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SOMMAIRE

RADIOPROTECTIVE AND RADIOREPAIRER EFFECT OF CYSTEINE ON IRRADIATED ROOT MERISTEM CELLS OF BROAD BEAN (*VICIA FABA L*)

CONSTANȚA SPÂRCHEZ*, C. CRĂCIUN**, V. SORAN*, Z. URAY***, VERONICA CRĂCIUN**

The radioprotective and radiorepairer effect of cysteine has been studied before and after irradiation with gamma radiations produced by a Co^{60} source. The effect of irradiation has been estimated taking into account the electronmicroscopical images of nuclei from the radicular meristem of broad-bean (*Vicia faba L*), during interphase. It has come out that irradiation with gamma radiations caused a decrease of the relative quantity of DNA, throughout all the phases of the cell cycle (see Table 1). The cysteine treatment had a more significant effect, when it was performed after gamma irradiation; on the other hand this effect was less obvious when performed before gamma irradiation (see Table 2). Consequently, the cysteine has rather a radioprotective effect than a radiorepairer one; this effects was more obvious during phase G1 and more diminished during phase G2 of the cell cycle.

In a previous study (19), we have investigated the effect of gamma irradiations on heterochromatine distribution, after, cysteamine differentiated treatment. As a result of the study of electronmicroscopial images, it has been found out that cysteamine had a significant radioprotective effect on the gamma radiations action, at a 100 r dose, and a more diminished one, at a 300 r dose. The radiorepairer effect was expressed by an extension of the cell division phase. The present study is the research of the cysteine radioprotective and radiorepairer effect on the root meristem cells of broad bean (*Vicia faba L*).

MATERIAL AND METHODS

The material and the methods have been minutely described in our previous study (19). However, it is worth mentioning that the electronmicroscopical investigation was performed with a BS 613 TESLA microscope, the planimetrical measurements for the quantitative determination of heterochromatine were performed only on nuclei microphotographs, with a 9500 × magnification. By means of this method, the following parameters have been recorded : the total surface of the nucleus section, the area of the nucleolus section, the area occupied by heterochromatine on the nucleus surface. The relative quantity of heterochromatine per nucleus surface was expressed in mm^2.

RESULTS AND DISCUSSION

The results of our investigations have been included in two drawings, a diagram and three tables.

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1. HETEROCHROMATINE DISTRIBUTION PER NUCLEUS SECTION ON CONTROL

In order to reveal the radioprotective and radiorepairer effects of cysteine, we have reported the registered data to two types of control : non irradiated and irradiated control with gamma radiations at 100 and 300 r, doses, not treated with cysteine.

Out of the results from tables 1 and 3, the diagram 1 and the drawing I, Figs 1, 2, 3, the following aspects may be revealed : a). at non irradiated control, the ultrastructure of the nuclei in the root meristem cells of broad bean is the normal one (drawing I, fig. 1) ; b) at irradiated control with gamma radiations at 100 and 300 r doses, some alterations both in the quantitative and the qualitative distribution of heterochromatine were to be noticed.

It has been found out that after gamma irradiation, the nuclei become picnotic, while their membranes, more or less undulated, as a result of some small inwards or small excrescences (drawing I, Figs 2 and 3). The area occupied by heterochromatine on the nucleus' section surface has also decreased, at different phases of the cell cycle (table 1, diagram 1) ; the most sensible one was phase G1, at 100 r, dose, and at 300 r dose.

Table 1

Variations of the relative heterochromatine quantity in the nuclei of the root meristem cells broad bean *(Vicia faba L)*, expressed in mm^2 per area of nucleus section, at non irradiated and irradiated control

Type of control	Phases of the cell cycle	Heterochromatine relative quantity, in mm^2
Non irradiated control	G^1	1070
	S	1650
	G^2	2220
Irradiated 100 r	G_1	580
	S	1180
	G^2	1890
Irradiated 300 r	G^1	754
	S	1460
	G^2	2160

The more drastic effect of irradiation with 100 r as compared to the 300 r dose has already been partially discussed in our previous study, (19) ; the supposition was that this unexpected result was due to a stronger radiorepairer response of the cells, at the 300 r dose than at the 100 r dose. This response would be caused by the increase in the activity of the radiorepairer enzymes of DNA damaged by irradiation. Still, L. H. Eidus (5) has noticed that the cell's response or reaction to the action of ionization radiations is always non-specific ; after any irradiation, if it does not reach the lethal doses, there always take place repairing processes (7), (9). These are better represented in procaryotes (bacteria) and almost unknown in eucaryote cells (8), (13), though the plant material used by us was quite well studied (5).

In this respect, we must add the fact that the effect of small doses (1), (4), (14), (15), (17) is yet little known. As it comes out of the data in table 1, diagram 1, drawing I, Figs 2 and 3, the action of repairer endonucleases might be included only after the level of post-irradiation damages exceeds a certain value. The study of the plant material response would call upon investigations at small irradiation doses, also taking the time into account (14).

An interesting result of our investigation was the fact that it reveals that the nuclei from the root meristem cells of broad bean were more sensible to the same irradiation dose, in phase G 1 than in phase G2, especially after the 300 r dose, when the repairer endonucleases reactions are supposed to have been set free, the quantity of heterochromatine (DNA) per nucleus surface is not significantly different from the non-irradiated control. This behaviour is similar to that of the yeast cells, to the gamma irradiation. The authors who has studied this kind of fungi, (2), (10), (11), (12) as well as the cultivated mammalian cells (15), (16), (17), have found out that the haploid cells are more sensible to irradiation than the diploidones. Taking into account that the cells at phase G1 of the cell cycle also possess a smaller DNA quantity, about 1/2 of the quantity belonging to phase G2, the mechanism or the case for the sensitivity at gamma irradiation must be the same : the smaller DNA quantity. It is known that the haploid cells posses half of the existing number of chromosomes in the diploid cell.

2. RADIOPROTECTIVE AND RADIOREPAIRER EFFECTS OF CYSTEINE

The radioprotective and radiorepairer effects of cysteine were relatively well studied both from a biophysical and biochemical (1), (3), (6), (10), (13), (18), (20) point of view ; still so far they have not been investigated at the level of electronimcroscopical ultrastructure.

Tables 2, 3, diagram 1, the microphotographs in drawing I, Figs 4, 5, 6, 7, 8 represent the results of our measurements and investigations concerning the radioprotective and radiorepairer effects of cysteine, administered for 1 hour, at 300 mg. l^{-1} concentration, before gamma irradiation, as radioprotective and, at the same concentration, for 1 hour, after gamma irradiation, as radiorepairer.

The data from Tables 2 and 3 reveal the level of damages caused by gamma irradiations, in heterochromatine distribution, DNA fragments, per nucleus section surface. The repair level of the effects caused by irradiation, after cysteine treatment, was also revealed in detail. It has been found out that cysteine has a weak radioprotective effect and a strong radiorepairer one. The radioprotective effect of cysteine became obvious only at phase G1 of the cell cycle and was more pregnant in the plant material irradiated with 100 r. The radiorepairer effect of cysteine was obvious in all the phases of the cell cycle and at almost the same intensity both in the cells irradiated with gamma radiations with 100 r dose and 300 r dose.

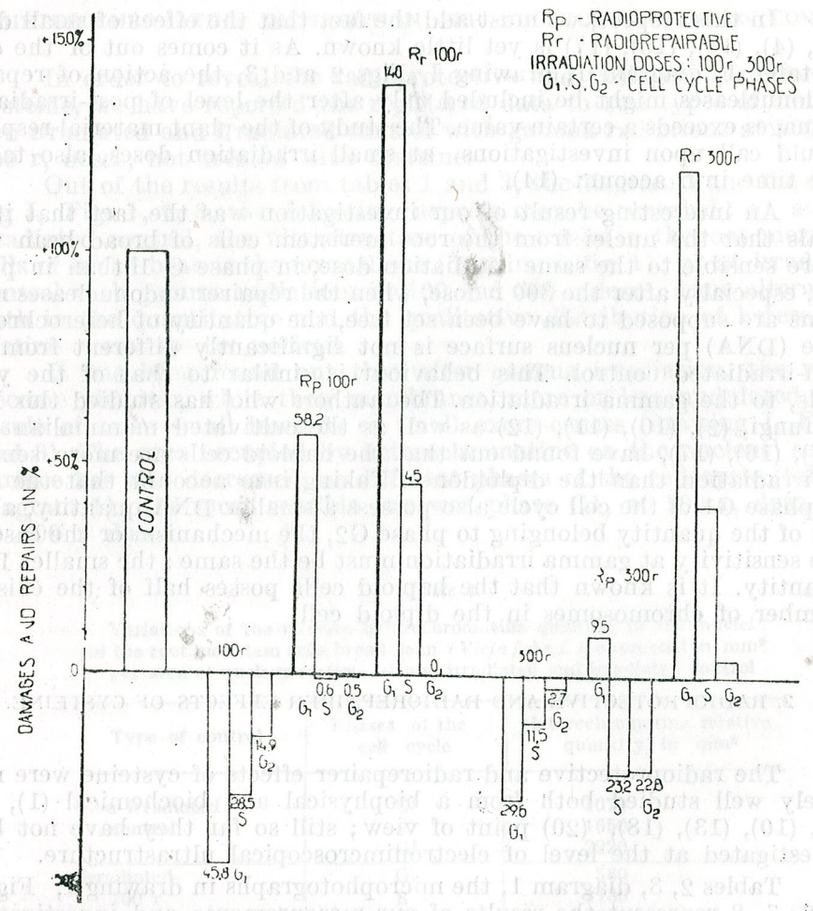
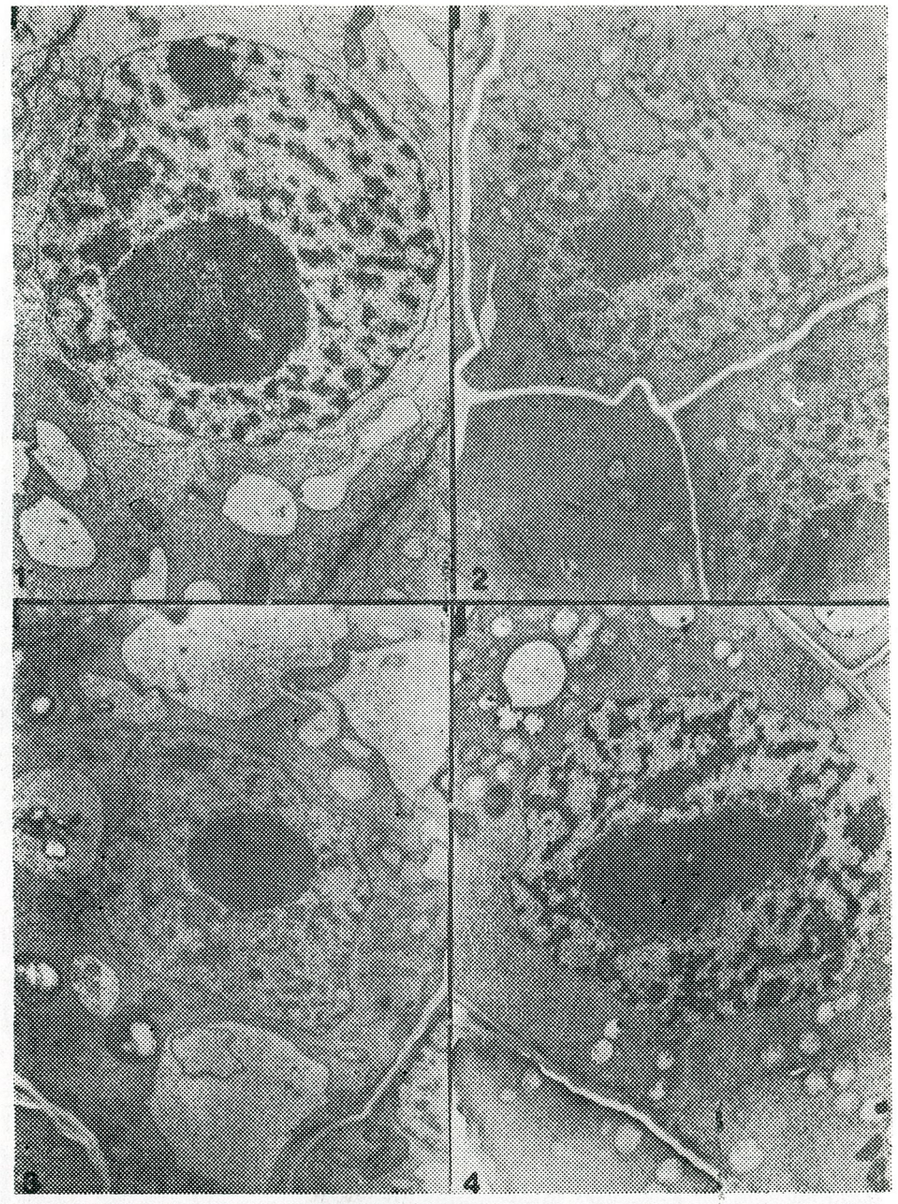


Fig. 1. — Radioprotective and radiorepairer effects of cysteine expressed in % of damages and repair of heterochromatine and of the nucleus structure from root meristem cells of broad bean *(Vicia faba L.)*.

Drawing I, Fig. 4, 5—8 represent the electronmicroscopical microphotographs after cysteine pre-treatment (amino-acid as radioprotector, drawing I, fig. 45) and post-treatment (amino-acid as radiorepairer-drawing I, Fig. 6—8).

In the case of cysteine radioprotective treatment a paradoxical phenomenon took place. At the 100 r dose (drawing I, Fig. 4) the cysteine radioprotection seems to be smaller than at the 300 r dose (drawing I Fig. 5). This results from the fact that the nuclei had an undulated membrane, whereas the qualitative heterochromatine distribution was far from the normal conformation at non irradiated control. On the other hand, at the 300 r dose, the radioprotective effect was obvious, as the aspect of nuclei was close to that at the non irradiation control.



Drawing I, Fig. 1. — The ultrastructure of the nucleus from the cells of root meristem broad beans *(Vicia faba L.)*, at non irradiated control, magnification 4750 +.

Fig. 2. — The ultrastructure of the nucleus from the cells of root meristem broad beans, after gamma irradiation, 100 r, dose, magnification 4750 ×.

Fig. 3. — The ultrastructure of the nucleus from the cells of root meristem broad beand, after gamma irradiation, 300 r, dose, magnification 4750 ×.

Fig. 4. — The radioprotective effect of cysteine on the ultrastructure of the nucleus from root meristem cells of broad bean, at the 100 r dose, magnification 4750 ×.

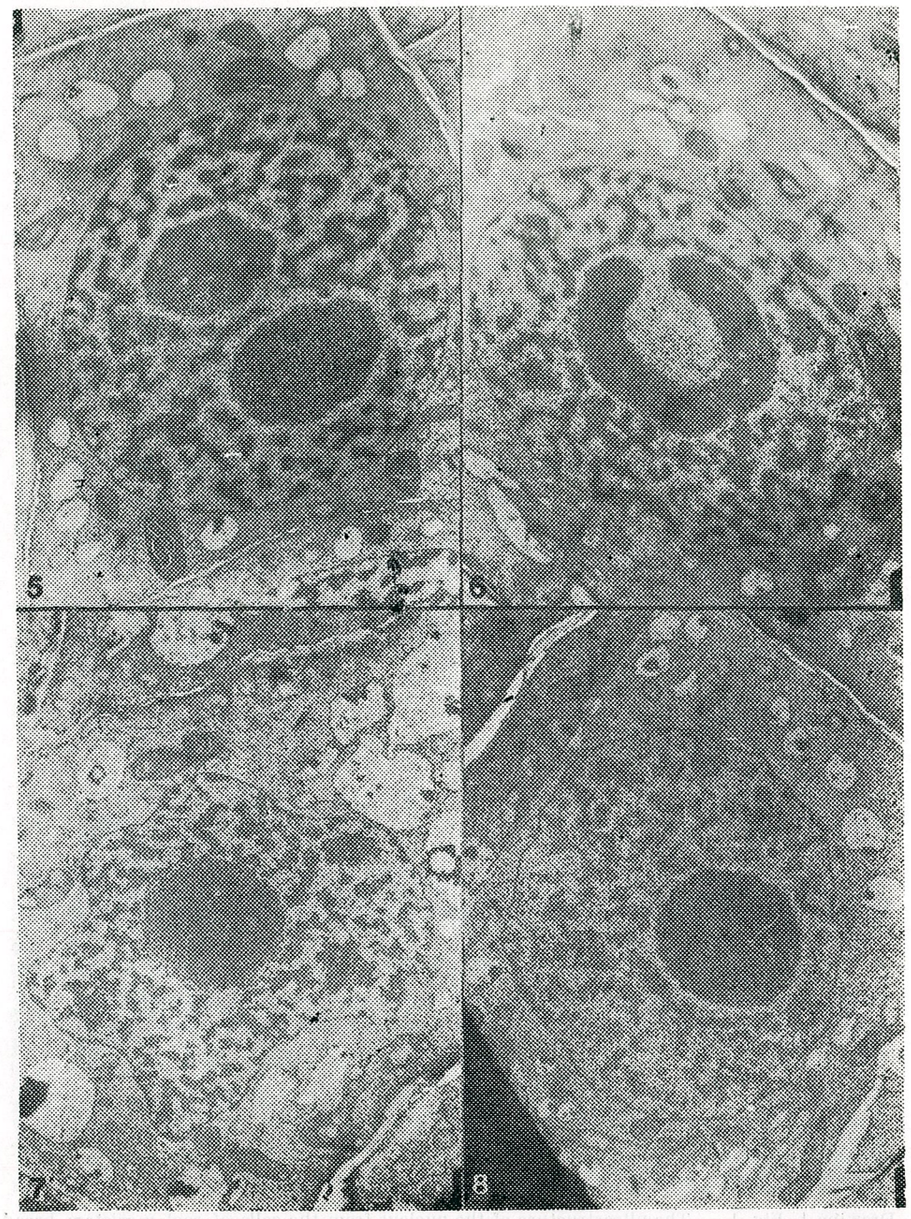


Fig. 5. — The radioprotective effect of cysteine on the ultrastructure of the nucleus from the root meristem cells of broad bean *(Vicia faba L.)*, at the 300 r dose, magnification 4750 ×.
Fig. 6. — The radiorepairer effect of cysteine on the ultrastructure of the nucleus from the root meristem cells of broad bean, at the 100 r dose, magnification 4750 ×.
Figs 7 and 8. — The radiorepairer effect of cysteine on the ultrastructure of the nucleus from the root meristem cells of broad bean at the 300 r, dose, magnification 4750 ×.

Table 2

Variations of the relative heterochromatine quantity in the nuclei of the root meristem cells of broad bean *(Vicia faba L)* expressed in mm² per area of nucleus section, after the radioprotective and radiorepairer action of cysteine

Cysteine action and irradiation dose	Phases of the cell cycle	Heterochromatine relative quantity, mm²
A. RADIOPROTECTIVE		
Irradiated 100 r	G1	918
	S	1173
	G2	1198* (approximate)
Irradiated 300 r	G1	826
	S	1122
	G2	1167
B. RADIOREPAIRER		
Irradiated 100 r	G1	1394
	S	1716
	G2	1890
Irradiated 300 r	G1	1659* (approximate)
	S	2052
	G2	2238

* The values are missing, as on the electronmicroscopical microphotographs did not appear the nuclei at the phase of the cell cycle. The approximate values have been estimated in accordance with the DNA quantity at different cell cycle phases.

Table 3

Radioprotective and radiorepairer effects of cysteine in damage (—) and repair (+) percentange ot the non irradiated control and the irradiated material. The estimation is in accordance with heterochromatine distribution in the nuclei of meristem cells of broad bean *(Vicia faba* L)

Cysteine action and irradiation dose	The damage (—) and repair (+) level in %, as a result of heterochromatine distribution per nucleus section surface and phases of the cell cycle		
	G1	S	G2
Non irradiated control	100	100	100
Irradiated 100 r	—45.8	—28.5	—14.9
Irradiated 300 r	—29.6	—11.5	—2.7
A. RADIOPROTECTIVE			
To non irradiated control	—14.2	—28.9	—19.0
To irradiated 100 r	+58.2	—0.6	—0.5
To non irradiated control	—22.8	—32.0	—24.9
To irradiated 300 r	+9.5	—23.2	—22.8
B. RADIOREPAIRER			
To non irradiated control	+30.3	+4.0	—14.9
To irradiated 100 r	+140.0	+45.0	0
To non irradiated control	+55.0	+24.3	+0.8
To irradiated 300 r	+120.0	+40.5	+3.6

In the case of cysteine radiorepairer treatment, the microphotographs (drawing I, Figs 6—8) showed a more distinct radiorepairer effect at the 100 r irradiation dose (drawing I, fig. 6), as compared to the 300 r irradiation dose (drawing I, Figs 7—8).

The above data could have several plausible explanations. As far as the radioprotective effect is concerned, there are two suppositions : a). the time interval of one hour, while cysteine penetrated the root meristem of broad bean, was not sufficient for the amino-acid absorption in an optimum concentration, so as to take part in the radioprotective proteinic metabolism, and b). cysteine, after its absorption in the cell, as a free amino-acid, had a less efficient radioprotective action. As far as the radiorepairer effect is concerned, there are also two suppositions : a). the time interval for including cystein in the structure of some enzymes was sufficient to start the radiorepairer processes and b). besides the radioprotective effect of cysteine, whichever it would have been, the natural repairer mechanisms of the cell also took part.

Certainly, the time [9], [14], mentioned above, during the repair processes of the damages as a result of gamma irradiation, was of main importance. Therefore, the question arises, why the time was in favour of cysteine, as radiorepairer agent, and was less favourable for the radioprotective action of the same substance. For the time being there is no explanation for this differentiated behaviour, but we must emphasize that cysteine is rather a radiorepairer substance than a radioprotective one.

CONCLUSIONS

1. Cysteine treatment applied to meristem cells of broad bean (*Vicia faba L*), before and after irradiation, revealed that the substance has radiorepairer and radioprotective effects.

2. The electronmicroscopical microphotographs showed more significant damages of the nuclei and disturbances in the heterochromatine distribution per nucleus section surface, at the 100 r dose than at the 300 r dose. The measurements of heterochromatine surface per nucleus section showed a decrease in the heterochromatine relative quantity (DNA) after gamma irradiation at 100 r dose.

3. The radioprotective effect of cysteine was lower and became obvious only in the nuclei of the cells at phase G1 of the cell cycle. On the other hand, the radiorepairer effect of cysteine was obvious in all the cases and was more significant in the nuclei of the cells at phase G1 of the cell cycle, moderate at phase S and non-significant at phase G2.

4 The electronmicrophotographs showed the distinct radiorepairer effect of cysteine and have confirmed, to a certain degree, its radioprotective effect.

5. We have not found a plausible explanation for the differentiated radioprotective and radiorepairer action of cysteine.

REFERENCES

1. Alper, T., *Cellular Radiobiology*, Cambridge University Press, Cambridge, 1979.
2. Bertsche, U., Radiat. Res., 1978, **76**, *2*, p. 349—367.
3. Bresler, S. E., Noskin, L. A., i Stepanova I. M., Molec. Gen. Genet., 1978, *163*, **1**, p. 75—85.
4. Christensen R. C., Tobias C. A. and Taylor W. D., Int. J. Radiat. Biol., 1972, **2**, *5*, p. 457—477.
5. Eidus L. H., *Nespetsificheskaya reaktsija klotok i radiochuvstvitelnost*, Atomizdat, Moskva 1977.
6. Graevskii E. Ja., *Sulfgidrilnye grup i radiochustvitelnosti*, Atomizdat, Moskva, 1969.
7. Haynes R. H. and Stelow R. E. (eds.), *Molecular Mechanism for Repair of DNA*, Plenum Publ. Comp., New York, 1975.
8. Hesslewood I. P., Int. J. Radiat. Biol., 1978, **34**, *5*, p. 451—469.
9. Korogodin V. I., *Problemy postradiatsionnogo vosstanovleniye*, Atomizdat, Moskva, 1966.
10. Maisin J. R., Studia Biophysica, 1977, **53**, p. 121—124.
11. Mortimer R. K. and Wolfe R. C., Arch. Biochem. Biophys., 1954, **49**, *1*. p. 110—122.
12. Petin V. G. i Matrenina V. L., Molec. Gen. Genet., 1981, **183**, *1*. p. 152—157.
13. Petin V. G., *Geneticheskii kontrol modificatsii radiochuvstvitelnosti kletok*, Energoatomizdat Moskva, 1987.
14. Raevskii B. N., *Dozy radioaktivnykh izluchenii i ih deistvie na organizm*, Medgiz, Moskva, 1959.
15. Raju M. R., *Heavy Particle Radiotherapy*, Academic Press, New York, 1980.
16. Revesz L., Edgren M. and Nishida T., *Modification and Radiosensitivity in Cancer Treatment*, Academic Press, Tokyo, 1984.
17. Rossi H. H., *Third Symp. on Microdosimetry*, Luxemburg, 1972, p. 1—12.
18. Sawada S., and Okada ., Radiat. Res., 1970, **44**, *1*, p. 116—132.
19. Spârchez C., Crăciun C., Soran V. and Uray Z., Rev. Roum. Biol. — Biol. Végét., 1990, **35**, *2*, p. 121—125.
20. Yarmoshenko S. P., Vainson A. A. i Magdon E., *Kislorodnyi effekt i luchevaya terapiya opuholei*, Meditsina, Moskva, 1980.

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EVIDENCE ON THE CORRELATION BETWEEN PLASMA MEMBRANE ELECTRICAL PARAMETERS AND THE STRESS RESISTANCE IN *NICOTIANA TABACUM* PROTOPLASTS

ANIELA STINGA, AURELIA BREZEANU*, GINA COGĂLNICEANU*

The present paper reports on electrorotation experiments done with foliar mesophyll protoplasts isolated from *Nicotiana tabacum* cv. Xanthi. We were interested in possible correlations between passive electrical properties of plant protoplasts and their functional state resulting from a stress injury. Exposure to stress was achieved by preserving the protoplasts for either 24 hrs or 48 hrs at 4°C in an osmotic stabilising medium (0.7M mannitol in deionized water) unsupplemented with nutrients. Electrorotation measurements were performed at a field strength of 8 kV/m, in the frequency range 5—200 kHz. The population of protoplasts in each sample was analysed with respect to individual values for the membrane electrical parameters (relative dielectric constant and specific conductivity) that were evaluated by least-squares fitting of the experimental data to a theoretical electrorotation curve. For freshly isolated protoplasts, we found a wide range of values for the relative dielectric constant and the specific conductivity of the membrane, while for stress-injured samples the corresponding values were situated in a narrower range. Protoplasts that had values (either very low orvery high) for any of the passive electrical parameters under consideration, proved to be the most stress sensitive.

INTRODUCTION

Electrical phenomena are known to accompany many of the vital cellular events (mitosis, fertilization, cell growth, cell movement, metabolite transport etc.). There is also experimental evidence showing that low level electromagnetic fields can induce stimulating effects on some cellular functions.

In order to get further insight into these aspects of bioelectricity, it is useful to have information on the passive electrical properties of cells and to study the way they reflect a given functional state. The electrorotation method proves to be convenient for this approach, since it is a sensitive, noninvasive research tool well suited for unicellular measurements (1, 2).

Plant protoplasts represent a convenient experimental system for studies on the correlation between electrorotation derived electrical parameters and some morpho-physiological characteristics. They are extremely stress-sensitive, their response being readily expressed at the morphological level. Different degrees of stress-induced physiological alterations can thus be easily monitored by light microscopy examination and can subsequently be correlated to changes of the cellular electrical parameters, evaluated from electrorotation spectra.

The present paper reports on electrorotation experiments done with *Nicotiana tabacum* cv. Xanthi protoplasts exposed to the stress of preservation in cold, on a medium without nutrients. The population of protoplasts was analyzed with respect to individual values for the

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membrane relative dielectric constant and specific conductivity. These values were situated within a range whose width can be correlated to the stress-sensitivity of protoplasts.

MATERIALS AND METHODS

ISOLATION OF PROTOPLASTS

We used, as a protoplast source, foliar mesophyll cells from *Nicotiana tabacum* cv. Xanthi plants, obtained by aseptic germination of seeds and grown on agar culture medium. Protoplasts' isolation was achieved according to the method described in (3) with slight modification. Mesophyll fragments were incubated in an enzyme solution without shaking, for 16 hrs at 24° C in the dark. The enzyme medium was 0.5% Cellulizin (Serva) and 0.5% Macerozyme (Serva) in 0.7 M mannitol solution, prior to use, it was sterilized by filtration. After the incubation, the protoplasts were isolated by passage through four layers of nylon sieve (62—70 μm), followed by three successive centrifugations (5 min at 100 × g) and resuspended in osmotic stabilizing solution (0.7 M mannitol, pH 5.8, in deionized water). Further purification was achieved by another centrifugation, for 8 min at 100 × g on a 23% sucrose gradient. Intact protoplasts formed a light-green layer at the mannitol-sucrose interface, where from they were recovered and subsequently used in our experiments. Cellular debris and damaged protoplasts sedimented at the bottom of the centrifuge tube.

The protoplasts obtained as stated above were preserved at low temperature (4° C) for two days in osmotic sabilizing solution without nutrients. Before performing electrorotation measurements, the samples were again purified on sucrose gradient, in order to remove the damaged protoplasts and the debris resulted from protoplasts that had burst during exposure to stress.

Protoplasts' viability was assessed by the methylene blue exclusion test. For each sample, an average of 400 protoplasts were examined, the percent of protoplasts that have excluded the dye within 2 min being taken as a viability index.

ELECTROROTATION MEASUREMENTS

The measurements were performed at room temperature, in a cylindrical four-electrode chamber as described in (4). The vertical needle electrodes were driven by square-topped pulses phase-shifted by 90° to each other. The key ratio was 1 :3 and the strength of the rotating field was 8 kV/m. Measurements were carried out over the frequency range 5—200 kHz. Suspending media (isoosmotic mannitol solution 0.7 M, pH 5.8) with specific conductivity of 2×10^{-3} S/m or 1.75×10^{-2} S/m were used.

For each sample, the period of rotation versus the frequency of the applied field was measured for at least ten individual protoplasts.

The characteristic frequency of rotation, the relative dielectric constant ε_m and the specific conductivity of the membrane σ_m were evaluated by least-squares fitting of the experimental data to a theoretical electrorotation curve with the aid of an adapted form of the CURFIT programme (5) run on a M 118 computer. The function describing the electrorotation of one-shell spherical bodies was taken from (b), with the assumption that the protoplasts have a membrane thickness of 50 Å, an internal conductivity of 0.3 S/m and a relative dielectric constant of the internal medium equal to 58. The dynamic viscosity of the suspending solution was taken to be 10 P and for its relative dielectric constant a value of 70 was assumed.

RESULTS AND DISCUSSIONS

Our first approach to the characterisation of the protoplast samples was the examination of their morphological appearance under the light microscope. We identified several tyes of protoplasts, that are summarized and described in Table 1.

The freshly prepared samples showed a large morphological diversity with respect to shape size, disposition of chloroplasts and of the vacuole, the dominant type being the 'normal' protoplast (type I in Table 1).

Table 1

Cumulative data regarding the morphological peculiarities of the protoplasts used in the experiments

Type	Shape	Size (average diameter)	Internal constitutive elements	Sample (**)		
				Fresh protoplasts	Protoplasts preserved in cold for:	
					24 hrs	48 hrs
I	spherical	large (7—9 μm)	— small central vacuole; — uniformly distributed chloroplasts	+	—	—
II	spherical	medium (5—7 μm)	— asymmetrical vacuole; — polar distribution of chloroplasts	+	+	+
III	oval	large (7—9 μm)* medium (5—7 μm)*	— large, central or asymmetrical vacuole; — plastidial elements randomly distributed or polarized	+	+	—
IV	irregular	medium (4—6 μm)	— central vacuole; — plastidial elements randomly distributed	—	—	+
V	spherical	small (3—5 μm)	— large central vacuole; — scarce chloroplasts, randomly distributed	+	+	+

(*) Longest axis length.
(**) The (+) and (—) symbols denote the presence and respectively the absence of a given protoplast type in the sample.

For the protoplasts exposed to cold stress, the dominant features were, small or medium size, random or polarized disposition of chloroplasts and a large vacuole, frequently situated eccentrically (see Table 1).

A further step in our analysis was the viability test, that gave the results presented in Table 2.

The data in Table 2 show that after the first day, there is a drop in the viability index. After 48 hrs, the protoplasts have reached a steady-state from the point of view of the viability test, with almost half of the population being metabolically active.

Table 2

The time evolution of the viability index for protoplasts samples

	Fresh protoplasts	Protoplasts preserved in cold for			
		24 hrs	48 hrs	144 hrs	168 hrs
Viability index	81%	74%	47%	48%	44%

Some typical electrorotation spectra are shown in Fig. 1. The main features of the spectra — the characteristic frequency and the maximum angular velocity — were dependent on the electrical and geometrical characteristics of the protoplasts and on the conductivity of the suspension medium, as predicted by the theory (1).

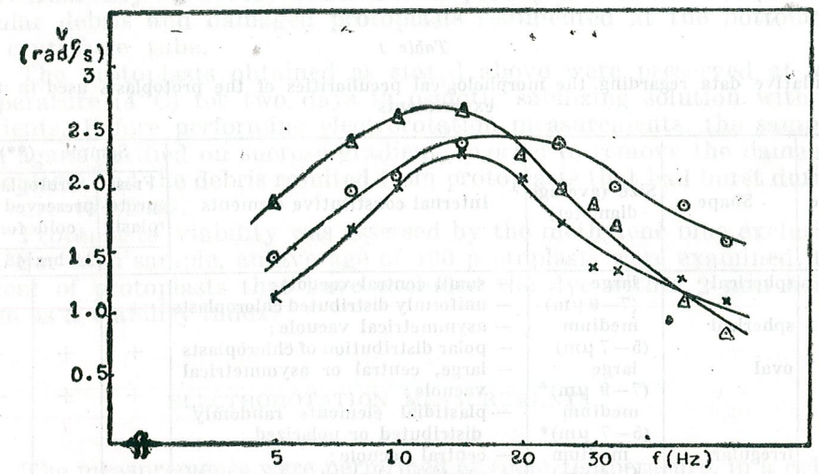
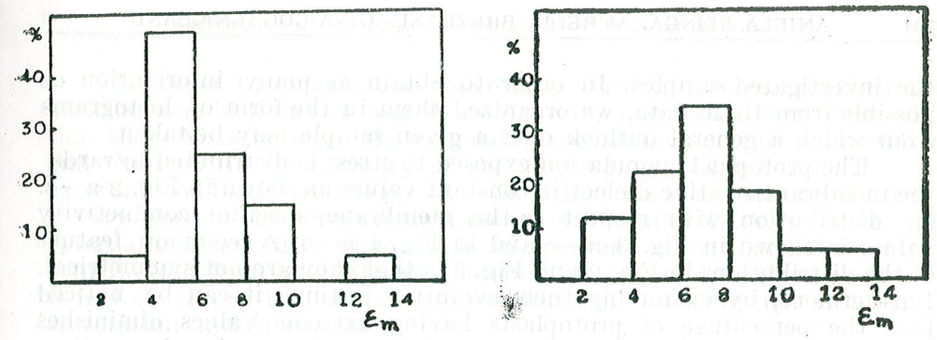


Fig. 1. — Electrorotation spectra of protoplasts. (×) — fresh protoplasts; (0)—protoplasts preserved for 24 hrs; (D)—protoplasts preserved for 48 hrs.

The 'best fit' values for the membrane dielectric constant and the specific conductivity were determined for individual protoplasts from their corresponding electrorotation spectra, as stated in MATERIALS AND METHODS. These values showed considerable spreading, for all



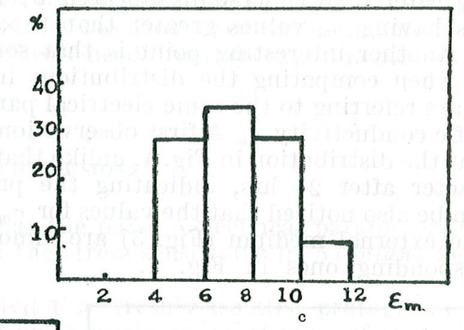


Fig. 2. — Distribution of protoplasts with respect to the relative dielectric constant values. (a) — fresh protoplasts; (b) — protoplasts preserved for 24 hrs; (c) — protoplasts preserved for 48 hrs.

Fig. 3. — Distribution of protoplasts with respect to membrane specific conductivity values. (a) — fresh protoplasts; (b) — protoplasts preserved for 24 hrs; (c) — — protoplasts preserved for 48 hrs 48 hrs. The conductivity of the external medium was 20 μ S/cm.

the investigated samples. In order to obtain as many information as possible from these data, we organized them in the form of histograms from which a general outlook over a given sample may be taken.

The protoplasts population exposed to stress is distributed towards the membrane relative dielectric constant values as shown in Fig. 2 a—c. Its distribution with respect to the membrane specific conductivity values is shown in Fig. 3 a—c and in Fig. 4 a—c. A common feature of the distributions in Fig. 2 and Fig. 3 is that they are not symmetrical. Furthermore, by examining their evolution in time, it can be noticed that the percentage of protoplasts having extreme values diminishes or even vanishes. For example, the protoplasts with ε_m values lower than 4 or greater than 12 have disappeared after 48 hrs. So did also the protoplasts having σ_m values greater than 14×10^{-6} s/m (Fig. 4 c).

Another interesting point is that some dissimilarities become evident when comparing the distributions in Fig. 3 and Fig. 4 although they are referring to the same electrical parameter, namely the membrane specific conductivity σ_m A first observation to be made in this connection is that the distribution in Fig. 4, unlike that in Fig. 3, acquires a bimodal character after 24 hrs, indicating the presence of two subpopulation. It can be also noticed that the values for σ_m at low specific conductivities of the external medium (Fig. 3) are almost six times smaller than the corresponding ones in Fig. 4.

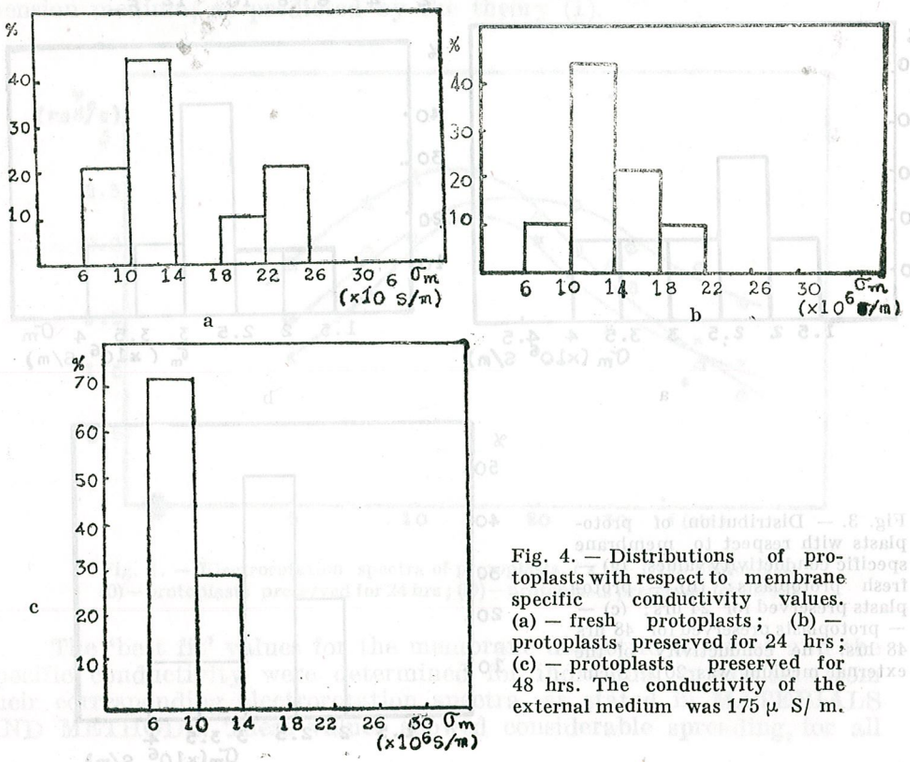


Fig. 4. — Distributions of protoplasts with respect to membrane specific conductivity values. (a) — fresh protoplasts; (b) — protoplasts preserved for 24 hrs; (c) — protoplasts preserved for 48 hrs. The conductivity of the external medium was 175 μ S/ m.

In order to explain the above mentioned differences between the distributions in figures 3 and 4, we have to take into account the relation that exists between the calculated membrane specific conductivity and the specific conductivity of the suspending medium. At very low external conductivities (Fig. 3), the value that we are determining is very close to the actual membrane specific conductivity, while at high external conductivities we evaluate an apparent value, that has a certain contribution from the surface conductance. At present, there is no satisfactory theory that allows to quantitate this contribution from electrorotation measurements.

These findings give us reasons to believe that what is actually changing during exposure to stress is the outer side of the plasma membrane, containing the structures that have resulted after the detachment of the cell wall. However, this hypothesis needs further experimental verification.

CONCLUSIONS

We were able to show that the passive electrical parameters of the membrane are correlated with the stress sensitivity in *Nicotiana tabacum* cv. *Xanthi* protoplasts.

Our results have indicated that freshly isolated protoplasts prove to be a heterogeneous population from the point of view of the morphological characteristics and also as regards the electrical parameters deduced from electrorotation spectra.

From the time evolution of the population of protoplasts during exposure to stress, we identified the stress-sensitive ones as being those having extreme values for either the relative dielectric constant or the specific conductivity. The population of stress-resistant protoplasts proved to be more homogeneous, the corresponding range for the electrical parameters being considerably narrower.

REFERENCES

1. Arnold W. M., Zimmermann U., J. Electrostatics, 1988, **21**, 151.
2. Glaser R., Fuhr G., *Electric Double Layers in Biology*, ed. M. Blank, Plenum Press, New York, 1986.
3. Power J. B., Cocking E. C., J. Exp. Bot., 1970, **21**, 64.
4. Radu M. et al., 1991, preprint Inst. Atomic Physics RB—38—1991.
5. Bevington P., *Data Reduction and Error Analysis for the Physical Sciences*, Academic Press, 1969.
6. Radu M., Rev. Roum. Phys., 1990, **35**, 507.

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SPHEROPLAST ISOLATION, FUSION, REVERSION AND GENETIC TRANSFORMATION IN *ESCHERICHIA COLI*

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A technique for rapid and efficient isolation of spheroplasts in *E. coli* by penicillin treatment is presented. Spheroplast reversion is tested in different regenerating media. Spheroplast isolation, fusion and reversion are analysed on microelectronographs. PEG-induced transformation of *E. coli* spheroplasts by pBR322 is achieved too.

The information concerning the spheroplasts of Gram negative bacteria are characterized by paucity compairing with those on Gram positive bacteria protoplasts (Hopwood, 1981). The notion of protoplast itself is disputed in Gram negative bacteria, because of the impossibility of removing all the components of the cell wall. The special structure which cannot be totally eliminated is the outer membrane. As a consequence, the term protoplast is more suitable for Gram positive bacteria and the term spheroplast for Gram negative one, in which fragments of the outer membrane still remain (Birdsell, 1967).

Similar with Gram positive bacteria, it is possible to obtain recombinants after PEG-induced spheroplast fusion, as proved Tsenin' studies (1978) on *E. coli* and Coetzee's (1979) on *Providencia alcalifaciens*. It is also possible to transfer the plasmids by spheroplast fusion (Vorobjeva, 1979), even if genetic transformation of Gram negative bacteria spheroplasts has not been reported yet.

The aim of the present study was to establish a simple and rapid technique for spheroplast isolation in *E. coli* by penicillin treatment. Spheroplast reversion in different regenerating media is tested too. Spheroplasts isolation, fusion and reversion are analysed on microelectronographs. PEG-induced transformation *of. E. coli* spheroplasts by pBR322 DNA was achieved too.

MATERIAL AND METHODS

The following bacterial strains were used : *E. coli* HB101 Sm^R pro^- leu^- thy^- $lacY^-$ recA gal , *E coli* HB101 Tn5 Sm^R Km^R pro^- leu^- thy^- $lacY^-$ recA gal^- and *E. coli* 354 (prototroph).

SPHEROPLAST ISOLATION

— Bacteria were cultivated in TYN broth (1% triptone, 0.25% yeast extract, 0.5% NaCl) for sixteen hours.

— 10 ml TYN broth containing 0.2% $MgSO_4$, 20% sucrose and 1 mg penicillin per ml were inoculated by 3 ml bacterial culture and cultivated five hours with shaking.

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— Spheroplasts obtained were centrifuged at 2,500 rpm and washed in TYN broth osmotically stabilised by 20% sucrose.

SPHEROPLAST REVERSION

Three different nutritive media were used for reversion :

— The first was TYN broth with 2% agar and osmotically stabilised by 20% sucrose and 0.2% $MgSO_4$ (Olasz, 1983). The spheroplasts were mixed with a top layer medium containing TYN broth with 0.4% agar, osmotically stabilised by 20% sucrose and spread on the plates with the first medium.

— The second regenerating medium contained normal broth with 2% agar and osmotically stabilised by 20% sucrose.

— The third reversion medium was a minimal medium (OLASZ, 1983) with 2% agar and osmotically stabilised by 20% sucrose.

GENETIC TRANSFORMATION OF *E. COLI* SPHEROPLASTS BY pBR 322

— pBR322 was obtained by the method of Birnboim and Dolly (1979) ;

— in order to achieve genetic tansformation 1ml spheroplast suspension with 1×10^2 cells was mixed with 100µl plasmidial DNA. Polyethyleneglycol 4,000 at a final concentration of 40% was added.

— after one minute the suspension was ten fold diluted and centrifuged at 2,500 rpm. The pellet was suspended in TYN broth osmotically stabilised and incubated for two hours at 37° C.

— After this the transformed spheroplasts were incubated in TYN reversion medium to which ampicilin (Ap) (50 µg/ml) and tetracycline (Tc) (20 µg/ml) were added.

Protoplast fusion was achieved by 40% PEG 4,000 after a method described before (Zarnea et al., 1988).

The electronmicroscopic study was carried out by fixing the material consisting of isolated, fused and reversed protoplasts by 3% glutaraldehyde, followed by postfixing with 2% OsO_4 and embeding in Epon. The ultrathin sections obtained with a TESLA ultramicrotom were stained by uranyl acetate and lead citrate and examined under a Philips electronmicroscope.

RESULTS AND DISCUSSIONS

SPHEROPLAST ISOLATION IN *E. COLI*

The spheroplasts were obtained by penicillin treatment. Compaired to OLASZ's method (1983), the concentration of penicillin was three times increased and the time of incubation was prolonged from three to five hours. The frequency of spheroplast isolation was nearly 100% in all tested strains (Table 1).

Table 1

Frequency of spheroplast reversion in different regenerating media

The Strain	The regenerating medium	Protoplasts frequency	Protoplasts per ml	Regenerated cells per ml	The frequency of regeneration %
Escherichia coli 354	TYN+TYN 0.4% agar, 20% sucrose 0.2% $MgSO_4$	100%	1×10^9	25×10^6	0.25
	agarised nutrient broth + 20% sucrose	100%	1×10^9	62×10^6	0.62
	agarised minimal medium 20% sucrose	100%	1×10^9	81×10^6	0.081
Escherichia coli HB101 Sm^R	TYN+TYN with 0.4% agar, 20% sucrose 0.2% $MgSO_4$	100%	1×10^9	6×10^6	0.06
	Agarised nutrient broth + 20% sucrose	100%	1×10^9	3×10^6	0.3
Escherichia coli HB101 Sm^R KM^R	TYN+TYN with 0.4% agar, 20% sucrose 0.2% $MgSO_4$	100%	1×10^9	3×10^6	0.03
	agarised nutrient broth + 20% sucrose	100%	1×10^9	3×10^7	0.3

As is known the cell wall of Gram negative bacteria consists of peptidoglycan and outer membrane, generating a periplasmic space (Fig. 1). The outer membrane, which is the special structure of Gram negative bacteria cell wall, is a selective membrane which does not permit the access of lytic enzymes to peptidoglycan. Before applying the lysozyme, spheroplast suspension is pretreated with EDTA in order to destabilise the outer membrane and to facilitate the access of lysozyme to peptidoglycan (WEISS, 1976, Witholdt, 1976). Another procedure for spheroplast obtaining is the treatment of the cells, which actively grow, with an antibiotic-like phosphomycin or penicillin which acts by inhibioing the cell wall formation (Lederberg, 1956 ; Olasz, 1983). In this situation the protoplast appearance is a consequence of the imposibility of cell wall formation.

In our study we choose the penicillin method because it is simpler and more efficient.

In some instances, the spheroplast formation starts by retracting the protoplast from both poles of the cell (Fig. 2). The retraction could progress and the protoplast could be completely eliberated from the outer membrane (Fig. 3) or the protoplast could remain attached to the outer membrane (Fig. 8). At the same time, the outer membrane could be broken (Fig. 3 and Fig. 8) or could remain entirely (Fig. 8 and Fig. 9). Sometimes the outer membranes form vesicles in the neighbourhood of the protoplast (Fig. 8 and Fig. 9). Inside the protoplast, on its outskirts, vesicles resulting from the membrane could be observed, too (Fig. 3 and Fig. 8). We suppose that these inner vesicles are a consequence of the disturbances caused by penicillin treatment.

In most intances, the spheroplasts have double membranes on some portions, formed by the cytoplasmic and outer membrane (Fig. 3 and Fig. 9).

The electronmicroscopic analysis permits us to conclude that the method used here for spheroplast isolation is very efficient.

SPHEROPLAST FUSION

In the case of *E. coli* spheroplast fusion a detailed analysis on microelectrographs, similar to that made by Frehel, (1979) for *B. subtilis*, was not made, even though recombinants were obtained by spheroplast fusion (Tsenin, 1978). For this reason we have considered useful such a study.

Similar to the protoplast fusion in *Bacillus*, in the first stage, simple or multiple adherences of *E. coli* spheroplasts could be observed. The adherence could take place between two different outer membranes (Fig. 8), between one outer membranes and a cytoplasmic one (Fig. 9), as well as between two cytoplasmic membranes (Fig. 11).

In the next stage the bispherical forms appear and the contacts between spheroplasts implied in fusion stretch on higher surface (Fig. 11). In some instances the cytoplasmic membranes of each spheroplast implied in fusion are waved and interconnected (Fig. 12). The space between these membranes is generally electrondense (Fig. 10), loaded by phospholipidic and proteic micelles. This is similar to what happens in the case of protoplast fusion in *Bacillus* (Frehel, 1979, Zarnea, 1988). Sometimes, vesicles could be seen in the space between the spheroplasts implied in fusion (Fig. 12).

In the final stage of fusion, the boundaries between the fused spheroplasts disappear, leading to the cytoplasm intermixing (Fig. 13).

The conclusion of the fusion process in the case of *E. coli* spheroplasts is that the same stages take place as in *B. subtilis* with some differences which are the consequence of the special structure of *E. coli* spheroplasts.

PEG-INDUCED SPHEROPLAST TRANSFORMATION BY pBR322

Certain data concerning *E. coli* spheroplast transformation by plasmid DNA do not exist as for our information. Vorobjeva et al. (1981)

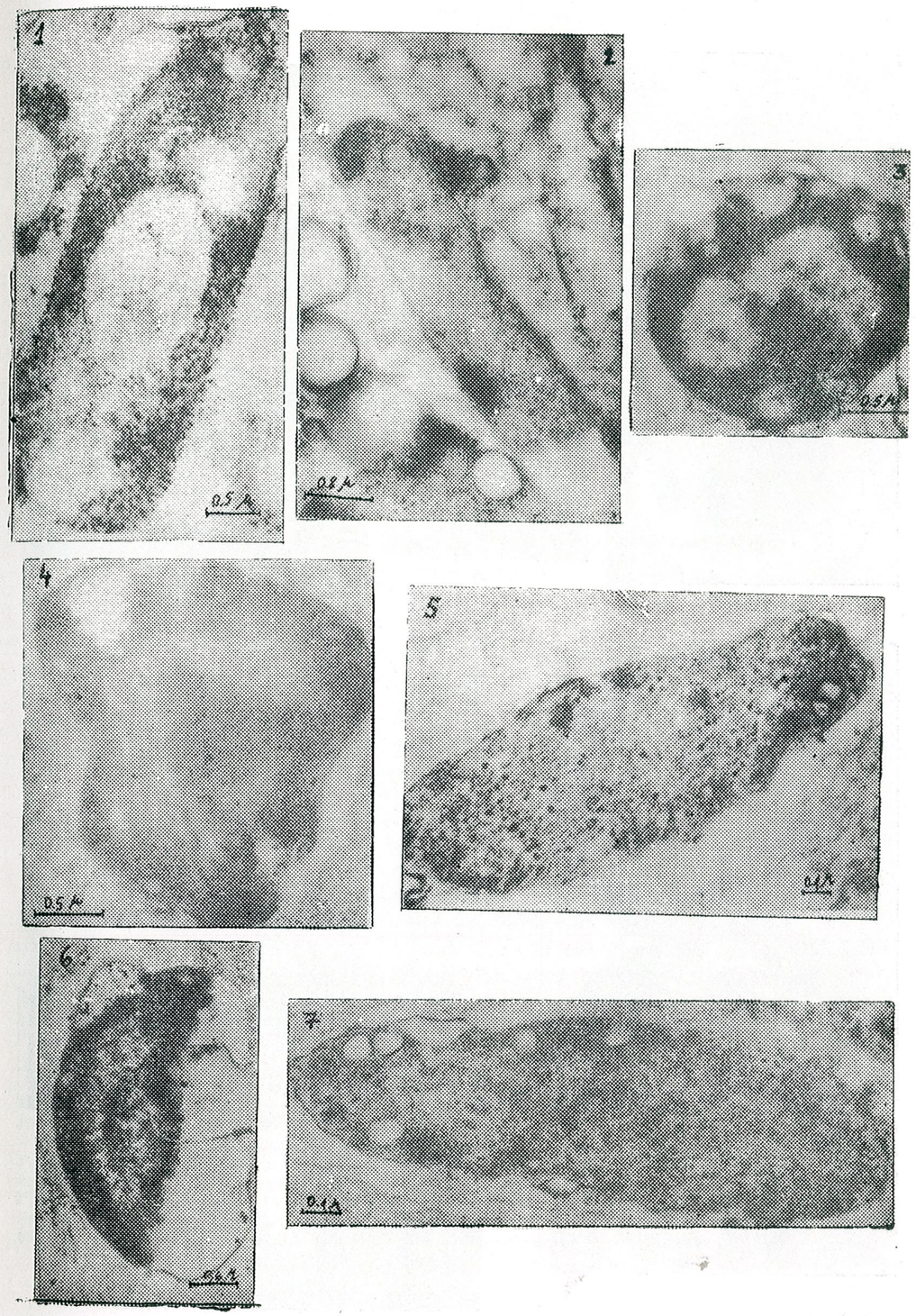

PLATE I. Ultrastructural aspects of *E. coli* spheroplast isolation and reversion processes:
Fig. 1. — The ultrastructural peculiarities of an *E. coli* cell.
Fig. 2. — Initial stage of protoplast isolation- Retracting of the protoplast from both poles of the cell.
Fig. 3. — A typical *E. coli* spheroplast.
Fig. 4. — Initial stage in reversion — Amoeboidal form.
Fig. 5. — Revertant having a bacillary form.
Fig. 6. — Revertant in which fibrilar material could be observed.
Fig. 7. — Revertant in which outer and cytoplasmic membranes could be distiguished.

PLATE I

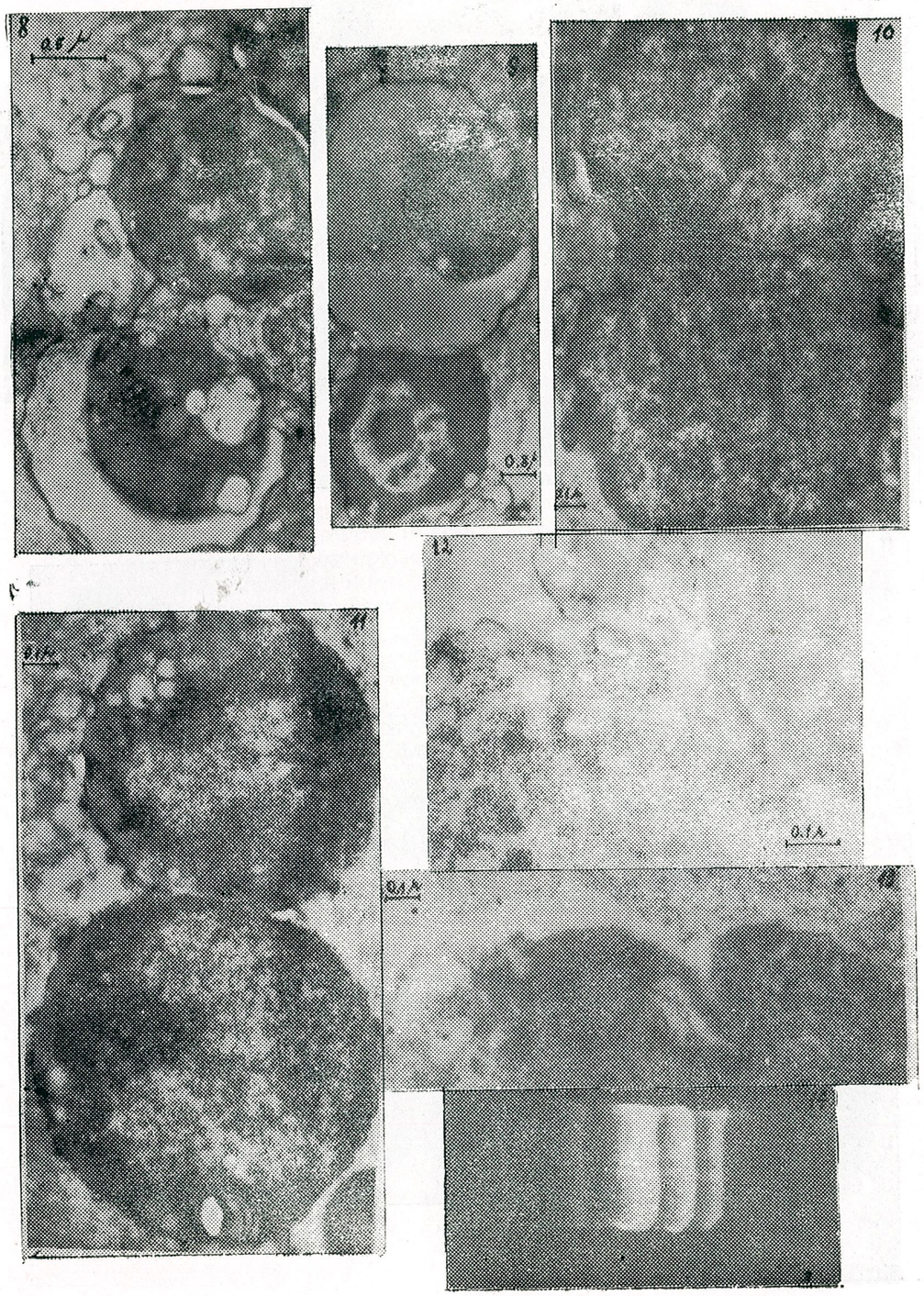

PLATE II

achieved the transfer of plasmids by protoplast fusion, but the transformation with plasmid DNA was unsuccessful. Olasz (1983) supposed that protoplasts obtained after penicillin treatment could be transformed but she did not publish any experimental data.

We did transform spheroplasts obtained by penicillin treatment in the conditions shown above by plasmid pBR322. The transformed colonies appeared on the regenerating medium, supplemented by ampicilin (Ap) and tetracyclin (Tc), were transferred on normal broth with 2% agar and the same antibiotics, to confirm the phenotypes acquired as a consequence of tansformation. At the same time, the plasmid DNA was isolated from the transformed cells and identifiend by agarose gel electrophoresis (Fig. 14).

We could appreciate that the transformation frequency was 5×10^{-6} transformed spheroplasts per ml, for a regeneration frequency of 0.06%.

We consider that, besides the genetic transformation of competent cells, PEG-induced transformation of spheroplasts could be a method for exogen DNA introducing in *E. coli* cells.

E. COLI SPHEROPLAST REVERSION

The reversion process was followed using different regenerating media (Table 1).

The results obtained showed that the frequencies of reversion for different strains of *E. coli* are comprised between 0.03—0.62%, comparable with those obtained by Tsenin (1978). The frequencies of regeneration varied depending on the regenerating medium and on the strain studied. The reversion had the heighest value on nutrient agar broth osmotically stabilised and the lowest one on minimal medium. The reversion in *E. coli* is lower as compared with that noticed in *B. subtilis*, may be because of the complexity of the cell wall in *E. coli*.

We considered opportune an analysis of the reversion process on microelectronographs. In Gram negative bacteria, the study of the spheroplast reversion is more difficult because the peptidoglycan is very thin and its regenerating could hardly be detected.

Similar to *B. subtilis*, in the first stage of reversion the change of the spheroplast shape can be observed with the appearence of the amoeboidal forms (Fig. 4). The change of the cell shape indicates the differentiation of the cell superficial tensions as a consequence of the beginning

←

PLATE II. Ultrastructural aspects of *E. coli* spheroplast fusion.
Fig. 8. — Adherence after PEG treatment between two different outer membranes.
Fig. 9. — Adherence between cytoplasmic and outer membranes after PEG treatment.
Fig. 10. — Bispherical form with an electrondense space between the protoplasts
Fig. 11. — Fused protoplasts after PEG treatment exhibiting a bispherical form.
Fig. 12. — Interconnections between the membranes implied in fusion.
Fig. 13. — Cytoplasm intermixing in fused spheroplasts.
Fig. 14. — Agarose gel electrophoresis of pBR322 DNA extracted from transformed spheroplasts

of cell wall biosynthesis. In the next stage the cells become longer and keep their irregular shapes (Fig. 5). Finally, the cells become bacillary (Fig. 6). At the outskirts of the cells two membranes will be distinguished : the cytoplasmic and the outer membrane (Fig. 6 and Fig. 7). Fibrilar material, indicating the synthesis of the peptidoglycan, could be noticed too (Figs 6 and 7).

The reversion of the cell wall in Gram negative bacteria seems to be specifically, because of the peculiar structure of the cell wall in these microorganisms.

REFERENCES

Birdsell, D. C., E. H. Cota-Robiel, J. Bacteriol., 1967, **93**, 427.
Birnbiom, H. C., Dolly J., Nucleic Acid Res., 1979, **7**, 1513.
Coetzee, J. N., F. A. Sirgell, G. Lecatsas, J. Gen. Microbiol., **114**, 313.
Frehel, C., Lheritier A. -M., C. Sanches-Rivas, P. Schaeffer, J. Bacteriol., 1979, **137**, 1354
Hopwood, D. A., Ann. Rev. Microbiol., 1981, **35**, 237.
Lederberg, J., J. St. Clair, J. Bacteriol., **75**, 143.
Olasz, K., in "Protoplasts, 1983"-Poster Proceedings, Experientia Suplimentum, **45**,
Potrykus I. et al. eds., Birkhauser Verlag, Basel, Boston, Stutgardt, 103, 1983.
Tsenin, A. M., C. A., Karimova, U. N. Rybchin, Dokl, Acad. Nauk. SSR, 1978, **243**, 1066.
Vorobjeva S. P., I. A. Khmel, in Advances in Protoplast Research, Procedeings of the 5th International Symposium, Budapest, Eds. Ferenczy L. and Farkas G., Akadémiai Kiadó, 1979, 37.
Weiss, R., J. Bacteriol., 1976, **128**, 668.
Witholdt, B., M. Boekhouti, M. Brock, J. Kingma, H. Van Heerikhuizen, L. De Leij, Analyt. Bichem, 1976, **74**, 160.
Zarnea, G., I. Vatafu, D. Avram, A. Brezeanu, P. Cornea, D. Moldoveanu, D. Vintilia, Rev. Roum. Biol., 1988, **33**, 51.

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PRELIMINARY STUDIES ABOUT HUMAN BIOENERGY EFFECT "ON IN VITRO" VEGETAL CULTURES

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Explants from *Chrysanthemum hortorum* HV Lamet (stem fragments with internodes), *Hyacinthus orientalis* (scale fragments) and *Mamillaria* sp. ("in vitro" neoformed plantlets) were treated with human bioenergy emitted from the two experimental subjects. The explant response is dependent on genotype, treatment time, explant type a.o. The human bioenergy had determined an intensification of the development rhythm, the enhancement of the caulogenesis points a.o. In *Hyacinthus orientalis* the rhizogenesis and caulogenesis took place on the same culture medium for 135 days (in control on two culture media for 170 days) on both faces of the scale fragments (in control only on the scale abaxial face).

The organisms usually emit electromagnetic radiations which induce a power field (biofield) around them. Each organism could be considered like a very complex biopower field, which could be recepted by other organisms around them. When a stress stage exists the bioenergy level becomes lower, leading to that organism exhaustion. Many experiments about bioenergy were effected with man and other organisms, but no data about bioenergy effect "in vitro" culture conditions were found.

In the present experiment there are presented preliminary data about the bioenergy effect, emitted by two experienced human subjects on some "in vitro" vegetal cultures.

MATERIAL AND METHOD

The effect of human bioenergy emitted by two subjectes (I.U. and E. P.) was studied at the "in vitro" development of different types of explants from *Chrysanthemum horiorum* HV Lamet (stem fragments with internodes), *Hyacinthus orientalis* L. (scale fragments inoculated in adaxial and vertical position given the culture medium surface) and *Mamillaria* sp. ("in vitro" neoformed plantlets). Part of *Chrysanthemum hortorum* explants originated in normal plants from "in vitro" subculture) and an other part in plants which were "in vitro" irradiated with X-rays (a unique, acute dose of 80 Gy, dosis rate being of 2 Gy/min).

All used explants were inoculated and developed on a MS basal medium with 2.5 mg/l KIN, 0.5 mg/l NAA and 0.3 mg/l thiamine-Hl.

The bioenergetic treatment was made two days after explants inoculation. With *Ch. hortorum* a chronical treatment was applied with bioenergy emitted by the I. U. subject, in two variants :

(a) twice a day, for 1 min each, six days long (12 × 1 min) ;

(b) alternatively, once at two days, for 1 min each, twice a day (6 × 1 min).

The subject E. P. applied an acute bioenergetic treatment with *Hyacinthus orientalis* and *Mamillaria* sp., for 2—4 minutes, until she "felt" that the culture bottles "charged" with bioenergy.

The explant development took place in the growth chamber at a 24° ± 2° C temperature and a light time of 16 hours/day (2400 lux).

The phenotype and biometric observations have been effectuated 65—135 days after treatment. Each treatment variant has 5—7 Erlenmayer bottles with 4—5 explants on each glass.

RESULTS AND DISCUSSIONS

Chrysanthemum hortorum HV Lamet. After the bioenergetic treatment took place an enhancement of the plantlets size and more organogenesis points appeared as confronted by control (Table 1). The biggest effect was stated at a longtime treatment (12 × min). With the explants from the "in vitro" irradiated subculture (with 80 Gy), the stem height was enhanced with 160% as compared with the control (72.0 mm as confronted by control, 45.0 mm) at 80 days after treatment. At the same time a caulogenesis process started, that is absent in the control (Table 1).

Table 1

The effect of the treatment with human bioenergy on the *Chrysanthemum hortorum* HV. Lamet explants, 80 days after treatment

Mother plant	Treatment	Plantlets height (mm) $\bar{x} \pm s_{\bar{x}}$	Shooting percentage	Shoots length (mm) $\bar{x} \pm s_{\bar{x}}$
Control	Control	83.1±15.0	57.5	26.8±16.0
	6×1 min	85.0±14.0	50.0	52.8±24.0
	12×1 min	89.2±6.0	115.4	41.3±27.0
Irradiated 80 Gy	Control	45.0×11.0	0.0	—
	12×1 min	72.0±13.3	120.0	45.0±12.6

Mamillaria sp. The bioenergetic treated plantlets by E. P. subject, presented, after 65 and 135 days of treatment, an intensification in the growth, caulogenesis and rhizogenesis processes (Table 2), in comparison with the control untreated.

Hyacinthus orientalis. Previous experiments (1), (2) made with *H. orientalis* have revealed that organogenesis has been produced on the abaxial face of the scale only (1). The NAA hormone presence in culture medium inhibits the organogenesis (1). The caulogenesis (130 days) and rhizogenesis (35—40 days) processes required the presence of two culture media with a different hormone content.

The stimulation with bioenergy of explants (scale fragments), two days after inoculation, determined modifications in the "in vitro"

Table 2

The effect of treatment with human bioenergy on *Mamillaria* sp. plantlets "in vitro" development

Treatment	65 days		135 days		
	Height (mm) $\bar{x} \pm s_{\bar{x}}$	Diameter (mm) $\bar{x}$	Root length (mm) $\bar{x} \pm s_{\bar{x}}$	Stem length (mm) $\bar{x} \pm s_{\bar{x}}$	Stem diameter (mm) $\bar{x} \pm s_{\bar{x}}$
Control	10.0±0.9	2.0	19.4±4.4	16.4±0.8	5.1±0.2
Bioenergy	11.8±2.0	2.5	21.0±1.1	19.0±0.6	5.5±0.6

explant development. The NAA hormone presence in the culture medium determines, in these conditions, the organogenesis processes starting both on the abaxial face, and on the adaxial face of the scale fragments, inoculated with the adaxial face in relation to the medium surface (Figs. 1, 2). The caulogenesis and rhizogenesis took place on the same culture medium, in only 135 days (Table 3). The obtained plants, with 2—3 leaves and 2—3 roots, are vigorous by well developed, capable for acclimatisation.

Table 3

The effect of the treatment with human bioenergy on *Hyacinthus orientalis* L. scale fragments

Treatment	Scale position	Shooting percentage	Number of shoots on scale $\bar{x} \pm s_{\bar{x}}$	Shoots length (mm) $\bar{x} \pm s_{\bar{x}}$	Leaves number $\bar{x} \pm s_{\bar{x}}$	Roots length (mm) $\bar{x} \pm s_{\bar{x}}$
Control	Adaxial	—	—	—	—	—
	Vertical	50	1.0±0.0	8.0±3.0	2.0±0.0	—
Bioenergy	Adaxial	50	4.0±0.0	12.9±5.2	2.0±0.9	5.0±2.2
	Vertical	50	2.5±1.5	18.8±8.4	2.2±0.7	—

CONCLUSIONS

1. The vegetal explant response to the bioenergetic treatment depends on the genotype, explant type, source of bioenergy, administered treatment a.o.

2. An increase of the metabolic processes which determines both the enhancement of the explant growth rhythm (with the three tested species) and of the organogenesis points has been found.

3. The treatment with human bioenergy, shortened with 35 days the caulogenesis and rhizogenesis processes in *Hyacinthus orientalis* eliminating a part of the work stages, all the organogenesis processes taking place in the same culture medium.

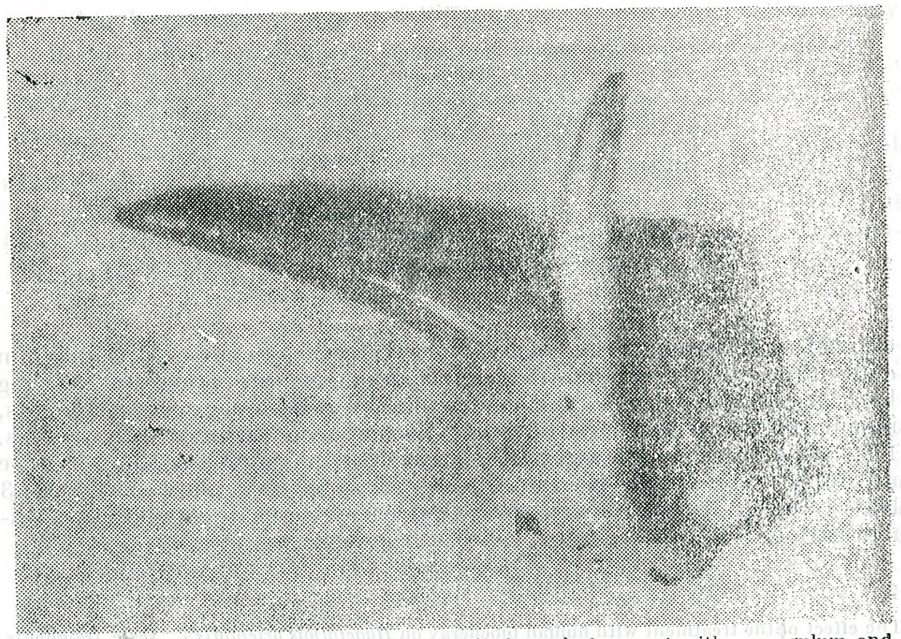

Fig. 1. — Organogenesis in *Hyacinthus orientalis* L. scale fragment with propagulum and shoot.

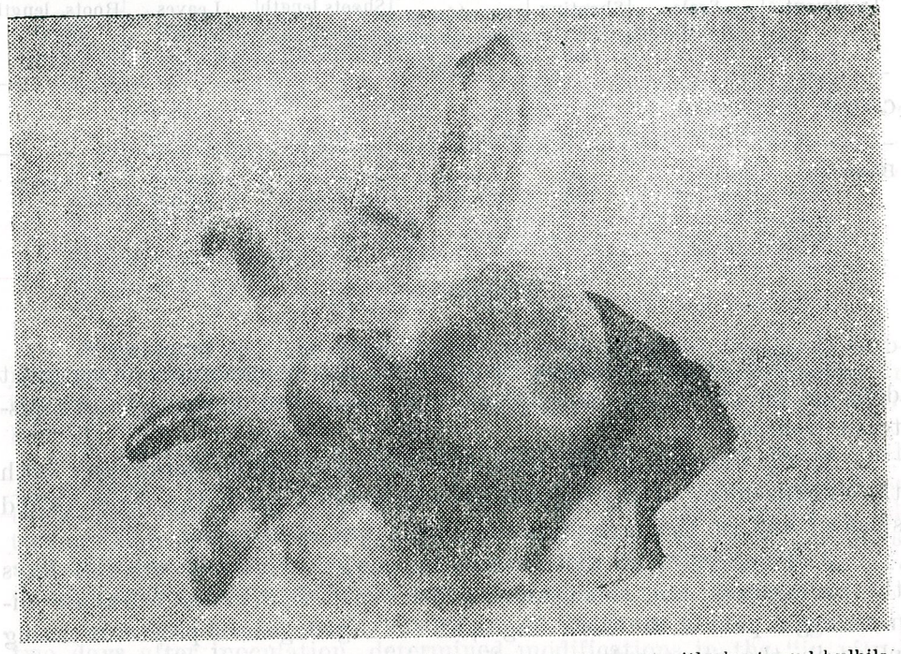

Fig. 2. — Organogenesis in *Hyacinthus orientalis* L. scale fragment with shoots and bulbils.

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REFERENCES

1. Corneanu Mihaela, In vitro clonal multiplication at *Hyacinthus orientalis* L., 1991 (in press).
2. Paek, K. Y., Thorpe, T. A., Hyacinth. In: *Handbook of Plant Cell Culture*, vol. 5, pp. 479—508 (P. V. Ammirato, D. V. Evans, W. R. Sharp, Y.P.S. Bajaj, Eds.), McGraw-Hill Publ. co., New York.

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L'ASSIMILATION SÉLECTIVE DES FACTEURS ABIOTIQUES PAR LES SYSTÈMES VÉGÉTAUX DE NIVEAU INDIVIDUEL

ION I. BĂRA, CONSTANTIN TOMA, IULIAN ALBU, MIHAELA NIȚĂ, SIMONA AIZICOVICI

Le processus de l'évolution et de l'adaptation des systèmes vivants s'est déroulé dans une continuelle et indispensable interaction avec le milieu ambiant (la totalité des facteurs cosmiques qui inondent en permanence la Terre). Le spectre des espèces qui existent actuellement dans la biosphère représente la résultante d'une longue pression sélective, de la confrontation entre les tendances héréditaires conservatrices et la capacité intrinsèque de variabilité, augmentée par l'impact aléatoire de quelques facteurs perturbateurs dans le canal de transmission de l'information génétique.

Dans le processus de l'évolution, l'information génétique détenue par les systèmes vivants ancestraux s'est enrichie sans cesse. Par conséquent, l'hérédité représente la quintessence du mélange des éléments génétiques et écologiques. Le rôle de la sélection consiste également dans la perpétuation et le perfectionnement des systèmes individuels à potentiel d'adaptation élevé, à savoir ceux aptes à saisir, assimiler et utiliser les facteurs abiotiques aux qualités les plus propices pour leurs autoperfectionnement et évolution.

Un exemple en est donné par la capacité des systèmes individuels végétaux de convertir l'énergie cosmique au bénéfice de leur propre développement. Dans ce contexte, nous rappelons que le bilan positif de l'impact de l'irradiation solaire sur la végétation est localisé entre 300—800 nm, à un rendement de 0,86% (3). Les processus énergétiques des biosystèmes végétaux reposent sur l'émission et l'absorption de quanta d'énergie électromagnétique. Tout le phénomène représente une résultante de la pression de la sélection, dans le contexte de l'évolution sous l'impact des radiations. Mais il y a encore un aspect complémentaire. À l'irradiation du monde vivant s'est ajoutée une irradiation du substrat inorganique, suivie de modifications qualitatives. En se diversifiant et en s'adaptant continuellement, les systèmes vivants sont devenus capables de déceler parmi les éléments du substrat inorganique ceux dont l'état est le plus compatible avec leurs nécessités et de les assimiler de façon sélective.

Les recherches relatives à l'assimilation des bio-informations par les structures biologiques, en interaction avec les substances inorganiques irradiées dans le spectre monochromatique de 546 nm, ont ouvert un nouveau chapitre dans la « biophysique des radiations » et sont groupées sous le générique d' «effet Comoroşan » (3).

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Vu que l'effet des radiations est en grande mesure connu (14, 20, 16, 9, 2, 12, 18), nous nous sommes proposé de poursuivre des investigations sur l'effet de l'irradiation indirecte, à savoir les radiations électromagnétiques dans le domaine visible, afin de surprendre l'existence d'un récepteur biologique pour le substrat inorganique « excité » par l'irradiation.

MATÉRIEL ET MÉTHODES DE TRAVAIL

Pour l'irradiation dans le spectre monochromatique de 546 nm, on a utilisé une lampe UV de 125 W, alimentée à 220 V (5, 6, 7, 8). L'intensité de la lumière au niveau de la surface irradiée a été de 700 lx. L'irradiation a visé le KNO_3 cristallin, en couche mince (0,1 g ; 0,2 g ; 0,3 g).

L'effet indirect de l'irradiation a été testé sur du blé *(Triticum aestivum*, cultovariété Moldova 8371, super-élite).

Le test, répété 6 fois, a consisté dans une variante contrôle général 3 variantes contrôle spécifique et 6 variantes d' irradiations (de 5 et, respectivement, 10 secondes). Chaque variante a comporté 6 boites Petri à 100 plantes, disposées en cercles concentriques. Les données ont été interprétées statisquement par un programme sur l'ordinateur TI 51 III.

Le contenu d'acides nucléiques totaux des racines des plantules a été établi par lecture sur Spekord UV—VIS. La quantité de pigments assimilateurs a été établie par extraction d'après méthodes spécifiques et lecture sur SPEKOL-20.

Les tests histo-anatomiques ont été effectués sur de coupes faites au microtome à la main et par coloration vert iode et carmin aluminé.

RÉSULTATS ET DISCUSSION

L'impact de certains facteurs biotiques ou abiotiques sur des systèmes vivants peut modifier le taux des processus de transcription et translation avec des répercussions dans la phénotypisation du génotype (dans le cadre de la norme de réactions spécifiques — champ d'action de la selection).

On connaît que le KNO_3 a des effets inhibiteurs sur l'activité de l'ADN (19). On peut apprécier que l'effet respectif s'étend sur le processus de transcription, impliquant tant l'activité de l'ADN que celle de tous les types d'ARN. D'autre part, le K (représentant 25—30% des cendres de plantes) est considéré comme un macro-élément indispensable à la croissance et au développement, impliqué surtout dans les processus métaboliques des cellules méristématiques. Le K intervient dans la synthèse des glucides et des protéines (11) et peut accélérer les processus de germination des graines, ayant des implications positives dans la protéosynthèse à cause de ses qualités radioactives.

Grâce à son rôle dans la structure du protoplasme, par sa présence dans les chaînes latérales des molécules protéiques, le K a la possibilité de circuler d'un bout à l'autre de l'organisme végétal, des tissus vieillis

à ceux méristématiques. Le fait que les ions de potassium augmentent le degré d'hydratation des colloïdes plasmatiques, en liant les molécules d'eau est important lui aussi.

En même temps, l'azote est indispensable à la bonne croissance et au développment des plantes, étant connu que le plus haut rendement dans l'utilisation des deux ions est obtenu lorsqu'on les administre sous forme de KNO_3.

Pour estimer correctement l'effet de l'irradiation indirecte sur les processus impliqués dans la croissance du blé dans les 10—14 premiers jours du cycle ontogénétique, nous avons assuré une variante à laquelle l'arrosage s'est effectué seulement avec de l'eau distillée (témoin général), trois variantes dans lesquelles l'arrosage s'est effectué avec des solutions de KNO_3 en concentrations de 0,1%, 0,2% et 0,3% (témoins specifiques) et deux groupes à trois variantes chacune dans lesquelles le KNO_3, dans la même concentration que pour les témoins spécifiques, a été administré après des irradiations de 5 ou 10 secondes.

Le premier jour des mesurages (cinquième après ensemancement des caryopses en vue de germination), on a constaté des différences entre les variantes, tant sous l'aspect de la longueur des épicotyles que sous celui de l'accumulation de la biomasse (tableau 1). Il faut souligner que les variantes obtenues par irradiation se sont nettement détachées tant par rapport au témoin général que par rapport aux témoins spécifiques. Ultérieurement, la situation se complique et se diversifie toujours, le seul trait général qui reste étant l'effet stimulant de l'irradiation, surtout pour la concentration de 0,2% et l'irradiation pendant 10 secondes, jusqu'au cinquième jour des mesurages. Plus tard, surtout pour la longueur de l'épicotyle, l'irradiation induit des effets répressifs de sorte que, finalement, à une seule exception près, les variantes arrosées avec du KNO_3 irradié ont enregistré des valeurs inférieures à celles des témoins spécifiques.

La situation est beaucoup plus évidente si on exprime en pourcentages la longueur finale comparée de toutes les variantes (tableau 1). Donc, si on inscrivait sur une courbe la dynamique de l'allongement, on constaterait que, chez les variantes arrosées avec du KNO_3 irradié, l'asymptote supérieure est atteinte dans un temps plus court que chez les témoins correspondants et, implicitement, le plateau s'installe plus vite. Par conséquent, l'effet bénéfique du KNO_3 ainsi que l'effet toxique dû au dépassement d'un certain niveau d'accumulation dans la plante se manifeste beaucoup plus rapidement chez les variantes traitées avec du KNO_3 irradié.

Mais quels sont donc les mécanismes intimes par lesquels la plante saisit l'effet de l'irradiation sur le KNO_3? Evidemment, la dynamique de la pénétration du KNO_3 dans la plante est modifiée après l'irradiation. Par conséquent, la mobilisation des effets de l'endosperme s'accélère. La plante pousse plus rapident. Mais les réserves de l'endosperme s'épuisent elles aussi plus vite. Alors, naturellement la stagnation de la croissance survient plus vite chez les variantes irradiées. Cette supposition est partielle ment argumentée par le comportement de quelques

Tableau 1

Comportement des caractères quantitatifs sous l'influence du traitement indirect avec les phases ontogénétiques prématurées

Variante		Dynamique de la hauteur et du					
		I		II		III	
		mm	mg	mm	mg	mm	mg
Témoins	H_2O	51,25	133,3	82,2	147,6	114,5	188,6
	KNO_3 1‰	57,1	153,5	100,1	178,8	133,6	230,0
	KNO_3 2‰	57,6	144,8	98,2	166,7	127,2	197,6
	KNO_3 3‰	54,6	128,3	91,0	158,5	126,4	189,9
KNO_3 irradié 5″	1‰	63,2	158,7	101,5	171,1	137,1	226,0
	2‰	61,5	154,4	99,4	167,1	135,1	227,4
	3‰	57,0	134,2	94,3	167,7	130,3	206,5
KNO_3 irradié 10″	1‰	60,9	153,4	99,3	182,2	135,4	215,0
	2‰	61,8	138,4	101,3	180,0	136,6	203,7
	3‰	58,2	138,9	95,4	178,0	133,4	195,7

caractères physiologiques et biochimiques (le contenu en acides nucléiques et en chlorophylle).

On peut avancer encore une hypothèse. Sous l'impact de l'irradiation, le KNO_3 pénétre (passivement ou par absorption active) plus rapidement dans la cellule. Implicitement, pour réaliser l'équilibre des paramètres chimiques, la plante absorbe plus d'eau — les vacuoles augmentent, es tissus deviennent plus turgescents. Les données histo-anatomiques offrent des arguments pour cette supposition.

La dynamique de la biomasse (fraîche et sèche) offre, également, quelques explications (tableau 2). Le dernier jour de l'observation, la

Tableau 2

Comportement des caractères quantitatifs sous l'influence du traitement indirect avec des

Variante		Biomasse initiale	Biomasse accumulée			
			Fraîche			
			Nette		Rapportée aux témoins	
		mg	mg	%	H_2O	KNO_3
Témoins	H_2O	23.03	204,60	889,60	100,00	—
	KNO_3 1‰	24.43	262.30	1073.70	128.20	100.00
	KNO_3 2‰	23,88	223,90	937,60	109,43	100,00
	KNO_3 3‰	23.77	230,63	970,30	112,72	100,00
KNO_3 irradié 5″	1‰	24,71	239,49	969,20	117,10	91,30
	2‰	25,63	224,37	875,40	109,70	100,20
	3‰	25,10	217,40	866,10	106,30	96,24
KNO_3 irradié 10″	1‰	24,28	276,40	1138,40	135,10	105,40
	2‰	20,53	255,17	1242,90	124,70	114,00
	3‰	24,66	227,80	923,76	111,30	98,80

des radiations électromagnétiques dans le domaine du visible chez. *Triticum aestivum* durant

poids pendant 6 jours						Hauteur finale par rapport aux témoins avec :	
IV		V		VI		H_2O	KNO_3
mm	mg	mm	mg	mm	mg	%	%
139,2	191,0	152,2	203,6	164,8	227,6	100,00	—
161,6	237,6	173,8	255,9	207,3	286,7	125,80	100,00
160,6	225,3	190,4	239,0	202,7	247,8	123,00	100,00
146,7	197,9	187,5	241,9	198,0	254,4	120,10	100,00
164,8	234,3	185,4	247,2	204,7	264,2	124,21	98,74
167,0	229,8	189,4	249,5	197,9	250,0	120,10	97,63
161,8	221,8	186,3	235,5	192,5	242,5	116,08	97,22
163,8	237,1	184,4	263,2	190,9	300,7	115,83	92,08
159,6	232,1	187,9	256,0	211,5	275,7	128,33	104,34
161,3	224,2	184,2	243,2	194,6	252,5	118,10	98,28

biomasse fraîche a enregistré, pour la concentration optimale de 0,2%, des valeurs supérieures à celles des témoins spécifiques, chez les deux type d'irradiation (d'ailleurs, tout comme la variante 01%, irradiée pendant 10″ .

Qui donne la différence — l'eau du tissu ou la substance synthétisée *de novo*? Intéressantes et partiellement explicables sont les données relatives à 'accumulation de substance sèche.

radiations électromagnétiques dans le domaine du visible cheu *Triticum aestivum* pendant les phases ontogénétiques prématurées

pendant l'expérience				Cendre			
Sèche							
Nette		Rapportée aux témoins avec :		Nette		Rapportée aux témoins avec :	
mg	%	H_2O	KNO_3	mg	%	H_2O	KNO_3
26,1	113,30	100,0	—	0,7	2,68	100,00	—
25,1	102,70	96,200	100,00	1,4	5,57	200,00	100,00
25,1	105,10	96,20	100,00	1,6	6,37	228,60	100,00
25,2	106,00	96,60	100,00	1,9	7,53	271,50	100,00
25,5	103,20	97,70	101,60	1,5	5,88	214,30	107,10
25,3	98,71	96,90	100,80	1,7	6,71	242,90	106,30
26,9	107,20	103,10	106,70	2,8	10,40	400,00	147,40
26,6	109,60	101,90	106,00	1,4	5,26	200,00	100,00
27,2	132,50	104,20	108,40	2,0	7,35	285,70	125,00
27,8	112,70	106,50	110,30	2,2	7,91	314,30	115,80

Les témoins spécifiques et deux variantes irradiées sont au niveau du témoin général. Comparées aux témoin spécifique, les variantes irradiées ont réalisé des valeurs supérieures à l'accumulation de substance sèche. Donc, la quantité de substance nouvellement synthétisée est plus grande, la quantité d'eau libre étant implicitement plus petite. Une nouvelle question se pose alors. Le surplus de substance sèche est dû aux composés organiques ou minéraux? La quantité de cendre est plus grande chez toutes les variantes d'irradiation.

Outre les réserves nutritives de l'endosperme, les plantes n'ont reçu que l'eau et du KNO_3. Les différences dans la biomasse fraîche sont peut-être dues à la substance organique ou minérale. Les différences dans la quantité de cendre ne peuvent être dues qu'aux substances minérales (le K en dernière instance). Donc chez les variantes arrosées avec du KNO_3 irradié, il y a plus de K. Pour les hypothèses avancées sur la base des données analysées, on a cherché également des arguments d'ordre histo-anatomique (tableau 3).

Tableau 3

Données numériques moyennes (en μm) concernant quelques traits histo-anatomiques des

Variantes	Racine			épaisseur de l'écorce
	Ø section	Ø cylindre central	Ø vaisseau de xylème méta	
Témoins				
H_2O	1600	587	43	105
KNO_3 $1^0/_{00}$	1750	527	50	133
KNO_3 $2^0/_{00}$	1640	507	45	125
KNO_3 $3^0/_{00}$	1330	540	47	95
Traitements 5 sec.				
KNO_3 $1^0/_{00}$	1266	433	50	92
KNO_3 $2^0/_{00}$	1733	580	47	133
KNO_3 $3^0/_{00}$	1533	513	47	117
Traitements 10 sec.				
KNO_3 $1^0/_{00}$	1933	647	52	150
KNO_3 $2^0/_{00}$	1707	527	40	137
KNO_3 $3^0/_{00}$	1866	547	48	150

Les investigations de ce type ont surpris des aspects généraux conformes aux données de la littérature de spécialité (4, 10, 13, 15, 21, 22, 23).

Chez les variantes irradiées, la radicule avait les poils absorbants plus grands, des couches corticales plus nombreuses et avec des cellules plus grandes. L'endoderme est passè tôt du stade primaire à celui tertiaire, ayant les parois intérieures plus épaisses. Le cylindre central a des cellules dont les parois sont modérément épaisses et lignifiées. Le nombre des vaisseaux de protoxylème a augmenté et les vaisseaux de bois ont eu les parois plus minces. L'épaisseur des racines a augmenté chez quelques-unes des variantes irradiées.

Le coléoptile des variantes iradiées a 5—6 couches de parenchyme, par rapport à 3—5 couches chez les témoins. Les cellules parenchymatiques sont plus grandes. Le grand diamètre d'une section romboïdale est supérieur par rapport aux témoins, en moyenne de 7 cm, tandis que le petit diamètre arrive à 6—7 cm. L'épaisseur du parenchyme a enregistré des augmentations de 1—1,6 cm. Le limbe foliaire est étroit et tordu chez les témoins, large et rarement tordu chez les variantes de traitement. La plus grande largeur a été enregistrée aux concentrations de 0,1% et 0,2%, irradiées pendant 10". La largeur du limbe foliaire a enregistré, sans exception, les plus grandes valeurs chez les variantes irradiées. Les éffets bénéfiques des irradiations indirectes ont été saisis également par les déterminations du contenu en acides nucléiques et en protéines (tableau 4).

Il est évident que les quantités les plus grandes d'acides nucléiques et de protéines se trouvent chez les variantes irradiées (2—3 fois plus de protéines). Nous nous demandons comment s'explique cette situation,

organes végétatifs des plantules (dans des coupes transversale)

Coléoptile			Limbe de la feuille		
Ø longitudinal	Ø transversal	épaisseur aux pôles	largeur	épaisseur	
				au niveau de la nervure médiane	au niveau des vallecules
1140	700	320	2113	200	70
1227	767	314	2267	242	83
1466	1060	340	2867	238	105
1467	900	334	3000	190	92
1380	1093	314	3280	233	117
1447	1000	320	3133	240	142
1347	1080	327	2833	192	83
1527	1120	373	2627	177	88
1580	1087	387	3240	242	127
1667	1200	400	2687	247	133

tout en tenant compte du fait que le K est considéré comme un inhibiteur de l'activité de l'ADN (19). La présence du K, par ses propriétés légèrement radioactives, est stimulante pour la biosynthèse de l'ARN et des protéines. Comme hypothèse de travail, on peut considérer que le traitement avec des radiations électromagnétiques a stimulé l'effet bénéfique de la radioactivité du K. Ce qu'il faut souligner c'est le fait que la présence des acides nucléiques et des protéines en quantités plus grandes sous l'impact de l'irradiation entre en corrélation avec le contenu plus grand de substance sèche et de cendre.

Tableau 4

Quantités d'acides nucléiques totaux (AcN) et de protéines, déterminées d'après le monogramme d'Adams

Variante	T_{260} nm	T_{280} nm	260 nm	280 mm	AcN (mg/ml)	Protéines (mg/ml)
Témoins H_2O	91	89	0,040	0,050	0,003	0,02
KNO_3 2‰	86	80	0,065	0,100	0,005	0,02
Traitement 5 sec. KNO_3 2%	78	70	0,110	0,150	0,006	0,07
Traitement 10 sec. KNO_3 2‰	79,5	75	0,100	0,125	0,0052	0,05

Les investigations concernant l'établissement de la dynamique du contenu des pigments assimilateurs (tableau 5) ont relevé des données dont l'interprétation est (du point de vue des hypothèses exposées dans le présent travail) difficile. D'ailleurs, si l'on tient compte du fait que, dans les cadre **de** l'idiotype d'un individu végétal, le génome chloroplastique a une **origine** différente du reste des génomes (1), le comportement différent des chloroplastes (sous l'impact de l'irradiation) semble naturel. En outre, on apprécie que les facteurs qui favorisent l'activité du protoplasme accélèrent la biosynthèse des pigments chlorophylliens aussi (17), car le K intervient dans le métabolisme des hydrates de carbone par le maintien des colloïdes plastiques en état hydraté favorable aux processus d'oxydo-réduction et peut avantager la biosynthèse de la chlorophylle. Dans ce cas, le contenu de pigments chlorophylliens n'a pas un comportement linéaire, mais ondulatoire. D'un jour à l'autre il enregistre des baisses et des croissances alternatives. Ce qui différencie les variantes témoins des variantes irradiées c'est l'amplitude des maxima et des minima quotidiens d'un côté, et celle du rythme de l'alternance de la hausse et de la baisse d'autre côté. Le caractère cyclique du comportement, dans le cas des chloroplastes, peut être expliqué par l'alternance lumière—obscurité (12 h/12 h) dans l'expérimentation ci-présente.

On connaît que les plantes synthétisent la chloropyhlle seulement en présence de la lumière et, de plus, que le rendement de la biosynthèse augmente beaucoup dans une lumière de petite intensité.

En mettant en corrélation la synthèse des pigments chlorophylliens avec la dynamique de l'accumulation de la biomasse fraîche, on constate des interdépendances claires. Le taux d'accumulation de la biomasse est maximum chez les variantes auxquelles la chlorophylle enregistre les oscilations quantitatives les plus petites.

Les tests d'ordre cytogénétique (tableaux 6 et 7) n'ont pas surpris d'aberrations induites par le traitement à radiations électromagnétiques. Sous l'aspect de l'indice mytotique, les variantes arrosées avec du KNO_3 irradié ont enregistré des valeurs nettement supérieures.

Tableau 5

Influence des radiations électromagnétiques du spectre visible sur la synthèse de la chlorophylle a et b chez *Triticum aestivum* L., 2n = 42, durant les phases ontogénétiques prématurées

Jours	I		II		III		IV		V		VI	
Variante	Chlorophylle (mg/g)											
	a	b	a	b	a	b	a	b	a	b	a	b
Témoins H_2O	0,410	0,774	0,357	0,762	0,652	1,330	0,649	2,299	0,743	1,593	0,650	1,330
KNO_3 1‰	0,454	0,942	0,281	0,705	0,689	1,534	0,645	1,428	0,766	1,606	0,743	1,534
KNO_3 2‰	0,393	0,780	0,410	0,699	0,820	1,826	0,624	1,328	0,781	1,623	0,865	1,826
KNO_3 3‰	0,349	0,687	0,354	0,791	0,740	1,670	0,767	1,619	0,904	1,948	0,868	1,817
Traitements 5 sec. KNO_3 1‰	0,315	0,612	0,303	0,612	0,687	1,489	0,574	1,270	0,705	1,512	0,789	1,719
KNO_3 2‰	0,408	0,788	0,304	0,648	0,899	1,887	0,657	1,413	0,734	1,562	0,754	1,578
KNO_3 3‰	0,408	0,801	0,322	0,770	0,823	1,755	0,767	1,619	0,904	1,948	0,868	1,817
Traitements 10 sec. KNO_3 1‰	0,379	0,754	0,339	0,764	0,734	1,603	0,544	1,178	0,726	1,503	0,715	1,490
KNO_3 2‰	0,344	0,683	0,322	0,732	0,884	1,957	0,739	1,545	0,787	1,664	0,757	1,544
KNO_3 3‰	0,370	0,705	0,474	0,992	0,474	1,728	0,685	1,496	0,730	1,592	0,816	1,656

Tableau 6

Indice mitotique des radicules de *Triticum aestivum*, 2n = 42, sous l'influence du KNO_3 irradié en lumière monochromatique (λ = 546 nm)

Variante	Ensemble des cellules analysées	Ensemble des cellules en interphase		Ensemble des cellules en division		Cellules en mitose					
						Prophase		Métaphase		Anaphase		Télophase	
	N	N	%	N	%	N	%	N	%	N	%	N	%
Témoin	1383	419	30,29	964	69,70	832	86,30	49	5,08	30	3,11	53	5,49
Traitement KNO_3 2‰ 5''	1441	341	23,66	1100	76,33	937	85,18	37	3,36	46	4,18	80	7,27
Traitement KNO_3 2‰ 10''	1354	330	24,34	1024	75,62	938	91,60	24	2,34	24	2,34	38	3,71

Tableau 7

Fréquence des aberrations des chromosomes pendant l'anaphase et la télophase dans les radicules de *Triticum aestivum*, 2n = 42, sous l'influence du KNO_3 irradié

Variante	Ensemble A et T étudiées	A et T normales		A et T aberrantes		Types des aberrations			
						Fragments		Ponts simples	
	N	N	%	N	%	N	%	N	%
Témoin	343	333	97,08	10	3,0	5	50,0	5	50,0
Traitement KNO_3 2‰ 5″	381	375	109,32	6	1,6	2	33,3	4	66,6
Traitement KNO_3 2‰ 10″	327	325	94,75	2	0,6	—	—	2	100,0

BIBLIOGRAPHIE

1. Antohi, S., Gavrilă, L., 1981, *Progrese în genetica moleculară*, Ed. Ştiinţifică şi Enciclopedică, Bucureşti.
2. Bowen, H. J. M., 1962, Rad. Bot., **1** (3): 223—228.
3. Celan, N., 1985, *Materia vie şi radiaţiile*, Ed. Ştiinţifică şi Enciclopedică, Bucureşti.
4. Champagnat, R., Ozenda, P., Baillaud, L., 1969, *Biologie végétale, III. Croissance, morphogenèse, reproduction*, Masson, Paris.
5. Comoroşan, S., Murgoci, D. S., Cru, N., 1971, Physiol. Chem. Physics, **3**: 343—352.
6. Comoroşan, S., 1974, Int. J. Quantum Chem. Quantum Biol., **1**: 221—228.
7. Comoroşan, S., Hristea, N., Murgoci, P., 1980, Bull. Math. Biol., **42**: 107—117.
8. Comoroşan, S., Tiron, V., Hristea, M. Cincă, S., Pîslaru, L., Popescu, V., 1980. Physiol. Chem. Physics, **12**: 497—508.
9. Dubinin, N. P.,1966, *Genetica moleculară şi acţiunea radiaţiilor asupra ereditătii*. Ed. Ştiinţifică, Bucureşti.
10. Fritsch, R., 1977, Flora, **196**: 285—326.
11. Jeanrenaud, E., 1973, *Curs de fiziologia plantelor*, Univ. Iaşi.
12. Kaindl, K., Linser, H., 1961, I.A.E.A. — Viena, 10: 1—30.
13. Matienko, B. T., 1984, *Ekologo-anatomiceskie osobennosti izmenchivosti kulturnth rastenii*, Izd. Ştiinţa, Kischinev.
14. Milcu, Şt., Nicu, M., Racoveanu, N,. 1966, *Doze mici de radiaţii în medicină, biologie şi ştiinţe agricole*, Ed. Academei, Bucureşti.
15. Napp-Zinn, K., 1984, *Anatomie des Blates*, Berntraeger, Berlin.
16. Nicolae, I., Nasta, A., 1975, *Radiogenetica*, Ed. Ştiinţifică, Bucureşti.
17. Péterfi, S., Sălăgeanu, N., 1972, *Fiziologia plantelor*, Ed. Didactică şi Pedagogică, Bucureşti.
18. Popp, F. A., Becker, G., König, H., Peschka, E., 1979, *Electromagnetic Bio-Information* Urban und Schwerzenberg, München.
19. Portocală, R., Popa, L., 1966, *Acizii ribonucleici celulari şi virali*. Ed. Academiei, Bucureşti.
20. Sax, K., 1955, Amer. J. Bot., **42** (4): 360—364.
21. Takhtadzhyan, A. L., 1985, *Sravnitel'naya anatomya semyan*, Tome I, Izd. Nauka, Leningrad.
22. Toma, C., Niţă, M., Tănase, C., Toma, L., 1983, Cercet. Agron. în Moldova, **2** (62): 119—124.
23. Zanoschi, V., Toma, C., 1985, *Morfologia şi anatomia plantelor cultivate*, Ed. Ceres, Bucureşti.

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LA VARIATION DU COMPLEXE D'ENZYMES OXYDO-RÉDUCTRICES DURANT LA PÉRIODE DE REPOS DE LA VIGNE

ANCA ANTOHE

In this paper researches are a continuation of previous ones (1, 2, 3, 4) carried out in the north-east of Moldavia, with the following sorts of *Vitis vinifera*: Aligoté (sort originating from France) and black Fetească (autochthonous sort). The resistance of plants to environment unfavourable conditions is also given by the enzymatic complex.
In the profound and forced repose the activity of polyphenoloxidase increases especially in buds and their nodes.
The dynamics of polyphenoloxidase and of peroxidase is influenced by the position of the internode or of the buds on the branches.
Ascorbinoxidase has a more reduced activity during the repose, comparatively with other enzymes.

La résistance des plantes aux conditions défavorables du milieu est déterminée par la quantité du complexe enzymatique ainsi que par la capacité des plantes de reconstituer d'une manière plastique le système enzymatique dans des conditions de milieu modifiées.

La polyphénoloxydase est le ferment caractéristique pour le moment de l'entrée des organes en état de repos. Son activité est particulièrement intense dans les tissus formés d'éléments vivants (8).

La péroxydase représente une sorte de ferment de défense contre l'intoxication des plantes avec de l'eau oxygénée, le péroxyde d'hydrogène formant la réaction d'auto-oxydation des galvanoprotéines (6, 8).

L'ascorbinoxydase catalyse l'oxydation de l'acide ascorbique à l'aide de l'oxygène. Son rôle, comme celui de la polyphénoloxydase, est de servir d'intermédiaire pour la transmission des électrones (9).

La cathalase a dans les plantes un rôle discutable. On condidère que ce ferment a le rôle de défendre la cellule contre l'eau oxygenée, de même que de décomposer les alcools secondaires (5, 7).

MATÉRIEL ET MÉTHODE

On a effectué l'étude dans la Station expérimentale horti-viticole de Iaşi, dans des conditions d'expérimentation identiques à celle rappelées dans les travaux antérieurs (1, 2, 3, 4).

Pendant la période de repos on a étudié les sarments d'une année chez les deux espèces.

Les sarments ont été divisés en : base (comprenant les internœuds 1—4), milieu (internœuds 4—9) et sommet (intrnœeuds 10—18). On a analysé les internœuds divisés en : écorce avec liber et, séparement, le bois. Les bourgeons ont été analysés avec leur nœuds.

La variation du complexe d'enzymes oxydo-réductrices a été déterminée de la manière suivante :

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Polyphénoloxydase (PFO), péroxydase (PO) et ascorbinoxydase (AO) titrymétrique avec le réactif 2,6 dichlorphénole, acide ascorbique. et eau oxygénee sur un matériel fraîchement mélangé, au sable quartzifère dans une solution tampon. Les résultats ont été exprimés en milligrammes d'acide ascorbique/un gramme subsance fraîche/heure.

La cathalase a été déterminée par la méthode monométrique Warburg. Les résultats ont été exprimés en mm^3O_2/gramme substance fraîche/heure.

RÉSULTATS ET DISCUSSIONS

L'activité de la polyphénoloxydase (fig. 1,2) se maintient à un niveau élevé pendant toute la période de repos. Elle ne diminue pas sous la valeur de 100—150 mg. acide ascorbique/gramme substance fraîche/heure. Au moment de l'entrée dans la période de repos profond, au moins de décembre, l'activité de la polyphénoloxydase se manifeste plutôt dans l'écorce et dans le liber, et spècialement dans les bourgeons et dans leurs nœuds, que dans le bois.

Pendant le repos forcé, au mois de février, l'activité de la polyphénoloxydase augmente surtout dans les bourgeons et les nœuds. Au mois de mars, le niveau énergétique de la polyphénoloxydase diminue.

La dynamique de la polyphénoloxydase est influencée par la position des internœuds ou du bourgeon sur le sarment. Les internœuds 1—4 de la base du sarment ainsi que les bourgeons et les nœuds respectifs présentent chez les deux espèces de vigne une activité réduite de ce ferment, par rapport aux internœuds et aux bourgeons situés vers le milieu du sarment (7—11). Il y a aussi des différences entre ceux-ci (7—11) et les bourgeons de les internœuds du sommet du sarment (12—18), mais ces différences sont plus réduites.

Parmi ces deux espèces, l'espèce Fetească neagră a une activité plus intense de la polyphénoloxydase.

La péroxydase (fig. 1, 2) a une dynamique pareille à celle de la polyphénoloxydase, mais à un niveau énergetique plus diminué. Le ferment est plus actif dans l'écorce et le liber, de même que dans les bourgeons avec leurs nœuds et moins acif dans le bois.

L'activité de la péroxydase est influencée par la position de l'internœuds et du bourgeon le bong du sarment. Dans les bourgeons et les internœuds situés vers le sommet du sarment et surtout vers la base de celui-ci, l'activité de la péroxydase est plus réduite que dans ceux du milieu du sarment.

L'espèce Fetească neagră présente une activité plus intense du ferment par rapport à l'espèce Aligoté.

L'activité de la cathalase (fig. 1, 2), observée dans les mêmes organes, relève pour le mois de février pendant le repos forcé et pour le mois de mars pendant le début de la croissance des bourgeons, une activité plus grande que pour le mois de décembre, pendant le repos obligatoire. Cette activité dépend aussi de la partie de l'organe étudié.

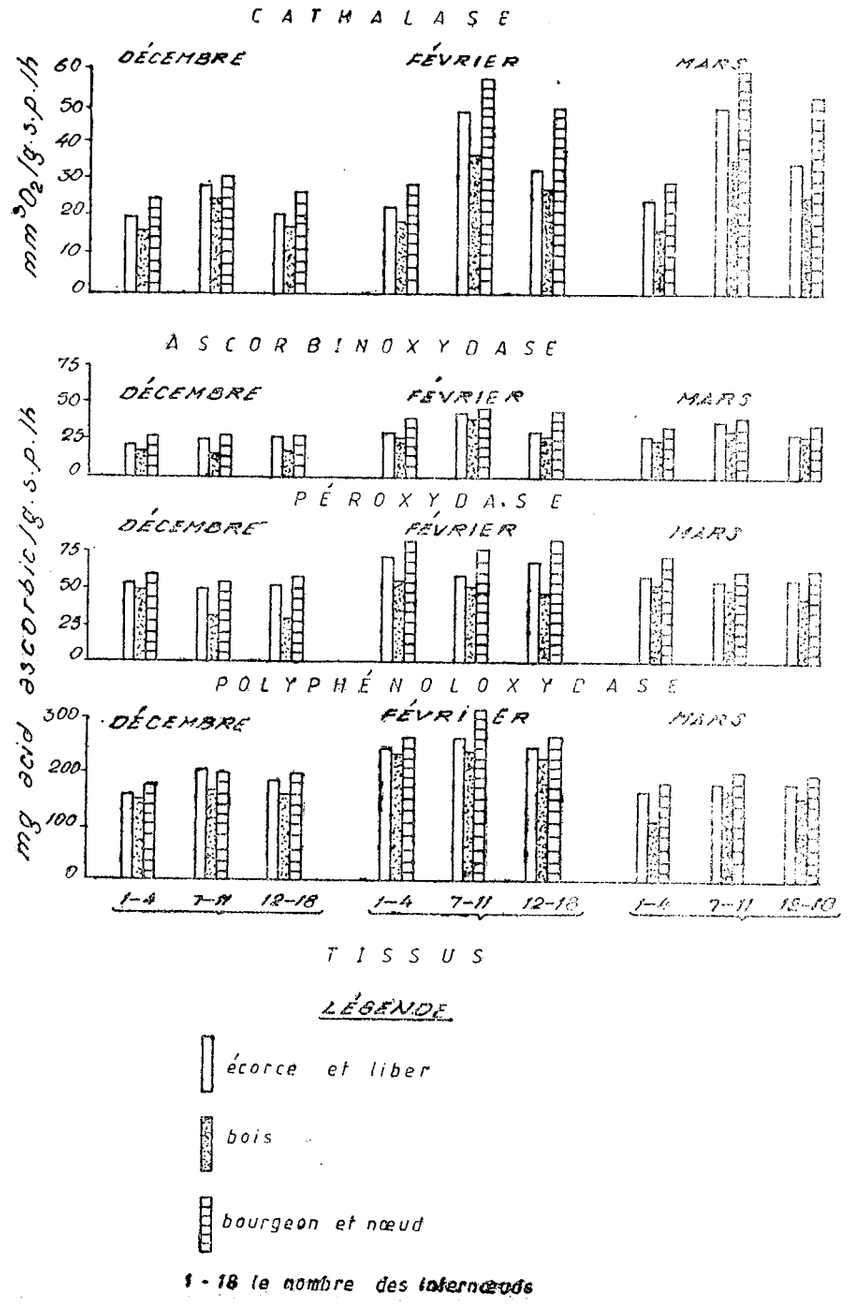

Fig. 1. — Activitè du complexe d'enzymes oxydo-réductrices durant la période de repos chez l'espèce Aligoté

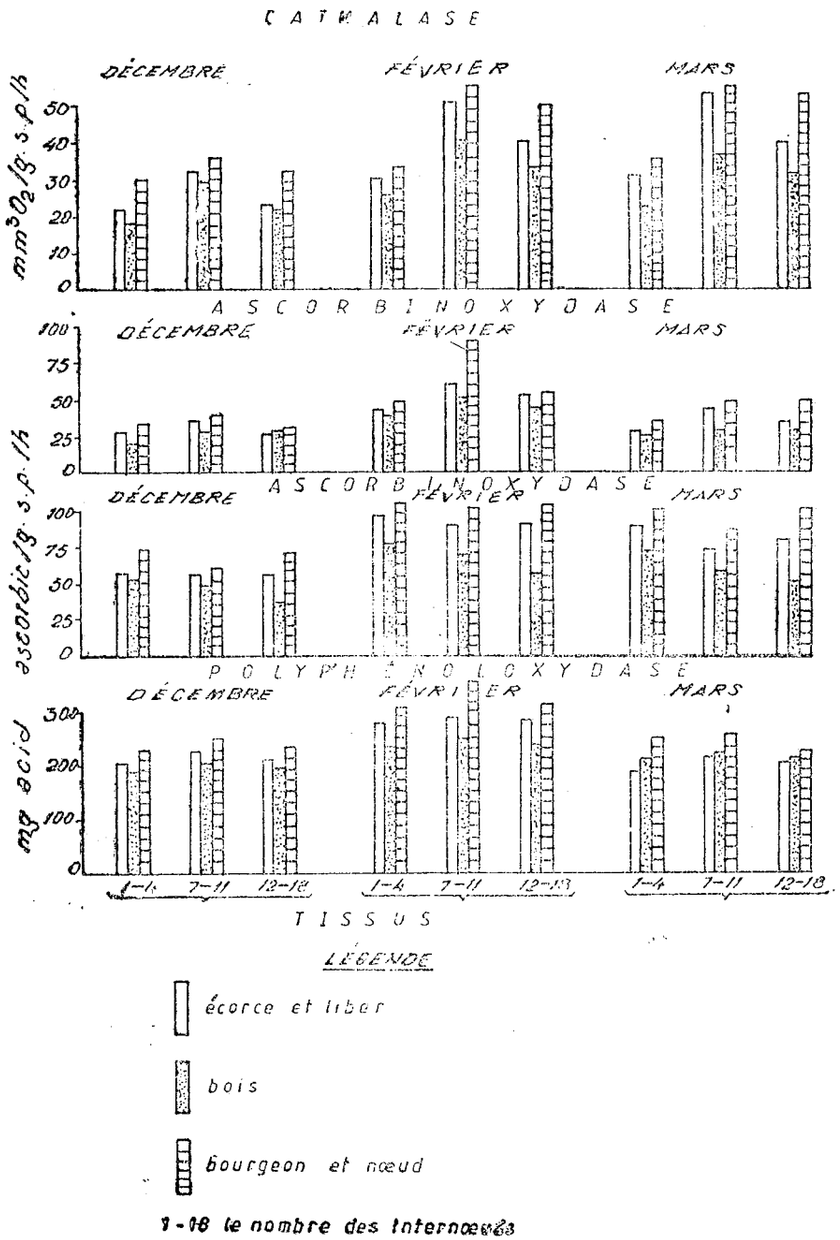

Fig. 2.— Activité du complexe d'enzymes oxydo-réductrices durant la période de repos chez l'espèce Feteasca neagră.

Dans le bois, la cathalase a toujours un niveau énergétique plus reduit que dans l'écorce et spécialement dans les bourgeons. De même, l'activité de la cathalase dépend aussi de la position de l'internœud ou du bourgeon sur le sarment. Les bourgeons situés vers la base ou vers le sommet du sarment présentent une activité plus réduite de la cathalase par rapport à ceux situés au milieu du sarment de reproduction.

Parmi les deux espèces, l'espèce Aligoté se caractérise par une activité plus intense de ce ferment.

CONCLUSIONS

1) L'activité de la polyphénoloxydase se maintient à une niveau élevé pendant toute la période de repos.

2) La péroxydase présente une dynamique semblable à la polyphénoloxydase, mais à un niveau énergétique plus réduit.

3) Le ferment avec l'activité la plus réduite est l'ascorbinoxydase.

4) L'activité de la polyphénoloxydase, de la péroxydase et de l'ascorbinoxydase se manifeste plus intensément dans les bourgeons et leurs nœuds, dans l'écorce et liber et ensuite dans le bois.

5) Pendant la période du repos obligatoire, l'activité de la cathalase est réduite. Son niveau énergétique augmente pendant le repos forcé et parallèlement au développement des bourgeons.

6) La position des internœuds et des bourgeons sur le sarment exerce une influence sur l'activité du complexe de ferments oxydoréducteurs, de même que sur l'activité de la cathalase. Le plus haut niveau énergétique se trouve dans les bourgeons et les nœuds situés vers le milieu du sarment de reproduction.

7) Parmi les deux espèces, l'espèces Feteasca neagră a un complexe de ferments oxydoréducteurs plus actifs que l'espèce Aligoté (à l'exception de la cathalase).

8) En ce qui concerne l'activité de la cathalase, celle-ci est plus intense chez l'espèce Aligoté que chez l'espèce Feteasca neagră.

BIBLIOGRAPHIE

1. Antohe, Anca Lidia, Trav. du Museum d'Hist. naturelle « Gr. Antipa », 1984, **XXV**, 379.
2. Antohe, Anca Lidia, Rev. Roum. Biol. — Biol. végét., 1984, **28**, 2, 77.
3. Antohe, Anca Lidia, Rev. Roum. Biol. — Biol. véget., 1984, **29**, 2, 177.
4. Antohe, Anca Lidia, volume solennel 150 ans depuis la fondation du Musèe d'Histoire naturelle, fondé en 1834, Iași, 1984.
5. Cernomoreț, M. V., Pomic., vitic et vinif., 1966, **10**, 26.
6. Dvornic, V., Pomohaci, N., Dvornic, Valentina, Ilie, Maria, 1966, Lucr. St. Inst. Agr. București, 205.
7. Hanin, D., Pomic., vitic. et vinif. Moldovei, 1963, **2**, 33.
8. Molceanova, Z., J., Vitic. et vinif. Moldovei, 1965, **6**, 339.
9. Molceanova, Z., J., Vitic. et vinif., București, 1966, **5**, 265.

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BIOLOGICAL RESPONSES IN THE LICHEN *XANTHORIA PARIETINA* TRANSPLANTED IN BIOMONITORING STATIONS

KATALIN BARTÓK, ANA NICOARĂ, BERCEA VICTOR, OSVÁTH TIBOR

In the heavy polluted area of FMR, Dej town, where had not been found lichens in natural conditions, samples of *Xanthoria parietina* were transplanted at different distance around the polluting source (MgO, Fe_2O_3, Al_2O_3, CeO_3 etc.). After 150 days of exposure (11 November 1988 — 11 April 1989), both the assimilation pigments content (mostly the *a* chlorophyll) and the lichen's respiration have diminished; at the same time an exaggerated accumulation of Mg, Cr, Fe and Cd ha been recorded, depending on the distance from the polluting source and the wind direction.

To transplant healthy lichens into polluted areas and to measure deterioration and physiological changes of thalli is a monitoring methos strongly recommended as a highly practical procedure (12, 13).

We have also been using this method to measure pollution in the Dej industrial area (Cluj county). Our investigations started as soon as 1987 round the cellulose manufactures (2,3), and they continued during 1989 around the Refractory Material Plant (FMR), in the same town.

The main source of FMR emission was considered to be the chromite dryer which releases MgO (30.68%), Fe_2O_3 (3.52%), Al_2O_3 (10.95%) and CrO_3 (3.09%).

In the proximity of the plant there is the Bungăr forest, an oak and hornbeam forest aged 120. The first pollution attestation was in 1978; in 1983 the pine, sycamore maple, larch plantation was compromised, although these trees were considered to be resistant to pollution. In 1984 the reforestation had the same ending.

For the vegetation, the most dangerous are the suspension powers released by FMR, their quantity being of 1.17 mg/mc compared to the allowable limit of 0.1 mg/mc. The deposited powders reached an 813 t/km²/year value, the allowable limit being 200 t/km²/year.

The Dej climate, characterized by calm-stable stratificaion (24.6%), that is poor conditions for vertical dispersion of noxae as well as the atmospheric calm which is characteristic for 50% of the number of the days, determine the spreading of the FMR emission on a small area, but in a high concentration. Both the relief forms and the topographical and meteorological factors represent a complex playing an unfavourable part in the selfpurifying process. Consequently, all the pollution sources in the twon of Dej are more aggressive (4).

METHOD OF WORK

The *Xanthoria parietina* samples having a surface of 20×20 cm have been picked from the poplars by the Someş river, "Victor Babeş"

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park in Cluj (control) together with the bark on which they vegetate. These samples have been transplanted in different directions and different distances from the pollution source (FMR) in the stations P_1, P_2, P_3, P_4 and P_5 on the 11th of November 1988 (Fig. 1). The lichens have been sampled after 5 months on the 11th of April 1989.

We must mention that the plant has been functioning all this period in a discontinuous manner and with a low efficiency.

The following physiological indicators of lichens have been studied : the assimilatory pigment content (Arnon 1949 and Davies 1965 method) ; the viability (Bergmann 1974 method) and the biogene element content (Perkin-Elmer 1971, atomic absorption spectrophotometry).

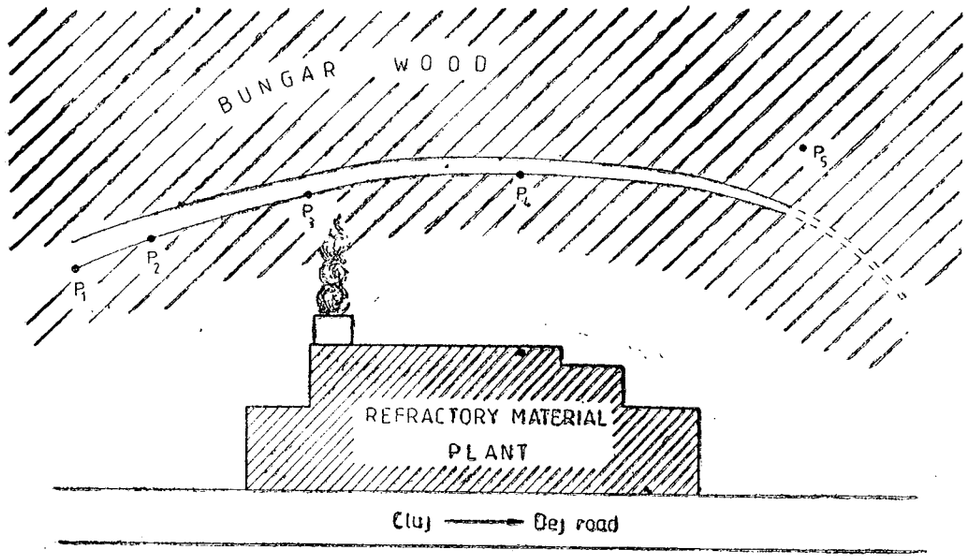

Fig. 1. — Emplacement of stations.

RESULTS AND DISCUSSIONS

I. THE ASSIMILATION PIGMENT CONTENT

In all the places of study after 5 months of exposure, both the total pigment content and that of chlorophylls *a* and *b* is lower than that of the control, a fact which is kwnown from the literature (10,14), as well as from our own experiments (2,3). On the other hand the carotenoid content is higher than that of the control in almost all the polluted points.

As it can be noticed in Fig. 2, the most polluted point is P_3 which is to be found exactly in the direction of the FMR exhaust chimney. The analyses have thrown into relief there the lowest pigment quantity, not only the chlorophyllian ($a = 0.326$ and $b = 0.145$) but also the carotenoidicone (0.153).

The values of the ratio between photosynthetic elements of the species *Xanthoria parietina* and absorption spectra D_{435}/D_{414} depend on the different position of stations as compared to the studied pollutants and point out to a marked sensitiveness of the *a* chlorophyll (that is to be considered as the main constituent on which the pollutants operate) at the same time with the accumulation of pollution products.

II. *The viability*, measured by a respiratory process, is an indication of lichen sensitivity to pollution (2). According to the available data (Fig. 3) there is a large scale adaptation. The differences in lichen respiration depending on the place of studied points are not too important and this is likely due both to the cold season when the metabolic processes are comparatively slow and the discontinuity in FMR activity.

As in the case of pigment content, in the P_3 investigation point the respiration is the lowest (8.99 mg formasan g of fresch substance). Pollution becomes more prominent corresponding to the direction of the western wind. In P_1 where pollution is lower and the physiological processes are amplified on account of stress, lichen respiration exceeds that of the control (13.74 compared to 11.44) ; in P_2, on account of a greater damage, respiration is decreasing (13.00) while in P_4 and P_5 it becomes smaller than the control (9.84 and 9.13 g formasan/g, respectively), the plants showing signs of exhaustion.

Following Selye's theory (1956) explaining the mechanism of the disease appearance, the following three critical moments have been distinguished with the *Xanthoria parietina* species transplanted in the neighbourhood of FMR :

— the stage of alarm during which the species tried to reject the stress by an intense activity

— the stage stability or accommodation to stress, in fact a partial, potentially reversible, exhaustion.

— the stage of total exhaustion followed by the death of the plant.

Within 5 months, the species transplanted from the study point P_3, the area directly polluted, reached the total exhaustion stage manifested by a minimum pigment contents and a very low respiration.

III. ELEMENTS CONTENT

In order to determine the distance which FMR metal pollution was operating we included, besides the investigation points P_1 to P_5 placed in the Bungăr forest, the points P_8, P_9, P_{10}, and P_{11}, stations placed around the Cellulose Factory in sej, used in the experiments of 1987—1988.

Our investigations concerned 10 elements (Cr, Pb, Cu Zn, Cd, Fe, Ni, Mn, Ca and Mg). The macroelements were expressed in percentages and the microelements in ppm referred to dry substance (Table 1).

Iron is the metal with the highest concentration in *Xanthoria parietina* the value lying between 547 and 5349 ppm. The species transplanted from points 2 and 5 contained the highest concentration (2489 ppm and 2744 ppm). However, having in view that P_5 is the most distant station from FMR and that near, the Dej Cellulose Factory in the stations

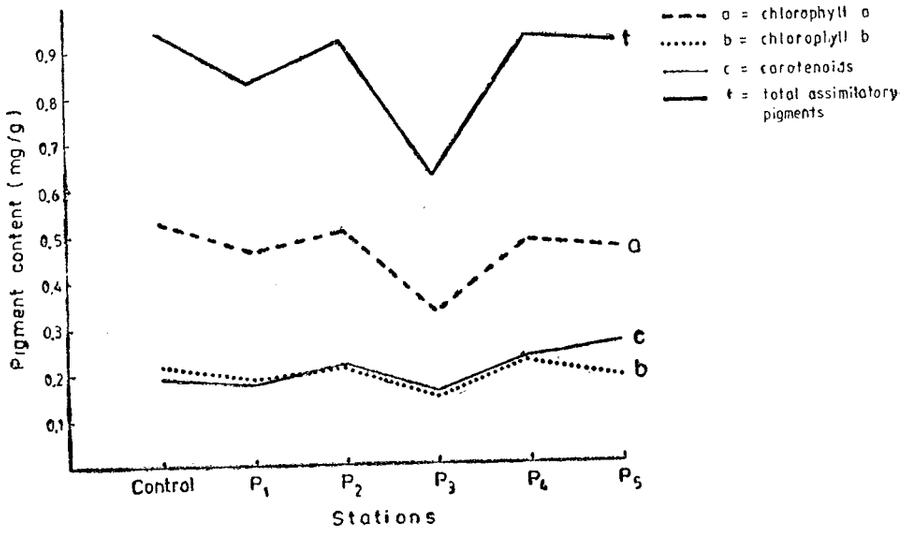

Fig. 2. — Respiratoric (dehydrogenasic) activity in *Xanthoria parietina*, studied around the FMR

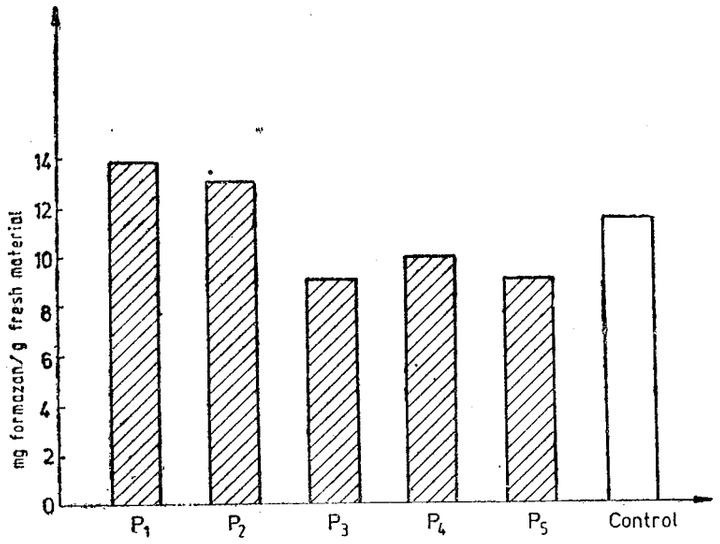

Fig. 3. — Changes of assimilatory pigments in *Xanthoria parietina* sampled around the plant.

Table 1

Biogene elements content in *Xanthoria parietina*

Stations	Cr	Pb	Cu	Cd	Zn	Fe	Ni	Mn	Ca	Mg	Total
				ppm					%		
Control (1988)	11.89	46.29	22.51	0	315.0	520.3	16.88	81.2	0.74	3.16	4.91
P_1	157.05	57.46	8.65	2.27	162.9	547.1	20.84	51.4	0.73	8.01	9.75
P_2	346.04	229.24	15.92	8.14	352.8	2489.5	49.47	68.7	0.51	12.00	16.06
P_3	207.23	45.12	17.98	0.74	584.4	905.6	41.52	50.2	1.97	9.13	12.95
P_4	147.03	89.62	16.50	2.57	267.1	1250.2	24.45	139.2	1.41	6.22	9.57
P_5	255.78	47.43	23.66	3.24	901.4	2743.7	29.61	97.8	0.70	4.23	9.03
Control (1987)	37.13	31.16	26.10	0	100.9	825.7	19.70	47.7	2.06	1.35	4.50
P_8	140.01	62.45	32.02	1.14	120.8	825.0	31.79	75.9	0.70	1.16	3.15
P_9	115.46	44.26	25.46	1.81	272.3	5359.4	67.13	153.3	3.55	2.58	12.16
P_{10}	110.14	62.75	28.87	3.63	208.0	3995.2	23.35	116.7	1.00	3.17	8.72
P_{11}	114.08	6.85	17.74	1.81	188.6	536.9	8.59	27.8	1.18	1.76	3.90

Note : Stations P_1 — P_5 in the proximity of the FMR
Stations P_8 — P_{11} in the proximity of the Cellulose Factory

P_5 and P_{10}, iron concentration reached 5349 ppm, we could suppose that the main pollution source was not FMR.

Chromium. The results obtained by us show that the most pollutant metal in the area is chromium. Its quantity increased by 300% (Control = 11.89 ppm, P_2 = 346.04 ppm Cr). Our data correspond to those reported in the literature (11, 5, 7), where chromium accumulations reached 2 to 10 times near industrial centres as compared to the situation noticed 80 years ago. The chromium is contained in the ash exhausted by the FMR chimneys and it reaches to long distances. Its concentration usually exceeds 110 ppm in the most distant point from the pollution source.

Zinc. According to Hale (8) the lichens have a 30—80 ppm content in unpolluted conditions and the concentrations over 200 ppm are noxious to lichens.

The values detected by us lie between 120.8 and 901.4 ppm. The maximum has been recorded in the observation point P_5. The values are also high in P_3 and P_2 and they are lower in the more distant stations. However, they exceed the zinc values found near the main highways around Washington (150 ppm).

Copper is not pollutant in the examined points as the values have not significantly exceeded those recorded in the control analysis.

Lead, the most important pollutant in the atmosphere and thus studied to the greatest extent did not play an eminent part in this case, its accumulation being put the shadow by other pollutans.

Nickel content data about lichens in the United State, according to Hale (8), lie between 3 and 9 ppm while in the areas of circulated highways they reach 30 ppm; in Israel, for *Squamarina* species, even to 40 ppm (5,6). Nickel determinations for lichens have not been yet made in this country. Our analyses point out the nickel increase in all points of invesigation as compared to the control. The same is true for manganese.

The smallest quantity of metal is that of *cadmium*, which could not be detected in the control. However, during the 150 days of exposure, 8.14 ppm have been accumulated in *Xantohria parietina* (highest value), the same quantity is present in the lichens in the most distant investigation points in the Dej town. Similar data were cited for Israel (6), where the cadmium content increased in the *Ramalina duriae* species from 1 ppm to 9.35 ppm in the polluted area as a result of a 8 months transplantation.

Amoung the macroelements, the *calcium* and *magnesium* content has been studied. It has been ascertained that the ratio $\frac{Ca}{Mg} \sim \frac{1}{10}$ characteristic of the vegetable matter holds good for lichens, too; the analyses of lichens included in familias *Parmeliaceae* and *Usneaceae* colleced in the Retezat Mountains lead to the same conclusion (1).

Surprisingly, the ratio is not applicable to *Xanthoria parietina* even in the control. The magnesium content exceeds by far the calcium one, the values lying between 1.16 and 12.00% for magnesium and from

0.57 to 3.35% for calcium. It is in the station P_2 that the highest magnesium and the lowest calcium contents have been recorded.

It has been ascertained that magnesium oxides are released and dispersed in great quantities from the FMR chromite dryer and this fact explains the exaggerated magnesium content in the transplanted species. The inversion of the relation Ca/mg also ascertained in the control is an indication for a specific affinity of the *Xanthoria parietina* species for magnesium.

The amount of the 10 pollution elements investigated (Fig. 4) is the highest in the station P_2, exceeding by 3.3 times the control content; then it lowers both downstream and upstream.

Comparing the values of the investigated elements concentration we obtained the following rank:

Mg > Ca > Fe > Zn > Cr > Mn > Ni > Cu > Pb > Cd

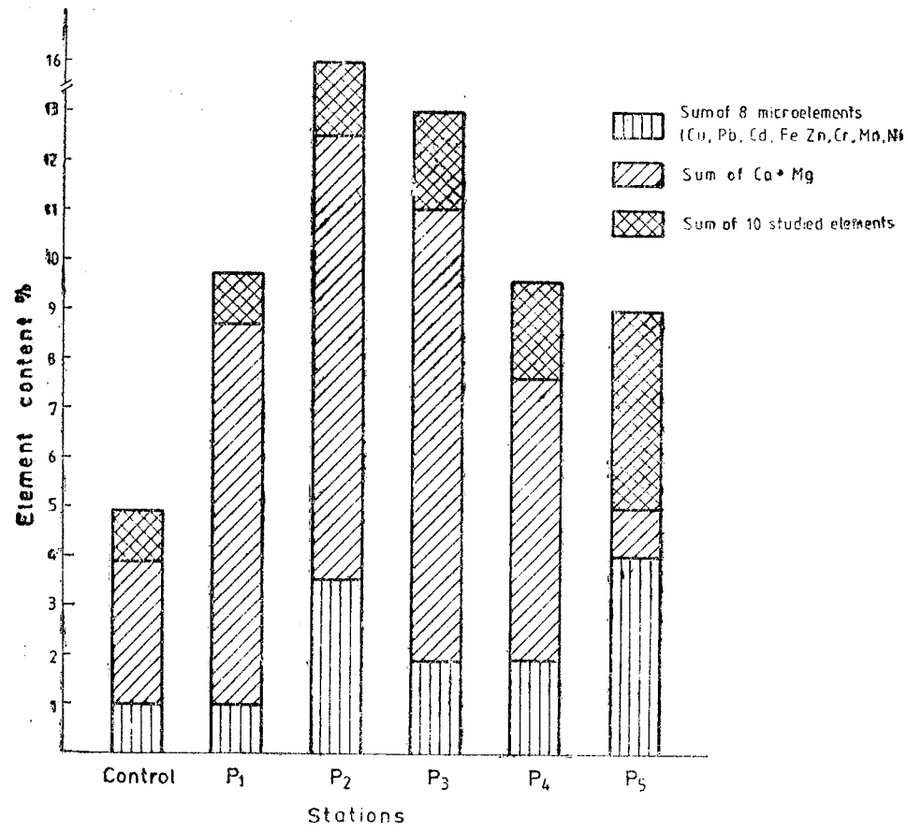

Fig. 4. — Elements content modifies after transplanting (150 days) in different stations.

CONCLUSIONS

The total absence of lichens in the investigated area (setlement of "lichen desert"), which is the indication of a severe pollution, has been confirmed by the transplantation of the *Xanthoria parietina* species from an unpolluted area in different investigation places around FMR. The exposure lasted for 150 days (from the 11 th of November 1988 to the 11th of April 1989).

The pollutants emanated by FMR Dej affected *Xanthoria parientina* as follows :

— diminution of assimilation pigments content, the most sensitive being the *a* chlorophyll

— diminution of respiration, i.e. of the dehydrogenasic activity with signs of plant total exhaustion dependent on the distance from the pollution source

— exaggerated accumulation of Mg, Cr, Fe and Cd whereas Cu, Pb, Ni, Zn and Mn do not play an important part in the pollution of the area.

The heavy metals pollution alters not only the FMR precinets but also the distant stations in the proximity of the Cellulose Factory.

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REFERENCES

1. Bartók Katalin, Studii şi Cerc. Biol. ser. Biol. Veget., 1985, **35**, 2, 140—144.
2. Bartók Katalin, Oscath, T., *Influence of pollutants emanated from Dej Cellulose Manufactures over lichens* (under press).
3. Bartók Katalin, Bercea V., *Efectul poluanţilor din zona industrială a Dejului asupra conţinutului de pigmenţi asimilatori ai speciilor de licheni Physcia aipolia şi Xanthoria parietina* (under press).
4. Berindean Cornelia, *Estimarea valorii urbane a pădurii Bungăr în vederea recomandării privid protecţia mediului* (unpublished).
5. Garty J., Environ. Pollut. Ser. B, 1985, **10**, 287—300.
6. Garty J., Fuchs C., Water, Air and Soil Pollution, 1982, **17**, 175—183.
7. Garty J., Ammann K., Environ Exp. Bot., 1987, **27**, 2, 127—138.
8. Hale M. J., *The biology of lichens*, Third Edition, Edicard Arnold, 1983
9. Marti J., Can. Jour. Bot., 1983, **61**, 1964—1969.
10. Ronen R., Garty J., Galun M., in *Developments in Ecology and Environmental Quality*, 1983, Ed. Shuval H. I., II, 167—176.
11. Schutte A. Julia, *The bryologist*, 1977, **80**, , 279—283.
12. Seaward M. R. D., Nature, 1986, **320**, 581—584.
13. Seaward M.R.D. in *Plants and Pollutants in Developed and Developing Countries*, 1989, Irmir, Zurkey, 307—319.
14. Von Arb Ch., in *Progress and Problems in Lichenollogy the Eighties*, Edit. Cramer, Berlin-Stuttgart, 1987, 343—347.

STRUCTURE AND COENOTAXONOMY OF *SISYMBRIETALIA* ORDER IN THE ROMANIAN VEGETATION

V. SANDA, A. POPESCU

The paper analysed a number of 26 associations of the *Sisymbrietalia* J. Tx. 61 order with the 3 alliances : *Sisymbrion officinalis* Tx., Lohm. et Prsg. 50, *Convolvulo (arvensi)* — *Agropyrion repentis* Görs 66 and *Artemisio-Agropyrion intermedii* Muller et Görs 69, under various aspects (structure, dynamics, evolution, arealography, etc.).

The *Chenopodietea* Br. —Bl. 51 emend., Lohm., J. Tx. et Tx. 61 class groups the vegetation characteristic to the waste lands on vacant grounds and borders with soils rich in organic matters in a decomposition condition. The species constituting the vegetation of this class are nitrophilous in their great majority, therophytes and therohemicryptophytes with adaptations that allow them to spread quickly on lands left unworked.

The species characteristic to the class are : *Amaranthus albus, A. retroflexus, Chenopodium album, Ch. opulifolium, Ch. polysperum, Cardaria draba, Capsella bursa-pastoris, Datura stramonium, Echinochloa crus-galli, Rumex conglomeratus, Sonchus oleraceus, Solanum nigrum, Stellaria media, Senecio vulgaris, Verbena officinalis, Xanthium spinosum, X. strumarium* etc.

SISYMBRIETALIA J. Tx. 61

Gathers the associations on the dry lands around the houses where accumulations of organic matters in the layers exist. The most representative recognizing species are : *Berteroa incana, Echium italicum, Chenopodium urbicum, Erysimum repadum, Hordeum murinum, Lappula myosotis, Malva sylvestris, Lactuca saligna, Sisymbrium altissimum, Thlaspi arvense.*

Sisymbrion officinalis Tx., Lohm. et Prsg. 50.

Comprises ruderal associations that develop especially on drained lands that dry quickly after the rainy season. As a result of these conditions, the great majority of the species within the associations of the alliance *Sisymbrion* are annual spring plants.

The representative species are : *Bromus tectorum, Hordeum murinum, Erigeron canadensis, Chenopodium opulifolium, Lepidium ruderale, Malva neglecca, Sisymbrium loeselli, S. officinale.*

1. *Hordeetum murini* Libbert 32 em. Pass. 64

(Syn. : *Bromo-Hordeetum* Lohm. 50; *Hordeo murini-Chenopodietum albi* Timár (55) ex Timár-Bodrogk. 59 ; *Bromo-Chenopodietum albae* Timár (55) ap. Soó 61).

It is an association greatly spread in localities, road borders on the earthwork of railways where the characteristic species creates a rather compact carpet. It develops well on dry lands lightly beaten where it abundantly fructifies. On dry lands with sandy texture, within the *Hordeum murinum* phytocoenosis, *Bromus tectorum*, develops abundantly and becomes sometimes codominant while on beaten, clayey lands, *Polygonum aviculare* appears in a rather high quantity. Within the *Hordeum murinum* phytocoenosis, the species *Cardaria draba*, *Capsella bursa-pastoris*, *Verbena officinalis*, *Sisymbrium officinale*, *Malva neglecta*, *Lepidium ruderale*, *Erodium cicutarium*, *Lactuca serriola*, *Polygonum aviculare*, *Bromus sterilis*, *B. tectorum*, *Chamomilla recutita* etc, are never failing. The association is spread all over the country. The presence in high quantity of some species, within the *Hordeum murinum* phytocoenosis, made possible the description of some subassociations such as: *brometosum arvensis* Grigore 68, *lepidietosum drabae* Grigore 68 and *matricarietosum chamomillae* Grigore 68. All these sousassociations have been signaled out only in Banat, up to the present.

2. *Brometum arvensis* (Şerbănescu 57 n.n.) Kiss 64

(Syn.: Ass. of *Bromus arvensis* Şerbănescu 57 n.n.).
It develops on unworked grounds, spongy and dug up, at the edge of cultures forming phytocoenosis well organized. Along the characteristic species there have been also identified: *Cirsium arvense*. *Convolvulus arvensis*, *Matricaria perforata*, *Ciochorium intybus*, *Centaurea cyanus*, *Convolvulus arvensis*, *Consolida regalis*, *Cirsium lanceolatum*, *Echinm vulgare*, *Lolium perenne*. As the ground fallow there appear a series of perennial species and the association develops towards *Lolio-Plantaginetum majoris*. Although the association is spread in the Romanian Plain, the specialized literature mentions only that in Oltenia and Muntenia (Slatina).

3. *Atriplicetum nitentis* Knapp 45

It is an association little known in Romania being singnaled out by A. Kovács (1968) in Tg. Secuiesc and by Florița Diaconescu (1978) in the Bahluiul Basin. The floristic composition of the *Atriplex nitens* phytocoenosis is composed by ruderal species characteristic to the *Chenopodietalia* order and the *Sisymbrion* alliance from which the most representative are: *Descurainia sophia*, *Lepidium ruderale*, *Sisymbrium altissimum*, *Bromus tectorum*, *Lactuca serriola*, *Atriplex hastata*, *Capsella-bursa-pastoris*, *Chenopodium viride*, *Ch. album*, *Matricaria perforata*, *Solanum nigrum*, *Agropyron repens*, *Convolvulus arvensis*. The coenotaxonomic position of the association is still in discussion, some authors consider it like a subassociation to *Atriplicetum tataricae* then they synonymize it with this one while E. Oberdorfer (1957) includes it in *Sisymbrietum sophiae* Krech 35.

4. *Atriplicetum tataricae* (Prodan 23) Borza 26, Ubrizsy 49

(Syn.: As. of *Polygonum aviculare* with *Atriplex tatarica* and *A. littoralis* Todor 48). The characteristic species is very spread in Romania;

it installs on dry grounds but with high content of nitritious substances. The association is met on hardly saline grounds but with organic material in decomposition. It is a pioneer association that occupies the dug up grounds preparing the layer for installing the perennial species (Table 1).

In *Atriplex tatarica* phytocoenosis, nitrophilous species may be found characteristic for the order and class where they are placed from which the most representative are: *Hordeum murinum*, *Chamolilla recutita*, *Cirsium lanceolatum*, *Xanthium spinosum*, *Polygonum aviculare*, *Sisymbrium officinale*. On saline grounds *Atriplex tatarica* associates with some halophilous and subhalophilous species among which we may mention: *Puccinellia distans*, *P. limosa*, *Aster tripolium*, *Spergularia media*, *Acorellus pannonicus*, *Juncus gerardi*, *Hordeum hystrix*, *Taraxacum bessarabicum* and more seldom *Suceda maritima* and *Salicornia europaea*. Some species appear in high quantities such is the case of *Cynodon dactylon*, *Equisetum arvense* or *Ecbalium elatrium* constituting subassociations or facies such as: the *cynodontetosum* (Morariu 43) Popescu, Sanda, Doltu 78 (Syn.: *Cynodonto-Atriplicetum tataricae* Morariu 43) facies with *Equisetum arvense* Popescu, Sanda, Doltul 80 (Syn.: *Cynodonto-Atriplicetum tataricae* Morariu 43 *equisetetosum arvense* Grigore 71) and facies with *Ecbalium elatrium* Popescu, Sanda, Doltu 80 (Syn.: *Cynodonto-Atriplicetum tataricae* Morariu 43 *ecbalietosum elateriae* Grigore 71).

5. *Chenopodio (boni-henrici)-Urticetum urentis* Tx. 31 em. Lohm. 50 (= *Chenopodietum boni-henrici* Klika 48).
It represents the vegetation of low weeds, frequent around the sheepfolds and mountain villages. The association has been signaled out by D. Mititelu and N. Barabaș (1973) in the Suceava and Bacău districts. The most representative species are: *Urtica dioica*, *Lamium maculatum*, *L. album*, *Silene alba*, *Rumex obtusifolius*, *Cirsium arvense*, *Senecio vulgaris*, *Stellaria media*, *Poa annua*. The association differs from *Arctio-Chenopodietum* = *Arctio-Ballotetum* by the lack of the *Arctium tomentosum*, *Urtica dioica*, *Ballota nigra* etc. species and by the presence of the *Urtica urens* species in a great quantity.

6. *Chenopodio-Urticetum urentis* (Tx. 31) Siss. 46

(Syn.: *Chenopodietum muralis* Slavnić 51 non Br. —Bl. 36; *Chenopodio vulvariae muralis-Urticetum urentis* Soó 71). It is an association of ruderal plants, signaled out by Al. Borza (1959) in the Sebeș Valley under the name of *Chenopodietum muralis* Br. —Bl. 36 and by D. Mititelu and N. Barabaș (1973) in Moldavia. The recognition species are: *Chenopodium murale*, *Ch. vulvaria*, *Urtica urens*. Within the association there participate *Amaranthus lividus*, *Atriplex patula*, *A. tatarica*, *Chenopodium opulifolium*, *Ch. serotinum*, *Ch. strictum*, *Geranium pussilum*, *Hordeum murinum*, as well as other numerous species characteristic of the *Chenopodietea class*.

Table 1

Atriplicetum tataricae (Prodan 23) Borza 26

Number of survey	1	2	3	4	5	6	7	8	9	10	11	12	13	AD
Area (sq.m)	100	150	100	150	150	100	1	150	100	100	150	200	150	
Cover area (%)	90	95	85	95	100	100	90	80	85	95	100	90	75	
Vegetation height (cm)	10	15	15	10	10	15	20	5	10	15	10	5	5	
Sysimbrion officinalis	3	4—5	5	5	5	4	4—5	5	4—5	5	4—5	+1	4	+1—5
Atriplex tatarica	+	. .	. .	. .	. .	. .	. .	. .	. .	. .	++	++	+ .	+++
Hordeum murinum	. .	. .	. .	. .	. .	. .	+	. .	. +	. .	+ . +	+	1 . .	+—1
Lepidium ruderale	. .	. .	. .	. .	. +	. .	. .	. .	. .	. .	. .	. .	. .	++
Chenopodietea	. .	. .	. .	+	. .	. +	. .	+	.	.	.	.	.	+
Matricaria chamomilla	. .	. .	. .	. .	. +	. .	. .	. .	. +	. .	+ .	+1	1 . .	+—1
Cirsium lanceoladum	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. +	. .	. .	++
Xanthium spinosum	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	+
Plantaginetea	. .	. .	. .	+	. .	. .	. .	. .	. +	. .	+ .	4—5	. + .	+—5
Polygonum aviculare	. .	+	.	.	. .	. .	. .	. .	. .	. .	. .	. .	. .	++++
Festuco-Brometea + Festucion rupicolae	. .	. .	. .	. .	. .	. .	. .	. .	. .	+ .	+ . .	. .	. + .	+—1
Cynodon dactylon	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	++++
Artemisia absinthium	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	+
Plantago lanceolata	. .	. .	. .	+	. .	. .	. .	. .	. .	. .	. .	. .	. .	++
Taraxacum serotinum	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	+
Puccinellion limosae + Puccinellietalia	. .	. .	+	. .	. .	+	. .	. .	+	1 +	. .	.	. .	+—1
Puccinellia limosa	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	+
Puccinellia distans	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	+
Artemisia maritima	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	+
Aster tripolium	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	+
Cypero-Spergularion	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	+
Spergularia media	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	+
Acorellus pannonicus	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	+
Juncion gerardi	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	+
Juncus gerardi	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	+
Thero-Salicornion	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	+
Suaeda maritima	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	+
Salicornia europaea	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	+
Puccinellio-Salicornietea	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	+
Hordeum hystrix	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	+
Taraxacum bessarabicum	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	+

Present species in a survey: Artemisia austriaca (13), *Bromus hordeaceus* (2), *Crypsis aculeata*(3) *Chenopodium album* (11), *Camphorosma annua* (4), *Cardus nutans* (13), *Erysimum repandum* (2), *Eryngium campestre* (13), *Erodium cicutarium* (13), *Heleochloa schoenoides* (12), *Halimione pedunculata* (7), *Onopordon acanthium* (1), *Pastinaca graveolens* (9), *Phragmites australis* (8), *Spergularia salina* (11).
The place and data of surveys: 1—10, Traian, 15—18. VI. 1976; 10—13, Movila Miresii, 15—17. VII. 1977 (Brăila district).

7. *Chenopodietum urbici* Soó 33

It is an association with a strong nitrophilous character, it develops on grounds where animals have stopped on waste lands with accumulations or organic material as well as in localities on layers rich in nitritious substances. In the floristic structure there participate nitrophilous species among which most constant are: *Atriplex tatarica*, *Chenopodium album*, *Datura stramonium*, *Xanthium spinosum*, *Amaranthus retroflexus*, the last two ones being able to make up facies within the association. It is known in Moldavia from several localities and in Muntenia it is cited in Ruşeţu.

8. *Cannabinetum ruderalis* (Morariu 43) corr. Morariu 70; Fijalkovschi 67; Turenschi et Zanoschi 70 (Syn.: *Cannabinetum sativae* Morariu 43; Soc. *Cannabis sativa* Morariu 43).
Much spread at the edge of culture on lands rich in organic maters, *Cannabis ruderalis* is a nitrophilous species well represented in Banat, Muntenia, Dobrogea and Moldavia. The association described by I. Morariu (1943) in the surroundings of Bucharest under the name of As. of *Cannabis sativa* has been then signaled out in all south regions of the country where it extends largely.

The characteristic species *Cannabis ruderalis* by its high standing and by its large number of individuals that develop, hinders the development of other species so that few taxons appear in its phytocoenosis; we may mention among these taxons: *Polygonum aviculare*, *Chenopodium album*, *Stellaria media*, *Capsella bursa-pastoris*, etc. These species that are comprised in the structure of the association are usually net towards the periphery of the *Cannabis ruderalis* phytocoenosis.

9. *Malvetum sylvestris* Todor et al. 71

The coenosis of the association are spread in yards, unworned gardens grounds with accumulations of organic subsances. The *Malva sylvestris* phytocoenosis has few component species and mostly nitrophilous plants characteristic to the *Chenopodietea* class and to the order *Onopordetalia* among which the most frequent are: *Ballota nigra*, *Sisymbrium loeseli*, *Solanum nigrum*, *Kochia scopria*, *Chenopodium vulvaria*, *Ch. album*, *Datura stramonium*, *Artemisia annua*, *Sonchus oleraceus*, *Descuriania sophia*, *Atriplex hastata*, *Capsella bursa-pastoris*, *Polygonum aviculare*.
The association is cited only in Banat (Old Moldavia and the Locvei Mountains).

10. *Xerantemetum annui* (Borza 31 n.n.; Prodan 39) Dihoru 70

The characteristic species *Xaeranthemum annum* is rather spread in the country at the edge of cultures, roads and commons. It is a xerophilous species that composes more seldom compact phytocoenosis on the grounds where the initial vegetation has been destroyed. It is a pionneer association; in its floristic composition many ruderal species are com-

prised such as: *Bromus sterilis*, *Sisymbrium orientale*, *Papaver dubium*, *Urtica dioica*, *Capsella bursa-pastoris*, *Convolvulus arvensis*, *Stellaria media*, *Nepetea cataria*, etc.

Within the *Xeranthemum annum* phytocoenosis, some species of lawns in the initial vegetation such as *Euphorbia agraria*, *Thalictrum minus*, *Erysimum cuspidatum*, *Agrimonia eupatoria*, *Carex praecox*, *Botriochloa ischaemum*, *Crupina vulgaris* etc. are also maintained.

In the case when man's intervention is diminished or ceases, the association develops towards *Medicagini-Festucetum valesiacae* or *Botriochloetum ischaemi*.

11. *Polygono (avicularis)-Amaranthetum crispi* Vicol, E. Schneider-Binder et Täuber 71 (Syn.: *Scherochloo-Polygonetum avicularis* (Gams 27)Soó 40 *amaranthetosum crispi* Szabó 71; *Amaranthetum crispi* Mititelu 72). It develops on soils rich in nitrates, slightly acid, argillous-sandy or even sandy and possibly temporary damp. It occupies the beaten grounds at the edge and the slope or the roads and railways on strongly used commons around the villages.

The *Amaranthus crispus* and *Polygonum aviculare* phytocoenoses have few component species among which the recognition ones are: *Amaranthus albus*, *A. blitoides*, *Portulaca oleracea*, *Malva pusilla*, *Sisymbrium officinale*, *Urtica urens*.

The association is signaled out in Moldavia, Transilvania, Banat and Muntenia (Pietroasele, Buzău district).
The variant with *Urtica urens* is described within the association by Erika Schneider-Binder (1976) in Sibiu district.

12. *Malvetum neglectae* Aichinger 33 em. Pass. 66

(Syn.: *Urtico-Malvetum neglectae* Lohm. 50; *As*: *Datura stramonium-Malva neglecta* (Athenstädt 41) Lohm. 50).

The characteristic species is rather spread in the country, being comprised by different phytocoenoses. It is an association in connection with beaten places around the houses, on the lands where animals stopped and around the places of deposits of garbage. The dominant species develops abundantly achieving a medium coverage of 80—90% from the surface of the land. In the structure of *Malva neglecta* phytocoenoses also participate: *Sisymbrium officinale*, *Descurainia sophia*, *Urtica dioica*, *Urtica urens*, *Chenopodium hybridum*, *Ch. murale*, *Ch. album*, *Atriplex patula*, *Sonchus oleraceus*, *Capsella bursa-pasttoris*, *Geranium pusillum*, *Solanum nigrum*, *Polygonum aviculare*, *Plantago major*, etc.

Spreading: Transylvania, Banat, Muntenia, Moldavia.

The subassociation *malveto (neglectae)-lolietosum* Resmeriță et al. 71 is described in the Danube Pass.

13. *Chenopodietum stricti* Oberd. 57

(Syn.: *Chenopodietum ruderale* Oberd. 57).
A pioneer association that develops on a layer with several accumulations of organic material. It is made of ruderal annual plants but also

of those growing in cornfields. As the organic matter decomposes, the perennial species begin to appear in a constant larger number and the association evolves towards *Arctio-Balloletum nigrae* with numerous taxons specific for the *Artemisietea* class.

Recognition species: *Chenopodium strictum*, *Amaranthus albus*, *Chenopodium opulifolium*, *Amaranthus hybridus*, *Xanthium strumarium*. In the *Chenopodium strictum* phytocoenoses the *Erigeron canadense*, *Hordeum murinum*, *Sisymbrium officinale*, *Lactuca serriola*, *Bromus tectorum*, *Datura stramonium*, *Chenopodium album*, *Sonchus oleraceus*, *Solanum nigrum*, species as well as other species appear frequently.
Spreading: Muntenia and Moldavia.

14. *Erigero-Lactucetum* Lohm. 50 mscr. apud Oberd. 57

It is signaled out by Florița Diaconescu (1978) in the Bahluilui Basin. The two characteristic species *Erigeron canadensis* and *Lactuca serriola* are extremely spread on waste lands at the edge of cultures or in cultures poorly kept.

Within the phytocoenoses of the association there is a rather large number of species characteristic to the alliance *Sisymbrion* and to the *Chenopodietea* class that take part in and mong which the most frequent are: *Bromus tectorum*, *Hordeum murinum*, *Chenopodium strictum*, *Ch. album*, *Sonchus oleraceus*, *Capsella bursa-pastoris*, *Senecio vulgaris* etc.

It is a pioneer association made out mostly of annual species but as the ground gets fallowed, conditions for the installation of perennial species are being created and the phytocoenoses evolve towards the constitution of the *Lolio-Plantaginetum majoris* association.

15. *Descurainietum sophiae* Krech 35 corr. Oberd. 70

(Syn.: *Descurainio (sophiae)-Brometum tectori* Burduja et al. 69 ined. apud Horeanu 75; Burduja et al. 76).
It installs on the uncultivated grounds, debrises, fallow grounds, there where the soil is rich in organic substances. Within the phytocoenoses of *Descurainia sophia* the characteristic species has an extremely high density, stopping thus the development of the species pretentious to light. As it is a pionneer association, the majority of the species comprised by their phytocoenoses are annual plants among which *Hordeum murinum*, *Malva sylvestris*, *Matricaria perforata*, *Atriplex tatarica*, *Erigeron canadensis*, *Lactuca serriola*, *Chenopodium album*, *Sonchus oleraceus*, *Capsella bursa-pastoris*, *Bromus tectorum* etc.; are never missing. The association is spread all over the country. As coenotaxons of inferior rank the followings are described: *laminetosum (amplexicauli-purpurei)* Resmeriță et Roman 75, *brometosum squarrosi* Horeanu 75, *sisymbrietosum orientale* Horeanu 75, *asperugetosum* (Grigore 71) comb. nova (Syn.: *Sisymbrio-Asperugetum banaticum* Grigore 71, *Sisymbrietum sopiae banaticum* Grigore 68), facies with *Sisymbrium loeselii* Burduja et al. 76.

16. *Marrubium vulgare-Atriplex rosea* ass. Slavnić 51

It is a nitrophilous association that develops on slightly sandy grounds, with small gravel but with accumulations of organic material. The *Atri-*

plex rosea and *Marrubium vulgare* phytocoenoses are characteristic to the grounds slightly beaten near the lodgings. The component species are generally therophytes among which *Sisymbrium officinale*, *Lepidium ruderale*, *Erodium cicutarium*, *Chenopodium album*, *Ch. murale*, *Urtica dioica*, *Amaranthus albus*, *Polygonum aviculare*, *Ballota nigra*, *Xanthium spinosum*, *Malva sylvestris*, *Hordeum murinum*, *Lolium perenne*, *Artemisia annua*, etc. are not missing.

The association is known only in Dobrogea up to the present (Old Customs and Casimcea Plateau).

17. *Sisymbrio(altissimi)-Brassicetum nigrae* Kruseman 41

(Syn.: *Brassico-Atriplicetum hastatae* Pass. 64).
The association is characteristic to the unworked grounds with accumulations of organic matters. The phytocoenoses are made out of the species characteristic to the *Chenopodieta* class and to the alliance *Sisymbrion* among which most representative are: *Brassica nigra*, *Sisymbrium loeselii*, *S. altissimum*, *Atriplex hastata*, *Descurainia sophia*, *Sisymbrium officinale*, *Lepidium campestre*, *Creps tectorum*, *Bromus tectorum*, *Lactuca serriola*, *Cardaria draba*, *Bromus hordeaceus*, *Chenopodium album*, *Capsella bursa-pastoris*, *Sinapis arvensis*, *Convolvulus arvensis*, *Sonchus arvensis*.

It is signaled out by D. Mititelu and N. Barabaș (1972) and D. Mititelu (1975) in Moldavia (Bacău and Vaslui districts).

18. *Diplotaxietum tenuifoliae* Burduja et Horeanu 76

Occupies rather large surfaces on the terraces and abrupt slopes strongly eroded in Salygni. It is a pioneer association that installs after an almost complete or a complete destruction of the xerophilous initial meadows fact than can by seen from the presence of some species characteristic to the *Festuco-Brometea* class within the phytocoenoses of *Diplotaxis tenuifolia*. The recognition species are: *Diplotaxis tenuifolia*, *Euphorbia sequerana*, *Cleistogene bulgarica*, the last one being indicatory for the grounds strongly eroded. The association evolutes as the lying fallow of the layer grows towards the vegetation of xerophilous meadows with *Festuca valesiaca*, *Botriochloa ischaemum* and *Stipa capillata*.

Spreading: Dobrogea (Salygni)

19. *Malvetum pusillae* Morariu 43

It is a lot more spread in the perimeter of localities, at the border of roads around the houses, in yards there where the soil contains organic substances in decomposition. It develops well on dry ground and on those strongly sunlit summer beaten by animals and birds.

The *Malva pussilla* phytocoenoses are made out of a rather small number of species, almost all ruderal from which *Malva neglecta*, *Amaranthus crispus*, *Xanthium spinosum*, *Sisymbrium officinale*, *Polygonum aviculare*, *Urtica urens*, *Bromus tectorum*, *Lepidium ruderale*, etc. are never missing. The association plays a pioneer's work by the therophytes that compose this vegetal grouping.

Spreading: Transylvania, Banat, Oltenia, Muntenia, Moldavia, Dobrogea. As coenotaxons of inferior rank, the subassociations: *verbenetosum officinalis* Grigore 68, *polygonetosum avicularis* Coldea 72, *amaranthetosum crispi* Mč., Gč. 66, *chenopodietosum vulvariae* Grigore 68 and *typicum* Mč., Gč. 66.

20. *Xanthio spinosae-Amaranthetum* Morariu 43

It develops on cultivated grounds, on fallow grounds, unirrigated gardens and unworked places close to human lodgings. The *Xanthium spinuosm* and *Amaranthus retroflexus* phytocoenoses are mostly made of annual plants, nitrophilous with a pioneer's part. The recognition species are: *Xanthium spinosum*, *Amaranthus retroflexus*, *A. lividus*, *Chenopodium album* along which *Sisymbrium officinale*, *Malva neglecta*, *Solanum nigrum*, *Urtica urens*, *Polygonum aviculare*, *Capsella bursa-pastoris*, *Stellaria media*, may also be found. The association is known from all the parts of the country.

— subassociation *xanthietosum spinosi* (Ubrizsy 49) Grigore 68 (Syn.: *Xanthietum spinosi* Ubrizsy 49)

Convolvulo (arvensi)-Agropyrion repentis Görs 66

The alliance comprises ruderal groupings of places recently dug up, situating at the limit between the *Secalietea* phytocoenoses and those of *Chenopodietea*. The recognition species: *Convolvulus arvensis*, *Agropyron repens*, *Equisetum arvense*, *Cirsium arvense*, *Bilderdykia convolvulus*, *Capsella bursa-pastoris*, *Cardaria draba*.

21. *Cardarietum drabae* Timár 50

(Syn.: *Capsello-Cardarietum drabae* Resmeriță et Roman 75)
It is the association characteristic to sunny slopes, to uncultivated and slightly beaten grounds in the neighbourhood of villages, being largely spread all over the country. The characteristic species *Cardaria draba* appears frequently in a large number of phytocoenoses never missing from those growing in cornfields and from ruderal ones. Being largely spread, *Cardaria draba* takes great part, together with other annual species in the consolidation of dams of recent embankments where the layer has accumulations of organic matter. The most representative species in *Cardaria draba* phytocoenoses are: *Marrubium vulgare*, *Lamium maculatum*, *L. amplexicaule*, *Capsella bursa-pastoris*, *Arctium lappa*, *Carduus nutans*, *Veronica persica*.

The facies *ceratocephalosum orthoceri* Resm. et Roman 75 is described within the association.

22. *Agropyretum repentis* Felföldy 42

(Syn.: *Convolvuletum arvensis* Felföldy 42 emend. Pass. 64; *Agropyron repens-Convolvulus arvensis* ass. Felföldy 43; *Elytrigietum repentis* Felföldy 42 emend. Dihoru 70). The characteristic species of the associations of *Agropyron repens* and *Convolvulus arvensis* are perennial plants which means that the fallowing stage of the layer is rather advanced. The asso-

ciation was largely spread in the past but the extension of agricultural cultures and the planning of the lands led to the slow restriction of these phytocoenoses. *Agropyretum repentis* installs preferentially at the borders of ploughed fields, on ground dug up at longer periods of time. The recognition species are: *Convolvulus arvensis*, *Agropyron repens* and *Cynodon dactylon*. Within the association we may meet more frequently the species *Cardaria draba*, *Setaria pumila*, *Hibiscus trionum*, *Stachys annuua*, *Cirsium arvense*, *Sinapis arvensis*, *Polygonum aviculare*, and when the fallowing is more advanced, the perennial, lawn species as: *Poa pratensis*, *Medicago sativa*, *Agrostis stolonifera*, *Festuca pratensis* appear. They indicate the evolution of the phytocoenoses towards the meadow associations. The following subassociations are known: *convolvuletosum arvensis* Grigore 71, *cardarietosum (lepidietosum) drabae* Grigore 68, *cirsietosum arvense* (Burduja et Fl. Diaconescu 76) comb. nova (Syn.: *Cirsio-arvense-Convolvuletum arvensis* Burduja et Fl. Diaconescu 76).

St. Grigore (1971) mentions in his doctor's thesis summary the association *Chondrillo-Agropyretum* Oberd. 67 in Banat. The lack of data refeirring to this coenotaxon does not allow us to describe it and we think he is talking about the *Agropyretum repentis* Felföldy 42 here, too.

23. *Rubo (arvalis)-Calamagrostetum* Coste 75

It represents the *Calamagrostis epigeios* phytocoenoses on the cultivated grounds, boundary paths in the vine abandoned plantations. The floristic heterogeneous composition is influenced by the vigorous growing of the dominant species *Calamagrostis epigeios* which does not allow the installing of other species but in a small quantity.

In the phytocoenoses of the association, the presence of some species characteristic to grounds about to be fallowed that make together with the edifying one a nucleus of recognition taxons is rendered evident, such as: *Rubus caesius* var. *arvalis*, *Convolvulus arvensis*, *Chondrilla juncea*, *Agropyron repens*, *Agrimonia eupatoria*, as well as numerous growing on fields species. By the floristic composition the association resembles pretty well *Agropyretum repentis* both occupying the same kind of ground and having a rather common evolution towards the installing of the meadow vegetation. Spreading: Banat (the Locvei Mountains).

24. *Agropyro-Salvietum verticillatae* Szabó 71

The *Salvia verticillata* phytocoenoses with the participation of *Agropyron repens* in a rather small proportion have been described by V. Soran (1962) under the name of *Salvia verticillata-Daucus carota* ass. The lack of material referring to *Agropyro-Salvietum verticillatae* (published annotations) does not give us the possibility of synonymising this coenotaxon with the one described by V. Soran (1962) although we think that, in this case, we have two different synonymous groupings.

25. *Potentilletum repentis* P. Ekiás 74 emend. I. Pop 78

The association finds optimum conditions for growing on barren moist grounds or subjected to periodical inundations, covered with soils rich in nitrates, in the flower transplants with small gravel, moderately sunny

as well as at the borders of forests and edges of walls. It is a pionner association fact certified by the numerous annual species that make the floristic composition of its phytocoenoses. The number of species taking part in the *Potentilla reptans* phytocoenoses is rather large; we remind among the most representative ones: *Convolvulus arvensis*, *Anagallis arvensis*, *Verbena officinalis*, *Erigeron canadensis*, *Medicago lupulina*, *Prunella vulgaris*, *Taraxacum officinale*, *Polygonum aviculare*, *Lolium perenne*, *Achillea millefolium*, *Agrostis stolonifera*. The appearance of perennial species indicates the direction of evolution towards the installing of mesophyll meadows.

Artemisio-Agropyrion Intermedii Müller ET Görs 69

It groups the semiruderal associations in the xerothermic stations onatilt slopes, strongly eroded and levigated.

Recognition species: *Agropyron intermedium*, *Artemisia absinthium*, *A. compestris*.

26. *Artemisio compestris-Agropyretum intermedii* E. Schneider-Binder inscr. 74 cf. E. Schneider-Binder 76.

The association is signaled out as nomen nudum in the Sibiu depression with the mention that it is spread in the area of xerothermic steppe groupings and develops on precipices atilt slopes strongly eroded and levigated.

The coenoses where there are rendered evident *Agropyron repens* and *Artemisia compestris* correspond to the stages of *Artemisia* in the Plain of Transylvania (Şt. Csűrös, Margareta Csűrös-Káptalan, 1953) and are related with the groupings of the *Artemisietum pontico-sericeae* Soó (27) 42 association.

REFERENCES

1. Borza Al., *Flora şi vegetaţia văii Sebeşului*, Edit. Academiei, Bucureşti, 1959.
2. Burduja C., Horeanu Cl., Muz. Deltei Dunării. Peuce, Tulcea, 1976, 5, 321—334.
3. Diaconescu Florica, Rezumatul tezei de doctorat, Iaşi, 1978.
4. Kovács A., Studia Univ. Babeş-Bolyai, Ser. Biol. Cluj, fasc. 1, 1968, 51—55.
5. Mititelu D., Barabaş N., Analele Univ. "Al. I. Cuza", Iaşi, Serie nouă, Secţ. II-a, Biol., 1973, 19 (2), 427—431.
6. Morariu I., Bul. Grăd. Bot. de la Univ. Cluj la Timişoara, 1943, **23** (3—4): 131—212.
7. Popescu A., Sanda V., Muz. Brukenthal. St. şi Comunic. Şt. Nat. Sibiu, 1980, **24**, 147—314.
8. Schneider-Binder Erika, Mu. Brukenthal. St. şi Comunic. Şt. Nat. Sibiu, 1976, **20**, 15—45.
9. Şerbănescu I., Dări de Seamă ale Şed. Com. Geol. Bucureşti, 1957, 41: 181—188.
10. Todor I., Gergely I., Bârcă C., Contrib. Bot. Cluj, 1971, 203—256.

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SPECIES OF PLANTS CONSUMED IN WINTER BY *PHASIANUS COLCHICUS* IN ROMANIA (II). QUANTITATIVE RELATIONS OF THE VEGETAL FOOD

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The paper presents the quantity of food swallowed by pheasant (5.42±0.2482g mean) and the rate of different species participation; the most numerous seeds consumed are of: *Amaranthus crispus, Setaria glauca, Robinia pseudaccacia* (136.86—65.35 mean); the most weight of consumed seeds belongs to species *Zea mays, Prunus mahaleb, Robinia pseudaccacia Crataegus monogyna* (7.43—0.94). The pheasant may be considered a predator that controls the potential density of a great number of vegetal populations among which the most affected are the weeds of agricultural cultures. The energy quantity obtained by the pheasant from food is ensured in the first place by the seeds of some culture plants and of some species of trees and shrubs.

The previous papers showed the great diversity of vegetal food eaten by the pheasant during winter; the heterogeneity index varies between 0.99415, taking into account the list of plants identified by the authors, and 0.99554, if we consider the species cited by other authors too (Almășan, Scărlătescu 1961, Kiss, Rékási, Sterbetz 1976, 1985). Although the list of plants is very long (219 species) only 10 species are most frequently consumed (10—40%). All parts of the plant can be consumed, but the seeds are most frequently used (84%), then the leaves 7% and finally the rest of the identified vegetal material composed of fruit, seedlings, bulbs and rhizomes. Although it has been established that 69% of the pheasant food is of vegetal type for the whole year and that during winter it can reach 80—97% (Almășan, Scărlătescu 1961), only partial data upon the gravimetric or energetic weight of the plants used by the pheasant have been presented and this led to our interest in studying this aspect.

MATERIAL AND METHODS

The material extracted from the crop and stomach belonging to pheasants hunted during the winter of 1989—90 and used for the identification of the consumed species has been both totally and on certain categories weighted. From 360 samples only the quantity of seeds from the pheasant food has been established and only by the species with high frequency or gravimetrically representative. The determination of the biomass quantity of seeds was established as the product of the number of seeds in the samples and the mean weight of a seed in these samples (dry weight at 85° C). We noticed the fact that these seeds identify themselves in a different category of weight as compared to those gathered on plants. The data were analyzed and calculated on a dBase programme.

The pheasants come from 72 hunting funds belonging to 30 forest districts in the 14 districts mentioned in the first part.

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RESULTS AND DISCUSSIONS

The food quantity of the pheasant during winter reveals the energetic necessity for its survival, on the one hand and the possibilities of obtaining this food, on the other hand. The fact that the material of vegetal origin in this period is prevailing in its nourishment may be due to its greater availability during the analyzed period. The character of "predator" of the pheasant in the ecosystem seems to be kept in the case of its nourishment preponderantly granivorous (Barbour, Burk, Pitts 1987) because the seeds represent whole plants that they eliminate. The measures we made show that a pheasant swallows on the average 5.42± 0.2482 g of nourishments. The limits of variation are much larger, the weight of the swallowed food being sometimes of 0.0100 g other times reacheing 37.85 g. The variance is of 22.20 and the variation coefficient is very high, that is 86.93%. We may consider that this average quantity represents the energetic support for the existence of a pheasant even if in certain periods it is strongly underfed and in others excessively fed.

The quantity of food is made only of vegetal material in 81.25% of cases, only of insects in 0.5% and of mixed food (plants and insects) in the rest of 18.28%.

The most frequently consumed plants, respectively the most frequently met in the analyzed samples, are 15 species (from 171 identified by us) (Table 1). All these plants are consumed as seeds. They represent the basic contents of the pheasant's food and mark the vegetal formations and even the ecosystems where they ensure their own food supply. The skirt of locust forest and the border of the agricultural cultures with developed shrubs among them represent the land mostly frequented by the pheasants that breed freely.

The number of seeds is very different and is important both for the plant and pheasant because it regulates the potential density of their

Table 1

The list of the most frequently met species in the samples of food swallowed by the pheasant

Species	Frequency in the sample (%)
Robinia pseudaccacia	36.94
Cornus sanguinea	24.00
Rosa canina	20.27
Zea mays	17.77
Crataegus monogyna	15.55
Ligustrum vulgare	15.27
Sorghum halepense	10.55
Triticum vulgare	10.55
Sambucus ebulus	9.44
Setaria glauca	8.33
Crataegus pentagyna	7.50
Prunus mahaleb	6.94
Amaranthus crispus	6.94
Amaranthus retroflexus	5.55
Setaria verticillata	4.44

populations but also for the pheasant because they can ensure to a smaller or greater extent the essential food for its existence.

The number of seeds identified as being swallowed by the pheasant is most different; one single crop gathered up to 900 seeds of *Amaranthus crispus* for example or 250 seeds of *Robinia pseudaccacia* but only ten of seeds are usually consumed. The medium number of seeds for the analysed samples, that is the seeds swallowed by a single pheasant is of 22.88± 1.81 with a great variability of course ($S^2 = 4987.71$).

More eloquent seems to us the medium value of the number where the different seeds of different plants from the whole analysed food can be found (Table 2a, b).

Table 2a

The mean number of seeds of the main plants consumed by the pheasant (related to the whole number of analysed samples)

Species	Mean number of seeds $\bar{x}$	Error of the mean $S\bar{x}$	Variance S^2
Robinia pseudaccacia	23.96	3.7160	4955
Amaranthus crispus	8.36	3.2200	3754
Setaria glauca	7.26	1.3252	25.14
Sorghum halepense	6.06	1.9207	1327
Zea mays	5.36	0.6755	164.24
Triticum vulgare	4.62	1.2450	557.85
Amaranthus retroflexus	4.71	2.1200	1628
Rosa canina	4.55	0.5394	104.72
Cornus sanguinea	3.98	0.4976	89.19
Ligustrum vulgare	3.54	1.1636	487.26
Sambucus ebulus	2.41	0.4520	87.05
Crataegus pentagyna	2.30	0.3922	55.35
Crataegus monogyna	2.09	0.2796	28.146
Setaria verticillata	1.52	1.0569	401.99
Brasica nigra	1.42	0.3928	55.53

The number of seeds from each species related to all the analyzed samples indicate *Robinia pseudaccacia* on the first place among the consumed plants; it is a plant with the highest frequency among those chosen as nurishment by the pheasant and beside it, with a much less representation the weeds of *Amaranthus crispus*, *Setaria glauca* and *Sorghum halpense* that the pheasant has no uniform food or its whole area and that in many cases it lives in areas where, for example, there are no locust forests or no areas with shrubs, the number of seeds must be related to the number of samples where the species was present, thus only there where the pheasant may choose.

In this situation, although the same 10—15 species of plants have a significant numerical contribution, their degree of importance is changing, the first place being taken by the species of weeds and only then the locust tree, the species of culture, shrubs and other weeds came (Table 2b). Also in addition to the trophic relation plant-animal with a general

Table 2b

The mean number of seeds of the main plants consumed by the pheasant (related only to the samples where they have been identified)

Species	Mean number of seeds $\bar{x}$	Error of the mean $S\bar{x}$	Variance S^2
Amaranthus crispus	136.86	53.93	61440
Setaria glauca	87.11	5.03	7587
Amaranthus retroflexus	84.85	38.300	29319
Sorghum halepense	68.22	12.05	14935
Robinia pseudaccacia	65.35	10.117	13515
Triticum vulgare	43.79	11.79	5284
Setaria verticillata	36.47	25.38	9648
Brasica nigra	31.33	23.568	3331
Crataegus pentagyna	30.70	5.235	738
Zea mays	30.17	3.798	923
Sambucus ebulus	27.15	2.893	756
Ligustrum vulgare	24.52	8.055	3373
Rosa canina	22.45	2.667	516
Cornus sanguinea	16.87	2.094	377
Prunus mahaleb	15.88	6.30	1011
Crataegus monogyna	13.14	1.756	180

purpose of limiting the density of the above mentioned species its specific part for agriculture and thus for man — in limiting by natural means the weeds — must be rendered evident for the same unit of agricultural surface controlled by pheasants — minimum 470 and only 75 seeds of cultivated plants are destroyed from the supply of weed seeds in the soil.

The great number of seeds is most often not correlated with the seed size. Most of the time the plants with seeds of small size produce a greater number of seeds and they are also available for the consumers. For the pheasant the final quality that it can use becomes essential. We are presenting a few data (Table 3a, b) concerning the mean weight of the quantity of consumed seeds by the quantity where these were found in the analyzed samples. Owing to the great size of the maize seeds, this species is the most important for the pheasant's food; among the spontaneous plants the first place is taken by the locust tree followed by numerous species of shrubs and subshrubs that form thus the basic nourishment of the pheasant under the aspect of vegetal biomass and respectively of energetic contribution. The weed species in a great number, as we have seen, have a more reduced weight in the proper feeding of pheasants.

Even if we take into account (for a mean reckon) only the tests where the species are present, the relation among the species changes very little under the gravimetric aspect; the herb species still remain on an interior place as compared to those of trees and shrubs.

The individual weight of seeds is most variable; it differs a lot from the weight of the seeds harvested on the plant and, generally, the swallowed owes are heavier. During winter a great part of the consumed seeds are lacking the germ. At the same time seeds of wheat, for example,

were however identified not only as being viable, but they were preferred after the germination of the seed.

Table 3a

Mean weight of the seeds of the main plants consumed by the pheasant (related to the whole number of analyzed samples)

Species	Mean weight of seeds g d.w. $\bar{x}$	Error of the mean $S\bar{x}$	Variance S^2
Zea mays	1.3200	0.1663	9.955
Robinia pseudaccacia	0.5130	0.0794	2.269
Crataegus monogyna	0.1460	0.0200	0.146
Cornus sanguinea	0.1440	0.0179	0.340
Prunus mahaleb	0.1040	0.0418	0.629
Sambucus ebulus	0.0817	0.0132	0.480
Rosa canina	0.0770	0.0092	0.304
Ligustrum vulgare	0.0550	0.01787	0.114
Triticum vulgare	0.0520	0.0143	0.073
Crataegus pentagyna	0.0390	0.066	0.016
Sorghum halepense	0.0346	0.0102	0.037
Vitis vinifera	0.0115	0.0026	0.002
Setaria verticillata	0.0050	0.0026	0.002
Setaria glauca	0.0037	0.0015	0.0008
Amaranthus crispus	0.0037	0.0053	0.0006
Brasica nigra	0.0025	0.0179	0.0001
Amaranthus retroflexus	0.0022	0.0012	0.0005

Table b

Mean weight of the seeds of the main plants consumed by the pheasant (related only to samples where they have been identified)

Species	Mean weight of seeds g d.w. $\bar{x}$	Error of the mean $S\bar{x}$	Variance S^2
Zea mays	7.43	0.9350	56.00
Prunus mahaleb	1.50	0.3000	9.07
Robinia pseudaccacia	1.40	0.2167	6.19
Crataegus monogyna	0.94	0.1296	0.94
Cornus sanguinea	0.61	0.0754	0.49
Crataegus pentagyna	0.52	0.1000	0.21
Triticum vulgare	0.50	0.1364	0.70
Sambucus ebulus	0.46	0.0048	0.56
Sorghum halepense	0.39	0.0689	0.42
Ligustrum vulgare	0.38	0.1234	0.79
Rosa canina	0.38	0.0456	0.15
Setaria verticillata	0.09	0.0026	0.06
Setaria glauca	0.06	0.0219	0.01
Amaranthus crispus	0.06	0.0260	0.01
Amaranthus retroflexus	0.04	0.0170	0.01

The variated food the pheasant is consuming is probably owed to the limited offer of every species in the first place. Generally, the

content of the crop is almost wholly limited to the species (perhaps associated to a weed) where the pheasant found the seeds of *Zea mays* or *Triticum vulgare*.

This does not happen to other culture plants. For the other cases the number of species consumed is 2 or 3 but it is possible to reach 10. The samples analyzed in region contain the most diverse composition (about 3.3 species for an individual pheasant) and in Banat and Crișana regions the most uniform one (1.9 species); in Moldavia and Transylvania regions the situation is intermediaryan a mean of 2.2—2.3 species in a sample). No relation between the region and certain food was identified.

The species largely consumed have been used all over the country. Those very seldom met in the food samples cannot be connected to the area where they were identified but to the fact that they were found and used.

In conclusion, the pheasant may be considered a predator that regulates the potential density of a great number of vegetal populations, among which the most affected are the weeds of agricultural cultures. The quantity of energy obtained by the pheasant from food is ensured in the first place by the seeds of some culture plants, by the locust tree and shrubs taking into account the fact that from the medium consumption of 5.42 g done by the pheasant the 4th part may be ensured by the medium consumption of *Zea mays* (1.399 g) or the 10 th part by the medium consumption of *Robinia pseudaccacia* (0.51 g).

REFERENCES

1. Almășan H., Scărlătescu G., *Cunoașterea hranei naturale a fazanului în RPR, mijloc pentru sporirea producției de vînat*, Rev. Păd., 1961, **76**, 3, 185—188.
2. Barbour M. G., Burk J. H., Pitts D. Wanna, *Terrestrial plant ecology*, Benjamin/Cummings Publishing Company, Inc. 1987.
3. Kiss J. B. Rékási J., Sterbetz I., *Date privind hrana de iarnă a fazanului în pădurea Letea*, Rev. Păd., 1976, **91**, 4, 243—246.
4. Kiss J. B., Rékási J., Sterbetz J., *Date noi privind hrana fazanului în Delta Dunării*, Delta Dunării St. și Com. Ecol., 1985, 115—121.
5. Mihaela Paucă-Comănescu, Almășan H., Popescu A., *Species of plants cosumed in winter by Phasianus colchicus in Romania I. Diversity of vegetal food*, Rev. Roum. Biol. — Biol. Végét., 1991, **36**, 1—2, 101—108.

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PECULIARITIES OF PHYTOPLANKTON AND PHYTOPLANKTON PRIMARY PRODUCTIVITY IN THE MUSURA GULF (THE DANUBE DELTA)

EUGENIA STANCU-STOIANOVICI

As concerns the phytoplankton, the Musura Gulf is like an unstable ecosystem where the influences of the Danube and of the sea may be felt alternatively depending on the water quantities carried by the river, meteorological factors and biogen elements. The phytoplankton of the Musura Gulf develops under the conditions of water sweetening and of the increased contribution of nutrients as a consequence of the influence exerted by the overflows of the Danube.

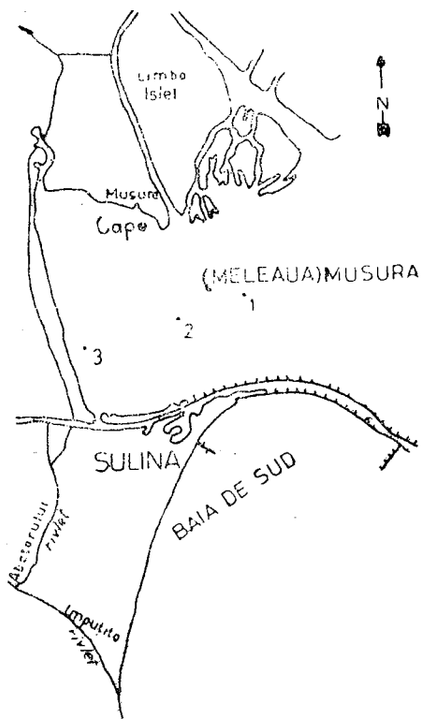
The Musura Gulf is a marine gulf in course of clogging, with a surface of 3500 ha and depths up to 5—6 m, situated at the mouths of the Danube, between the delta of the Chilia arm, the bank and the dam of the Sulina channel. Its opening towards the sea becomes more and more limited as a result of the advance of the delta of the Chilia arm in the gulf perimeter, the change of the running course of the waters belonging to the mentioned arm in the area of flowing and the creation of a new offshore bar by the decantation of the alluvional deposits drawn by the river and of the sand deposited by the marine current in the new hydrological configuration.

The process initially grasped at the end of the last century is extremely dynamic. Thus, from 1883 up to 1992 the mentioned arm entered the sea 7.5 km approximately, and 12 km around the secondary arms of Old Stambul and Musura, leading to the formation of the Chilia delta. After 1922, the land gains 70 ms each year. The Musura Gulf has been suffering an intense process of clogging for the latest 15—20 years, characterized by the decrease of the levels of the basin and the appearance of a bar of emersed vegetation, natantly broad of a few hundered meters and populated with avifauna of delta type, along the shores. The water salinity marks the important decreases from 5 — 10% as registered before to 1—2% except the outer area of the gulf where salmaster water infiltrates in the deep stratum under the conditions of a certain type of flood and level of the Danube waters. In the gulf represented in the past an area of strong impact for the riverine and marine waters with organisms comprised in their mass, now the shock force of the water mixture is a lot more reduced and the ecologic spectrum of the plankton organisms is mainly made of frech water, during long periods of the year. On the whole, Musura Gulf remains on area of ecotone between the categories of ecosystems.

MATERIAL AND METHODS

To study the phytoplankton of this ecosystem three stages of reaping the samples were established Fig. 1 : one out in the sea, the second in the area of contact of marine and fresh waters and the last one at the

shore. The samples were drawn seasonally. Samples of one liter were drawn to analyse the numerical density; they were fixed in formol and left to be deposited for two weaks; after that, the samples were concentrated to 100 ml from which a volume of 0.05 ml was microscopically examined; the results are given in thousant ex/liter. The phytoplankton biomass was estimated by the use of the volumetrical method based on a numerical analysis of the phytoplankton using specific medium volumes and considering the specific density of the cellular content as equal to one. The expression of the results was done in mg of wet substance/liter. To determine the phytoplankton primary productivity the Graan-



Gardner method was used and for the dosing of the dissolved oxygen the Winkler method was used. The studied biological material was collected from two horizons: surface and prebottom. The assembling on the spot of the white and black little bottles was made in two repetitions at depths corresponding to the depth where the sample was drawn from. The obtained values were expressed in mgs O_2/l/24 hours that may be transformed in mgs C/l/24 hours or Cal/l/24 hours. The estimation of the net productivity was made by reducing with 20% the values of the gross plankton primary productivity (1), (2), (3), (4).

RESULTS AND DISCUSSIONS

As concerns the phytoplankton, the Musura Gulf (avandelta) represents an unstable ecosystem where the influence of the Danube and of the sea may be felt alternatively having in view the quantities of water carried by the river, the wind direction. If the influence of one or the other is prevailing and according to their length of time, the phytoplankton modifies its qualitative and quantitative structure. The vertical circulation of water, that leads to the enrichment in biogenic elements of the superficial strata, is here doubled by the contribution of the high floods of the Danube that present great variations. The Danube carries huge quantities of suspensions that modify the transparence condition of the water. The waters enriched by biogenic elements and burdened by suspensions may be pushed by the currents in very inconstant directions which make sometimes the plankton associations to change their quality and quantity very quickly at the same point.

Generally, the phytoplankton in the Musura Gulf presented itself as a well diversified coenosis during the studied years (1988—1989), with 164 taxons and algal infrataxons in 1988 and 159 in 1989 distributed in five taxonomic groups: Cyanophyta Englenophyta Pirrophyta, Bacillariophyta, Chlorophyta. A number of 234 species has been identified on the whole; from these species only six are marine species, all others are ubiquist, with large tolerance from the salinity point of view, these species appear only when the hydrological front changes, when the contribution of fresh waters diminishes leading to a slight increase of water salinity. During spring when the waters of the Danube reach maximum levels, the pressure exerted on marine waters is strong and the contact front is out in the sea. At the some time with the lowering of the river levels and thus with the attenuation of the pressure exerted on sea waters, the contact front is getting near the shore. This is evidently mirrored in the numerical density of the phytoplankton organisms (Table 1) that show higher values in the distant station during spring (st. 1) and after this, the highest number of taxons is registered in the next seasons in the centre station (st. 2). The phytoplankton showed a qualitative structure similar to that met in the marshes of the Danube Delta while the sea influence was not felt in this area, in the shore station and during all seasons (st. 3). As concerns the taxonomic groups that possess the highest shore in belting the taxonomic spectrum, they correspond to the season changings.

As concerns the dynamics of the phytoplankton biomass and of the relative abundance of the biomass (Table 2) it mirrors the changes in space and time of the contact area of waters. During spring, this area places itself far in the sea, where a larger biomass (for example, in 1988 a value of 15.276 mg wet substance/liter in station 1 in contrast with 8.027 mg wet substance/liter was recorded) is registered. The stop of floods brings along the shifting of the contact area of waters towards the shore, a fact proved by biomass values that increase a lot in the centre area (st. 2) in contrast with the far area. Once the season is changed, the highest biomass is recorded in the centre station, the values being several times smaller here than in station 1. The biomass density is situa-

Table 1

Density (thousand ex/l) and numerical abundance (%) of the phytoplankton in the Musura Gulf (the Danube Delta) during 1988–1989

During	Month	Station	Total number	Taxonomic groups							
				Cyanophyta		Euglenophyta		Pirrophyta		Heterocontae	
				Nr.	%	Nr.	%	Nr.	%	Nr.	%
1	2	3	4	5	6	7	8	9	10	11	12
1988	IV	1 surface	4204	0	0.00	4	0.10	36	0.86	0	0.00
		1 bottom	4616	4	0.09	4	0.09	40	0.86	0	0.00
		2 surface	6014	0	0.00	0	0.00	4	0.07	0	0.00
		2 bottom	2448	0	0.00	6	0.00	36	1.47	0	0.00
		3	3532	4	0.11	12	0.34	0	0.00	0	0.00
	VI	1 surface	1004	144	14.34	4	0.40	0	0.00	0	0.00
		2 bottom	2070	78	3.78	10	0.48	2	0.10	0	0.00
		2 surface	1357	248	18.28	128	9.43	2	0.15	0	0.00
		2 bottom	1950	170	8.72	132	6.77	4	0.21	0	0.00
		3	2819	460	16.31	42	1.49	110	3.90	36	1.27
	VIII	1 surface	1846	444	24.05	22	1.19	140	7.58	0	0.00
		1 bottom	3170	1880	59.31	0	0.00	72	2.27	0	0.00
		2 surface	6708	3842	57.27	8	0.12	260	3.87	0	0.00
		3	2220	826	37.21	120	5.41	80	3.60	0	0.00
1989	III	1 surface	1316	65	4.94	6	0.46	0	0.00	0	0.00
		1 bottom	936	34	3.63	70	7.50	0	0.00	0	0.00
		2 surface	3996	6	0.15	0	0.00	0	0.00	0	0.00
		2 bottom	2856	4	0.15	28	1.08	0	0.00	0	0.00
		3	1870	74	3.96	8	0.43	18	0.96	0	0.00
	VI	1 surfaec	892	24	2.69	2	0.22	0	0.00	0	0.00
		1 bottom	586	4	0.68	2	0.34	26	4.44	0	0.00
		2 surface	3262	26	0.80	32	0.98	16	0.49	0	0.00
		2 bottom	2982	90	3.02	10	0.34	2	0.08	0	0.00
		3	1986	98	4.94	0	0.00	0	0.00	0	0.00

Bacillariophyta		Chlorophyta	
Nr.	%	Nr.	%
13	14	15	16
4160	98.95	4	0.10
4564	98.89	4	0.09
5858	97.41	152	2.53
2376	97.06	36	1.47
3512	99.43	4	0.11
194	19.32	662	65.93
914	44.15	1066	51.50
340	25.05	639	47.10
240	12.31	1404	72.00
890	31.57	1281	45.44
220	11.92	1020	55.25
414	13.06	804	25.36
2086	31.10	512	7.63
676	30.45	518	23.33
1172	89.06	73	5.55
774	82.69	58	6.20
3858	96.55	112	2.80
2444	94.51	110	4.25
1544	82.57	226	12.09
568	63.68	298	33.41
408	69.63	148	25.26
1216	37.28	1972	60.45
1374	46.08	1506	50.50
1446	72.81	442	22.26

Table 2

Density (mg net matter/l) and abundance (%) of phyteplankton biomass in the Musura Gulf (the Danube Delta) during 1988–1989

During	Month	Station	Biomass mg/l	Taxonomic groups							
				Cyanophyta		Euglenophyta		Pirrophyta		Heterocontae	
				mg	%	mg	%	mg	%	mg	%
1	2	3	4	5	6	7	8	9	10	11	12
1988	IV	1 surface	18·150	0·000	0.00	0.018	0.10	1.080	5.95	0.00	0.00
		1 bottom	12.402	0.072	0.58	0.014	0.11	0.320	2.58	0.00	0.00
		2 surface	9.910	0.000	0.00	0.000	0.00	0.012	0.12	0.00	0.00
		2 bottom	6.652	0.000	0.00	0.000	0.00	0.954	14.34	0.00	0.00
		3	8.657	0.180	2.08	0.028	0.32	0.000	0.00	0.00	0.00
	VI	1 surface	8.653	0.530	6.11	0.007	0.81	0.000	0.00	0.00	0.00
		1 bottom	11.322	0.086	0.76	0.058	0.51	0.053	0.47	0.00	0.00
		2 surface	48.257	0.278	0.58	1.282	2.66	0.006	0.12	0.00	0.00
		2 bottom	48.975	1.952	3.99	2.045	4.16	0.088	0.18	0.00	0.00
		3	20.495	0.414	2.02	0.322	1.57	3.180	15.52	6.84	33.37
	VIII	1 surface	14.231	6.934	48.73	0.053	0.37	4.200	29.51	0.00	0.00
		1 bottom	12.152	6.195	50.98	0.000	0.00	2.160	17.78	0.00	0.00
		2 surface	41.699	36.249	86.93	0.085	0.20	0.780	1.87	0.00	0.00
		3	20.494	11.631	56.75	1.197	5.84	2.400	11.71	0.00	0.00
1989	III	1 surface	7.608	3.721	48.91	0.048	0·63	0.000	0.00	0.00	0.00
		1 bottom	4.404	0.008	0.18	0.292	6.63	0.000	0.00	0.00	0.00
		2 surface	6.447	0.038	0.59	0.000	0.00	0.000	0.00	0.00	0.00
		2 bottom	4.979	0.036	0.72	0.162	3.25	0.000	0.00	0.00	0.00
		3	5.257	0.783	14.89	0.048	0.91	0.540	10.27	0.00	0.00
	VI	1 surface	12.379	0.013	0.10	0.004	0.03	0.000	0.00	0.00	0.00
		1 bottom	7.318	0.032	0.44	0.048	0.66	0.019	0.26	0.00	0.00
		2 surface	15.621	0.323	8.05	0.400	2.56	0.012	0.08	0.00	0.00
		2 bottom	15.124	1.257	8.31	0.082	0.54	0.006	0.04	0.00	0.00
		3	3.083	0.262	8.50	0.000	0.00	0.000	0.00	0.00	0.00

Bacillariophyta		Chlorophyta	
mg	%	mg	%
13	14	15	16
17.048	93.93	0.004	0.02
11.966	96.73	0.000	0.00
9.859	99.49	0.039	0.39
5.500	82.68	0.198	2.98
8.455	97.55	0.004	0.05
2.089	24.09	6.047	69.72
1.454	12.84	9.671	85.42
9.555	19.80	37.136	76.96
0.772	1.52	44.148	90.14
2.130	10.39	7.609	37.13
0.510	3.58	2.534	17.81
1.153	9.49	2.644	21.76
1.580	3.79	3.005	7.21
2.392	11.67	2.877	14.04
3.751	49.30	0.088	1.16
3.411	77.45	0.693	15.74
5.547	86.04	0.858	13.31
4.608	92.55	0.173	3.48
3.724	70.84	0.162	3.08
1.125	59.09	11.051	89.27
0.846	11.56	6.455	88.21
2.954	18.91	11.932	76.38
3.022	19.98	10.757	71.13
2.233	72.43	0.588	19.07

Table 3

Primary phytoplankton production in the Musura Gulf (the Danube Delta) during 1988—1989

During	Month	Station	mg O_2/l/24h organic	mg C	Cal/day	mg O_2/l/24 h organic	mg C	Cal/day
1988	IV	1 surface	8.852	0.757	7.083	0.369	0.138	1.295
		1 bottom	0.000	0.000	0.000	0.784	0.294	1.031
		2 surface	9.846	0.842	7.876	0.323	0.121	0.104
		2 bottom	0.260	0.022	0.207	0.742	0.278	0.551
		3	4.595	0.393	3.675	0.529	0.191	1.857
	VI	1 surface	42.471	3.630	33.977	5.370	2.014	18.849
		1 bottom	32.468	2.775	25.974	5.460	2.048	19.165
		2 surface	46.051	3.934	36.820	5.080	1.905	17.831
		2 bottom	37.452	3.203	29.975	4.680	1.755	16.427
		3	44.051	3.765	35.240	7.550	2.831	26.501
	IX	1 surface	25.602	2.188	20.481	1.832	0.687	6.430
		1 bottom	22.187	1.896	17.750	2.391	0.897	8.392
		2 surface	26.216	2.241	7.866	2.016	0.756	7.076
		2 bottom	18.371	1.570	14.696	2.382	0.893	8.361
		3	24.503	2.094	19.603	1.729	0.644	6.069
1989	III	1 surface	—	—	—	0.165	0.062	0.579
		1 bottom	0.519	0.044	0.414	0.595	0.223	2.088
		2 surface	11.021	0.942	8.817	0.654	0.245	2.296
		2 bottom	0.495	0.042	0.397	0.844	0.317	2.969
		3	8.280	0.333	3.120	0.327	0.123	1.148
	VI	1 surface	37.101	3.171	29.681	2.488	0.932	8.733
		1 bottom	3.579	0.321	3.005	2.547	0.955	8.940
		2 surface	39.414	3.369	31.530	3.709	1.391	13.019
		2 bottom	6.606	0.565	5.286	4.433	1.662	15.560
		3	56.915	4.865	45.552	3.049	1.143	10.702
	X	1 surface	18.568	1.586	14.847	2.970	1.114	10.425
		1 bottom	13.542	1.157	10.832	3.257	1.221	11.432
		2 surface	36.174	3.092	28.936	3.741	1.403	13.131
		2 bottom	3.580	0.306	2.864	3.918	1.469	13.752
		3	36.915	3.155	29.530	1.764	0.662	6.192
						2.522	0.946	
						0.000	0.000	
						2.805	1.052	
						0.074	0.028	
						1.309	0.491	
						12.100	4.538	
						9.250	3.469	
						13.120	4.920	
						10.670	4.001	
						12.559	4.706	
						7.294	2.735	
						6.321	2.370	
						7.469	2.805	
						5.234	1.963	
						6.981	2.618	
						—	—	
						0.148	0.056	
						3.140	1.176	
						0.141	0.053	
						2.359	0.885	
						10.570	3.964	
						1.071	0.402	
						11.229	4.211	
						1.882	0.706	
						16.215	6.077	
						5.290	1.984	
						3.858	1.447	
						10.306	3.865	
						1.020	0.383	
						10.517	3.944	

ted at values characteristic for the delta marshes in the shore station (3). Analysing the values of the phytoplankton primary productivity in the Musura Gulf (Table 3) we conclude that during spring months, when the temperature is a limiting factor of photosynthesis, we may consider this basin as eutrophic. In exchange, during summer months, high values of the primary productivity are registered; they allow us to frame the Musura ecosystem in the high eutrophic-polytrophic category.

Analyzing the values of the phytoplankton primary productivity vertically, we ascertain, without exception, the dramatic worsening of the life conditions in the profound strata of the basin. In stations 1 and 2 where water depth allowed and also imposed the installing of the experiment in two horizons, a clear differentiation between the productive capacity of the phytoplankton in the deep strata and the superficial stratum is observed which may be explained by quantitative differentiations rendered evident by the vertical study of the phytoplankton biomass, on the one hand, and also by the worsening of the physical-chemical conditions in the deep strata, on the other hand. Thus, the impact of marine waters with fresh waters leads to a horizontal stratification meaning that saltier waters are heavier: this impact leads to the increase of turbulence, fact mirrored in the low transparence here recorded. In addition to these physical-chemical factors in the Musura Gulf, the processes of microbial pollution are extremely active, fact that led to an oxygen shortage in the deep strata and, implicitly, to changes in the photosynthetic capacity of the phytoplankton, that is to its decrease.

In the shore station (3), where all these disadvantages are not present, only the temperature plays a limiting part in the photosynthesis, fact demonstrated by the curve of values of primary productivity that increases from spring to summer and then decreases during autumn months.

The curve of values of phytoplankton primary productivity enters in all the stations taken into study in the Musura Gulf; their amplitude oscillations are given by the season changes, meaning the values increase from spring towards summer then decrease towards autumn; we notice a concordance with the curves of phytoplankton density in the same period of time.

CONCLUSIONS

1. We may ascertain that the Musura Gulf is much stronger influenced by the Danube waters in contrast with the marine ones except for the periods with storms from the far out sea.
2. Our study confirmed the existence of a hydrologic front in the contact area of Danube and marine waters and this area does no longer represent a sorting of the phytoplankton organisms in these two planes, but an area of accumulation as this is where the biggest number of taxons and the highest phytoplankton density were found, with reference to their number and biomass.

3. The algal composition determined in the Musura Gulf renders clearly evident the change in the physical-chemical conditions, in the line of evolution of this ecosystem towards a fresh water, the taxonomic structure being distinctly dominated by limnological algal forms with a large tolerance as concerns salinity.
4. As concerns the phytoplankton primary productivity the studied ecosystem frames in the eutrophic category towards the high eutrophic polytrophic one during the hot season of the year as a result of its great dependence on the Danube waters contribution.

REFERENCES

1. Hillbricht-Ilkowska Anna, Pol. ecol. Stud., 1977, 3, 1, 3–98.
2. Nakanishi M., Productivity of communities in Japanese inland waters, J. I. B. P. Synthesis, 1975, 10, 381–391.
3. Oltean M., Al 3-lea simpozion "Bazele biologice ale proceselor de epurare și protecția mediului", Arges, Iași, 230–237.
4. Oltean M., Nicolescu N., Ziridava XVII Cunoașterea și valorificarea optimă a resurselor, naturale, Arad, 1988, 299–302.

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