was designed to ensure both the digitization of the signals and their transmission to the PC.

As a digital voltmeter, the DAQ board receives the amplified signals and converts them into numerical values. This task is achieved by means of a 10-byte analog-to-digital converter, which is included into a 80C 552 Philips microcontroller.

As a transmission element, the DAQ board sends in real time the converted data to the PC by means of its serial interface. The communication protocol was by software established at 57600 kbytes/s, thus being transmitted 150 values per second.

The software package. The data acquisition and processing are performed by means of a self-designed, LabView-based software package which consists of two parts.

One of the programs receives the value strings sent by the serial interface and transforms them into analog graphic representations. Thus, the signals rule on the monitor almost in real time. Parallel to this processing task, the program also saves the data into dedicated files on the harddisk.

The software package also contains a program designed to display the recordings made by the first one. Thus, an evolutionary bioelectrical process determined, for example, by changing the Ringer solution with one containing a disturbing factor, may be seen from the beginning till the end. Due to the facilities of this program, any segment from such a recording may be zoomed and analyzed.

Recorded signals. To illustrate the performances of our DAQS, we shall further present some of the recordings obtained by using it.

As we have mentioned above, from a whole recording, which can be very condensed if the experiment lasted for a long period (minutes or even hours), one could choose and magnify only a small segment.

In Fig. 2, three steps of magnification - a, b and c - can be seen.

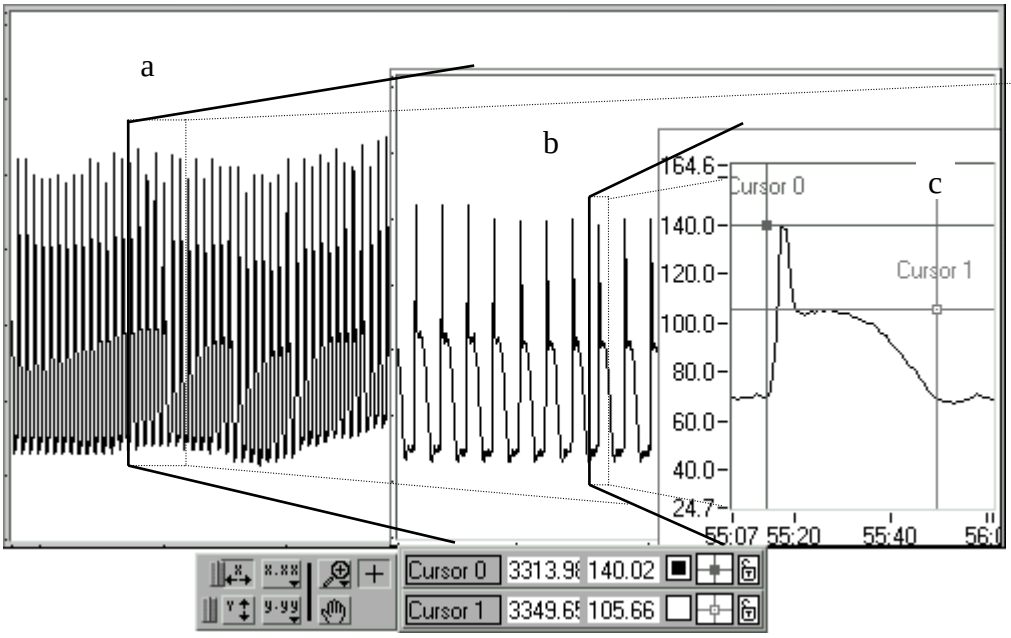


Fig. 2. *Three magnification steps of a bioelectric recording acquired from frog ventricular myocardium.*

In the cell c, a single zoomed wave is presented. Cursors 0 and 1 illustrate how the signal parameters can be measured both in amplitude and in duration. The numbers from the palette below the picture represent the units of the measured points, which are translated into absolute values of amplitude and time after the calibration.

The c cell shows a single bioelectrical wave recorded from the ventricular myocardium. According to Weidmann [15], this would represent a so called "injury action potential", which was for the first time photographically recorded in 1883.

More recently, in 1992, Neunlist and Zou [10], using an original optical method, obtained an integrated transmembrane potential recorded from frog ventricular epicardium. Although this signal, being acquired from a volume of tissue approximately 200 µm in diameter, may differ from that of individual cells [8], it expresses the main characteristics of a well known cardiac action potential. Thus, the authors describe a fast depolarization period with a rise time that can range from less then 1 ms to 20 ms, a first repolarization period followed by the depolarization plateau and, finally, the late repolarization phase. The maximal amplitude was reported to be of about 100 - 120 mV and the total duration of this integrated action potential ranged from 700 to 900 ms.

The individual signals acquired in our laboratory by means of the previously described DAQS also seem to present the characteristic steps of an action potential.

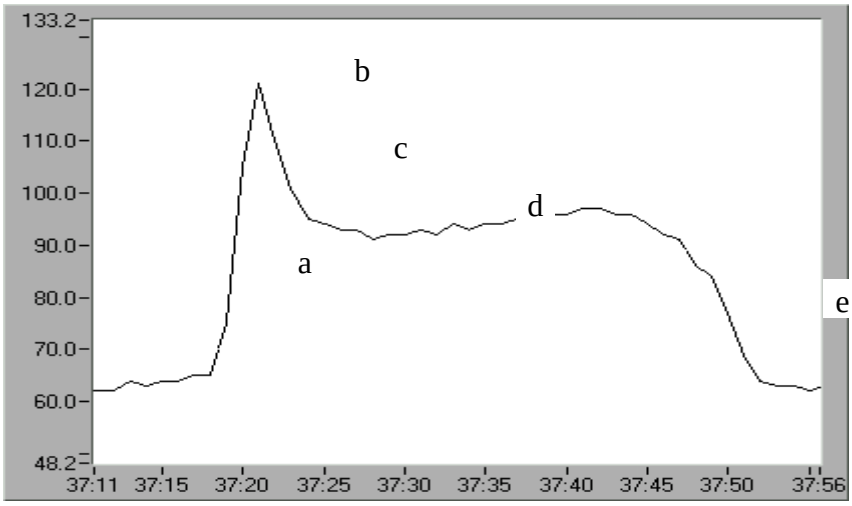


Fig. 3. *Individual signal acquired from self-pacing frog ventricular myocardium.* a; b; c; d; e - Specific phases in the development of the signal.

As can be seen in Fig. 3, the signal starts with a rapid up-stroke (phase a) that was found to last for 25 - 30 ms. The maximal spike amplitude (point b) range from 25 to 30 mV. After a rapid decrease of the amplitude (phase c), the subsequent characteristic plateau follows (phase d). Finally, the potential returns to its base-line value by means of a prolonged decreasing period (phase e).

As a characteristic of their recording devices, Neunlist and Zou [10] reported a peak-to-peak noise level of 2-7% from the action potential amplitude.

In our recordings, the maximum level of the peak-to-peak noise was 5% of the signal amplitude.

As we have already mentioned, a difficult recording problem is the fixing of the electrodes into/on the tissue when the bioelectrical signals are acquired from a moving organ.

The authors cited above have recorded their signals by means of an optical fiber which was positioned on the surface of the ventricle. When this fiber was not properly fixed by suction, a major motion artifact appeared some 50 - 100 ms after the upstroke of the action potential.

Although our signal is always followed by the ventricular contraction 90 - 100 ms after the spike, no motion artifact appears (see Fig. 3). Since the motion artifacts were totally eliminated, we may conclude that our technical solution related to the attachment of the electrodes is a valuable one.

Finally, we illustrate the capacities of our DAQS to reflect the effect of a disturbing factor on the cardiac bioelectric activity. As ca be seen in Fig. 4, a change in the signal shape, as well as in the frequency is developing next to the vertical bar. This sign marks the moment when the normal Ringer solution was replaced with the one containing the tested substance. Thus, the picture above shows the transition from a normal self-pacing ventricular activity to that determined by the administration of methanol solved in Ringer solution. Without discussing here the physiological mechanism of this effect, we only mention that our devices and methods seem to surprise well enough such a process during its development.

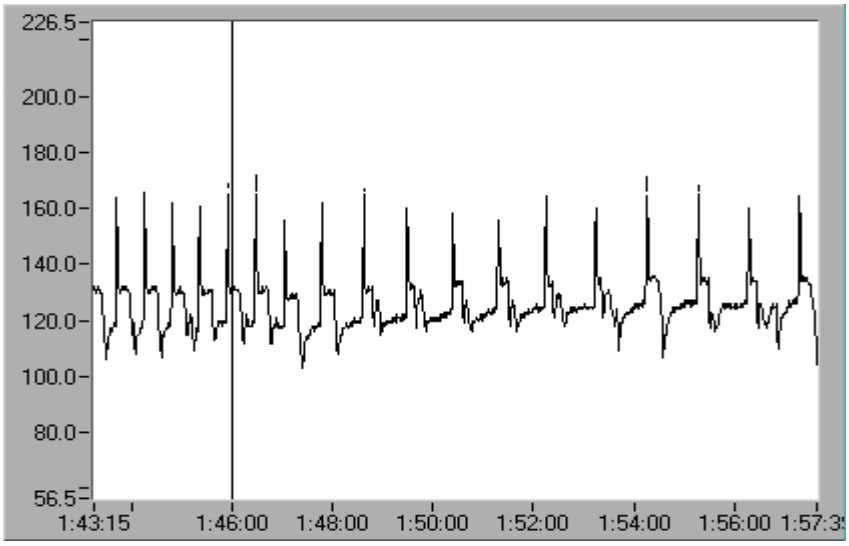


Fig. 4. *Effect of methanol on the bioelectrical activity of the frog ventricle.* The vertical bar marks the substance administration.

As a **conclusion** of the present work, we believe that our DAQS may be considered a valuable tool for the study of the frog heart bioelectrical activity either alone or in relation to other cardiac parameters.

REFERENCES

1. Ally, A., Murayama, S., *Cardiovascular effects of central administration of cholinomimetics in anesthetized cats,* "Neuropharmacology", **32**, 1993, 185-193.
2. Fares, N., Bois, P., Lenfant, J., Potreau, D., *Characterization of a hyperpolarization-activated current in dedifferentiated adult rat ventricular cells in primary culture,* "J. Physiol.", **506**, 1998, 73-82.
3. Goethals, M., Raes, A., van Bogaert, P., *Use-dependent block of the pacemaker current I_f in rabbit sinoatrial node cells by zatebradine (UL-FS 49),* "Circulation", **88**, 1993, 2389-2401.
4. Harra, Y., Ally, A., Murayama, S., *Central cardiovascular effects of physostigmine in anesthetized cats,* "Neuropharmacology", **31**, 1992, 923-928.
5. Kurachi, Y., *Voltage-dependent activation of the inward-rectifier potassium channel in the ventricular cell membrane of guinea-pig heart,* "J. Physiol.", **366**, 1985, 365-385.
6. Nakajima, T., Sugimoto, T., Kurachi, Y., *Effects of anions on the G protein-mediated activation of the muscarinic K^+ channel in the cardiac atrial cell membrane,* "J. Gen. Physiol.", **99**, 1992, 665-682.
7. Negoescu, R., *Instrumentaţia electronică biomedicală,* p. 141, Ed. Tehn., Bucureşti, 1985.
8. Neunlist, M., Tung, L., *Optical recording of ventricular excitability of frog heart by an extracellular stimulating point electrode,* "Pace", **17**, 1994, 1641-1654.
9. Neunlist, M., Tung, L., *Dose-dependent reduction of cardiac transmembrane potential by high-intensity electrical shocks,* "Am. J. Physiol. **273** (Heart Circ. Physiol. **42**)", 1997, H2817-H2825.
10. Neunlist, M., Zou, S., *Design and use of an "optrode" for optical recordings of cardiac action potentials,* "Pflügers Arch.", **420**, 1992, 611-617.
11. Rubenstein, D. S., Fox, L. M., McNulty, J. A., Lipsius, S. L., *Electrophysiology and ultrastructure of Eustachian ridge from cat right atrium: a comparison with SA node,* "J. Mol. Cell Cardiol.", **19**, 1987, 965-976.
12. Sevcencu, C., *Effects of certain cardioinhibiting and cardioactivating factors reflected in the total bioelectrical activity of frog isolated heart,* "Abstr., 4th Natl. Conf. Biohys."(Cluj-Napoca, 1997), P-33.
13. Sevcencu, C., Patriciu, A., Chihaia, E., *System for monitoring the total bioelectrical activity of frog isolated heart,* "Abstr., 4th Natl. Conf. Biohys." (Cluj-Napoca, 1997), P-34.
14. Vornanen, M., Tuomennoro, J., *Effects of acute anoxia on heart function in crucian carp: importance of cholinergic and purinergic control,* "Am. J. Physiol. **277** (Reg. Integr. Comp. Physiol. **46**)", 1999, R465-R475.
15. Weidmann, S., *Cardiac cellular electrophysiology: past and present,* "Experentia", **43**, 1987, 133-146.
16. Zhou, Z., Lipsius, S. L., *Na^+-Ca^{2+} exchange current in latent pacemaker cells isolated from cat atrium,* "J. Physiol.", **466**, 1993, 263-285.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, XLV, 1, 2000

CHARACTERISATION OF *NICOTIANA PLUMBAGINIFOLIA* TRANSFORMANTS, CONTAINING *METC* GENE FROM *ESCHERICHIA COLI*. I. ANALYSIS OF NUCLEIC ACIDS

GABRIELA ISPAS[*], LENUȚA RÁKOSY-TICAN[x], MICHAEL JACOBS[**] and VALÉRIE FRANKARD[xx]

SUMMARY. - *Nicotiana plumbaginifolia* plants, transformed with *metC* gene from *Escherichia coli* have been characterised at nucleic acid level. The transformed nature of the analysed plants has been proved.

Amino acid biosynthesis is an essential process for plant growth and development. The essential amino acids: lysine, threonine, methionine and isoleucine derive all from aspartate, via a branched biosynthetic pathway.The main sulphur- containing amino acids in plants and animals are methionine and cysteine.

Methionine is synthesised in plants and microorganisms *de novo*, from cysteine and O-phospho-homoserine (plants) and, respectively, from cysteine and O-succinyl-homoserine (bacteria). In contrast, in animals methionine represents the essential sulphur-containing amino acid and is the precursor of cysteine.

The steps of methionine biosynthesis in plants, downstream from cysteine are presented in Fig. 1. The precursor O-phospho-homoserine represents the crossing-point between aspartate, methionine and threonine pathway. *Cystathionine gamma synthase* (CS) may use either cysteine or sulphide as a sulphur supplier. The enzyme *cystathionine gamma synthase* (CS) catalyses the first committed step that consists in a transsulphurylation reaction between cysteine and O-phospho-homoserine to form the intermediate cystathionine and is regarded as a major regulatory point in the pathway [6]. Cystathionine cleavage by *cystathionine beta lyase* (Cbl) yields ammonia, pyruvate and homocysteine. In the next step, homocysteine is methylated by *methionine synthase* (MS) leading to methionine production. Most of the enzymes involved in methionine biosynthesis are localised in chloroplast. Only *methionine synthase* appears to be localised in the cytoplasm [4]. The localisation of *cystathionine beta lyase* enzyme is still a debate.

[*] *Babeş-Bolyai University, Faculty of Biology and Geology, Department of Ecology and Genetics, 3400 Cluj-Napoca, Romania*

[**] *Free University Brussels, Institute of Molecular Biology and Biotechnology, Laboratory of Plant Genetics, B-1640 Brussels, Belgium*

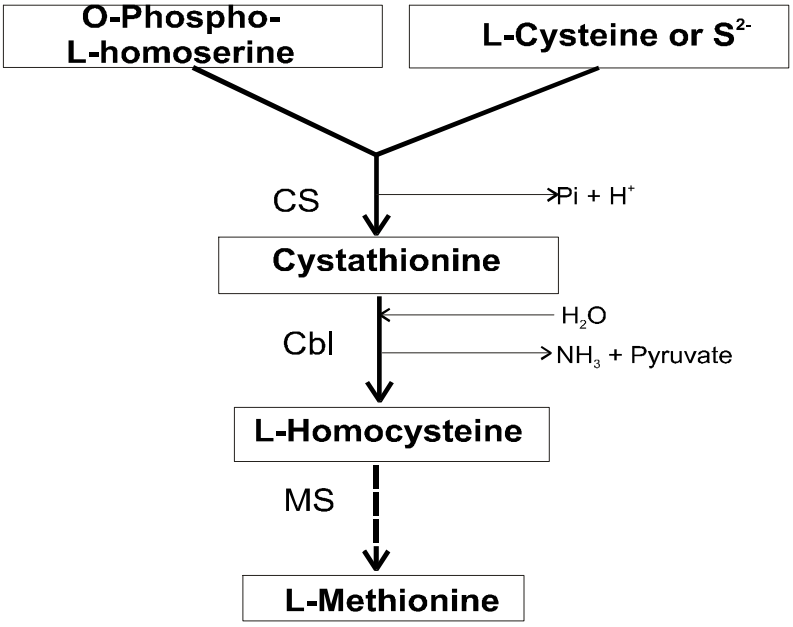


Fig. 1. *Branched pathway of methionine synthesis in plants.*

Enzyme assays performed on chloroplast-enriched fractions show that the majority of the Cbl activity, if not all, is localised in the plastid [2]. An attempt to identify the *cystathionine beta lyase* localisation in plant cells is the experiment initiated in the Laboratory of Plant Genetics (Free University Brussels).

The strategy of this work is based on the following idea: if a Met-auxotrophic mutation at the *cbl* level in plants may be complemented by a TP – *metC* construct, implying a chloroplastic localisation for MetC (cystathionine beta lyase enzyme in bacteria), the Cbl enzyme localised in chloroplast is necessary and sufficient for methionine biosynthesis.

The strategy followed in our experiments is presented in Fig. 2. The goal of our work is the characterisation of *metC* transformants, obtained in the Laboratory of Plant Genetics (Free University Brussels). In order to prove the successful complementation of the auxotrophic mutation, two transformants (T_1 and T_4) and the wild type (P_2) *Nicotiana plumbaginifolia* have been analysed at biochemical and nucleic acid level.

The work presented in this paper points on two main problems: 1. the presence of *metC* insertion(s) in analysed plants and 2. the *metC* transcription level in T_1 and T_4.

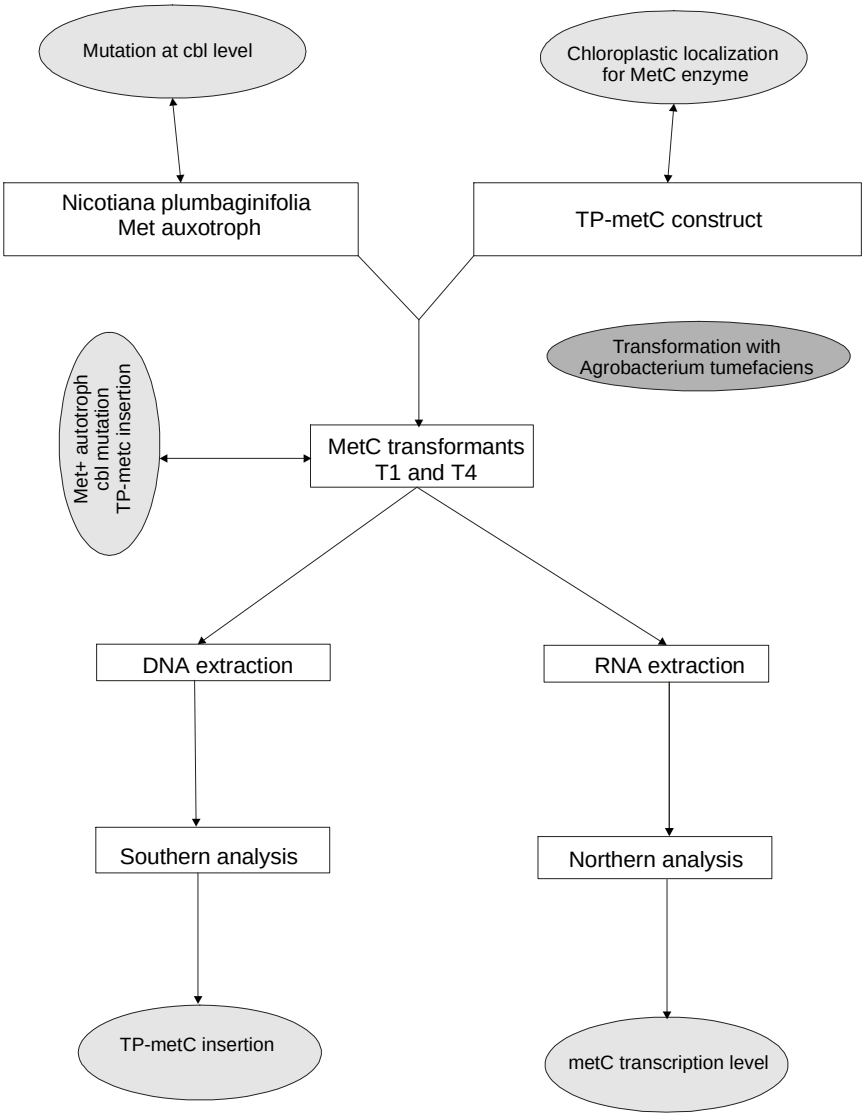


Fig. 2. Step by step strategy.

Materials and methods. *Plant material. Nicotiana plumbaginifolia* plants, auxotrophic for methionine, carrying a mutation at *cbl* level [5], have been transformed using *Agrobacterium tumefaciens* technique.

A pBIN vector has been used, containing: 1. the *metC* gene from *Escherichia coli* (coding for cystathionine beta lyase enzyme) under the control of a CaMV 35S promoter; 2. the *nptII* gene (neomycin phosphotransferase) for the selection of transformants, based on their resistance on kanamycin-containing medium; 3. the transit peptide (TP) corresponding to the spinach Rubisco small subunit, fused in frame with *metC* gene.

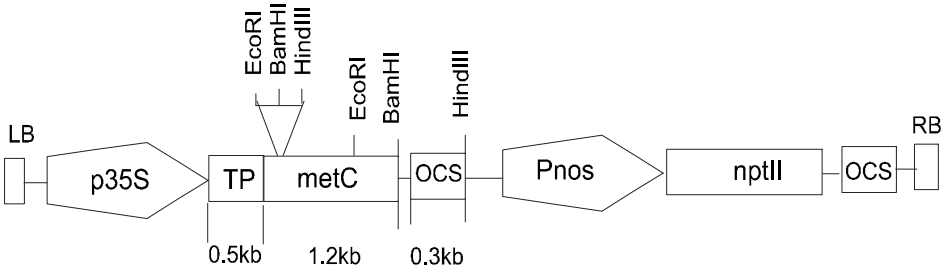


Fig.3. *The metC construct.*
LB - Left border. p35S - CaMV promoter. TP - Transit peptide. metC - Cystathionine beta lyase gene from *Escherichia coli*. OCS - Octopine synthase terminator. Pnos - Nopaline synthase promoter. nptII - Neomycin phosphotransferase gene. RB - Right border.

The recovered *metC* transformants (T_1 and T_4) are able to survive on medium without methionine. This is a direct proof that the exogenous *metC* gene complements the cbl mutation in auxotrophic *N. plumbaginifolia* mutants, due to the fact that the MetC enzyme targeted in chloroplast is sufficient to reinstall the autotrophy for methionine.

DNA and RNA analyses. *Nicotiana plumbaginifolia* genomic DNA was extracted from leaves using the technique described by Dellaporta *et al.* [1]. The DNA was digested using three different enzymes: EcoRI, BamHI and HindIII. On the agarose gel (0.8%), 10 μg DNA was loaded on each slot.The total RNA was isolated and RNA electrophoresis was performed according to Goldberg [3]. Standard Southern and Northern blot techniques have been used, with 0.4 N NaOH as a transferring agent. For DNA and RNA transfer, positively charged nylon membrane, purchased from Boehringer, has been used. The prehybridisation and hybridisation were performed in Church buffer (25% 1 M sodium phosphate buffer, 5% 5 M sodium chloride; 0.2% 0.5 M EDTA, 7% SDS).

The BamHI fragment of the *metC* gene (Fig.3) was used as a probe for Southern and Northern analyses. The BamHI fragment of the *metC* (1.2 kb) was separated on 0.8% agarose gel, electroeluted and further labelled with α-^{32}P-dCTP, 3000 Ci/mmol, using Rediprime Kit from Amersham.

Results and discussion. *Southern analysis.* For the analysed transformants (T_1 and T_4) three radioactive signals, corresponding to the three different restriction enzymes used, EcoRI, BamHI and HindIII, were obtained, at the expected locations: 1.2 kb for BamHI, 0.9 kb for EcoRI and 1.5 kb for HindIII (Fig.4).

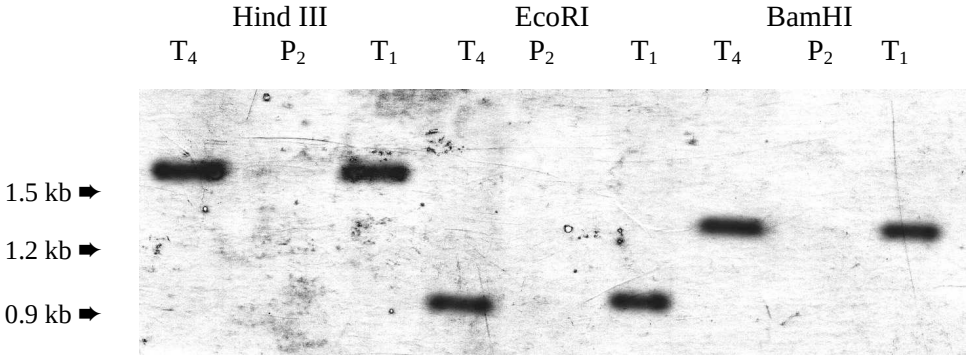


Fig. 4. *Southern analysis using* metC *probe.*

The restriction enzymes used for DNA analysis cut the BamHI fragment of the *metC* gene at the following positions:

Restriction enzyme	Restriction site in BamHI fragment (1.2 kb)
BamHI	0/1187 bp
EcoRI	891 bp
HindIII	12 bp

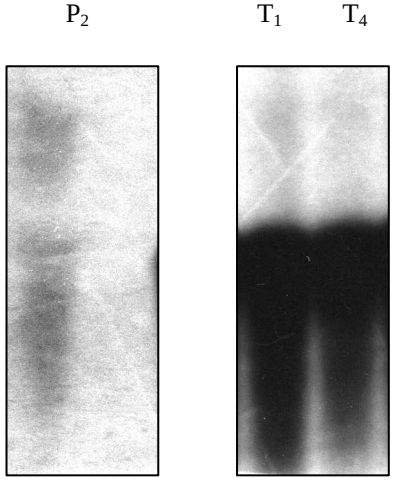


Fig. 5. *Northern analysis using* met C *probe.*

Corresponding to the cutting pattern of EcoRI and BamHI in the BamHI probe, the radioactive probe will hybridise with the two corresponding plant genomic DNA fragments : 0.9 kb for EcoRI and 1.2 kb for BamHI.

In the case of HindIII the first restriction site is located in the *metC* gene at position 12 bp, and the second restriction site is located after the *metC* terminator. The HindIII fragment will comprise the *metC* gene and *OCS* terminator, having a size of 1.5 kb. The corresponding radioactive signal is recovered after the hybridisation with BamHI probe at the expected location: 1.5 kb.

Northern analysis. A very strong signal was obtained for the analysed transformants, while no signal was present for the wild type (Fig. 5).

The transcription rate for *metC* gene in the transformants is imposed by the 35S promoter. This high inducible promoter allows a high transcription rate of the *metC* gene. As a fact, a large amount of mRNA coding for MetC protein will be produced and, consequently, the radioactive signal will be very strong in the Northern analysis, even after a short exposure time.

Due to the small homology between the *metC* gene and the *cbl* gene, the radioactive *metC* probe will not hybridise with the endogenous Cbl-mRNA from wild type. No signal for P_2 was obtained in the Northern analysis. Nevertheless, a small amount of Cbl-mRNA will be produced in wild type, under the control of the *cbl* natural promoter.

Conclusions. 1. The analysed T_1 and T_4 plants contain *metC* gene from *Escherichia coli*. The transcription rate of this gene in the transformants is very high, due to the presence of the 35S promoter.

2. The absence of hybridisation with the radioactive *metC* probe in the case of the wild type is most likely due to the fact that the homology between the *metC* bacterial gene and the endogenous *cbl* gene is not sufficient to allow the hybridisation under the established conditions.

R E F E R E N C E S

1. Dellaporta, S.L., Wood, J., Hicks, J.B.I., *A plant DNA mini preparation version II*, "Plant Molec. Biol. Reporter", **1**, 1983, 19-21.
2. Droux, M., Ravanel, S., Douce, R., *Methionine biosynthesis in higher plants. II. Purification and characterization of cystathionine beta lyase from spinach chloroplasts*, "Arch.Biochem.Biophys.", **316**, 1995, 585-595.

3. Goldberg, L., *RNA electrophoresis method*, "Proc. Natl. Acad. Sci. USA", **77**,1980, 5794.
4. Jacobs, M., Frankard, V., Ghislain, M., Vauterin, M. *The genetics of aspartate - derived amino acids in higher plants*, in Wallsgrove, R.M. (Ed.), *Amino Acids and Their Derivatives in Higher Plants*, pp. 29-50, Cambridge Univ. Press, Cambridge, 1995.
5. Negruțiu, I., De Brouwer, D., Dirks, R.,Jacobs, M., *Amino acid auxotrophs from protoplast cultures of* Nicotiana plumbaginifolia, *Viviani. 1. BUdR enriched selection, plant regeneration and general characterization*, "Molec. Gen. Genet.", **199**, 1985, 330-338.
6. Ravanel, S., Droux, M., Douce, R. *Methionine biosynthesis in higher plants. I. Purification and characterization of cystathionine gamma synthase from spinach chloroplasts*, "Arch. Biochem. Biophys.", **316**, 1995, 572-584.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, XLV, 1, 2000

CERCETĂRI ENZIMOLOGICE ASUPRA NĂMOLURILOR DIN LACURILE SALINE DE LA BAZNA ŞI BLAJ

MIHAIL DRĂGAN-BULARDA[*], IOAN-CĂLIN POPTELECAN[*], VASILE MUNTEAN[**], RADU CRIŞAN[**] şi DANIELA PAŞCA[**]

SUMMARY. – **Enzymological Research on Muds from the Salt Lakes in Bazna and Blaj**. Seasonal enzymological analyses were performed on muds from two salt lakes in Bazna (Sibiu county) and Blaj (Alba county). Four enzymatic activities (phosphatase, catalase, actual and potential dehydrogenase), as well as nonenzymatic catalytic H_2O_2-splitting capacity were measured quantitatively. The studied enzymatic and nonenzymatic catalytic activities were high through all seasons, with irregular seasonal variations. The enzymatic indicators of mud quality were calculated. The mud from the salt lake in Bazna had a higher enzymatic potential than that from the salt lake in Blaj, according to the values of the enzymatic indicator of mud quality. Other four enzymatic activities (maltase, cellobiase, invertase and lactase) were determined qualitatively. The results of the qualitative analyses confirmed those of the quantitative ones in respect of the higher enzymatic potential of mud from the salt lake in Bazna.

În țara noastră s-au efectuat numeroase cercetări enzimologice asupra sedimentelor lacurilor saline, în special asupra acelora folosite în balneoterapie [1-3, 5, 6, 8, 9, 11-16]. Lucrarea de față își propune să lărgească sfera cercetărilor întreprinse de autorii citați, prin studierea unui lac deja cunoscut pentru calitățile terapeutice ale nămolului său (Bazna) şi a unuia încă neintrat în circuitul balnear (Balta Sărată de la Blaj).

Sedimentul lacului salin de la Bazna este folosit de multă vreme ca nămol terapeutic, pentru tratarea unor afecțiuni reumatismale degenerative, inflamatorii, post-traumatice, neurologice periferice, ginecologice etc. Nămolul lacului Balta Sărată de lângă Blaj nu este exploatat într-un mod organizat în balneoterapie, dar calitățile lui curative sunt atestate de localnicii care în sezonul cald practică împachetări cu nămol, cu efecte benefice asupra stării lor de sănătate.

[*] *Universitatea "Babeş-Bolyai", Catedra de biologie vegetală, 3400 Cluj, România*
[**] *Institutul de Cercetări Biologice, 3400 Cluj, România*

Material şi metode. Asupra nămolurilor din lacurile de la Bazna (jud. Sibiu) şi Blaj (jud. Alba) au fost efectuate cercetări enzimologice cantitative şi calitative sezoniere în perioada 1996-1997. Din lacul de la Bazna au fost prelevate şi analizate probe de nămol din trei puncte: nord, centru (de unde se extrage şi nămolul pentru tratament) şi sud. Din lacul Balta Sărată de la Blaj eşantioanele au fost prelevate din două puncte: nord şi sud.

Au fost determinate cantitativ următoarele activităţi enzimatice şi catalitice neenzimatice: activitatea fosfatazică (metoda Krámer şi Erdei [10]); scindarea enzimatică (activitatea catalazică) şi neenzimatică a H_2O_2 (metoda Kappen [7]); reducerea clorurii de 2,3,5-trifeniltetrazoliu (TTC) în probe neautoclavate fără adaos de glucoză (activitatea dehidrogenazică actuală) sau cu adaos de glucoză (activitatea dehidrogenazică potenţială) (metoda Casida şi colab. [4]). Activitatea fosfatazică se exprimă în mg fenol/2,5 g nămol substanţă uscată; capacitatea de scindare a H_2O_2 se exprimă în mg H_2O_2 descompusă/1,5 g nămol substanţă uscată; activitatea dehidrogenazică se exprimă în mg formazan/0,5 mg nămol substanţă uscată.

Analizele enzimologice calitatitve au cuprins activităţile maltazică, celobiazică, zaharazică şi lactazică. Determinarea acestor activităţi enzimatice s-a efectuat prin cromatografie pe hârtie, tehnica circulară (Wright şi colab. [17]). Intensitatea activităţilor enzimatice stabilită pe baza spoturilor de culoare date de monozaharidele formate în urma hidrolizei substraturilor enzimatice a fost marcată cu semne de +.

Rezultate. Toate cele cinci activităţi determinate cantitativ au avut valori decelabile în toate sezoanele şi la toate punctele studiate. Excepţie face activitatea dehidrogenazică actuală, care nu a putut fi evidenţiată în punctele nordice de prelevare din ambele lacuri, în sezoanele de primăvară, toamnă şi iarnă, respectiv activitatea dehidrogenazică potenţială, fără valori decelabile în partea de nord a lacului Balta Sărată de la Blaj, primăvara şi toamna (Tabelul 1). Valoarea maximă a activităţii dehidrogenazice actuale este consemnată vara în partea de sud a lacului Balta Sărată (8,822 mg formazan/0,5 g nămol substanţă uscată). Valoarea maximă a activităţii dehidrogenazice potenţiale este înregistrată primăvara, în centrul lacului de la Bazna. Se poate afirma, deci, că în sedimentele ambelor lacuri studiate există o activitatea dehidrogenazică a cărei intensitate depinde mai puţin de adaosul unor surse suplimentare de carbon, probabil datorită prezenţei în aceste nămoluri a unei cantităţi suficiente de substanţe organice, care asigură o dezvoltare bună a microorganismelor a căror activitate respiratorie este măsurată prin testul reducerii TTC.

Activitatea fosfatazică înregistrează atât valoarea maximă (4,068 mg fenol/ 2,5 g nămol substanţă uscată – toamna) cât şi cea minină (0,111 mg fenol/ 2,5 g nămol substanţă uscată – iarna) în partea de sud a lacului de la Bazna. Desigur, valoarea maximă înregistrată toamna este datorată acumulării în sediment a resturilor organice, la sfârşitul perioadei de vegetaţie.

Tabel 1

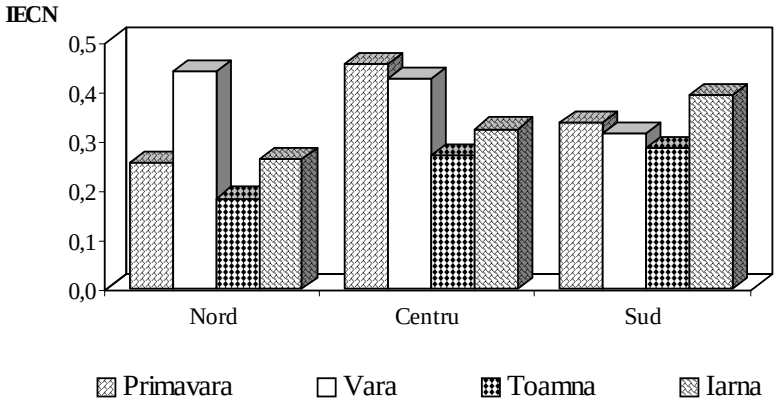
Rezultatele analizelor activităților enzimatice și catalitice neenzimatice cantitative

Lacul	Sezonul	Locul prelevării	Activitatea fosfatazică (mg fenol/2,5 g nămol s.u.)	Activitatea catalitică (mg H_2O_2 descompusă/1,5 g nămol s.u.)		Activitatea dehidrogenazică (mg formazan/0,5 g nămol s.u.)	
				Enzimatică	Neenzimatică	Actuală	Potențială
Bazna	Primăvara	Nord	1,535	28,591	30,306	1,000	1,954
		Centru	3,916	24,341	38,338	6,098	7,871
		Sud	1,430	34,190	23,393	3,455	5,290
	Vara	Nord	0,511	21,195	70,653	4,064	4,550
		Centru	0,186	12,792	66,654	4,951	5,597
		Sud	1,102	26,392	38,573	2,774	3,098
	Toamna	Nord	1,776	21,611	27,013	0	0,239
		Centru	2,750	28,970	37,662	0,226	1,174
		Sud	4,068	27,932	29,641	1,745	1,846
	Iarna	Nord	0,429	24,289	52,725	0,000	0,042
		Centru	3,098	18,964	45,304	2,245	3,068
		Sud	0,111	43,01	42,258	3,009	3,981
Blaj	Primăvara	Nord	2,295	28,188	39,796	0	0
		Sud	1,609	8,093	20,232	8,301	4,597
	Vara	Nord	3,386	26,889	57,853	4,747	6,165
		Sud	3,119	35,718	44,43	8,822	6,717
	Toamna	Nord	0,256	36,686	40,51	0	0
		Sud	1,213	25,694	30,721	4,622	3,373
	Iarna	Nord	2,617	17,134	33,163	0	0,196
		Sud	1,081	19,037	33,459	7,655	2,008

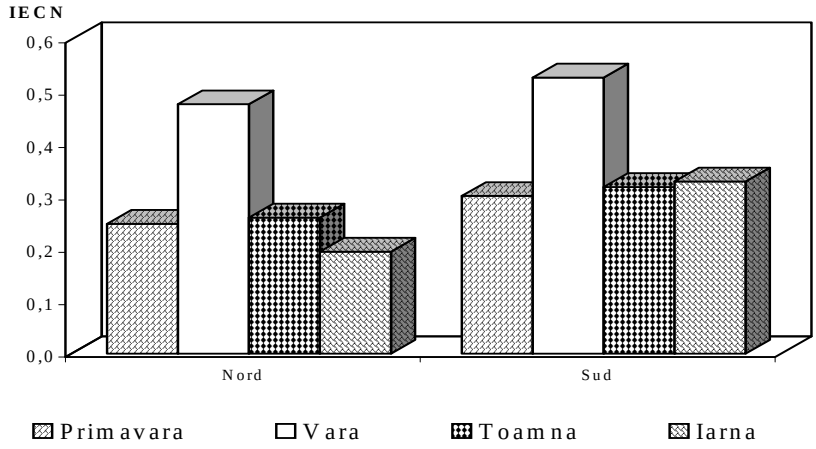
Intensitatea minimă a capacității de scindare a H_2O_2 este consemnată primăvara, în partea de sud a lacului Balta Sărată de la Blaj (8,093 mg H_2O_2 descompusă/1,5 g nămol substanță uscată – activitatea catalazică, respectiv 20,232 mg H_2O_2 descompusă/1,5 g nămol substanță uscată – scindarea neenzimatică). Valorile maxime ale acestor activități sunt înregistrate iarna în partea de sud a lacului de la Bazna (43,010 mg H_2O_2 descompusă/1,5 g nămol substanță uscată – activitatea catalazică), respectiv vara, în partea de nord a aceluiaşi lac (70,653 mg H_2O_2 descompusă/1,5 g nămol substanță uscată – scindarea neenzimatică). Aşadar, pe lângă o activitatea catalazică remarcabilă, notăm o şi mai intensă activitate de scindare neenzimatică a H_2O_2.

Pe baza valorilor absolute ale activităților enzimatice şi catalitice neenzimatice s-a calculat indicatorul enzimatic al calității nămolului (IECN), care rezultă din împărțirea la numărul activităților studiate a sumei raporturilor dintre valorile reale şi cele teoretice maxime [12].

În Fig. 1 şi 2 sunt reprezentate grafic valorile indicatorilor enzimatici sezonieri ai calității nămolurilor din fiecare punct de prelevare din cele două lacuri. Se poate observa că valoarea cea mai ridicată a potențialului enzimatic înregistrat sezonier în cele trei puncte de prelevare din lacul Bazna se întâlneşte primăvara, în zona centrală a lacului (IECN = 0,453). Valoarea cea mai scăzută a acestui potențial este consemnată toamna, în partea de nord a lacului (IECN = 0,182). Surprinzătoare este evoluția potențialului enzimatic în partea de sud a lacului, cu valori descrescătoare ale IECN dinspre primăvară spre toamnă, cu un maxim consemnat iarna.



F i g. 1. *Indicatorii enzimatici sezonieri ai calit¾ßii n¾molului din cele trei puncte de prelevare din lacul de la Bazna*.

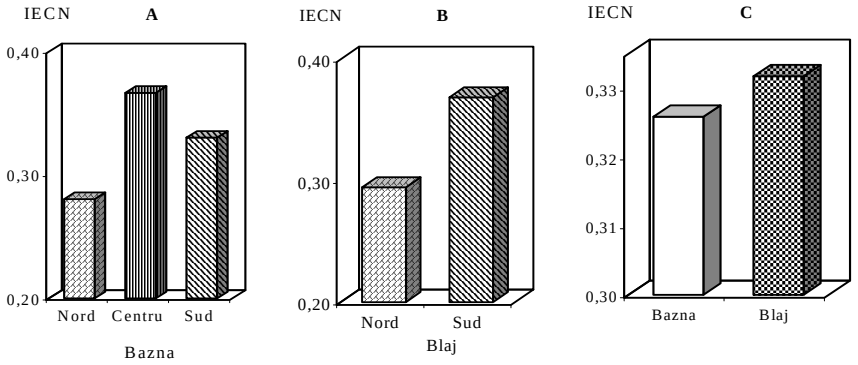


F i g. 2. *Indicatorii enzimatici sezonieri ai calit¾ßii n¾molului din cele dou¾ puncte de prelevare din lacul de la Blaj*.

În cazul lacului Balta Sărată de la Blaj, nămolul din partea de sud are un potențial enzimatic superior celui al nămolului din partea de nord. Valorile maxime ale IECN sunt înregistrate vara (0,527 – sud, respectiv 0,476 – nord). În celelalte sezoane indicatorii enzimatici ai calității nămolului depăşesc valoarea de 0,300 în sud, iar în nord se situează în jurul valorii de 0,200.

În Fig. 3 sunt reprezentate valorile indicatorilor enzimatici anuali ai calității nămolurilor din fiecare punct de prelevare, respectiv valorile indicatorilor enzimatici globali pentru cele două lacuri studiate. În lacul de la Bazna potențialul enzimatic cel mai remarcabil este consemnat în zona centrală (IECN = 0,367), de unde se şi extrage nămolul folosit pentru tratamente în stațiune. Potențialul enzimatic cel mai scăzut se înregistrează în partea de nord a lacului (IECN = 0,281), fapt explicabil şi prin impactul semnificativ al factorului antropic, în această parte a lacului deversându-se intermitent nămolul recuperat de pe pacienți, în urma tratamentelor aplicate în stațiune. Sedimentul lacului Balta Sărată de la Blaj are valori ale IECN mai apropiate (0,369 în sud, respectiv 0,295 în nord).

Calculați pe baza valorilor medii globale ale activităților enzimatice şi catalitice neenzimatice determinate în fiecare punct de prelevare din cele două lacuri, în fiecare sezon, indicatorii enzimatici globali ai calității nămolurilor din cele două lacuri (Fig. 3, C) caracterizează la modul cel mai cuprinzător potențialul enzimatic al sedimentelor lacurilor studiate. Pe această bază se poate afirma că potențialul enzimatic al nămolului din lacul Balta Sărată de la Blaj (IECN = 0,332) este superior celui al nămolului din lacul de la Bazna (IECN = 0,326), dar diferența este destul de mică.



F i g. 3. *Indicatorii enzimatici ai calit¾ßii n¾molului din cele dou¾ lacuri de la Bazna ¿i Blaj studiate*.
A ¿i B – IECN anuali pentru fiecare punct de prelevare. C – IECN globali.

Într-o lucrare citată anterior [12] s-a făcut o clasificare a 56 de lacuri saline din România, pe baza valorilor indicatorilor enzimatici ai calității sedimentelor lor. Deşi clasificarea s-a făcut în urma determinării a 7 activități enzimatice şi catalitice neenzimatice (față de cele 5 studiate în cazul de față s-a mai analizat capacitatea de reducere neenzimatică a TTC în amestecuri de reacție cu sau fără adaos de glucoză), modalitatea de calculare a indicatorilor permite raportarea la această clasificare. În acest sistem, lacul Balta Sărată de la Blaj s-ar situa pe locul 34, având aceeaşi valoare a IECN cu lacul Balta Albă, iar lacul de la Bazna pe poziția imediat următoare, înaintea lacului Nou Format de la Ocna Sibiului.

În ceea ce priveşte activitatea enzimelor determinate calitativ, rezultatele obținute sunt prezentate în Tabelul 2. Se poate constata că toate cele 4 oligaze determinate au putut fi evidențiate în nămolul celor două lacuri. Şi de această dată nămolul lacului Balta Sărată de la Blaj este mai activ pe plan enzimologic, comparativ cu cel din lacul de la Bazna, trei din cele patru activități enzimatice evidențiate fiind mai intense în primul.

Tabel 2

Rezultatele analizelor enzimatice calitative

Lacul	Locul prelevării	Activitatea enzimatică determinată			
		Maltazică	Celobiazică	Zaharazică	Lactazică
Bazna	Centru	++	+	+	+
Blaj	Sud	+++	++	++	+

Concluzii. 1. Este consemnată existența unor activități enzimatice destul de intense în sedimentele celor două lacuri studiate, care variază în funcție de anotimp și de locul de prelevare. De asemenea, se înregistrează un nivel ridicat al activității catalitice neenzimatice de scindare a H_2O_2.

2. Pe baza valorilor indicatorilor enzimatici globali ai calității nămolurilor din cele două lacuri studiate se poate afirma că nămolul din lacul Balta Sărată de la Blaj (IECN = 0,332) este superior celui din lacul Bazna (IECN = 0,326). Observația are o importanță deosebită, luând în considerare faptul că nămolul din lacul de la Blaj nu este exploatat științific în balneoterapie, ci numai empiric, de către localnicii care îi atribuie calități terapeutice.

3. Analiza calitativă a celor patru oligaze (maltază, celobiază, zaharază și lactază) crește complexitatea aprecierii potențialului enzimatic general al nămolurilor din cele două lacuri studiate, rezultatele analizelor calitative confirmându-le pe cele ale analizelor cantitative.

4. Cercetările efectuate ilustrează importanța sistemului de clasificare a lacurilor saline pe baza valorilor indicatorilor enzimatici ai calității nămolurilor, care permite identificarea și recomandarea folosirii în balneoterapie a nămolurilor mai active din punct de vedere enzimatic și care sunt terapeutic mai eficiente, fapt dedus empiric, în urma unei îndelungate tradiții. În acest sens se impune atenției lacul Balta Sărată de la Blaj.

B I B L I O G R A F I E

1. B u l g ă r e a n u, V.-A.C., K i s s, S., E n a c h e, G., C u ş m i r e n c o, G., U r c a n, T., P a ş c a, D., D r ă g a n - B u l a r d a, M., C r i ş a n, R., Z b o r o v s c h i, E., *Some data concerning the relations between limnological factors, mineralogical composition and enzymatic and nonenzymatic catalytic activities of pelogenous sediments from brackish and salt lakes*, "Stud. Univ. Babeş-Bolyai, Biol.", **34** (1), 1989, 69-81.
2. B u l g ă r e a n u, V.-A.C., K i s s, S., P a ş c a, D., D r ă g a n - B u l a r d a, M., C r i ş a n, R., P i n t e a, H., Z b o r o v s c h i, E., *The relations between the limnogeology and enzymology of sediments from some brackish and salt lakes*, "Rev. Roum. Géol., Géophys. Géogr., Géol.", 1987, **31**, 107-114.
3. 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.
4. 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.

5. C r i ş a n, R., P a ş c a, D., M u n t e a n, V., Z b o r o v s c h i, E., D r ă g a n–B u l a r da M., K i s s, S., *Enzymological study of muds from salt lakes in Turda (Cluj district)*, "Stud. Univ. Babeş-Bolyai, Biol.", **33** (2), 1988, 76-86.
6. D r ă g a n – B u l a r d a, M., K i s s, S., R ă d u l e s c u, D., C r i ş a n, R., P i n t e a, H., K o l o z s i, E., B u l g ă r e a n u, V.-A., *Studierea enzimologică a nămolurilor din lacurile de la Ocna Sibiului*, în *Lucr. celui de al III-lea Simp. Microbiol. Ind. (Bucureşti, 1981)*", p. 700-709, Inst. Centr. Biol., Bucureşti, 1982.
7. K a p p e n, H., *Die katalytische Kraft des Ackerbodens*, "Fühlings Landw. Ztg.", **62**, 1913, 377-392.
8. 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.
9. 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.
10. K r á m e r, M., E r d e i, G., *Primenenie metoda opredeleniia aktivnosti fosfatazî v agrohimiceskih issledovaniiah*, "Pocivovedenie", No.9, 1959, 99-102.
11. 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.
12. 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.
13. M u n t e a n, V., P a ş c a, D., C r i ş a n, R., K i s s, S., D r ă g a n – B u l a r d a, M., *Enzymological research on sediments from the Ursu and Negru salt lakes (Sovata, Mureş county)*, "Stud. Univ. Babeş-Bolyai, Biol.", **44** (1-2), 1999, 199-207.
14. P a ş c a, D., K i s s, S., P i n t e a, H., *Cercetări enzimologice la nămolul din lacul Băile de la Cojocna (jud. Cluj)*, în *Actualitate şi perspectivă în biologie. Structuri şi funcţii în ecosisteme terestre şi acvatice*, p. 265-274, Centr. Cercet. Biol., Cluj-Napoca, 1985.
15. P a ş c a, D., M u n t e a n, V., C r i ş a n, R., P i n t e a, H., Z b o r o v s c h i, E., I l i e s c u, M., I l i e s c u, C., D r ă g a n – B u l a r d a, M., K i s s , S., *Evolution of the enzymatic potential of mud in the Techirghiol salt lake*, "Evol. Adapt." (Cluj-Napoca), **4**, 1991, 119-123.
16. R ă d u l e s c u, D., K i s s, S., 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., *Studierea enzimologică a nămolurilor din lacurile de la Slănic-Prahova*, "Stud. Univ. Babeş-Bolyai, Biol.", **25** (1), 1980, 66-72.
17. W r i g h t, S.W., U l s t r ö m, R.A., S z e w c z a k, J.T., *Studies of carbohydrates in body fluids. I. Identification by means of paper chromatography*, "Am. Med. Assoc. J., Dis. Children", **93**, 1957, 173-181.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, XLV, 1, 2000

INDUCȚIA ENZIMATICĂ ÎN SOL CA TEST ECOTOXICOLOGIC PENTRU POLUANȚI ANORGANICI ŞI ORGANICI

ALIONA POPA*

SUMMARY. - Enzymatic Induction in Soil as Ecotoxicological Test for Inorganic and Organic Pollutants. In a laboratory experiment modelling soil pollution, 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 fuel oil and phenol), as potential pollutants, were added to samples of an alluvial soil for studying their effect on the induction of microbial synthesis of levansucrase. Sucrose, as specific substrate of levansucrase, served as inductor. Rates of the additions (per 100 g air-dry soil) were the following: 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; 0.001, 0.01 and 0.1 ml phenol; and 10 g sucrose. Soil samples, to which no pollutant or no sucrose was added, were the controls. All samples, representing 20 and 17 experimental variants (treated with heavy metal ions and potential organic pollutants, respectively) were moistened to 60% of water-holding capacity and incubated at room temperature for 18 days, then analysed to determine their levansucrase activity by means of circular paper chromatography.

The results have shown that the heavy metal ions inhibited activity and synthesis of levansucrase in the order: Hg^{2+}> Cd^{2+}> Co^{2+}> Zn^{2+}> Pb^{2+}>Cu^{2+}. Both detergents enhanced activity and synthesis of levansucrase, the enhancing effect of the detergent "Ariel" (manufactured with addition of enzymes) was more pronounced than that of the detergent "Rex" (containing no enzymes). 2,4-D strongly inhibited, while fuel oil and phenol slightly inhibited the activity and synthesis of levansucrase. The effect of heavy metal ions, detergents and 2,4-D increased with increasing rate of additions, but the effect of fuel oil and phenol did not change in dependence of the rate of additions.

The conclusion has been drawn that induction of microbial levansucrase synthesis in soil may be used as an ecotoxicological test for both inorganic and organic pollutants.

* *Universitatea "Babeş-Bolyai", Catedra de biologie vegetală, 3400 Cluj, România*

Ideea inducției secvențiale a sintezei enzimelor de către microorganismele din sol a fost formulată pe baza unor cercetări, în care probe de sol au fost compostate cu melasă. Zaharoza din melasă, acționând ca substrat enzimatic, a indus sinteza microbiană a levansucrazei şi dextransucrazei care au catalizat apoi sinteza polizaharidelor levan şi dextran. La rândul lor, aceste polizaharide au indus sinteza microbiană a levanazei şi dextranazei, polizaharidaze care catalizează hidroliza levanului şi, respectiv, a dextranului. Dat fiind că levanul şi dextranul contribuie la agregarea particulelor de sol, la formarea structurii favorabile a solului, levansucraza şi dextransucraza joacă un rol pozitiv, iar levanaza şi dextranaza au un rol negativ din punctul de vedere al fertilității solului [1]. La aceste cercetări se fac referiri şi într-o lucrare de sinteză asupra activității şi importanței agricole a polizaharidazelor din sol [2].

În prezenta lucrare s-a studiat posibilitatea folosirii inducției sintezei microbiene a levansucrazei în sol ca test ecotoxicologic pentru poluanți anorganici şi organici.

Material şi metodă. S-a efectuat un experiment de laborator pentru modelarea poluării solului. S-a lucrat cu acelaşi sol aluvial (probe de 100 g, uscate la aer) şi cu aceiaşi poluanți anorganici şi organici, care s-au folosit şi pentru studierea activității dehidrogenazice [3].

Ca inductor a servit substratul specific al levansucrazei, zaharoza, care s-a adăugat sub formă de pulbere în cantitate de 10 g/100 g sol uscat la aer.

Pentru studierea poluanților anorganici şi organici s-au realizat 20 şi, respectiv, 17 variante experimentale. Schema lor este prezentată în Tabelele 1 şi 2.

Solul fiecărei variante experimentale a fost umezit la 60% din capacitatea de reținere a apei. Compostarea a avut loc la temperatura camerei şi a durat 18 zile.

După compostare, probele de sol au fost lăsate să se usuce la aer, apoi li s-a determinat activitatea levansucrazică. În acest scop, s-au preparat amestecuri de reacție din câte 3 g sol + 2 ml toluen (antiseptic) + 10 ml soluție apoasă de substrat (zaharoză 10%, greutate/volum). Drept martori au servit amestecuri de reacție la care, în locul soluției de zaharoză, s-a adăugat apă distilată (10 ml); s-a preparat şi un amestec de reacție martor fără sol (constând numai din soluție de zaharoză şi toluen). Schema amestecurilor de reacție este redată în Tabelul 3.

Tabel 1

Schema variantelor experimentale realizate în vederea urmăririi inducției enzimatice în sol ca test ecotoxicologic pentru poluanți anorganici

Varianta	Sol (g)	Zaharoză (g)	Ionul metalic	Concentrația finală a ionului metalic (g/100 g sol)
V1	100	10	Hg^{2+}	0,001
V2	100	10		0,01
V3	100	10		0,1
V4	100	10	Cd^{2+}	0,001
V5	100	10		0,01
V6	100	10		0,1
V7	100	10	Zn^{2+}	0,001
V8	100	10		0,01
V9	100	10		0,1
V10	100	10	Pb^{2+}	0,001
V11	100	10		0,01
V12	100	10		0,1
V13	100	10	Co^{2+}	0,001
V14	100	10		0,01
V15	100	10		0,1
V16	100	10	Cu^{2+}	0,001
V17	100	10		0,01
V18	100	10		0,1
V19	100	10	Sol martor compostat cu zaharoză	-
V20	100	-	Sol martor necompostat cu zaharoză	-

Tabel 2

Schema variantelor experimentale realizate în vederea urmăririi inducției enzimatice în sol ca test ecotoxicologic pentru poluanți organici

Varianta	Sol (g)	Zaharoză (g)	Substanța organică	Concentrația finală a substanței organice (g sau ml/100 g sol)
V1	100	10	Detergent "Rex" (fără enzime)	0,1 g
V2	100	10		1 g
V3	100	10		2 g
V4	100	10	Detergent "Ariel" (cu enzime)	0,1 g
V5	100	10		1 g
V6	100	10		2 g

Tabel 2 (continuare)

Varianta	Sol (g)	Zaharoză (g)	Substanța organică	Concentrația finală a substanței organice (g sau ml/100 g sol)
V7	100	10	2.4-D	0,001 g
V8	100	10		0,01 g
V9	100	10		0,1 g
V10	100	10	Motorină	0,1 ml
V11	100	10		1 ml
V12	100	10		5 ml
V13	100	10	Fenol	0,001 ml
V14	100	10		0,01 ml
V15	100	10		0,1 ml
V16	100	10	Sol martor compostat cu zaharoză	-
V17	100	-	Sol martor necompostat cu zaharoză	-

Tabel 3

Schema amestecurilor de reacție pentru determinarea activității levansucrazice în solul variantelor experimentale realizate în vederea urmăririi inducției enzimatice

Numărul amestecului de reacție	Concentrația poluantului în variantele experimentale	Sol (g)	Toluen (ml)	Zaharoză, soluție 10% (ml)	Apă distilată (ml)
1	Minimă*	3	2	10	-
2		3	2	-	10
3	Mijlocie*	3	2	10	-
4		3	2	-	10
5	Maximă*	3	2	10	-
6		3	2	-	10
7	Sol martor compostat cu	3	2	10	-
8	zaharoză	3	2	-	10
9	Sol martor necompostat	3	2	10	-
10	cu zaharoză	3	2	-	10
11	Martor fără sol	-	2	10	-

* Vezi Tabelul 1 pentru concentrația ionilor metalici şi Tabelul 2 pentru concentrația substanțelor organice.

Amestecurile de reacție au fost incubate la 37^0C timp de 10 zile, apoi analizate pentru evidențierea levanului. Prezența levanului indică activitatea levansucrazică, deoarece levanul s-a format sub acțiunea levansucrazei. Analiza a fost efectuată prin metoda cromatografiei pe hârtie, tehnica circulară. S-a folosit hârtie Whatman 1. Din faza apoasă a amestecurilor de reacție s-au aplicat câte 14 µl (în câte două reprize a 7 µl) pe punctele de start ale hârtiilor cromatografice circulare. După uscare, cromatogramele au fost developate într-un sistem de dizolvanți alcătuit din *n*-propanol, acetat de etil şi apă distilată în proporție de 6:1:3 (volum/volum/volum). Developarea a avut loc la temperatura camerei şi a durat 2 ore. Final, cromatogramele au fost pulverizate cu un reactiv conținând uree şi acid *o*-fosforic; acest reactiv evidențiază, în mod specific, cetozele libere şi combinate, inclusiv levanul [4].

Rezultate. Pe cromatogramele redate în Fig. 1-6, levanul este uşor de identificat, fiind spotul observabil la punctul de start (R_f=0).

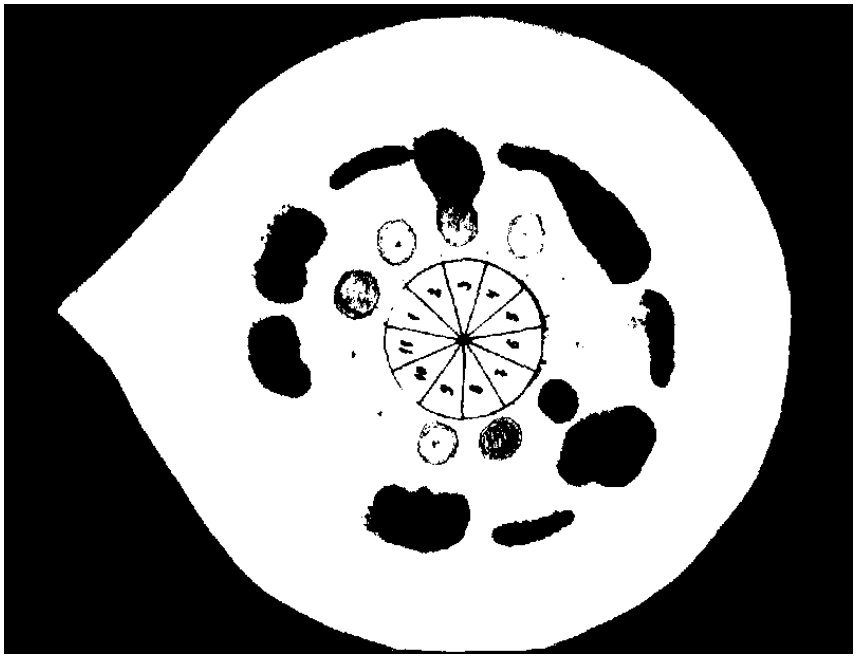


Fig. 1. *Activitatea levansucrazică în solul variantelor experimentale tratate cu* Hg^{2+}. Amestecurile de reacție 1-11: vezi Tabelul 3.

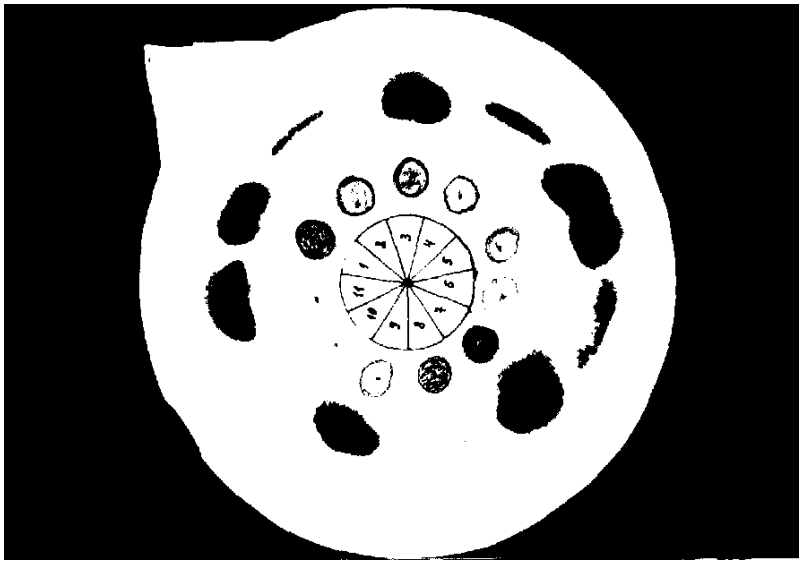


Fig. 2. *Activitatea levansucrazică în solul variantelor experimentale tratate cu* Cu^{2+}.
Amestecurile de reacție 1-11: vezi Tabelul 3.

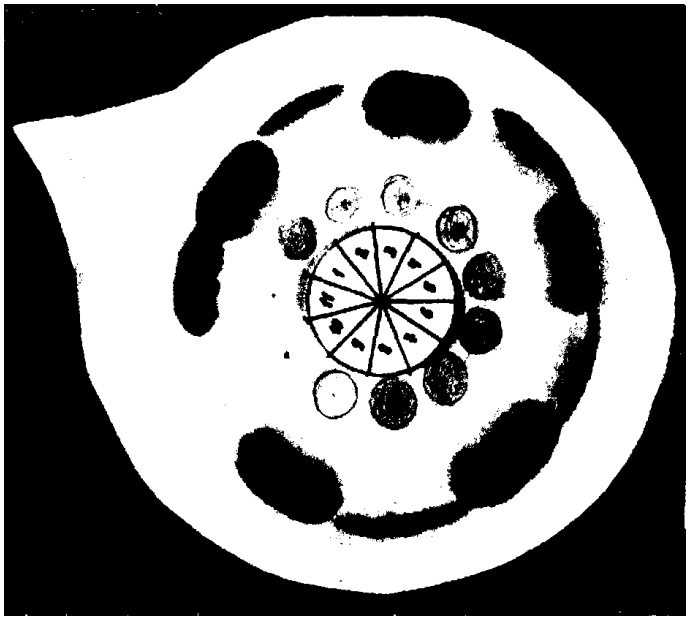


Fig. 3. *Activitatea levansucrazică în solul variantelor experimentale tratate cu detergentul "Rex"* (fabricat fără adaos de enzime).
Amestecurile de reacție 1-11: vezi Tabelul 3.

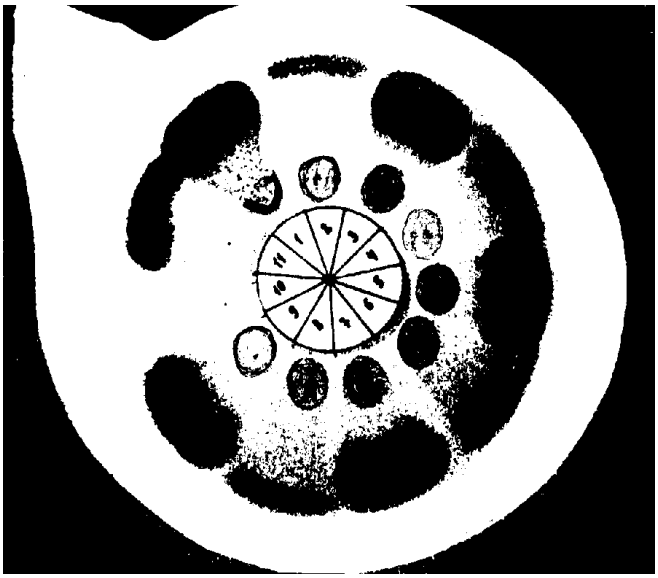


Fig. 4. *Activitatea levansucrazică în solul variantelor experimentale tratate cu detergentul "Ariel"* (fabricat cu adaos de enzime).
Amestecurile de reacție 1-11: vezi Tabelul 3.

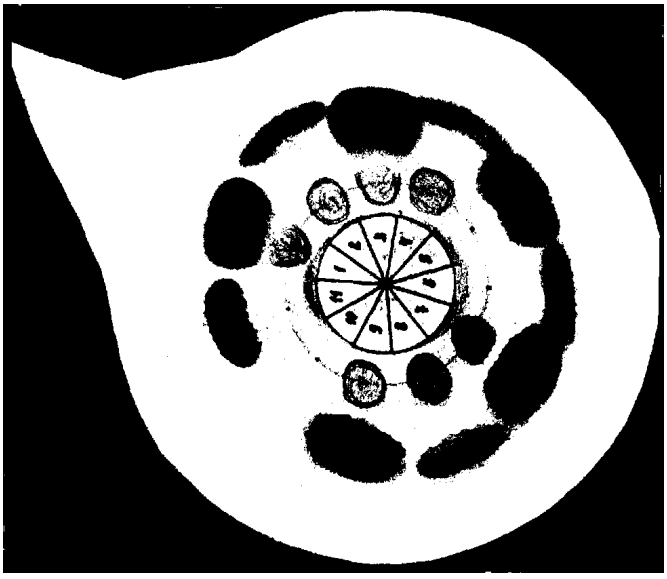


Fig. 5. *Activitatea levansucrazică în solul variantelor experimentale tratate cu ierbicidul hormonal 2,4-D.*
Amestecurile de reacție 1-11: vezi Tabelul 3.

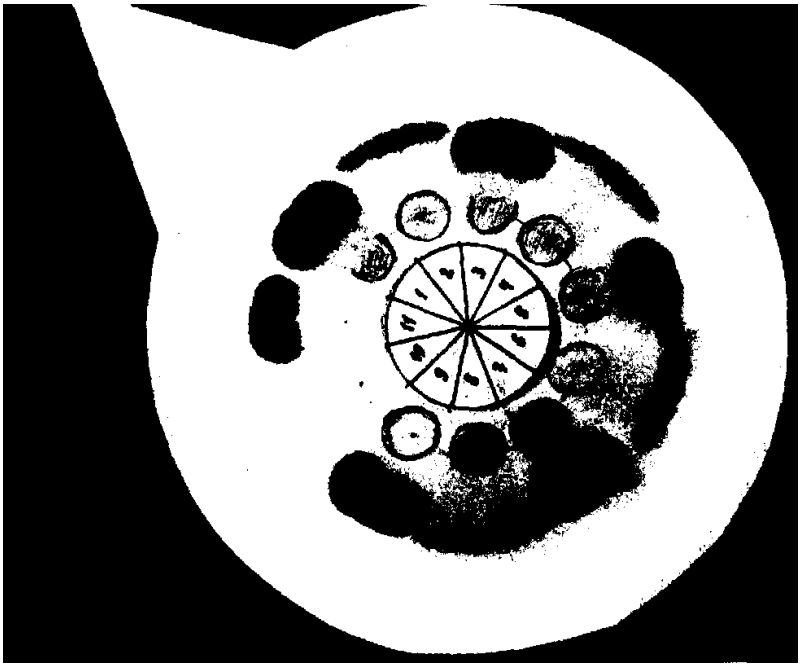


Fig. 6. *Activitatea levansucrazică în solul variantelor experimentale tratate cu motorină.* Amestecurile de reacție 1-11: vezi Tabelul 3.

Rezultatele s-au stabilit pe baza comparării spoturilor de levan la diferitele amestecuri de reacție.

A. Compararea amestecurilor de reacție cu sol martor 7-10 arată că spotul de levan este cel mai intens la amestecul de reacție 7, în care solul a fost compostat şi incubat cu zaharoză, ceea ce înseamnă că în timpul compostării a avut loc inducția sintezei microbiene a levansucrazei care şi-a păstrat activitatea şi după terminarea compostării. La amestecul de reacția 8, conținând sol compostat cu zaharoză, dar incubat fără zaharoză, se observă un spot mai slab de levan care s-a format în timpul compostării. Amestecul reacție 9, preparat din sol necompostat, dar incubat cu zaharoză, de asemenea arată prezența unui spot mai slab de levan care s-a format sub acțiunea levansucrazei preexistente (prezentă în sol înainte de compostare).În schimb, amestecul de reacție 10, în care solul nu a fost nici compostat şi nici incubat cu zaharoză, arată absența spotului de levan. Adăugăm că levanul este absent şi în amestecul de reacție 11 (martor fără sol), ceea ce dovedește că zaharoza folosită a fost un preparat pur, lipsit de levan.

B. Compararea amestecurilor de reacție 1, 3 şi 5 (conținând sol tratat cu cele trei concentrații de poluant, apoi compostat şi incubat cu zaharoză) cu amestecul de reacție 7 face posibilă stabilirea efectului poluantului asupra activității şi inducției sintezei microbiene a levansucrazei. Compararea amestecurilor de reacție 2, 4 şi 6 (în care solul a fost tratat cu poluant şi compostat cu zaharoză, dar incubat fără zaharoză) cu amestecul de reacție 10 de asemenea permite sesizarea efectului poluantului asupra activității şi sintezei levansucrazei.

Toți ionii de metale grele au inhibat activitatea şi sinteza levansucrazei, în următoarea ordine: Hg^{2+}> Cd^{2+}> Co^{2+}> Zn^{2+}> Pb^{2+}> Cu^{2+}. În Fig. 1 şi 2 redăm, pentru exemplificare, cromatogramele ilustrând activitatea levansucrazică în variantele experimentale tratate cu Hg^{2+} (ionul metalic cel mai inhibitor) şi în cele tratate cu Cu^{2+} (ionul metalic cel mai puțin inhibitor).

Efectuând comparările A şi B, putem constata că efectul inhibitor al Hg^{2+} este, în mod foarte evident, mult mai puternic decât cel al Cu^{2+}. Totodată, efectul inhibitor creşte cu creşterea concentrației de Hg^{2+} şi Zn^{2+}. Astfel, din amestecurile de reacție 5 şi 6, în care solul a fost tratat cu concentrația maximă de Hg^{2+} (0,1 g/100 g sol), levan nu s-a evidențiat (vezi Fig. 1), ceea ce dovedeşte că Hg^{2+} în această concentrație nu numai că a oprit inducerea sintezei microbiene a levansucrazei, dar a inactivat şi levansucraza preexistentă în sol.

Se poate vedea din cromatogramele redate în Fig. 3 şi 4 că detergenții "Rex" şi "Ariel" au stimulat activitatea şi sinteza levansucrazei. Efectul detergentului "Ariel" (fabricat cu adaos de enzime) a fost mai pronunțat decât cel al detergentului "Rex" (fabricat fără adaos de enzime). Efectul a crescut cu creşterea concentrației, fiind cel mai evident la 2 g detergent/100 g sol.

Ierbicidul hormonal 2,4-D (acid 2,4-diclorfenoxiacetic) a inhibat activitatea şi sinteza levansucrazei (Fig. 5). Gradul de inhibiție a crescut cu mărirea concentrației de 2,4-D. Astfel, amestecurile de reacție 5 şi 6, în care solul a fost tratat cu 2,4-D în concentrația maximă (0,1 g/100 g sol), arată absența levanului, ceea ce înseamnă că 2,4-D în această concentrație a inactivat levansucraza preexistentă şi a oprit inducția sintezei microbiene a levansucrazei.

Din Fig. 6 se poate deduce că motorina a cauzat o slabă inhibiție a activității şi sintezei levansucrazei, iar gradul de inhibiție nu s-a schimbat, în mod evident, în funcție de concentrația motorinei.

Efectul fenolului a fost similar cu cel al motorinei.

Concluzii. 1. Ionii metalelor grele au inhibat activitatea şi sinteza levansucrazei, în ordinea: Hg^{2+}> Cd^{2+}> Co^{2+}> Zn^{2+}> Pb^{2+}> Cu^{2+}.

2. Detergenții "Rex" şi "Ariel" au stimulat activitatea şi sinteza levansucrazei, efectul detergentului "Ariel" fiind mai pronunțat decât cel al detergentului "Rex".

3. 2,4-D a inhibat puternic, iar motorina şi fenolul au inhibat într-o măsură mică activitatea şi sinteza levansucrazei.

4. Efectul ionilor metalelor grele, al detergenţilor şi 2,4-D a crescut cu mărirea concentraţiei lor, în timp ce efectul motorinei şi fenolului nu s-a schimbat în funcţie de concentraţie.

5. Inducţia sintezei microbiene a levansucrazei în sol poate fi folosită ca test ecotoxicologic atât pentru poluanţi anorganici cât şi pentru cei organici.

BIBLIOGRAFIE

1. Drăgan-Bularda, M., Kiss, S., *Effects of molasses on microbial enzyme production and water-stable aggregation in soils*, în Szegi, J. (Ed.), *Soil Biology and Conservation of the Biosphere*, p. 397-403, Akad. Kiadó, Budapest, 1977.
2. Kiss, S., Drăgan-Bularda, M., Rădulescu, D., *Soil polysaccharidases: activity and agricultural importance*, în Burns, R. G. (Ed.), *Soil Enzymes*, p. 117-147, Acad. Press, London, 1978.
3. Popa, A., *Activitatea dehidrogenazică în sol ca test ecotoxicologic pentru poluanţi anorganici şi organici*, "Stud. Univ. Babeş-Bolyai, Biol.", **44** (1-2), 1999, 169-178.
4. Wise, C. S., Dimler, R. J., Davis, H. A., Rist, C. E., *Determination of easily hydrolyzable fructose units in dextran preparations*, "Anal. Chem.", **27**, 1955, 33-36.

RECENZII

Constantin Toma, Rodica Ruginã, **Anatomia plantelor medicinale. Atlas** (*Anatomy of Medicinal Plants. Atlas*), Editura Academiei Române, Bucureşti, 1998, 320 pages including 155 plates.

This book, the Authors of which are well-known and reknown plant anatomists, is the first treatise on anatomy of medicinal plants in the Romanian literature.

As the Authors emphasise in the Preface, the histo-anatomic analyses, by means of macroscopical and microscopical methods (the microscopical methods using sometimes microchemical reactions, too), play a key role in determination of the medicinal plant species, of their organs, fragments and powders, and present a major theoretical and practical importance for many human activities related *e.g.* to pharmaceutics, food industry, agronomy, silviculture, forensic science.

The book consists of two parts (the general and the special parts).

The General Part (pages 23 - 54) is entitled "General Histology and Anatomy" and comprises 12 chapters and 3 addenda. The chapter titles are the following: 1. Meristems and definitive primary tissues; 2. Meristems and definitive secondary tissues; 3. Primary structure of the root; 4. Transition from the structure of root to that of the stem; 5. Secondary structure of the root; 6. Anomalies in the structure of root; 7. Primary structure of the stem; 8. Secondary structure of the stem; 9. Anomalies in the structure of stem; 10. Structure of the leaf; 11. Structure of the seed; and 12. Structure of the fruit. One of the addenda deals with the chemical composition of the plant cell, whereas the other two are keys, namely for the determination of dried fragments of plants and plant organs and for the determination of strongly fragmented plants (plant powders).

The Special Part (pages 55 - 306) contains the description of 128 plant species, of which 111 are native and 17 are exotic. The structure of vegetative organs (root, rhizome, stem, leaf) and, for some plants, the structure of reproducing organs (flower, fruit) are described in the alphabetical order of the families, genera and species, starting with Pteridophytes (2 families, 2 genera, 2 species) and continuing with Gymnosperms (4 families, 4 genera, 4 species) and Angiosperms (44 families, 102 genera, 122 species). The descriptions are accompanied by 1154 original drawings and 285 drawings reproduced from papers of other authors. The drawings are grouped in 155 plates as already mentioned. Due to the rich illustrations, the book is really an atlas, beside being a treatise, too.

The bibliographical list comprises 78 titles of books and articles published in Romania or in many other countries.

The book ends with a 2-page abstract in English; Alphabetical index of the common names of the species cited in the text, with mention of the family; Alphabetical index of the genera and species, with mention of the family; and Etymological index of the scientific terms used.

Based on the excellent quality of this comprehensive and updated book, I warmly recommend its publication in world-wide spoken languages.

Anatomia plantelor medicinale is a valuable source of information for a broad circle of readers: students and researchers in biology, pharmacology, agronomy, silviculture, ecology, biochemistry, medicine; highschool teachers; experts in valorification of medicinal plants, in toxicology and legal medicine; and also for other readers interested in the medicinal plants.

CORNELIA DELIU

Cristina Dobrotă, Masamichi Yamashita, **Creşterea şi dezvoltarea plantelor** (*Growth and Development of Plants*), Casa de editură Gloria, Cluj, 1999, 223 pages with 41 figures and 5 tables in the text.

The book is structured into five parts and 17 Chapters which cover the topic of plant growth and development at four levels of organisation: cell, tissue, individual and community levels.

Part I, comprising Chapters 1-3, presents the processes expressed at cell level concerning growth and development such as: the unequal division, the cell polarity, the role of cytoskeleton, the physiological mechanism of cell expansion, the cell wall structure, the genic control and the influence of growth regulators on cell division and on cell expansion.

In Part II, Chapters 4-7 deal with aspects related to embryogenesis, meristem organisation and functioning, differentiation of vascular tissues, and the competence and determination processes in shoots, roots, leaf and flower development.

Part III, with Chapters 8-10, describes plant growth and development at individual level presenting: iterative growth through modules, the formation of branches, leaves and floral organs and the functional integration of plant organs.

Part IV, containing Chapters 11-15, concerns the environmental influences on plant growth and development. Photomorphogenesis, phototropism and photoperiodism are presented as growth and developmental responses to th1e light perception and transduction. The influence of low and high temperature, flooding and oxygen shortage, salinity and gravitation are also discussed.

In Part V, Chapter 16 deals with growth and development at community level in relation to the growth rate and the habitat productivity. The primary plant strategies, the morphological plasticity, the cellular acclimation and the stored growth are presented. Chapter 17 describes techniques for measuring plant growth such as: direct sampling techniques, relativised measures of growth, indirect sampling techniques, plastochron index, physiological measurement, remote sensing and modelling techniques.

The book written by Lecturer Cristina Dobrotă and Professor Masamichi Yamashita is remarkable through the integrating conception and the clarity of explanation of such complex processes as plant growth and development are. Each chapter ends with conclusions which are very useful for a better understanding of intimate mechanisms and processes. This comprehensive book, based on a rich and up-to-date bibliography comprising 239 titles, and also on personal investigations of the authors, is a very valuable source of information for students in biology, agronomy and forestry and experts working in different fields of plant sciences.

CORNELIA DELIU

L. Rákosy-Tican, **Utilizarea tehnicilor de electrofuziune în hibridarea somatică a plantelor** (*Electrofusion Techniques and Their Use in Plant Somatic Hybridization*), Presa Universitară Clujeană, Cluj-Napoca, 1998, 187 pages with 57 figures and 14 tables in the text and with 8 tables enclosed.

This comprehensive book reviews the state of the art of electrofusion techniques and their use in plant somatic hybridization. The book is highly original representing the Ph. D. thesis of the author (Babeş-Bolyai University, 1994). Its publication was made possible under a TEMPUS-PHARE project entitled "Masters' Degree Course in Plant Genetic Manipulation" (S-JEP-09697-95), funded by EU.

The book is structured into two parts.

Part I, "Electrofusion of higher plant protoplasts", presents: recent advances in plant protoplast electromanipulation (dielectrophoresis, electrorotation, electrofusion, electroporation and electrostimulation of plant protoplasts); basic studies on cereal mesophyll protoplast electrofusion and ultrastructural studies of electrofused plant protoplasts (transmission electron microscopy).

Part II, "Plant somatic hybridization through electrofusion techniques", deals with: theoretical aspects of higher plant somatic hybridization and its perspective in plant breeding; studies on plant regeneration from isolated protoplasts (potato mesophyll protoplasts, *Nicotiana africana* cell suspension-derived protoplasts); studies on asymmetric somatic hybridization of *Nicotiana africana* and *Solanum tuberosum* cv. Désirée, through mass electrofusion; studies on asymmetric somatic hybridization of *N. africana* and *N. tabacum* KY14; and technique used for electrofusion of preselected protoplast pairs to produce intraspecific somatic hybrids in *Nicotiana*.

Associate Professor Lenuța Rákosy-Tican's book, summarizing more than 11 years of research in plant protoplast electrofusion, is addressed to students and scientists in biology, biotechnology and agronomy, interested in the achievements of a modern and spectacular branch of plant genetic engineering.

The book may be characterised by a series of qualities: the novelty of the topic both for Romanian and international biological literature, basic and up-to-date information supported by 387 references, originality of research, logical division into parts, chapters and subchapters and clarity of the descriptions, simple, concise and attractive style, richness and originality of illustrations and a very beautiful cover.

The book has a table of contents in English. But taking into consideration its excellent qualities mentioned above, I strongly recommend its translation into world-wide spoken languages.

DORINA CACHIȚĂ-COSMA

Lenuţa Rákosy-Tican (Editor), **Plant Genetic Engineering - Lab Manual. Inginerie genetică vegetală - caiet de lucrări de laborator,** Presa Universitară Clujeană, Cluj-Napoca, 1998, 167 pages with 27 figures and 22 tables in the text.

The lab manual edited by Associate Professor Lenuţa Rákosy-Tican is a collection of methods described to be used for practical work in plant genetic engineering laboratories. It was published under the TEMPUS-PHARE project S-JEP-09697-95: Masters' Degree Course in Plant Genetic Manipulation. This manual is the result of a very successful co-operation of well-known specialists in plant genetic engineering and their Romanian colleagues, who joined their efforts in European spirit in order to set up a teaching laboratory in this modern field of biology.

The manual comprises four chapters. First, I present their authors:

Chapter I: Lenuţa Rákosy-Tican (Universitatea "Babeş-Bolyai", Cluj); Paul Anthony (University of Nottingham, UK) and Ioana Dinu (Institutul de Cercetare şi Producţie pentru Cultura Cartofului, Braşov);

Chapter II: Dr. Michael R. Davey, Paul Anthony and Nigel Blackhall (University of Nottingham, UK) and Călin Andraş (Ph.D. student, Universitatea "Babeş-Bolyai", Cluj);

Chapter III: Dr. Günther Hahne and Jean Molinier (CNRS, Strasbourg, France); and

Chapter IV: Professor Michel Jacobs and Fanny Frulleux (Vrije Universiteit, Brussel, Belgium).

The contents of the chapters (and subchapters) are specified below:

Chapter I: Plant tissue and protoplast culture (Plant genetic engineering laboratory design; Sterilization techniques; Inoculation of plant explants - organ culture; The use of hemocytometer to determine total cell counts in a cell suspension; The use of fluorescein diacetate (FDA) to determine cell viability; Protocol for sunflower hypocotyl protoplast isolation and culture; Isolation and culture of protoplasts from cell suspension of *Passiflora giberti*; Isolation and culture of protoplasts from seedling leaves of *Passiflora edulis*; Electrofusion of plant protoplasts - experimental protocol; Protocol for cereal protoplast electrofusion);

Chapter II: Mutagenesis - Transformation - Cryopreservation - Analysis of chromosomes and transgenes (Screening for mutation in *Arabidopsis thaliana*; Screening of *Arabidopsis thaliana* for barbiturate mutants; Leaf disk transformation of lettuce; Histochemical staining of tissues for GUS activity; Protocol for protoplast isolation and fusion in rice; Cryopreservation of rice cell suspension cultures; Examination of the chromosomes of cultured cells; Examination of the meiotic chromosomes of *Tradescantia* sp.; PCR analysis of transgenes);

Chapter III: Bacterial transformation and DNA analysis (Introduction of a plasmid into *Agrobacterium tumefaciens* and test in plants; Small-scale preparations of a plasmid DNA; How to write a Lab Report?); and

Chapter IV: Analysis of mutants (Callus initiation in *Daucus carota*; Influence of hormones on growth of cell suspensions; Protoplast isolation, transformation and fusion; Characterisation of biochemical mutants; Characterisation of resistant mutants of *Nicotiana sylvestris*; Segregation of kanamycin resistance in *Arabidopsis thaliana* transformants; Expression of endogenous and exogenous *Adh* genes in *Nicotiana plumbaginifolia* transformed with *Arabidopsis Adh*).

This manual in A4 format is combbound and its chapters are printed on different coloured papers. It was especially

designed to be used in the laboratory and to allow an easy way to find and follow practical information and also to stimulate the students' practical work and skill development.

Finally, I consider that the laboratory manual edited by Associate Professor Lenuța Rákosy-Tican is the best book of its kind in Romania. Therefore, I warmly recommend it to all laboratories of plant biotehnology for teaching and research work.

DORINA CACHIȚĂ-COSMA

Enzymes in the Environment: Activity, Ecology and Applications, Edited by Richard P. Dick, Oregon State University, Corvallis, OR, USA, 1999, 164 pages.

This volume comprises the Preface written by the Editor, and the abstracts of papers presented at the International Conference on Enzymes in the Environment, held in Granada (Spain), July 12-15, 1999. The abstracts are preceded by the Conference schedule.

As Professor R.P. Dick emphasises in the Preface "The core of the conference was on enzymes or enzyme-mediated processes as biological catalysts in soil, sediments and aquatic ecosystems. The conference had two broad themes: microbial ecology and environmental enzymology".

Ten symposia were organised. Their titles (and numbers of oral and poster presentations) are specified below: I. Enzymes in soil systems (3 and 32); II. Limnic systems (3 and 18); III. Plants and soil enzymes (3 and 15); IV. Enzymes in solid/liquid phases (4 and 7); V. Nutrient oyeling and organic matter decomposition (4 and 30); VI. Enzymic methodologies (3 and 12); VII. Bioremediation and extracellular enzymes (5 and 23); VIII. Bioremediation - genetically designed organisms and enzymes (3 and 1); IX. Marine ecosystems (4 and 4) and X. Enzymes as environmental sensors (4 and 30).

The past and present of environmental enzymology are outlined in the inaugural lecture "Biochemical context of enzymes in the environment" (J.N. Ladd) and its future is discussed in the closing lecture "Environmental enzymology in the 21th century" (R. G. Burns).

Two lists are enclosed to the volume. The first is a list with the names and addresses of the corresponding authors (166 persons from 32 countries, including Romania). The second list is the author index (457 authors).

This volume is a valuable source of information for all experts working in different fields of environmental science and technology.

ȘTEFAN KISS and ELENA MANOLACHE