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ULTRASTRUCTURAL MODIFICATIONS DETERMINED BY MUSTIN (CHLORETHYLMETHYLAMINUM) AT THE LEVEL OF WHEAT (*TRITICUM AESTIVUM* L.) MERISTEMATIC CELLS

BY

AURELIA CIOBANU

Dans le présent travail on met en évidence l'influence du mustin sur l'ultrastructure cellulaire en concentrations qui déterminent des effects inhibiteurs sur les processus physiologiques.
On a constaté que le mustin produit la désorganisation de l'infrastructure du cytoplasme et qu'en grandes concentrations il agit comme un fixateur toxique.

Taking into account that mustin manifested an inhibitory action on the protoplasm movement, germination, growth, and respiration [1], we intended to extend our investigations on the cell ultrastructure under the same cytostatic concentration conditions.

MATERIAL AND METHOD OF INVESTIGATION

We used as experimental material wheat seedlings treated, the same as in the case of physiology experiments, with mustin (chlorethylmethylaminum) (0.03% and 0.3%). Some seedlings were subjected to the cytostatic action for 3 hours, and others, without any treatment, served as a control material.

The fixation of the root and leaves meristems was made in a solution of glutaraldehyde 4%, in cacodylate buffer at pH = 7.4, during an hour, at the temperature of 4°C. After fixation, the material was washed in cacodylate buffer for 3 hours and then postfixed in a solution of osmium tetroxide 2%, in veronal buffer at pH = 7.4 (according to Palade [6]). After a postfixation, the samples were washed in distilled water and dehydrated in the alcohol series, then treated with propylene oxide for 3 hours and included in araldite (Davis technique). The staining of the sections was made with uranyl acetate (Watson technique), the observations and photographs being made under the electron microscope JEM 7 (functioning at 80 kw).

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RESULTS AND DISCUSSIONS

For comparative purposes we determinend the normal ultrastructure of the cell. In the following remarks we present first the results obtained in this respect.

The plasmalemma of the untreated cells is adherent to the pecto cellulose wall, and does not present morphological deformations which would indicate the existence of some abnormal physiological processes (Pl. II, fig. 2). The cytoplasm appears in our photos under the form of a network of fine sinuous microfibrils (Pl. II, fig. 1); in some portions we notice that these microfibrils carry ribonucleoproteic granules. The mitochondria have a long or oval shape (Pl. II, fig. 1), being surrounded by a double membrane cover. Inside, the mitochondria have cristae and a granular-dense stroma. The cytoplasm is crossed in different directions by a complex of tubules which form the endoplasmic reticulum (Pl. II, fig. 1). The Golgi apparatus is formed of dictyosomes which are widespread in the cytoplasm and made up of 4—7 saccules at whose ends emissions of Golgi vesicles are noticed (Pl. I, fig. 1; Pl. II, fig. 1). At the level of leaves meristems, the chloroplasts have an oval shape and are surrounded by a double membrane dense in electrons, among which a clear space exists (Pl. II, fig. 2). In the chloroplasts stroma, rich in a fibrogranular material, the grana lamellae are found longitudinally displayed in the form of packets of lamellae (thylacoids). The nucleus is separated from the cytoplasm by a nuclear envelope (Pl. I, figs. 1, 2) made up of two opaque membranes separated by a space of 250—400 Å. The external membrane of the nuclear envelope gets into contact with the cytoplasm and even with some cellular organelles, while the internal membrane, with the nucleoplasm, chromatin, and chromosomes. In some places the two constitutive membranes of the nuclear envelope can be interrupted forming pores, at whose level the communication between nucleoplasm and cytoplasm is possible.

Mustin manifests an evident action at a concentration of 0.03% (Pl. III, fig. 1), when the cytoplasm is affected, being impossible to point out a cellular organelle. The system of cytoplasmic membranes is destroyed, the same as the plasmalemma, so that the rests of cytoplasmic material come into direct contact with the pectocellulosic wall. The nuclear envelope is practically destroyed (Pl. III, fig. 1) and in the places where it is preserved it is transformed into vesicles. The destruction of the nuclear envelope determines the nucleoplasm and chromatin, structurally disorganized, to mix with the cytoplasm.

At a concentration of 0.3% mustin acted as a toxic fixing solution, affecting however the cell ultrastructure. Thus, the mitochondria have the cristae very dilated and the matrix destroyed. The dictyosomes present only some saccules with few vesicles in the process of disorganization and the cytoplasm is destroyed to a large extent (Pl. III, fig. 2; Pl. IV, figs. 1, 2). In the destroyed portions of the cytoplasm some profiles of endoplasmic reticulum are maintained, but are also in the process of destruction (Pl. IV, fig. 2). In this concentration mustin acted on the nucleus both by a process of destruction and as a toxic fixing solution (Pl. IV, figs 1, 2). At the chloroplasts level, the effect is manifes-

PLATE I

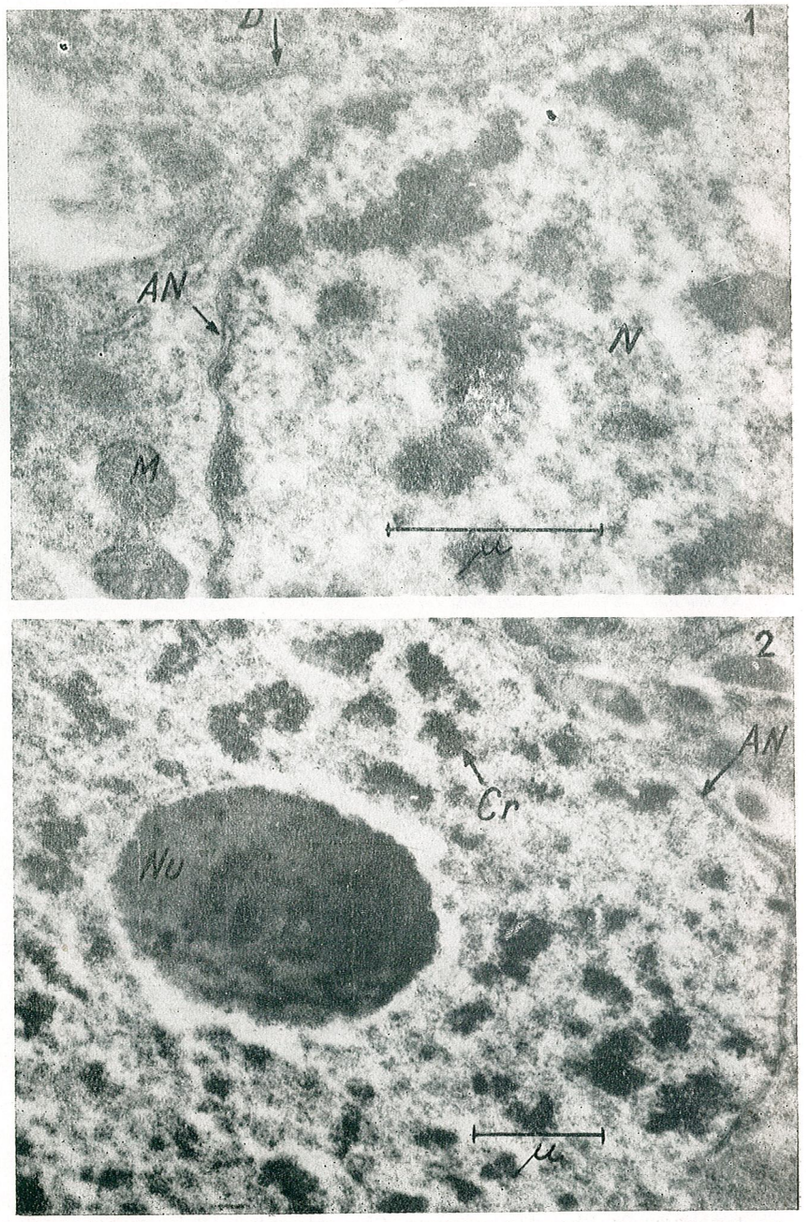
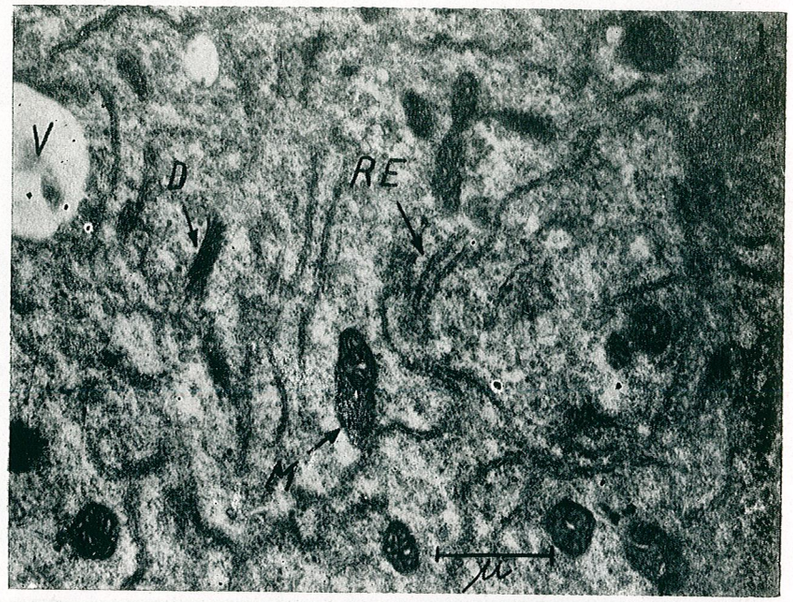


Fig. 1. — The ultrastructural aspect of a wheat untreated cell (the root meristematic zone): *D* = dictyosome, *N* = nucleus, AN = nuclear envelope, *M* = mitochondria.

Fig. 2. — The ultrastructural aspect of a wheat untreated cell (the root meristematic zone): *Nu* = nucleolus, *Cr* = chromatin, *AN* = nuclear envelope.



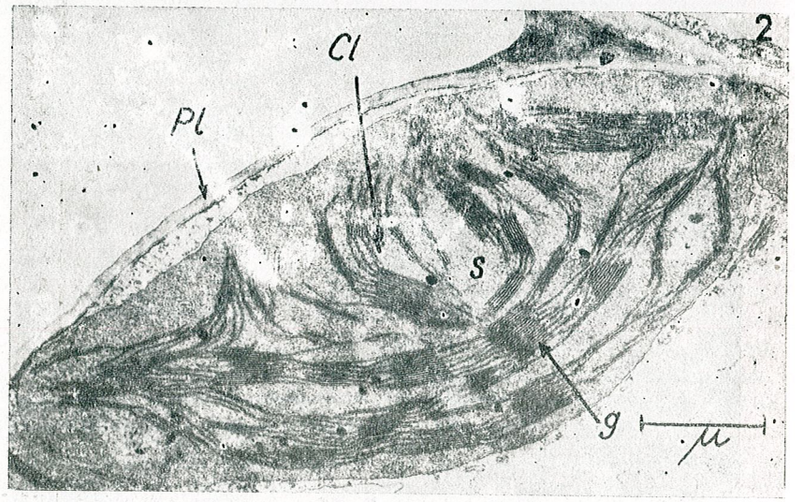
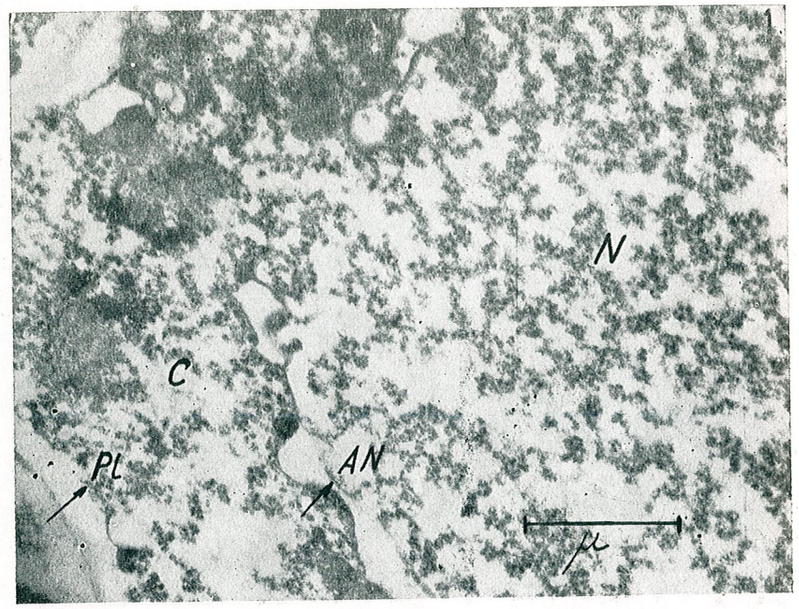


Fig. 1. — The ultrastructural aspect of a wheat untreated cell (the root meristematic zons) : V = vacuole, RE = endoplasmic reticulum, D = dictyosome, M = mitochondria.

Fig. 2. — The ultrastructural aspect of a wheat untreated cell (the leaves meristematic zone): Pl = plasmalemma, Cl = chloroplast, S = stroma, g = grana.



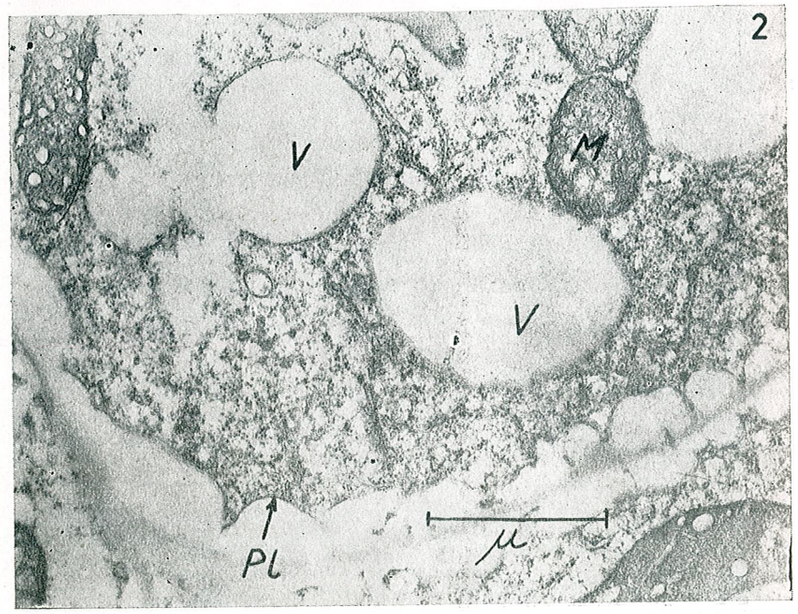
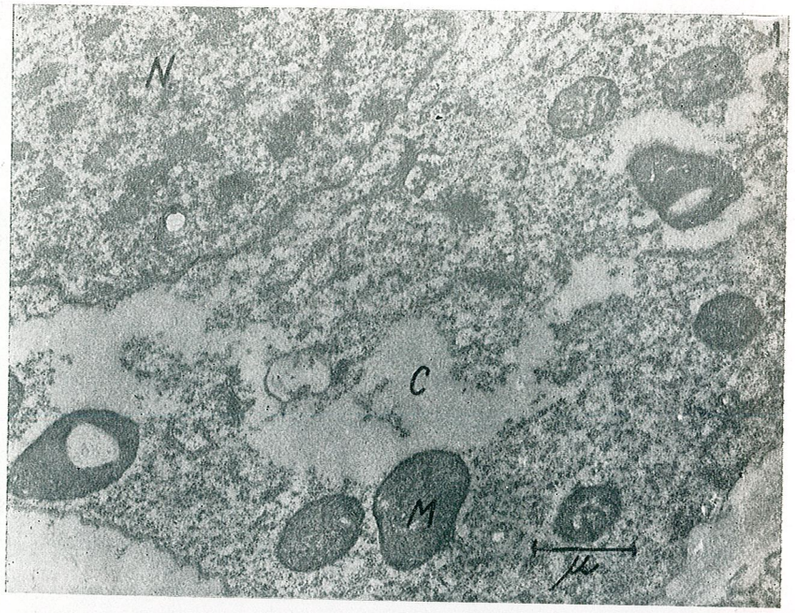


Fig. 1.—The ultrastructural aspect of a wheat cell treated with mustin 0.03 % (the root meristematic zone) : N = nucleus, AN = nuclear envelope, C = cytoplasm, Pl = plasmalemma.

Fig. 2. — The ultrastructural aspect of a wheat cell trated with mustin 0.3 % (the root meristematic zone) : V = vacoule, Pl = plasmalemma, M = = mitochondria.



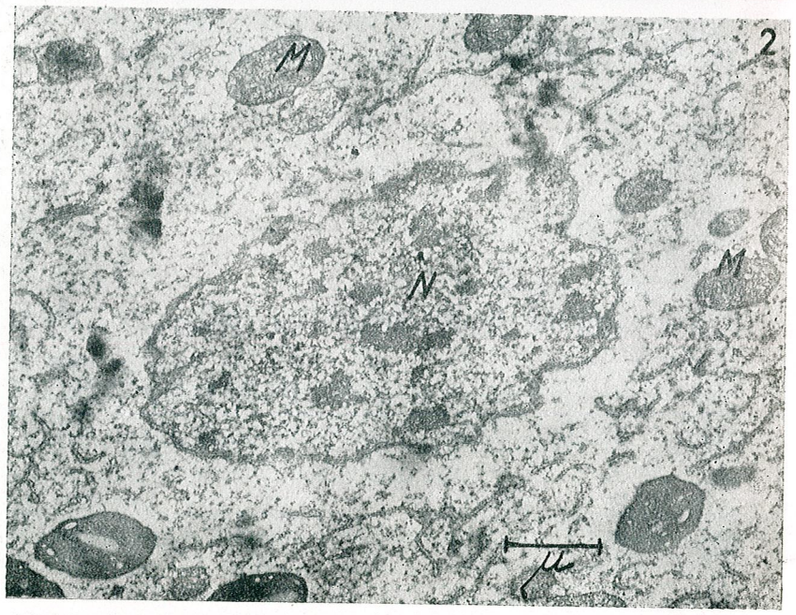
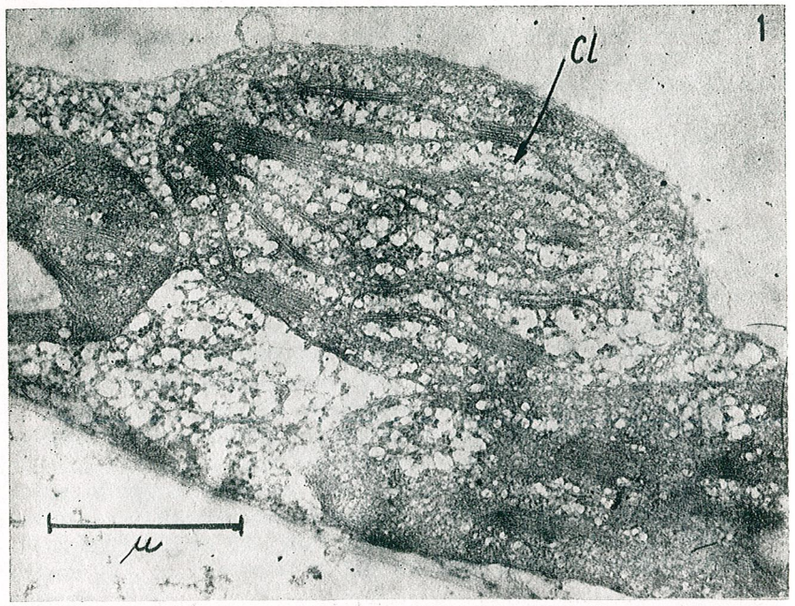


Fig. 1. — The ultrastructural aspect of a wheat cell treated with mustin 0,3 % (the root meristematic zone): N = nucleus, C = cytoplasm, M=mitochondria.

Fig. 2. — The ultrastructural aspect of a wheat cell treated with mustin 0,3 % (the root meristematic zone): N = nucleus, M = mitochondria.



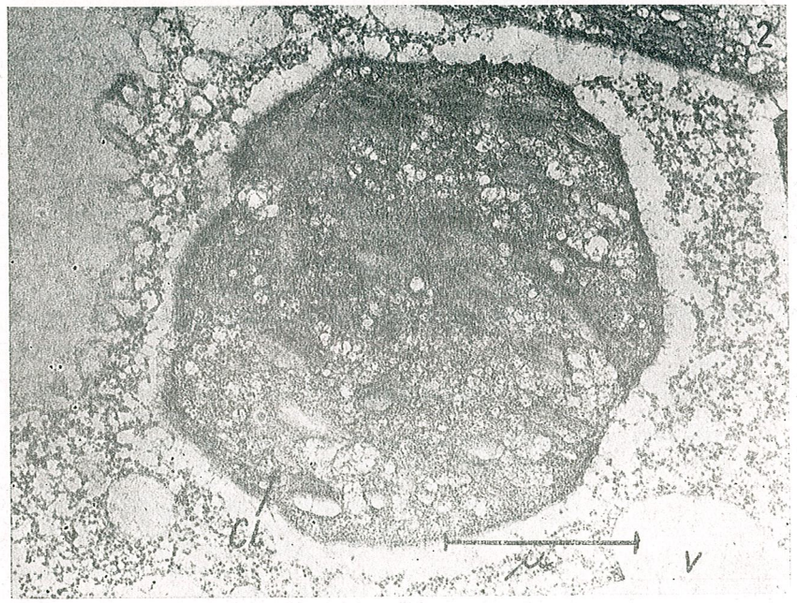


Fig. 1. — The ultrastructural aspect of a wheat cell treated with mustin 0.3 % (the leaves meristematic zone): Cl = chloroplast.

Fig. 2. — The ultrastructural aspect of a wheat cell treated with mustin 0.3 % (the leaves meristematic zone): Cl = chloroplast, V = vacuole.

ted by the disorganization of the stroma and the transformation into vesicles of their thylacoids (Pl. V, figs 1, 2).

In a previous paper, we noticed that at a concentration of 0.0003% mustin, the structure of the cytoplasm and cellular organelles is affected, the endoplasmic reticulum being almost completely destroyed. From the data presented in the present paper we notice that on increasing the concentration to 0.03% the investigated cytostatic substance manifested a strong action, materialized in the disorganization of the cytoplasm infrastructure, while at a concentration of 0.3% it acted like a toxic fixing solution.

Worth emphasizing is the fact that the above-mentioned ultrastructural modifications are determined by the concentrations (0.03% and 0.3%) which produced an irreversible inhibition of dinesis and strong inhibitory effects on growth, germination, and respiration [1]. Such ultrastructural modifications were also obtained under the influence of 1-carbamyl-2-phenyl-hydrazine [2], using the same experimental material.

From the consulted references one may see that other cytostatics determine similar effects; thus, azouracyl produces at the level of root meristems the premature vacuolization and hypervacuolization after the treating of the cytoplasm. The influence on mitochondria, endoplasmic reticulum, and dictyosomes results in ultrastructural modifications noticed by Mesquito [5] in the root meristematic cells and by Radkiewicz and Mikulsko [7], in the cells of the embryo saccule under the influence of colchicine.

In this respect, our results are a confirmation of these investigations, and prove that in high concentrations the cytostatic affects more markedly the ultrastructure, leading even to the destruction of the cell architectonics.

CONCLUSIONS

1) The mentioned ultrastructural modifications demonstrate that the cytostatic action is a complex process,because besides determining chromosomal aberrations they affect the cell organelles ultrastructure.

2) From the investigation carried out, we notice that the same concentrations of mustin which had a physiological influence on the vegetal cell determined at the same time deep modifications of an ultrastructural nature.

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THE MULTIANNUAL DYNAMICS OF THE PRIMARY PRODUCTIVITY OF THE HERBACEOUS LAYER FROM SOME MIXED FIR-TREE AND BEECH-TREE FORESTS

BY

AURELIA BREZEANU, FL. TĂCINĂ

and

MIHAELA PAUCĂ-COMĂNESCU

The article includes data recording the annual dynamics of superterraneous production and water content of herbaceous layer in different phytocenoses of *Pulmonario* (*rubro*) *Abieti-Fagetum*. The fact is presented that annual variations of climatic conditions influence the biomass accumulated both during a year and during every month. Though annual variations are very clear, stational differences are maintained in the same ratio, herbaceous layer production belonging to the forests of lower productivity having greater values than the production belonging to the forests of higher productivity, as a result of canopy opening differentiation.

The knowledge of ecologic laws requires long-term studies carried out throughout years, because of the biologic variability at all organization levels of the living world.

The accumulation of the herbaceous layer in forests and of the component populations is influenced by numerous factors — especially climatic ones —, which determine very important annual differences.

The present paper is aimed at pointing out the multiannual quantitative variations of the overground vegetable mass of the whole forest herbaceous layer, with a view to knowing its dynamics, the periods of maximum accumulation of the vegetable material as well as some factors that influence the overground yield of grasses from the phytocenoses under study.

MATERIAL AND METHOD

Investigations were carried out in 1971—1973, in different phytocenoses of the *Pulmonario* (*rubro*) *Abieti-Fagetum* (Knapp, 1942, Soo, 1962) association on two areas (at the foot of Mt. Piatra Arsă and on Platoul Izvor, characterized by different geomorphological and pedoclimatic conditions [2].

Determinations were performed monthly in 1971—1972 (in the May —September period), on 200 sample areas of 0.25 sqm within each station, according to a method described in a previous paper [2]. In 1973 data were added, recorded in the period of maximum development of the vernal and estival species. Research years evinced climatic differences in the months of the vegetation period, especially as concerns air temperature and precipitations. The year 1971 is generally characterized by somewhat higher values.

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Data on phytomass dynamics were correlated with some ecologic factors of peculiar importance — light — as well as with some elements of structure, such as populations density and covering.

RESULTS AND DISCUSSIONS

a) DYNAMICS OF PRIMARY PRODUCTION OF THE MAIN HERBACEOUS POPULATIONS

The results of researches recorded in table 1 and figures 1 and 2 point out that the superterraneous phytomass of grasses has peculiar annual variations, especially within estival species. These variations depend on the climatic conditions characteristic of every year. Thus, the increase of populations production throughout the vegetation season of 1972 took place according to a single-peak curve, in the case of all vernal species and of several estival species, and to a two-peak curve, in a reduced number of estival species. In this way, the season dynamics of the estival populations production differed in 1972 from 1971, by the absence (in most populations) of the two periods with maximum accumulation of vegetable material.

In vernal species, little pronounced annual differences are only of a quantitative order. The maximum production value is recorded in May, the same as in the previous year. It also coincides with the end of the vegetative growth, formation of the generative organs, an obtainment of maximum density.

Estival populations record maximum productions in most cases in mid-summer — June, July — and only in a few species (*Circaea lutetiana*, *Oxalis acetosella*, *Sanicula europea*), in August.

Thus, 1972 actually presents the disappearance of the spring maximum, as species do not find the most favourable conditions of temperature and moisture for intense vegetative growth or for ensuring high density. The populations where this maximum occurs are few and the latter characterized by a small amplitude. For both stations are common : *Mercurialis perennis*, *Geum urbanum*, *Lamium galeobdolon*, *Ranunculus repens*, *Petasites albus*, *Tussilago farfara*. In very few populations in that year, a slight increase of production occurred in autumn (*Lamium galeobdolon*) ; in this season conditions proved unfavourable for stimulating a supplementary sprouting at the end of summer, as it happened in 1971.

Annual differences appear in both stations also when comparing the value reached by the populations biomass in the period of maximum.

It is noticed (Table 1) that values are lower than in 1971, in the vast majority of cases. This is very obvious in *Carex sylvatica* for instance, where the maximum production varies from 439 kg. in 1971 to 107 kg. in 1972 and 58 kg. in 1973, as concerns green mass/ha, and from 126 kg. dry mass/ha to 22 and 15, respectively. The same differences are met also in other species, of which we mention *Athyrium filix-femina* (215 — 67—43 kg. green mass/ha, 33—14 — 6 kg. dry mass/ha in the Piatra Arsă station), *Dryopteris filix-mas* and *Petasites albus*, etc. There are few populations whose maximum production reach higher values than in

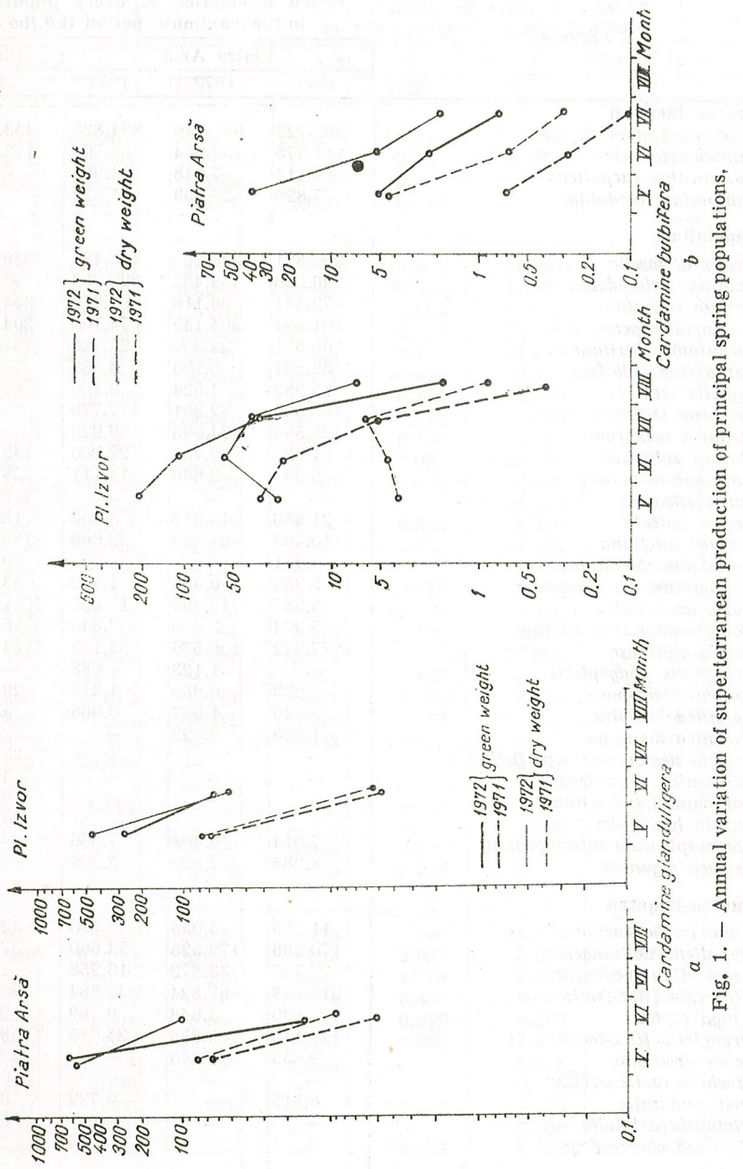


Fig. 1. — Annual variation of superterranean production of principal spring populations,

Table

Annual variation of primary production

No.	Species	Green production of every population in the maximum period (kg./ha)				Dry biomass of every population in the maximum period (kg./ha)							
		Piatra Arsă			Platoul	Izvor		Piatra Arsă			Platoul Izvor		
		1971	1972	1973	1971	1972	1973	1971	1972	1973	1971	1972	1973
	Fagion dacicum												
1	*Cardamine glanduligera*	592.722	671.346	394.828	453.129	262.737	443.618	80.784	66.897	36.344	71.949	63.426	16.794
2	*Pulmonaria rubra*	111.375	102.084	38.766	173.599	185.603	104.603	13.305	5.163	3.834	21.663	22.295	11.600
3	*Ranunculus carpaticus*	14.114	7.848	4.985	—	0.706	2.120	3.252	0.730	0.595	—	—	—
4	*Symphitum cordatum*	77.826	28.459	3.322	—	—	—	6.104	2.015	0.264	—	—	—
	Fagetalia												
5	*Oxalis acetosella*	200.821	347.263	136.488	339.238	266.344	176.620	20.790	47.738	11.374	31.954	49.804	17.662
6	*Lamium galeobdolon*	226.026	452.435	320.784	90.480	177.600	60.400	38.240	175.892	21.516	17.680	30.149	12.684
7	*Galium odoratum*	72.441	29.116	61.360	354.811	242.060	136.152	7.110	4.752	1.652	47.015	45.717	26.784
8	*Mercurialis perennis*	101.871	204.149	74.100	204.388	219.640	182.880	19.222	33.627	11.956	32.466	30.324	21.680
9	*Geranium robertianum*	68.671	22.350	23.126	281.882	176.369	211.448	12.231	2.120	2.728	31.478	15.498	25.088
10	*Cardamine bulbifera*	39.891	5.100	0.489	56.610	217.260	124.136	4.931	0.757	0.055	6.328	31.012	16.094
11	*Mycelis muralis*	15.982	4.524	2.160	62.426	40.729	25.290	2.079	0.354	0.276	6.154	3.471	2.970
12	*Isopyrum thalictroides*	189.500	72.291	117.779	25.160	19.514	18.018	19.481	12.122	14.638	3.454	3.148	2.356
13	*Stellaria nemorum*	125.584	74.095	59.920	5.816	0.201	1.260	27.905	0.495	6.136	0.524	0.037	0.080
14	*Stachys sylvatica*	61.920	37.748	22.760	142.800	130.476	127.820	8.532	5.628	3.034	24.744	13.015	20.172
15	*Anemone nemorosa*	9.231	2.666	14.111	38.057	45.374	66.444	1.626	0.187	2.083	6.706	7.490	8.542
16	*Carex silvatica*	—	—	—	439.018	107.328	58.028	—	—	—	126.022	21.964	14.996
17	*Actaea spicata*	21.656	15.318	3.752	18.152	7.200	23.384	1.976	2.376	0.600	3.350	1.506	3.706
18	*Circaea lutetiana*	10.463	21.583	22.066	123.366	58.720	59.840	4.187	2.007	2.950	21.865	8.915	4.476
19	*Epilobium montanum*	0.411	—	—	9.219	11.433	6.496	0.105	—	—	1.940	2.030	1.400
20	*Euphorbium amygdaloides*	5.382	0.428	1.532	53.788	37.897	10.230	0.229	0.081	0.685	9.283	5.808	1.980
21	*Paris quadrifolia*	5.687	18.496	13.633	1.617	1.134	6.116	3.230	1.815	1.167	0.220	0.114	0.588
22	*Polygonatum verticillatum*	5.934	2.440	3.840	16.184	2.526	4.590	0.668	0.310	4.800	2.836	0.280	0.630
23	*Salvia glutinosa*	77.922	106.575	54.152	184.481	275.360	273.800	9.651	5.048	6.104	27.555	24.460	33.600
24	*Dryopteris phegopteris*	—	1.123	1.088	—	—	—	—	0.212	0.128	—	—	—
25	*Sanicula europaea*	1.233	0.965	1.314	29.155	2.960	18.612	0.143	0.171	0.186	2.112	0.489	2.816
26	*Veronica latifolia*	2.640	1.087	0.068	5.692	8.840	7.080	0.331	0.113	0.019	1.121	1.456	1.200
27	*Veronica montana*	1.026	0.322	—	2.778	0.376	—	—	0.048	—	—	0.067	—
28	*Senecio nemorensis* ssp. *fuchsii*	—	—	—	23.657	50.808	39.942	—	—	—	3.253	8.079	2.970
29	*Cardamine impatiens*	—	—	—	4.390	3.200	—	—	—	—	0.085	0.380	—
30	*Campanula trachelium*	—	—	—	9.360	1.461	6.564	—	—	—	1.124	0.229	0.864
31	*Luzula luzuloides*	—	—	—	20.659	—	15.240	—	—	—	4.190	—	2.720
32	*Chrysosplenium alternifolium*	7.014	10.408	7.566	—	—	—	0.525	0.990	0.546	—	—	—
33	*Festuca gigantea*	8.285	7.520	5.206	—	—	—	1.476	1.000	1.216	—	—	—
	Querco-Fagetea												
34	*Viola reichenbachiana*	11.269	4.058	2.310	42.137	33.984	29.054	20.12	0.775	0.450	12.513	6.132	5.572
35	*Impatiens nolitangere*	170.366	179.525	33.660	57.443	105.840	62.160	28.867	0.335	2.832	3.927	9.680	6.216
36	*Aegopodium podagraria*	27.750	33.872	10.258	1.262	—	—	3.175	4.466	1.310	0.179	—	—
37	*Athyrium filix-femina*	215.988	67.824	43.684	90.808	66.144	36.720	33.027	14.731	6.808	16.783	10.218	5.780
38	*Ajuga reptans*	4.690	3.648	0.769	2.042	4.994	1.508	0.565	0.453	0.079	0.288	0.682	0.168
39	*Dryopteris filix-mas*	176.115	105.876	23.615	58.957	23.469	20.000	23.236	20.491	4.660	11.435	4.119	4.600
40	*Geum urbanum*	2.633	1.340	—	17.888	9.866	24.418	0.858	0.204	—	6.156	1.756	5.104
41	*Moehringia trinervis*	—	—	—	0.816	5.600	0.120	—	—	—	0.176	0.712	0.068
42	*Poa nemoralis*	6.815	—	0.722	6.391	4.984	1.960	1.095	—	0.228	2.273	1.202	0.340
43	*Platanthera bifolia*	—	—	—	6.376	5.440	1.986	—	—	—	0.408	0.550	0.168
44	*Veronica chamaedrys*	—	—	0.056	7.676	3.528	4.212	—	—	0.018	1.758	0.763	1.044
	Adenostiletalia												
45	*Myosotis sylvatica*	20.196	6.042	1.504	12.507	15.417	2.844	1.986	0.577	0.121	1.630	2.022	0.324
46	*Petasites albus*	69.841	130.883	47.412	82.346	16.000	3.948	5.742	7.955	3.896	7.898	0.256	0.330
47	*Urtica dioica*	26.827	22.080	30.948	30.212	33.958	19.140	3.600	3.412	1.714	22.618	3.583	2.920

of principal herbaceous populations

Table

No.	Species	Green production of every population in the maximum period (kg./ha)			
		Piatra Arsă			Platoul
		1971	1972	1973	1971
	Epilobiletalia				
48	*Fragaria vesca*	6.156	2.485	2.072	73.406
49	*Galeopsis speciosa*	3.940	8.437	2.673	7.599
	Arction				
50	*Lapsana communis*	—	—	—	65.520
51	*Tussilago farfara*	—	—	—	30.478
	Molinia Arenaterea				
52	*Prunella vulgaris*	—	—	—	—
	Filipendulo-Petasition				
53	*Ranunculus repens*	7.942	1.488	7.053	75.775
54	*Telekia speciosa*	—	—	—	380.200
	Cardamini-Montion				
55	*Cardamine amara*	3.046	1.466	3.268	—
	Carpinion				
56	*Stellaria holostea*	9.370	2.646	17.267	—
	Nardo-Callunetea				
57	*Veronica officinalis*	—	—	1.690	70.932
	Sparganio-Glycerion				
58	*Veronica beccabunga*	—	—	0.067	2.640

1971. Of these we mention *Lamium galeobdolon*, whose recorded values — especially at Piatra Arsă — are more than 3 times as high as in 1971 and 1973 (177 as against 38 kg. in 1971, 179 as against 21 kg. dry mass/ha in 1973) and *Oxalis acetosella* (47 as against 20 and 11 kg. dry mass/ha at Piatra Arsă, or 50 as against 31 and 17 kg./ha at Platoul Izvor).

Phenologic differences throughout years influence the moment of reaching maximum production. In most cases, in 1972 it was earlier by about one month (June — July) and in only a few populations it was in August.

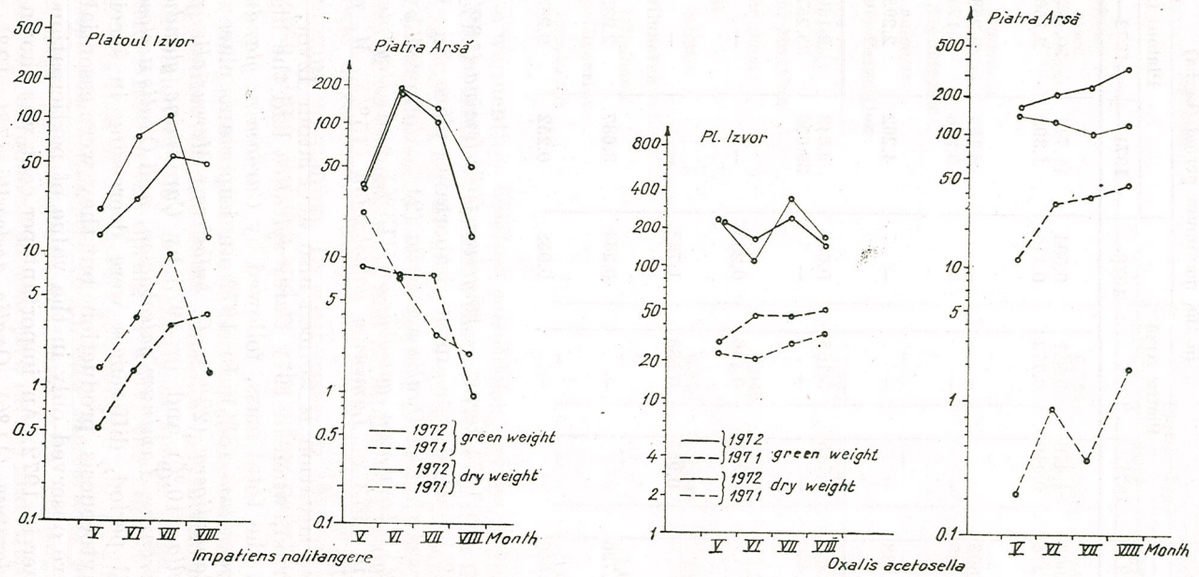
Peculiar annual variations are noticed as concerns the weight of dominant populations in the total mass of phytocenosis, throughout the vegetation period or only temporarily in spring (Figs. 3, 4 and 5). While at Piatra Arsă, in the spring of 1971, the highest percentage of the total mass was that of *Cardamine glanduligera* (33%), *Dryopteris filix-mas* (9%), *Impatiens nolitangere* (8%), and *Mercurialis perennis* (8%), in May 1972, high percentages were found with *Laminum galeobdolon* (53%), followed by *Cardamine glanduligera* (20%) and *Mercurialis perennis* (5%) and in 1973 with *Isopyrum thalictroides* (14%), *Laminum galeobdolon* (8%), and *Mercurialis perennis* (6%). Differences were maintained

1 *(continued)*

Izvor		Dry biomass of every population in the maximum period (kg.ha)					
		Piatra Arsă			Platoul Izvor		
1972	1973	1971	1972	1973	1971	1972	1973
109.450	22.068	1.476	0.551	0.504	11.748	20.197	2.232
3.785	3.276	0.410	0.772	0.140	1.395	2.156	0.236
1.615	5.808	—	—	—	5.189	0.253	0.924
69.350	72.168	—	—	—	3.276	6.174	6.200
13.996	6.888	—	—	—	4.262	2.293	1.148
38.700	58.360	0.715	0.165	0.607	9.843	2.160	4.766
394.400	22.480	—	—	—	39.696	35.257	3.260
—	—	0.345	0.136	0.258	—	—	—
—	—	0.955	0.588	1.763	—	—	—
13.311	3.726	—	—	0.264	3.687	2.235	0.920
2.666	5.560	—	—	0.058	0.252	0.283	0.670

also in summer; in 1971 ferns — *Athyrium filix-femina* (16%), *Dryopteris filix-mas* (10%) — held the most important place. In 1972, a huge participation was that of *Oxalis acetosella* (21%) followed by *Mercurialis perennis* (18%), *Laminum galeobdolon* (13%) and *Dryopteris filix-mas* (11%), and in 1973 of *Laminum galeobdolon* (15%), *M. perennis*, and *Oxalis acetosella* (8%).

A similar situation is noticed also at Platoul Izvor, especially in the vernal period, when in 1971 *Carex sylvatica* had the highest percentage (34%) in the total mass, followed by *Cardamine glanduligera* (16%) and *Galium odoratum* (8%). In 1972 an important place was held by *Cardamine glanduligera* (22%), *C. bulbifera*, *Mercurialis perennis*, and *Oxalis acetosella* (10%) and in 1973 by *Cardamine glanduligera* (8%), *Mercurialis perennis*, *Laminum galeobdolon*, and *Oxalis acetosella* (8—6%). In the estival period, differences were decreasing in species with high weight in phytocenosis production but they were essentially the same. Variations were observed only in the value of participation percentages, generally higher in 1972. An important per cent was shown in both years by *Galium odoratum* (11%), *Oxalis acetosella* (7%, 12% respectively), *Mercurialis perennis* and *Geranium robertianum* (6%, 8% respectively). In 1973, the situation was quite modified, the species with a high weight



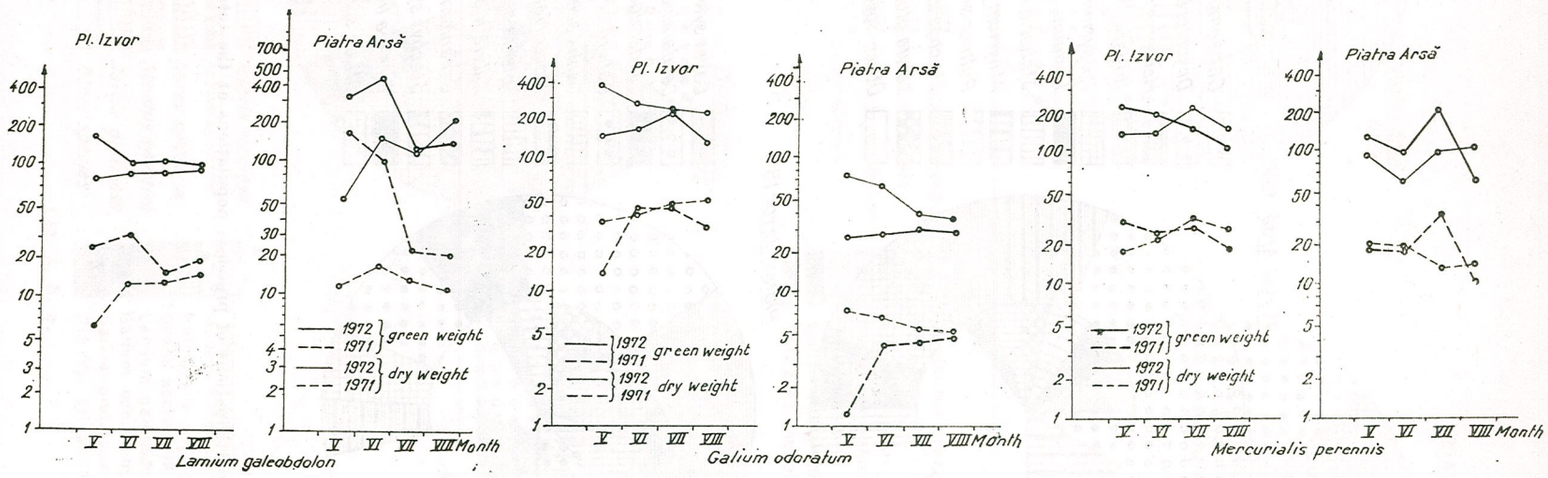
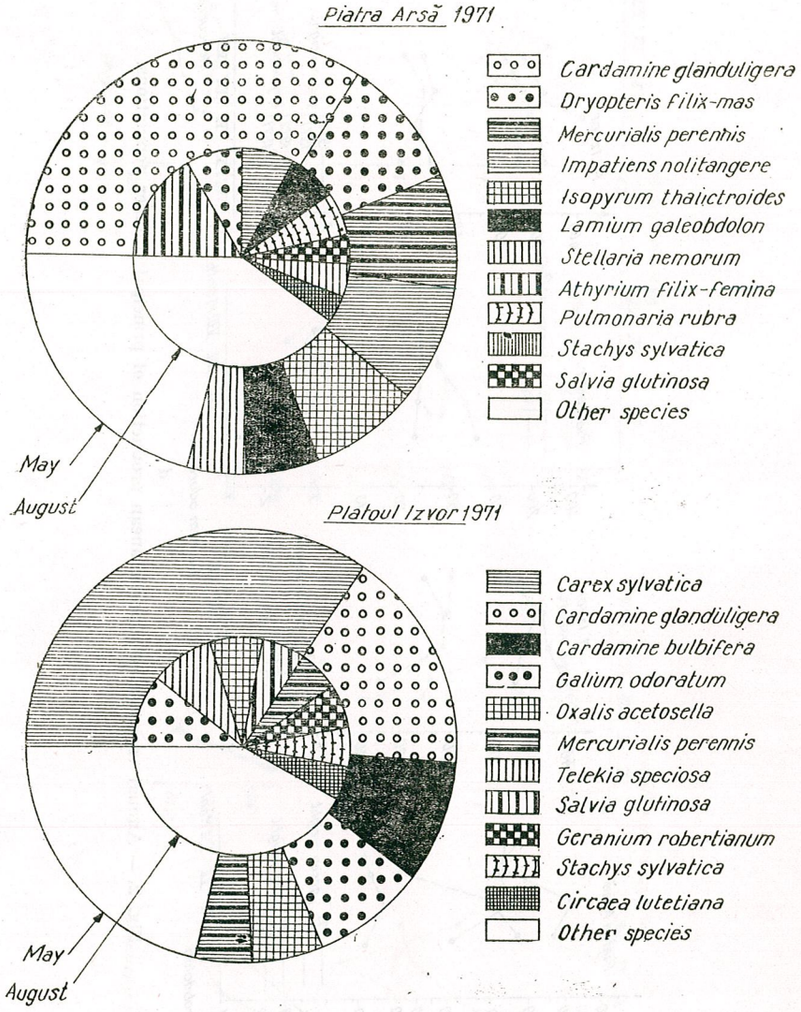
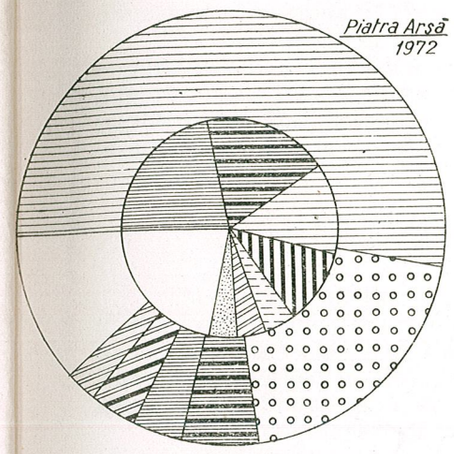


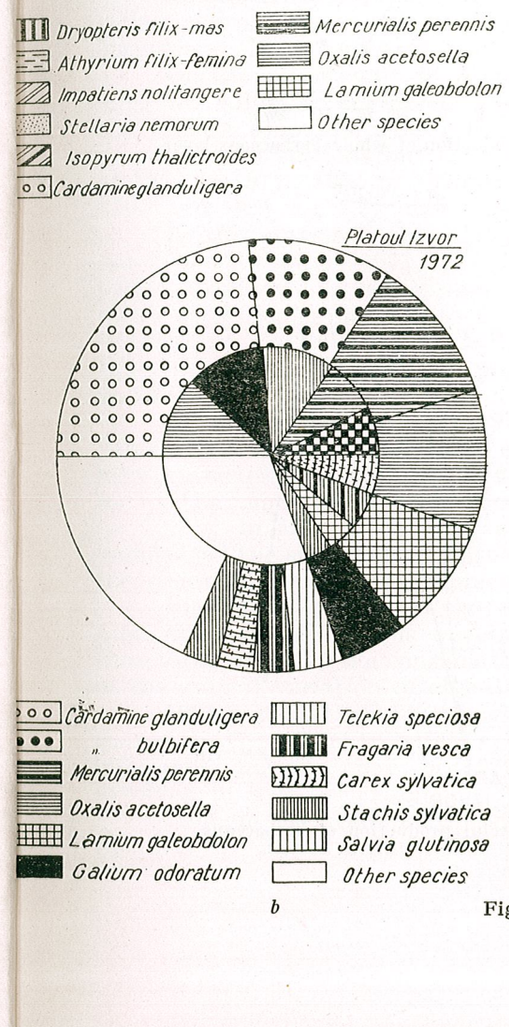
Fig. 2. — Annual variation of superterranean production of principal summer populations.

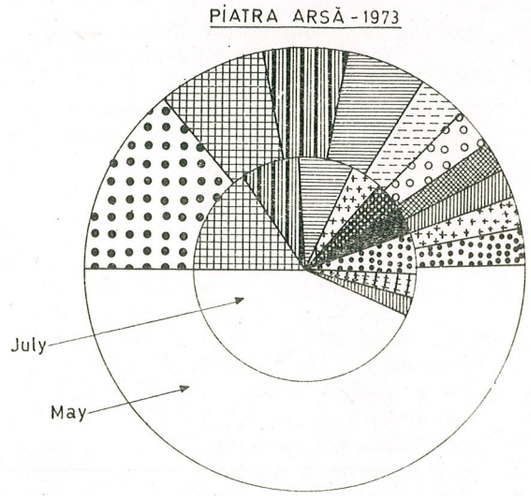


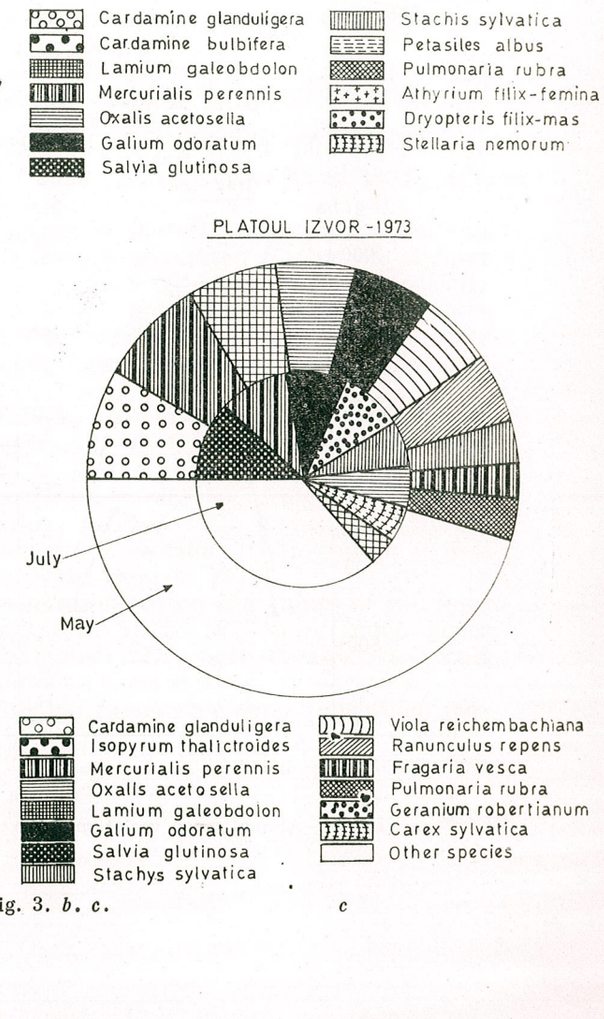
a

Fig. 3. — Per cent participations of principal populations at the total plant biomass.









b

Fig. 3. *b. c.*

c

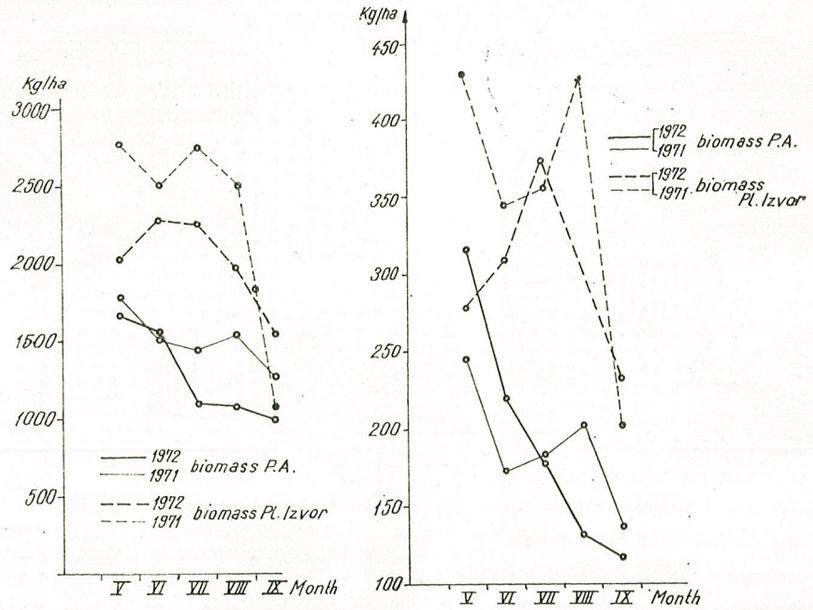


Fig. 4. — Annual variation of primary production of whole herbaceous layer.

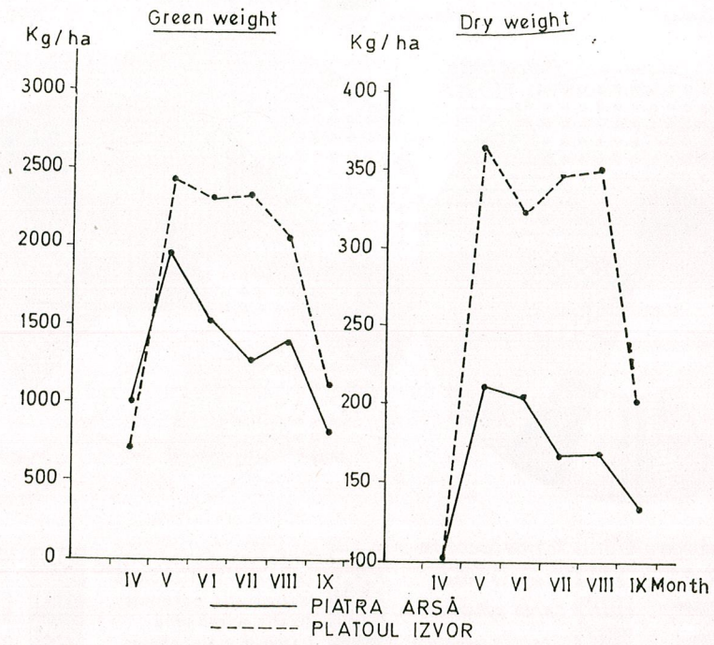


Fig. 5.—Annual mean variation of the total production of herbaceous layer.

in the production of the herbaceous layer being *Salvia glutinosa* (12%), *Mercurialis perennis* (11%), *Galium odoratum* (9%).

The knowledge of annual differences in the weight of some of the populations in the total mass of the herbaceous covering is of peculiar importance as this is mirrored in the values of the biomass of the whole phytocenosis and helps us to explain annual and stational variations occurring at the level of the grassy vegetable mass.

b) MULTIANNUAL DYNAMICS OF THE TOTAL PRODUCTION OF HERBACEOUS POPULATION

The increase of the overground production of the herbaceous layer in the two stations occurred in the vegetation season of 1972, according to the classical model of the curve with a single well expressed peak. That was recorded in May, both in the case of green and dry vegetable mass, at the Piatra Arsă station and in July — August at Platoul Izvor.

The differentiation of the herbaceous layer production from that of 1971 is thus great enough, going up to the modification of the accumulation curve. That curve had two annual peaks in the first year — a spring one (May) and a summer one (August).

On the other hand, in August 1972 and 1973, the production had very low values as compared to 1971, when the vegetable mass reached the highest amounts. This refers both to the green and dry vegetable mass, in both stations but especially at Platoul Izvor. The values were of 1954 in 1972; 1955 in 1975 and 2556 kg green mass/ha in 1971; 311, 279, and 425 kg. dry mass/ha, respectively.

In this station it was also observed that the May production was much lower than in 1971. This is largely accounted for by the slighter participation of vernal species with great vegetable mass, especially *Carex sylvatica* (from 34% to 2.7%). The individual variation of others (*Cardamine glanduligera*, *C. bulbifera*) was not modified. At the same time, the great majority of populations had lower productions than in 1971.

At Piatra Arsă in May 1972 one can exceptionally notice higher values than in 1971 and 1973, especially in the case of the dry vegetation mass (330 as against 240 kg./ha). This situation can be accounted for by the huge participation of *Lamium galeobdolon* (from 5.3% in 1971 to 52.9% in May 1972). The other species with higher percentages maintained the same value. Throughout the season, in this station as well the production values were much lower than in 1971.

Viewed as a whole, in the vegetation season the values of the total production of herbaceous vegetation in 1972—1973 were much lower than those of 1971. We consider that these differences represent the result of the annual variation of climatic conditions in those three years.

1971 is characterized as having had favourable conditions which permitted the vegetative growth of species, that in most cases reached higher individual weights than in 1972 and 1973. Populations density was involved to a lesser extent.

Though the accumulation curve of the herbaceous vegetable material had special variations throughout the different observation years,

the mean multiannual curve points out that the overground population production grows according to a curve with two well-defined peaks. One coincides with the upper period of the growth of vernal species and the other with that of estival species (Fig. 6). This proves that the typical course of the phenomenon is that of a two-peak curve. Deviations from this rule are exceptions, as a result of peculiar climatic conditions.The necessity of carrying out repeated investigations throughout several years is obvious in order to avoid unrealistic generalizations due to the description of singular situations.

In spite of the multiannual differences observed in the dynamics of overground vegetable mass production stational differences are maintained within the same limits as a result of the action of the same ecological factors with a peculiar role in the formation and development of the vegetable covering of these factors, among which light is most important. The two stations have forests of different tree productivity because of the different closure degree of the canopy and therefore the amount of light which reaches the level of grasses varies very much. It is higher in forests with low production classes.

In order to better explain the action of this factor, throughout 1972 and 1973, investigations were undertaken with a view to establishing the interrelations between the light amount received through the leaf canopy and production. Statistical data (Table 2) point out that at Piatra Arsă, where light greatly varied throughout vegetation seasons, a correlation was statistically ensured for these two parameters. Correlation indices have high values, especially in summer, proving a better correlation of light with summer production. At Platoul Izvor slighter correlations are observed between these two elements.

A low correlation, statistically not ensured, was also noticed between production and herbage covering. There was no correlation between plants density and vegetable mass production. Elements under study

Table 2

The correlations between production and light, covering, and density in the research surface values of correlation index *r*

Station	correlation index *r*					
	light		covering		density	
	V	VII	V	VII	V	VII
Piatra Arsă	0.785	0.890	0.610	0.605	0.430	0.455
Platoul Izvor	0.576	0.683	0.702	0.603	0.365	0.400

influence the crop of the herbaceous layer but play no essential role in the higher or lower accumulation of vegetable material. This proves that the production of the overground parts of the herbaceous layer from the studied forests is influenced by the great many factors among which light has an important role. It determines a certain specific composition in the studied areas as well as a more or less intense growth of individuals. Highly important are also other factors, such as the thermic or hydric regime, as well as the interrelations among them.

The idea of the influence of a multitude of ecologic factors on the primary production of the herbaceous populations is also supported by

Table 3

The categories of minimum and maximum values of populations water content (%)

Piatra Arsă							Platoul Izvor					
1971			1972			1971			1972		
Minim. categ.	% pop.	Maxim. categ.	Minim. categ.	% pop.	Maxim. categ.	Minim. categ.	% pop.	Maxim. categ.	Minim. categ.	% pop.	Maxim. categ.	% pop.
<50	2	61—70	51—60	2	76—80	<60	2	76—80	61—70	4	81—85	2
51—60	4	71—75	61—70	0	81—85	61—70	9	81—85	71—75	12	86—90	44
61—70	9	76—80	71—75	2	86—90	71—75	13	86—90	76—80	32	91—95	23
71—75	14	81—85	76—80	8	91—95	76—80	40	91—95	81—85	30	> 95	4
76—80	37	86—90	81—85	14	> 95	81—85	13	> 95	86—90	12		
81—85	30	> 90	86—90	30		86—90	17		> 90	2		
86—90	8		> 90	32		> 90	2					
> 90	2											

Table 4

Seasonal and annual variation of water content of herbaceous layer

Station	% water							
	V		VI		VII		VIII	
	1971	1972	1971	1972	1971	1972	1971	1972
Piatra Arsă	84.15	87.00	87.02	86.50	85.73	85.00	84.24	85.00
Platoul Izvor	87.25	87.00	86.09	86.00	84.44	84.00	80.79	80.00

the results of comparative investigations carried out in 1973 on annual variations of light intensity received by it. Observations performed on the 200 sample areas in the period of highest development of grasses (August) point out that in 1973 populations received a higher average light percentage than in the previous year (5.7% as against 3.0% total light at Platoul Izvor; 2.8% as against 1.5% at Piatra Arsă, respectively), as a result of the level of the tree layer.

Though the amount of light received by the herbaceous layer was considerably higher in 1973, the biomass accumulation was slighter since light alone did not succeed in ensuring a suitable development of grasses so long as the other climatic factors — temperature and moisture — which determined the decrease of the leaf covering of trees did not influence the increase of the amount of herbaceous vegetable material.

CONCLUSIONS

1) The production of the overground part of the herbaceous layer in the studied forests has particularly obvious multiannual variations determined by different climatic factors, specific of every year. This influences both the amount of vegetable material accumulated in a year and the seasonal course of the production increase throughout the vegetation period.

2) Despite particularly important annual variations, station differences are still maintained within the same limits, and the production of the herbaceous layer of forests with lower production classes has higher values than in those with higher production classes.

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ECOLOGIC RESEARCHES ON MACROSCOPIC FUNGI FROM *FAGETUM CARPATICUM* ASSOCIATION ON LEGHIN HILL (NEAMȚ COUNTY)

BY

TH. CHIFU

The paper presents the results of microstational researches bearing on *Fagetum carpaticum* association. References are made upon the natural framework, structure and floristical composition of the woody vegetation of Leghin Hill. The variations of the air and soil temperature as well as of the luminosity in several days of June-October were followed up in the permanent observation stations. At the same time sporiferous bodies of terricole macroscopic fungi were registered at each station, correlated with the climatic and stational conditions.

The investigated territory is situated in the SW part of Dobreanu summit, near Leghin village. The relief is divided into summits with various orientation and exposition, with slopes which reach 30—35°. The annual average temperature, registered at the Meteorology Station Tîrgu Neamț, is of 8.2°C [6]. The annual average of the atmospheric precipitation surpasses 546 mm, the highest values being recorded in summer. The registerings of the Secu rain-gauge station show that the precipitation average in 1967 and 1968 surpassed 700 mm. The dominant winds are those from NW followed by those from N and SW. The substratum is made up of a system of strata in whose constitution there are grey marls, organogenous limestones, gritstones, dysodylic schists, etc. belonging to the Senonian and Paleocene [5]. The soil is forest brown, in some places podzolized with much skeleton [7].

The slopes of Leghin Hill are covered by a woody vegetation, mostly represented by *Fagetum carpaticum* (Borza 30) Moor 38, Paucă 41 association, with a general covering of 90—100% and characterized by the domination of *Fagus silvatica*. There are also found: *Populus tremula*, *Betula verrucosa*, *Acer pseudoplatanus*, *Abies alba*, as well as *Carpinus betulus*, especially at the slopes foot [1] [2]. The regeneration layer is very active and is made up of plantlets of the above-mentioned species with the predominance of beech and hornbeam. The shrub layer is very poor, with specimens of *Corylus avellana* or *Crataegus monogyna* here and there. In general the herbaceous layer is poor in species, only *Luzula luzuloides*, *Rubus hirtus*, *Euphorbia amygdaloides*, *Fragaria vesca*, etc. being frequently met; here and there, due to the litter thickness, this layer is completely absent. The moss layer is scarce, with few species developing on trunks and around them or on barren stones.

In the association thus identified and delimited, there were fixed 4 permanent observation stations, within an area of 500 m². In these

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stations there were made hourly registerings on several days of June — October 1967 and 1968, bearing on the following microstational elements : air temperature at the soil surface, at 5 cm and 1 m height ; relative air humidity at 5 cm and 1 m height ; luminosity at 5 cm height ; soil temperature at 5 cm, 10 cm and 15 cm depth ; soil humidity in the layer comprised between 0 and 10 cm depth.

The 4 stations are characterized as follows :

Station	Altidude in m	Exposition	Inclination in degrees	Degree of layer covering				Kitter thickness in cm
				Tree	Shrub	Herbaceous	Moss	
I	525	SW	20	90	5	10	5	3
II	625	SW	25	95	40	—	—	15
III	725	SW	30	90	10	5	1	10
IV	725	NE	30	95	2	—	—	15

The data show that the soil is strongly acid, the acidity degree rising with altitude. The soil is also remarkably rich in total nitrogen, especially in the lower part of the slope, as well as in mobile potassium.

The chemical analysis of the soil tests gave the following results :

Station	pH	CO_3Ca %	Humus %	Total N %	P_2O_5 %	K_2O %	V %
I	5.2	0	4.55	0.270	1.8	21.7	68.75
II	5.0	0	4.32	0.188	3.4	20.4	59.25
III	4.0	0	5.28	0.130	5.4	16.0	18.35
IV	4.0	0	6.20	0.196	8.6	22.5	32.40

On the other hand, the phosphorus content varies from a low percentage in station I, to a high percentage in stations III and IV. As concerns the saturation degree, the investigations reveal that the soil is moderately saturated at the slope foot and strongly up to moderately unsaturated in the higher part of the slope.

The soil humidity, depending on the atmospheric precipitation, the vegetation consistency, orographic conditions, litter thickness, etc. presented the following variations :

Station	20. VI. 1967	23. VI. 1968	24. VII. 1967	26. VII. 1968	8. VIII. 1967	29. VIII. 1968	8. IX. 1967	25. IX. 1968	7. X. 1967	24. X. 1968
I	32.5	15.9	30.2	50.8	31.9	36.8	25.5	26.3	37.9	27.3
II	26.7	13.2	21.5	50.0	27.2	30.9	18.9	29.1	31.2	23.2
III	20.8	8.7	20.1	43.2	21.6	25.2	14.6	21.2	30.6	19.1
IV	25.2	10.6	23.4	49.1	26.3	28.4	20.2	26.7	31.5	20.5

These data show that there were registered low values at the beginning of summer and, generally, in autumn when the atmospheric precipitation was scarce. In summer the soil humidity was high. A slight decrease occurred in the higher part of the slope, as against its foot. Such little variations were likewise noticed depending on the slopes exposition, namely on the SW slope the soil humidity suffered a certain lowering in comparison with the NE slope.

The soil temperature presented, in general, a progressive growth up to 15—16 hrs, after which it gradually decreased or remained stationary. Characteristic is the lowering of the values at the same time with the growth of the depth. Thus, for example, on the 24. VII. 1967, in station II, the temperature average at the soil surface was of 17.4°C as against 15.1°C at 5 cm depth and 14.6°C at 15 cm depth. It may be appreciated that, in general, the temperature at 15 cm depth was, on an average, by 0.1—0.6 °C lower in comparison with that occurring at 5 cm depth and by 0.1—0.3°C in comparison with that at 10 cm depth. The soil temperature was also lower at 5 cm depth, on an average by 0.9—2.9°C in comparison with that at the soil surface. The highest maxima were at 5 cm depth in July and August (16.8—16.9°C), while the lowest minima in October, likewise at 5 cm depth (3.8°C). The amplitudes were, in general, higher at 5 cm depth in comparison with those at 15 cm depth ; they ranged between 3.3°C on the 28. VIII.1967 in station I and 0.6°C on the 8.IX.1967 in station IV.

The air temperature was influenced by the climate conditions of the day, the vegetation consistency, the slopes exposition, altitude, etc. An essential characteristic was that, in general, the air temperature registered higher values at about 1 m height and especially that at 5 cm height, the temperature was nearly all the time higher than that at the soil surface and at 1 m height. Thus, the temperature at 1 m height was, on an average, by 0.2—1.1°C higher than that at the soil surface, while the temperature at 5 cm height was, on an average, by 0.6—1.5°C higher than that at the soil surface and by 0.1—0.9°C higher than that at 1 m height. For example, on the 24.VII.1967, in station II, the temperature average at 5 cm height was 18.9°C as against 17.4°C registered at the soil surface and 18.3°C at 1 m height. The highest maxima were in July and August (23.0—23.4°C) and the lowest minima in October (3.4°C). The values were lower, on an average, with 0.5—1.0°C for each 100 m altitude. For example, on the 23.VI.1968 the air temperature at 5 cm height was, on an average, of 16.2°C in station I as against 15.2°C in station II and 14.3°C in station III. On the slope with SW exposition there were also registered higher values than on the NE slope. Thus, on the 24.VII.1967, the temperature at the soil surface was, on an average, of 17.6°C in station III as against 16.8°C in station IV.

The relative air humidity varied in relation with the air temperature, that is the temperature growth led to a relative air humidity lowering. That is why the curve of the relative air humidity registered a gradual decrease up to 14—16 hrs and then it grew gradually in the evening. The marked cloudiness also led to the relative air humidity growth. The highest values were registered in the morning (80—85%), the average ones ranging between 65 and 75%. Minimum differences occurred depending on the height above the soil, in the sense that at 1 m height the values were lower, on an average, by 2—4% than those at

5 cm height. Insignificant differences were also observed depending on the slopes exposition and altitude.

The luminosity, influenced by vegetation consistency and by nebulosity, registered an ascending curve until noon and then decreased till the evening. This general curve was disturbed either by a temporary nebulosity or by vegetation consistency. The luminosity values ranged between 100 and 2500 lx.

In parallel with the microstational observations there was followed up the frequency of the sporiferous bodies of each terricole species from each area of the observation stations. The microclimate data are insufficient, so that definitive conclusions cannot be drawn regarding the frequency and dynamics of terricole macroscopic fungi depending on the stational conditions. However, some appreciations may be made on the basis of the registered data. These ecologic researches, together with the mycocoenologic ones [1], showed that the macroscopic fungi had a non-uniform development during the year, registering an ascending curve up to July — August, after which it decreased in autumn (Fig. 1). On the general background of this development a series of exceptions occurred due to the microclimate conditions. Thus, in the drought conditions of June 1968 the reduced atmospheric precipitation (38.4 mm) determined a low soil humidity (8.7—18.9%), which hindered the macroscopic fungi development. As a result, on the 23.VI. 1968 no species of macroscopic fungi could be found on the test areas under study. On the other hand, though in July 1968 the soil humidity was one of the highest (43.2—50.8) owing to the abundant atmospheric precipitation (143.2 mm), the clouded sky and frequent precipitation (it rained during 21 days in the month) determined a relatively lower temperature in comparison with the same period of 1967. This led to a reduced development of macroscopic fungi, both as concerns the number of species and the number of sporiferous bodies.

Most frequently met were : *Cantharellus cibarius*, *Marsmius alliaceus*, *Mycena pelyanthina*, *M. pura*, *Amanita pantherina*, *Lactarius blennius*, *L. piperatus*, *L. vellereus*, *Russula cyanoxantha*, *R. xerampelina*, etc.

The highest development reached by macroscopic fungi was in August 1968, due to the existence of a sufficient quantity of water in the soil (21.6—31.9%), as a consequence of abundant precipitation (75.4 mm), as well as of a relatively sudden temperature increase. The most frequent species were : *Craterellus cornucopioides*, *Hydnum repandum*, *Hygrophorus eburneus*, *Laccaria laccata*, *Marasmius alliaceus*, *Collybia peronata*, *Mycena pelyathina*, *M. pura*, *Rozites caperata*, *Lactarius piperatus*, *L. vellereus*, *Russula cyanoxantha*, *R. foetens*, *R. xerampelina*, etc.

There was likewise established a certain inequality among the stations, in the sense that, in general, a higher frequency of macroscopic fungi was registered at the slope foot, as well as on the NE slope as against the corresponding station on the SW slope. Worth mentioning is the fact that out of the 102 terricole macroscopic fungi identified on the test areas of the observation stations, the forest mycorhizal species were predominant (57 species), followed by the humicolous saprophyte ones (31 species); the less numerous belonged to the foliicolous sapro-

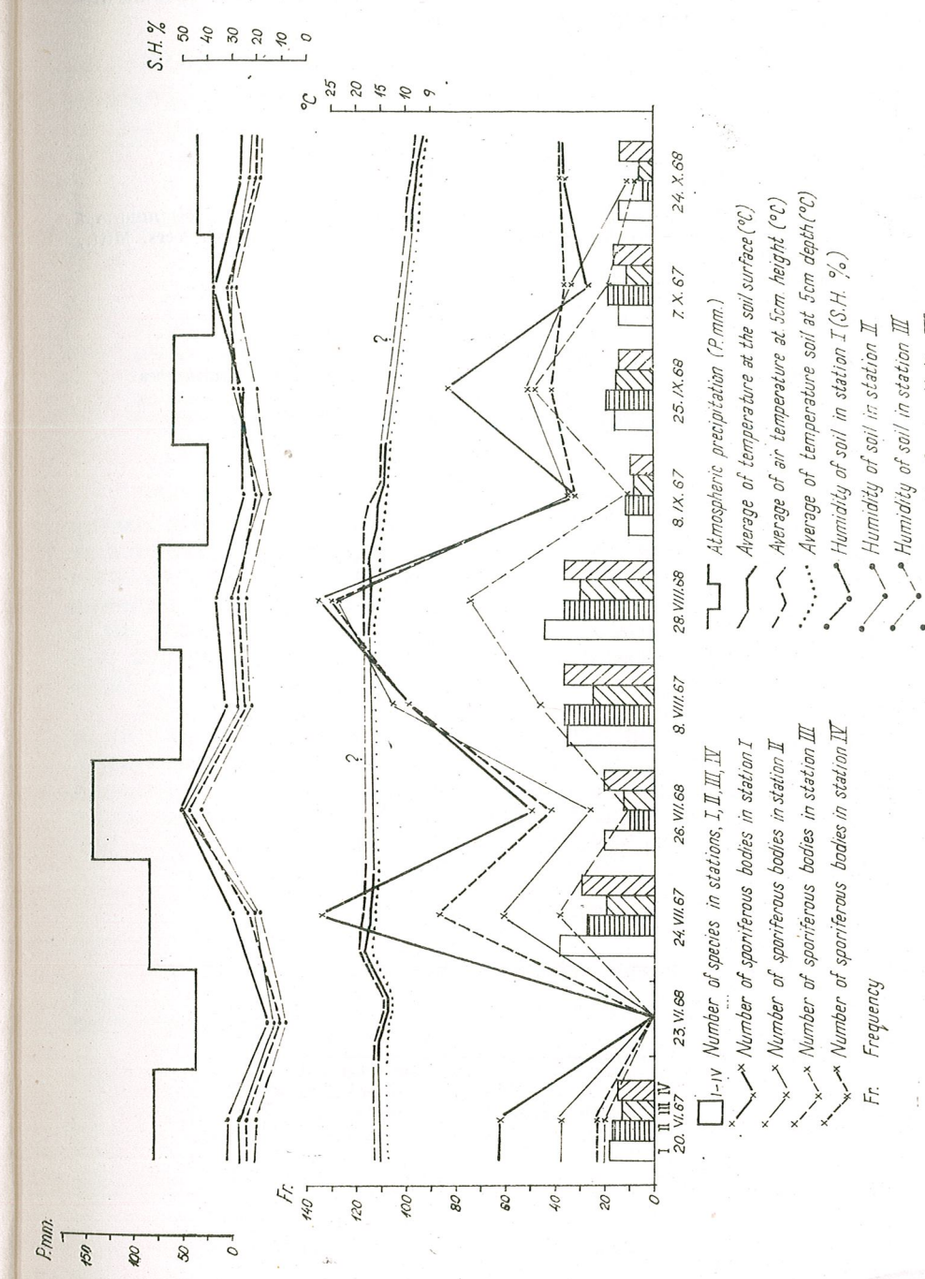

Fig. 1. — Frequency of terricole macroscopic fungi in accordance with microstational conditions.

phyte species as well as to intermediate categories. In general, this structure was maintained throughout the year.

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CONTRIBUTIONS TO THE KNOWLEDGE OF THE INFLUENCE OF NUTRITION ON THE GROWTH AND DEVELOPMENT OF THE ALGA *SELENASTRUM GRACILE* REINCH.

BY

RODICA BILCEA

Studying the physiological peculiarities of the green alga *Selenastrum gracile* Reinch., grown in various nutritive mediums with a supplement of soil extract in various concentrations, it was ascertained that the alga manifests preference for more concentrated nutritive mediums, with a N : P ratio of 5.5 : 1. The soil extract in concentration of 1 g% stimulates the multiplication of the alga in all the nutritive mediums.

The results obtained by Spoehr and Milner (1947–1948) in the guided growth of the alga *Chlorella* [3] and the possibility of using it as a source of proteins, lipids, carbohydrates generated an avalanche of investigations on the unicellular algae, followed by assiduous investigations for finding nutritive solutions which should allow the extension of the cultures of algae under favourable economic conditions. The attention and efforts of the researchers were directed on selecting the algae strains with increased productivity.

The investigations that make the object of the present paper have in view to determine the best conditions for the culture of the green alga *Selenastrum gracile* Reinch., in order to obtain increased quantities of biomass. For the same reason we made investigations on some factors which stimulate or inhibit the growth and development of algae on different nutritive mediums.

MATERIAL AND METHOD

The green alga *Selenastrum gracile* Reinch. (isolated on Snagov Lake) was cultivated on the following nutritive mediums, after preliminary experiments :

I. Teodorescu – modified II. Molisch-Ravin – modified III. Knop-Pringsheim – modified IV. Pirson et al. – modified	+ soil extract (soil 1g%)	series I
The same nutritive mediums	+ soil extract (4g% soil)	series II

The cultures were kept for 12 hours in the light and 12 hours in the darkness. The light intensity was of 4,100 lx at the culture level. Temperature was of 26±1°C (Ist series) and 27 ± 3°C (IInd series). The alga was cultivated in Erlenmeyer glass of 500 ml and the culture was aerated with a stream of atmospheric air, the nutritive mediums being sterilized beforehand by autoclaving at 1.2 kgf/cm² for 15 minutes. The soil extract was prepared out of 40 g soil in 400 ml water (in case of the IInd series : 160 g), the supernatant being used for completing the nutritive mediums. The appreciation of the effect of soil extract on the growth and division of the alga was made by determining the cell density of the cultures (every 2 days) with the hemocitometric method, on which the growth was based, and the pH values, as well as by microscopic observations on the morphostructural peculiarities of the alga at the beginning and end of the experiment. At the end of the cultivation period we also determined the quantity of biomass and the dry substance.

The dates and duration of experiments and the results obtained are presented in tables 1 and 2 and figures 1, 2, 3.

RESULTS

Analysing figures 1*A* and 2*A*, we notice the following : in the variant Knop-Pringsheim medium with 4 g% soil a slight increase of the alga occurs until the 5th day, after which the difference as compared to the control sample reaches about 19% growth increase. In the 2nd variant (1 g% soil) the influence of the soil extract on alga growth is much more important, reaching an increase of the cell density of about 33% as compared to the control sample, a percentage achieved towards the end of the experiment period.

The superiority of this variant is evident, taking into account the increased accumulation of dry substance. An easily detectable influence of the soil extract on the alga *Selenastrum gracile* was noticed when we used the Molisch-Ravin medium (Figs 1 *B*, 2 *B*). For the variant 1 g% soil, an increase of 146% was obtained as compared to the control sample; in the 2nd variant (4 g% soil) the increase was of 271%. In both concentrations of soil extract, at the beginning of the experiment the absorption of the substances from the soil extract was reduced; about 5 days after the beginning of the experiment it was more important. The Teodorescu medium (Fig. 1 *C*) supplemented with soil extract (1 g% soil) proved to be favourable for the alga growth, an increase of 23% being obtained after 12 days. The same medium with an addition of 4% soil (Fig. 2 *C*) presented a slightly inhibitory action on the alga growing process, as compared to the variant in figure 1 *C*. In the case of variant Pirson et al. [10] with soil extract in the concentrations indicated in figures 1 *D* and 2 *D*, we noticed an approximately constant growth of the number of algae, as compared to the control sample, an increase of 11% being obtained in case 1 *D* and respectively 17% in case 2 *D*. Our microscopic observations pointed to the fact that the algae grown in nutritive mediums supplemented with soil extract present

Table 1

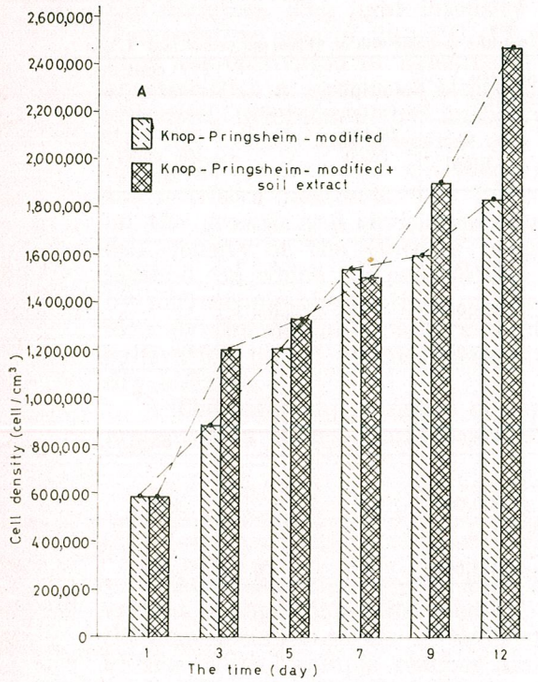
The growth of the alga *Selenastrum gracile* Reinch. in different nutritive mediums with or without soil extract (soil 1 %)

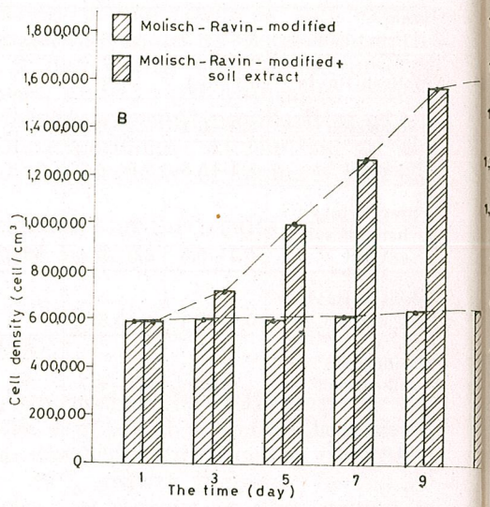
Variants	pH		Cell density (cell/cm³)		Multiplication factor N/N_0	Ratio N : P	Per cent increase of the cell number related to the control sample
	Initial	Final	N_0 Initial	N Final			
Knop-Pringsheim	5.8—6.2	6—6.2	584,320	1,850,000	3.1	5.5 : 1	
Molisch-Ravin	5.1—5.4	5.4	,,	650,000	1.1	3 : 1	
Teodorescu	6.2—6.4	6.4—6.7	,,	1,678,000	2.8	2.6 : 1	
Pirson et al.	5.4—5.8	5.8—6.2	,,	1,665,200	2.8	1.2 : 1	
Knop-Pringsheim + soil extract	5.4—5.8	6.2—6.4	,,	2,478,000	4.2		33%
Molisch-Ravin + soil extract	5.1—5.4	5.8	,,	1,600,000	2.7		146%
Teodorescu + soil extract	6.2	6.4—6.7	,,	2,073,600	3.5		23%
Pirson et al. + soil extract	5.4	5.6	,,	1,956,266	3.3		11%

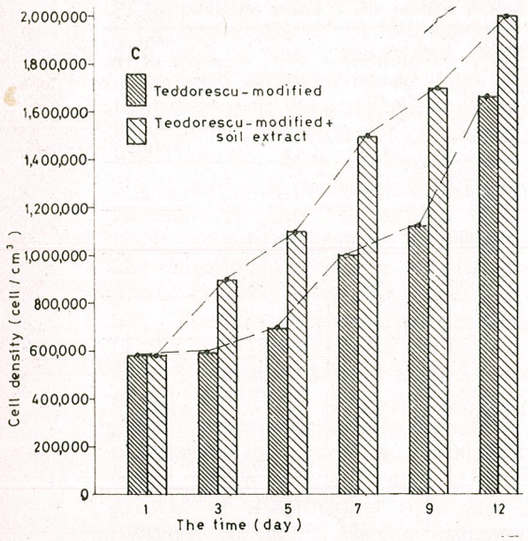
Table 2

The growth of the alga *Selenastrum gracile* Reinch. in different nutritive mediums with or without soil extract (soil 4%)

Variants	pH		Cell density (cell/cm³)		Multiplication factor N/N_0	Ratio N : P	Per cent increase of the cell number related to the control sample
	Initial	Final	N_0 Initial	N Final			
Knop-Pringsheim	5.8—6.2	5.8—6.2	246,666	1,618,666	6.5	5.5 : 1	
Molisch-Ravin	5.1—5.4	5.4	,,	350,000	1.4	3 : 1	
Teodorescu	6.2—6.4	6.4—6.7	,,	1,306,666	5.2	2.6 :1	
Pirson et al.	5.4—5.8	5.8—6.2	,,	1,293,333	5.2	1.2 : 1	
Knop-Pringsheim+ soil extract	5.8	5.8	,,	1,928,000	7.8		19%
Molisch-Ravin+ soil extract	5.4—5.8	5.4	,,	1,290,000	5.2		271%
Teodorescu+ soil extract	6.2—6.4	6.2—6.4	,,	1,209,000	4.9		—
Pirson et al.+ soil extract	5.4—5.8	5.4—5.8	,,	1,509,000	6.5		17%







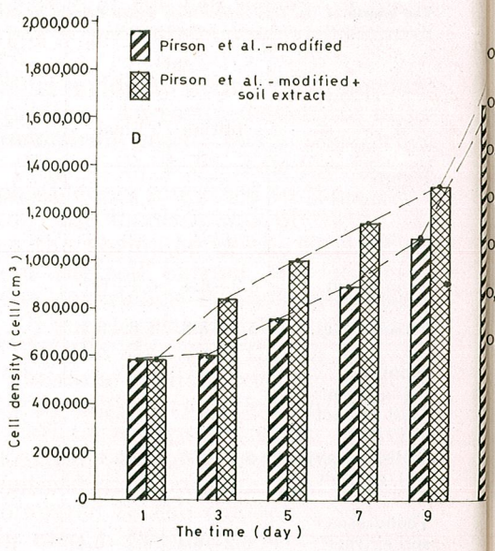
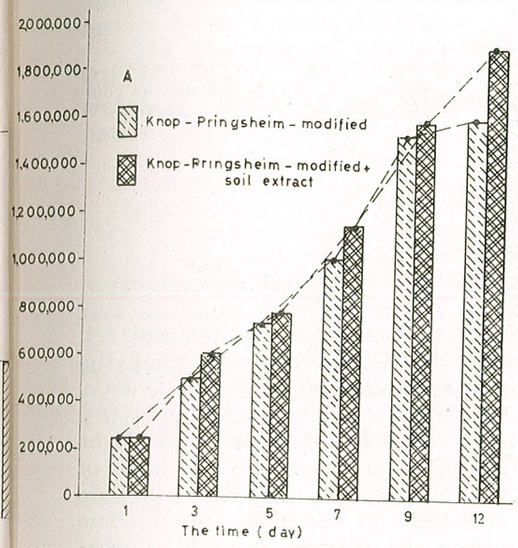
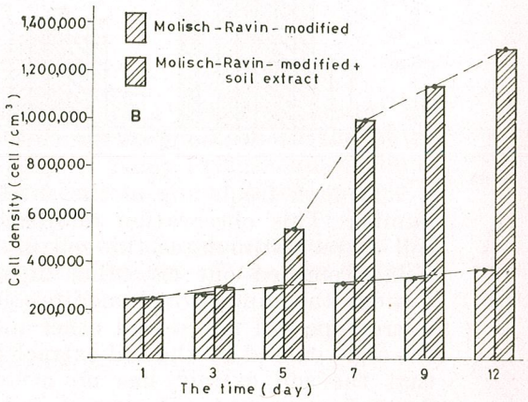
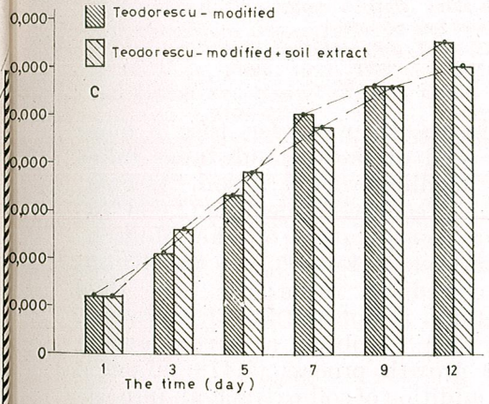


Fig. 1. — The dynamics of the growth of the alga *Selenastrum gracile* Reinch. on various nutritive s tions (*A*, *B*, *C*, *D*) with or without soil extract (soil 1 g%).







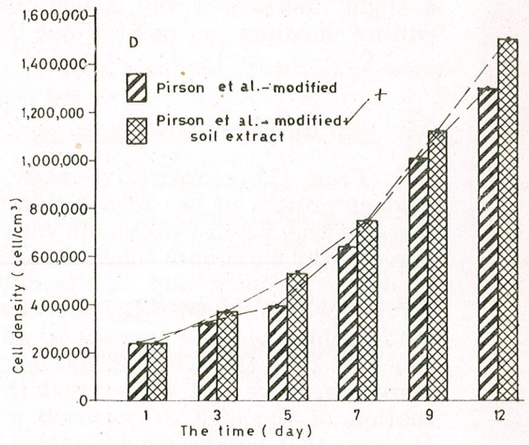


Fig. 2. — The dynamics of the growth of the alga *Selenastrum gracile* Reinch. in different nutritive solutions (*A*, *B*, *C*, *D*) prepared with or without soil extract (soil 4 g%).

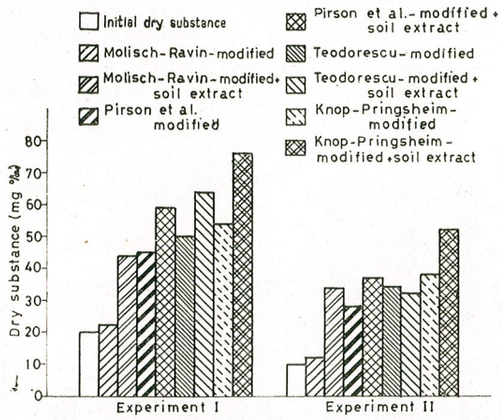


Fig. 3. — Dry substance (mg%) at the end of the experiments as compared to the initial moment.

a very high frequency of the small cells, as compared to the total cell number. This observation allowed us to draw the conclusion that the soil extract stimulates the cell division of the alga *Selenastrum gracile*, a fact pointed out for other algae by Droop [1] and others [7] [8]. Besides the dimensional modifications of the mentioned cells, the light microscope did not reveal other modifications at the structural level of the alga, caused by the soil extract in the medium; we may thus conclude that the soil extract has no other influence on the alga growth. The determinations of pH carried out during the culture growth showed a slight increase of the latter, due to the reduction of nitrates in the culture medium (as pointed out by Round).

DISCUSSIONS

From the comparative study of the results we infer that a more evident growth of the alga cell density and of the dry substance takes place in the Knop-Pringsheim nutritive medium with 1% soil. As compared to the maximum concentration of the Teodorescu medium (3.006 $^0/_{00}$) in our experiment and of the minimum concentation of the Molisch-Ravin medium (0.665 $^0/_{00}$), the concentration of 1.406 $^0/_{00}$ of the Knop-Pringsheim medium represents an intermediary value; we may thus conclude that the Knop-Pringsheim medium is optimal for alga growth. Moreover, if we add soil extract (1 g% soil) we obtain a marked accentuation of the alga *Selenastrum gracile* growth process (2.478 ml/cm^3). It seems that besides concentration and addition of soil extract, an important role in the optimal development of the algae is played by the ratio between the different nutritive elements contained in the medium, such as the relationship N:P. The same as Ryther [11], who experimented on chlorophytes *Nannochloris atomus* and *Stichococcus* sp., in our experiments on alga *Selenastrum gracile* in the Knop-Pringsheim medium we found the growth optimum at the N : P ratio of 5.5 : 1. The extra weight of the alga dry substance when soil extract was added to the

Knop-Pringsheim medium shows that the latter has an important role in the alga growth, which could be explained by the existence in the soil of humic acids, traces of nutritive substances, vitamins, etc. [1] [4] [5] [7] [12]. Neeb [5] shows that the humic acids from the soil extract may serve as an ion exchanging resin, releasing Ca and Mg and binding preferentially Fe, Mn, Zn, Co, Cu for which the chelators of metals have a great affinity.

In the case of Molisch-Ravin nutritive medium, where Fe was administered as SO_4Fe (in the other mediums Fe was given as a citrate), the results of our experiments are in accordance with the explanation given by Neeb.

CONCLUSIONS

1. The soil extract in a concentration of 1% soil stimulates the multiplication and growth of the alga *Selenastrum gracile* in all the nutritive mediums used, especially in the case of Knop-Pringsheim medium. In high concentrations the soil extract becomes an inhibitor, especially in the more concentrated nutritive mediums.

2. The multiplication process depends on the concentration of the nutritive solution, the N:P ratio and the weight of the soil extract.

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THE INFLUENCE OF PROCAINE UPON THE ^{32}P AND ^{45}Ca ABSORPTION INTO THE RED PEPPER SEEDS

BY

DORINA CACHIȚĂ-COSMA, A. IONICĂ, T. RĂDULESCU and GH. POPOVICI

The effect of procaine on the absorption and accumulation of ^{32}P and ^{45}Ca in pepper seeds was studied. The authors found out that procaine solution of 0.1—1 mg% concentration stimulated the absorption of the two radionuclides in the seeds. A prolonged submersion of the seeds in procaine (12—24 hours) diminished the absorption, particularly of Ca. Procaine hydrolysis products — the p-aminobenzoic acid and diethylaminoethanol — have a different effect on absorption as compared to the undivided procaine molecule.

The research regarding the effect of procaine hydrochloride attests its biostimulative influence upon growing and developing processes of the plants [5] [8]. The experiments carried out in this respect pointed out the favourable action of the diluted solution of procaine (1 mg % concentration) upon the germinative process [5] [14] [15], the multiplication and growth of some algae [6] [7] [10], and seedlings respiration [1] [11].

It was found that tomato seedlings treated with procaine in a concentration of about 1 mg% absorbed 19.9% more ^{32}P than the radicular system of the control lot [5].

In order to find out the way in which procaine affects the physiological processes of the seed we made investigations at the seminal level. We started the study of absorption phenomena because it was supposed that procaine generally increases cell permeability [2] [3] [4].

METHOD

Procaine, as well as p-aminobenzoic acid (PABA), respectively diethylaminoethanol (DE) were tested on red pepper seeds, Kalinkov type. The seeds were imbibed in water (for the control), procaine solution or its hydrolysis products (PABA, DE), for 6, 12, and 24 hours. The concentrations used were of 0.1 mg% and 1 mg%, the seeds being treated as follows :

6 hours imbibition in water (or in solution with tested effect)

12 ,, ,, ,, ,, ,, ,, ,, ,, ,,
24 ,, ,, ,, ,, ,, ,, ,, ,, ,,

We note that ^{45}Ca accumulation in seeds was studied by a combined treatment of equal parts of PABA and procaine. Then the seed

material was treated with a radioactive solution for 6, 12, or 24 hours, represented in the figures as 6/6; 12/12; 24/24. In procaine variants a complementary treatment was applied and some seeds were simultaneously treated with procaine and radioactive solutions, but only in 6 and 24 hours tests.

For each experimental variant 50 seeds as near in size as possible were used, the solution being poured in excess. The absorption of the radionuclides, ^{32}P out of a K_2HPO_4 solution and ^{45}Ca applied as $Ca(NO_3)_2$, was observed in the control seeds, respectively in the treated ones. The solutions activity was of 4000 p.Ci.

When the treatment interval was over, the liquids above the seeds were decanted and the seeds washed in running water. Then the seeds were immediately dried in an electric oven, at 105°C, to a constant weight and the dried weight of the material was exactly determined. The seeds were put in crucibles and calcined at 500°C (gradually) up to a complete mineralization of the tissues. The obtained ash was put on a metal support, previously weighed, and reweighed after loading. Ash activity was measured at a GM counter, having a 1.8 mg/cm² window (VA—Z—310 type) adapted to an ORION type counting machine.

For the counter calibration a ^{32}P standard source was used, with an efficiency of 18.5%.

The experimental data — radioactivity of the tests measured in impulses per minute — represent the average of 4 repetitions for each variant. The values in the figures represent the average of the 4 repetitions expressed in per cent as compared to the control lots (100%).

RESULTS AND DISCUSSIONS

Figures 1 and 2 show the relative values of ^{32}P and ^{45}Ca absorption in the pepper seeds previously treated with procaine solution. From the figures we can see that procaine in 0.1 or 1 mg% concentration caused

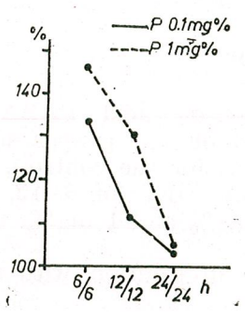

Fig. 1.—Per cent values representing the accumulation of ^{32}P in red pepper seeds treated with procaine (P) as compared to the control (100%).

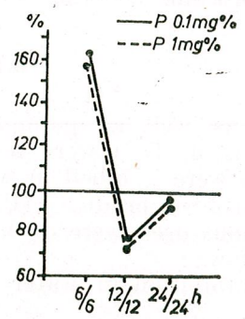

Fig. 2.—Per cent values representing the accumulation of ^{45}Ca in red pepper seeds treated with procaine as compared to the control (100%).

a substantial accumulation of radionuclides in seeds, especially with the variant of 6 hours procaine imbibition followed by 6 hours absorption in radioisotope solution (Fig. 1, 6/6). In 12/12 and 24/24 variants, the radionuclides absorption decreased with a prolonged treatment. We mention that in both procaine concentrations a significant stimulation of ^{32}P and ^{45}Ca absorption and accumulation was observed. In the variants where procaine and radioisotope solutions were simultaneously used for a period of 6 hours, the seeds treated with 0.1 mg% concentrations accumulated more than 13% ^{32}P as compared to the control seeds; at 1 mg% concentration it was more than 31%. In the variant of 24 hours treatment with procaine and radioisotope solution a lowering of ^{32}P absorption was observed at negative values; it was of 96% with 0.1 mg% concentration and of 93% with 1 mg% concentration. When both procaine and radionuclide solution were simultaneously used the ^{45}Ca uptake was inhibited. Procaine induced a decrease of this radionuclide absorption to 30%, with the 24 hours test, at 1 mg% concentration.

The uptake of radioactive tracers in pepper seeds treated with PABA or DE (Figs 3 and 4) was also studied. Figure 3 comparatively expresses the accumulation of ^{32}P. The DE solutions stimulated the ^{32}P absorption, having a maximum in the 12/12 hours variant. PABA had an unsignificant stimulation in ^{32}P absorption, excepting the 24/24 hours variant, but only at 0.1 mg% concentration. With ^{45}Ca absorption the situation was completely modified.

PABA stimulated the ^{45}Ca absorption in seeds especially in the 6/6 hours variant (but the values were by 10—15% lower than those obtained in the similar variant with procaine). Absorption decreased when the treatment period was longer, but maintained at higher values than the control tests. When PABA was combined with procaine (in equal

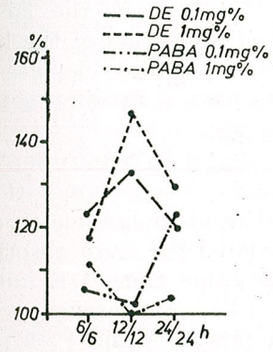

Fig. 3. — Per cent values representing the accumulation of ^{32}P in red pepper seeds treated with p-aminobenzoic acid (PABA) or diethylaminoethanol (DE) as compared to the control (100%).

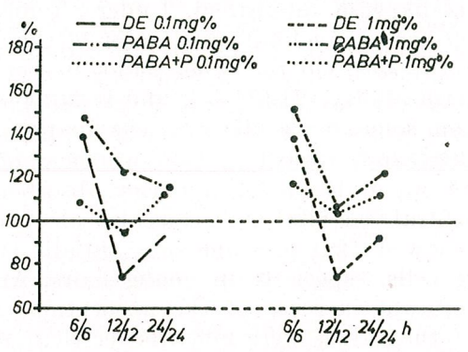

Fig. 4. — Per cent values representing the accumulation of ^{45}Ca in red pepper seeds treated with p-aminobenzoic acid (PABA) or p-aminobenzoic acid + procaine (PABA + P), and diethylaminoethanol (DE), as compared to the control (100%).

parts) the absorption of ^{45}Ca in seeds diminished, proving that procaine changed the permeability or metabolism of this element in the seminal tissues of the plant. DE stimulated only the 6/6 hours variant, the seeds having a reaction similar to the procaine-treated ones. Another important aspect was that of absorption modification not only according to the nature and concentration of the treatment solutions, but also to the physiological stage in which the treatment was applied, and to its duration.

Thus, the physiological state of the seeds was different for each variant. We refer to the inhibition stage of the seeds in the 6/6 hours variant, then to a release moment of the initial absorption for the 12/12 hours variant (a crucial moment for seed physiology in which the curves generally registered slight lowerings), and last to the 24/24 hours variant with which the seed entered a phase of active life.

The liquid in which the seeds were kept (for 48 hours) had a negative influence on the germination and growing of the seedlings, as it created anaerobic conditions which caused disturbances in the respiration processes. The treatment of the seeds with procaine, PABA, or DE influenced the cell permeability in the sense specific of each substance. Our previous research (in press) proved a stimulation of oxygen consumption in barley plantlets, as a consequence of the treatment with procaine. The respiration was only slightly stimulated in the caryopses treated with DE and even inhibited in the variants with PABA [12].

It is known that the procaine solutions (in 1 mg% concentration) determine an intensification of the respiration of yeast cells, a fact not proved with PABA treatment [1].

These data partly explain the absorption lowering according to the duration of submersion of the seeds (more than 12 hours) in procaine solution. Thus, the embryonic tissues of the pepper seeds, stimulated by procaine, intensify their respiration. As a result, the oxygen consumption is likewise intensified. Under submersion conditions, the lack of oxygen becomes a limitative factor of the normal evolution of the biological processes. On the other hand, it seems that procaine increases the cell permeability [2] [3] [4], and is supposed to have a favourable influence on some metabolic and enzymatic processes.

Research regarding the influence of procaine on absorption was carried out at the level of barley absorbent hairs. The accumulation of the neutral red vital stain was also observed. The histophotometric readings showed that procaine substantially stimulated the stain absorption in the cells, especially in young hairs. At the same time, DE induced a significant increase of the absorption, but of a low intensity. With PABA the values were much lower than with procaine, and even under those of the absorption in the control tests (in press).

As concerns the differentiated action of procaine on the anionic (P) and cationic (Ca) absorption at the seed level, our opinion is so far unsettled, but we mention some research carried out on a gelatine pattern membrane regarding the procaine effect on the fixing and mobilization of Ca ions. When treating such a membrane with procaine, that

part of it where the procaine solution was present was strongly charged with calcium ions. With a membrane of which a half was decalcified (using hydrochloric acid), the procaine treatment induced a redistribution of the calcium from the other half in the whole membrane [13]. On the other hand, an important reduction of anorganic phosphorus was observed in animal tissues, due to the treatment with procaine. The authors concluded that this phenomenon could be explained by the intake of phosphorous in the ATP macroergic links, with a stimulation of respiration processes and an increased oxygen consumption, as well as by the increased number of macroergic connections in the treated cells.

The negative influence of procaine on the absorption process when seeds were kept in solution for a longer period, might be accounted for by the accumulation of procaine in cells in superoptimal concentrations. Histological reactions with procaine on petal tissues [2] [3] [4] proved an evolution of cytological processes with the passing of time, i.e. an ever higher permeability of procaine up to the vacuole level. It seems that procaine treatment at the beginning of seeds germination releases in the embryonic cells a complex metabolic reaction, which intervenes in the regulation of numerous physiological phenomena.

Another hypothesis to be taken into account is the influence of the procaine hydrolysis products on the absorption. It is known that in animal tissues procaine can be divided into its components, which are also active from a physiological point of view. The p-aminobenzoic acid is in fact a vitamin found in all living organisms. At first sight, we could admit that procaine would act by releasing PABA from its molecule, which in its turn would stimulate the folic acid synthesis. The latter, a co-enzyme, would intensify some enzymatic reactions. Without excluding this hypothesis, we have arguments against it, the most important being that procaine hydrolysis at the vegetal cell level was not proved. The procaine hydrolysis products, PABA and DE, both in previous and in the present expriments indicated the different response of the plant treated with procaine, as compared to that induced by its hydrolysis products. The stimulative action of procaine on absorption processes at the seed level is specific of the procaine molecule and not of the PABA or DE.

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THE INFLUENCE OF N, S, AND Mg DEFICIENCY ON THE SYNTHESIS OF LEAF PROTEINS

BY

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Protein amino acids from leaves and their total radioactivity were determined in corn plants grown for 20 days under conditions of N, S, and Mg deficiency and in sunflower plants grown under the same conditions for 13 and 19 days. $^{14}CO_2$ was previously assimilated for 15 minutes.
Results have shown that the deficiency of these elements prevents protein synthesis either due to the absence of a normal synthesis of amino acids or of some of these, or due to the prevention of amino acids incorporation in complex substances.

The low radioactivity of the insoluble fraction from the leaves of plants grown under conditions of N, S, and Mg deficiency, as compared to that in the leaves of control plants, observed in previous experiments [5], determined us to suppose that the deficiency of these elements negatively influences many processes, among which protein synthesis.

The aim of the present work was the checking up of this hypothesis.

MATERIAL AND METHOD

The experimental material used was made up of corn and sunflower plants. In the experiment with the half-late hybrid corn H.D. 310 the plants were first grown on Knop full nutrient mineral solution and then, for 20 days, on N-, S-, and Mg-deficient solutions. The vegetable material (leaves) was gathered and fixed for analyses, when plants showed obvious signs of deficiency. In the experiment on "Smena" variety of sunflower, plants were also grown first on a Knop mineral solution and then for 13 days on N- and Mg-deficient solutions and for 19 days on S- and Mg-deficient solutions. After these 2 time intervals, non-detached leaves from the middle of the plant were left to assimilate in the sun (32,000—43,000 lx and 25—27°C temperature in the first experiment, and 55,000 lx and 28°C in the second experiment), in an atmosphere of $^{14}CO_2$ (with a concentration of 1% CO_2 and radioactivity of 100 Ci/l air) for 15 minutes.

After the fixation of the vegetable material (leaves) by boiling in methyl alcohol 85% for 5 minutes and the extraction of the soluble fraction (left material), the insoluble fraction was submitted to acid hydrolysis with a view to separating and determining protein amino acids. The hydrolysis of the insoluble fraction was performed with HCl

6n (by adding 10 ml HCl for each 0.1 g dry weight) at a temperature of 100°C, for 24 hours.

After hydrolysis, samples were brought to a neutral pH by adding bidistilled water and by repeated evaporations, in a water bath at 40°C. When samples reached pH 6, they were filtered, brought to 100 cm^3 and passed through an ion-exchanger column, Dowex 50 cationite, in order to separate amino acids from the other components resulting from hydrolysis.

Amino acids taken out of the cationite column with NH_4OH n were condensed with the help of a rotary evaporator at 20°C up to 10 cm^3. Amino acids obtained with the ion exchangers were separated into components by means of paper chromatography. Solvents : butanol-glacial acetic water (4 : 1 : 5). In sunflower plants with leaves which assimilated $^{14}CO_2$, the total radioactivity of amino acids was determined with a GM, B-34-M_2 IFA type counter. The differences of radioactivity in different amino acids separated by paper chromatography were revealed by autoradiography.

RESULTS

We shall further present results concerning the protein amino acids contents of corn leaves and the radioactivity of amino acids involved in proteins, during assimilation by sunflower leaves in $^{14}CO_2$ atmosphere, for 15 minutes.

Thus, as may be seen in figure 1, the amount of all amino acids in corn leaves decreases very much after an N deficiency of 20 days. Chromatogram shows only isoleucine, valine, alanine, threonine, glutamic acid, aspartic acid, and lysine. In S-deficient plants, the chromatogram shows the same number of amino acids as in control plants, with the difference that their amount is somewhat lower. More pronounced quantitative decreases may be observed in phenylalanine, in an amino acid with the Rf of the γ-aminobutyric acid, in proline, glutamic acid, and aspartic acid.

In the leaves of plants grown under conditions of magnesium deficiency, the same as of N deficiency, the amount of all amino acids obviously decreases. The amount of phenylalanine, of valine, and of an amino acid with Rf equal to that of γ-aminobutyric acid, the same as the amount of alanine, threonine, glutamic acid, aspartic acid, and lysine was more affected by the absence of magnesium than by that of the other identified amino acids.

Since the chromatogram of amino acids obtained by the hydrolysis of the insoluble fraction of sunflower leaves was generally similar to that described in the corn, it needs no longer be presented. We shall further refer to the autoradiogram of the chromatogram and to the total radioactivity of amino acids.

Thus, figure 1 shows that the radioactivity of protein amino acids from leaves of sunflower plants grown under conditions of nitrogen and magnesium deficiency for 13 days is five and four times, respectively, as low as that of control plants.

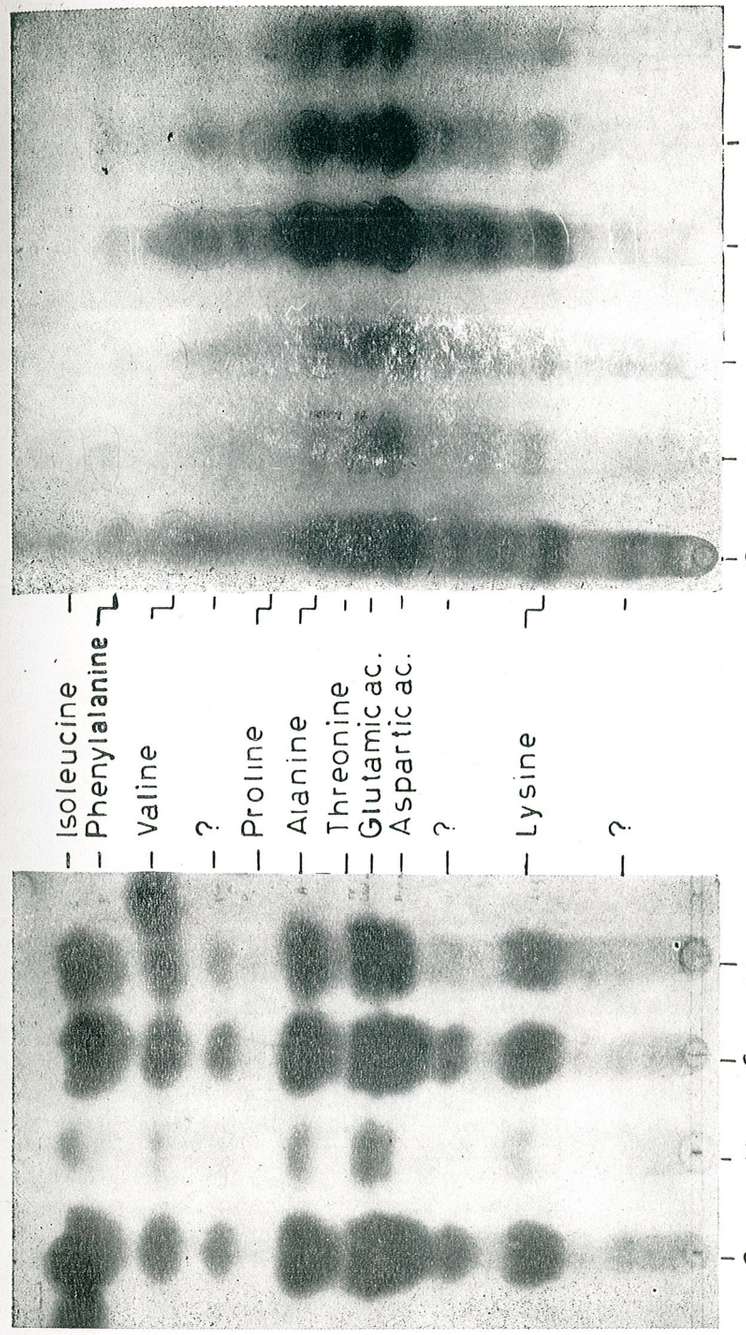

Fig. 1. — Protein amino acids from the leaves of corn plants grown under conditions of N, S, and Mg deficiency for 20 day.

Fig. 3. — Autoradiogram of the chromatogram of protein amino acids in leaves in sunflower grown under conditions of N, S, and Mg deficiency for 13 and 19 days.

For a longer Mg deficiency (19 days), the radioactivity of amino acids goes on decreasing.

As concerns the radioactivity of protein amino acids from the leaves of plants grown for 19 days without sulphur, a decrease of the involved ^{14}C may be also observed, yet it is less pronounced than in the case of N and Mg deficiency. These data point out that both N, Mg, and S deficiency negatively influences protein synthesis. The effects of the deficiency were more or less obvious after a shorter or longer period of deficiency, depending on the necessity for protein synthesis in these elements. In control plants of both experiments, all identified amino acids were radioactive to a greater or smaller extent (as shown by the degree of blackening of the Roentgen film and spot surface): isoleucine, phenylalanine, valine, proline, alanine, threonine, glutamic acid, aspartic acid, and lysine. Several non-identified amino acids also evinced radioactivity (one with the Rf of the aminobutyric acid and two with Rf ranging between those of aspartic acid and lysine).

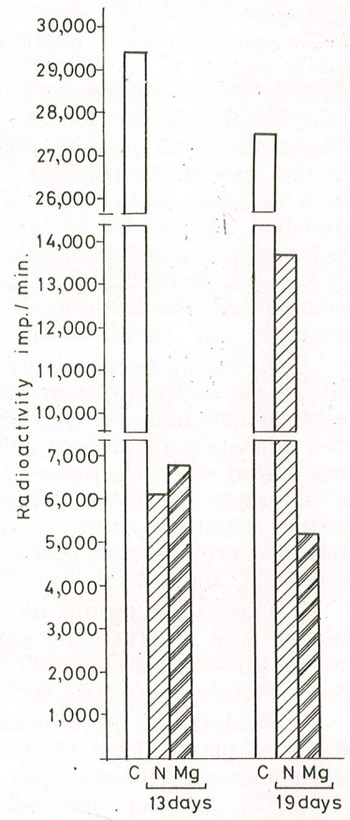

Fig. 2. — Total radioactivity of protein amino acids from sunflower leaves grown under conditions of N, S, and Mg deficiency for 13 and 19 days.

In N-deficient plants, aspartic acid, followed by glutamic acid, threonine, and lysine had low radioactivity. Alanine and phenylalanine evinced very low radioactivity.

In plants Mg-deficient for 13 days, aspartic acid and glutamic acid were radioactive ; lysine, threonine, and alanine evinced slighter radioactivity ; phenylanine and traces of valine and isoleucine were very slightly radioactive. In plants that were Mg-deficient for 19 days, radioactivity greatly decreased in all mentioned amino acids, as against those deficient for 13 days, and disappeared almost completely in isoleucine and valine.

In the leaves of plants grown on sulphur-less nutrient mineral solutions for 19 days, radioactivity of all amino acids also decreased compared to control plants. Radioactivity was visible in aspartic acid, alanine, and lysine, slight in glutamic acid and threonine and very slight in the other amino acids with higher Rf. It is interesting that in samples of leaves collected from N- and Mg-deficient plants the film is little impressed by sulphur amino acids, while in samples of leaves gathered from sulphur-deficient plants, the film is almost unimpressed.

DISCUSSIONS

The important decrease of the total radioactivity of amino acids from the insoluble fraction, their lower number and concentration in corn and sunflower leaves after a deficiency period of 13 days, points out that nitrogen deficiency deeply and quickly affects the synthesis of amino acids and, hence, of proteins. The inhibition of protein synthesis especially results in modifications of the fine structures of chloroplasts and, thus, in the disorganizing of physiological processes. Whatley [8] has shown that modifications of the thylacoid system occur in the absence of nitrogen. Under the same deficiency conditions, Hall et al. [6] noticed a reduction in the size of chloroplasts. Changes in the chloroplast structure as well as a decrease in the protein amount in fragment I were recorded in N-deficient plants also by Andreeva et al. [1]. Studies carried out so far point out that nitrogen deficiency induces modifications within cell microstructures, by inhibiting protein synthesis. In this connection one can wonder whether these modifications are reversible and if so, up to which stage is our interference still efficient.

As could be seen, the results obtained for protein amino acids in the leaves of Mg-deficient plants do not greatly differ from those obtained with N-deficient plants. The supposition that Mg deficiency results in the inhibition of protein synthesis [5] turns into certainty. Recent data by Duval and Duranton [4] have shown on the one hand, that Mg is necessary in the synthesis of magnesium-protein complexes from membrane structures, and on the other hand, that it is used as a cement between proteins and lipids. Travis et al. [7] have proved the favourable effect of magnesium on ^{14}C-phenylalanine uptake by polyribosomes.

These data permit us to state that, in addition to its role as a component and activator of some enzymes with a very important function (e. g. carboxylases, see Ditrich et al. [3]), magnesium also has functions more closely connected to the incorporation of amino acids in proteins.

Findings on protein amino acids obtained from leaves of sulphur-deficient plants point to the fact that the deficiency of this element also influences protein synthesis. This statement is supported by Biddulph's data [2], proving that sulphur deficiency prevents nitrogen transformation into protein substances. The deficiency of sulphur, as a component of a series of amino acids (e.g. cisteine, cistine, methionine) as well as of other compounds (glutathione) has also negative effects on the qualitative composition of proteins. In this respect, Willenbrink [9] observed an inhibition of protein synthesis with S in the nucleoplastic cytoplasm of cells from plants grown in the absence of this element. It is interesting that though S acts directly as a component element of some protein amino acids and indirectly, as a necessary element for the synthesis and activation of many enzymes, the influence of sulphur deficiency was not so obvious as that of the other two elements under study, N and Mg. This might be accounted for by the necessity of a longer period of deficiency to make effects more visible, or of the use of four typical plants in experiments for the deficiency period used by us. We think that one should emphasize both directions of this problem.

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DIE DYNAMIK DES KOHLENHYDRATGEHALTES BEI *PERILLA OCYMOIDES* WÄHREND DER PHOTOPERIODISCHEN INDUKTION

VON

CONSTANŢA OCHEŞANU, MARTIN KEUL und ROZALIA VINTILĂ

Untersucht wurde der Kohlenhydratgehalt, der Trockensubstanzzuwachs und die Transportgeschwindigkeit der Assimilate in den Blättern von *Perilla ocymoides* in Abhängigkeit von der photoperiodischen Induktionsdauer und der Entwicklungsphase.

Die Blätter der photoperiodisch behandelten Pflanzen enthalten während oder nach der Induktion größere Mengen an reduzierendem bzw., an löslichem Gesamtzucker und weisen einen geringeren Stärkegehalt auf. Die photoinduzierten Blätter haben eine wesentlich größere Synthese- und Speicherfähigkeit für Kohlenhydrate als die vegetativen Blätter gleicher Etagen und identischen Alters. Ihre Photosyntheseaktivität ist ungefähr 2mal größer, der Abtransport der Assimilate liegt bedeutend höher als bei vegetativen Pflanzen.

Die festgestellten Veränderungen in der Synthese-, Speicher- und Transportfähigkeit werden auch unter den der Photoperiode uneigenen Bedingungen (Postinduktion) aufrechterhalten.

Die Dynamik des Kohlenhydratstoffwechsels im Laufe der Ontogenese der Pflanzen ist noch ungenügend erforscht worden. Die ersten Angaben stammen von Krebs (1916), der wenige, aber äußerst wichtige Erkenntnisse brachte [11]. Er konnte feststellen, daß der Kohlenhydratgehalt in den Blättern während der Blütezeit ein Minimum verzeichnet, eine Tatsache, die er durch den Abtransport der Assimilate an die reproduktiven Organe der Pflanze erklärte.

Versuche dieser Art wurden später von Smirnov (1926, 1928), Okanenko (1931), Kursanov (1940), Rubin (1951) u.a. durchgeführt, die im allgemeinen zu ähnlichen Schlußfolgerungen gelangten [1] [3] [5] [6] [8] [9] [12] [17] [18] [19] [20] [21] [22]. Zwischen 1951—1952 führte Gorbunova (1956) weitreichende Versuche sowohl mit Langtags- (*Nicotiana rustica*) als auch mit Kurztagspflanzen (*Perilla ocymoides*) durch [7]. Sie konnte zeigen, daß der Gesamtkohlenhydratgehalt (reduzierende und lösliche Zucker, Stärke) in den Blättern mit der Entwicklung vom vegetativen Wachstum zum Blühen zunimmt. Nach Gorbunova ist der Höchstgehalt an Kohlenhydraten in den Blättern von *Perilla* während der Blütezeit festzustellen. Im Laufe der weiteren Entwicklung nimmt die Kohlenhydratmenge der Blätter zur Zeit der Fruchtbildung wieder ab, wobei der Stärkegehalt besonders stark, die löslichen Zucker in geringerem Maße reduziert werden.

In vorliegender Arbeit untersuchten wir die Dynamik der Kohlenhydrate in den Blättern von *Perilla ocymoides* nur während der

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photoperiodischen Behandlung, also vom Beginn der Induktion bis zur Knospenbildung, indem der Einfluß der Photoperiode und die Dauer der photoperiodischen Induktion auf die Speicherung der Kohlenhydrate verfolgt wurde. Eine besondere Aufmerksamkeit widmeten wir dabei dem Verhältnis zwischen dem Kohlenhydratgehalt und ihrem Transport, da die Speicherung bzw. die Weiterleitung der Assimilate in den Blättern nach Gorbunova für den Ablauf der Photosynthese eine bedeutende Rolle spielt [7].

MATERIAL UND ARBEITSMETHODE

Als Versuchsmaterial verwendeten wir die Kurztagspflanze *Perilla ocymoides*. Die Pflanzen wurden im Gewächshaus bei Dauerlicht (tagsüber natürliche Beleuchtung, nachts Glühlampenlicht) bis zur Ausbildung von 5 Blätterpaaren gezogen. Zu diesem Zeitpunkt wurden Pflanzen gleicher Größe und mit annähernd gleich großer Assimilationsfläche des obersten Blätterpaares ausgesucht, die Endknospe und die Blätter der I. und II. Stufe entfernt und die Pflanzen in zwei Gruppen geteilt. Eine Gruppe wurde für 9—10 Stunden der photoperiodischen Behandlung einer Kurztagspflanze unterworfen, während die andere Gruppe als Kontrolle bei Dauerbelichtung verblieb.

Die photoperiodische Behandlung dauerte 15 Tage.

Die Proben für die Analysen wurden 5, 10, 15 und 20 Tage nach Beginn der Induktion eingesammelt, indem um 8 Uhr 30 eine Blatthälfte, um 12 Uhr 30 die an der Pflanze verbliebene andere Blatthälfte abgetrennt wurde.

In einer Versuchsserie wurde der Gehalt an reduzierendem und löslichem Zucker in den Blättern verschiedener Etagen, in einer zweiten Versuchsreihe der Gehalt an reduzierendem bzw. löslichem Zucker und Stärke der gesamten Pflanze bestimmt. Die Bestimmung der Zucker erfolgte direkt oder nach Hydrolyse mit HCl mit der Methode mikro-Bertrand nach Bierry [2]. Die Ergebnisse wurden in g/100 g Frisch- bzw. Trockengewichte/4 Stunden ausgedrückt.

Die Photosynthiseassimilation wurde gleichzeitig mit der Blattscheibenmethode bestimmt und die Ergebnisse auf das Trockengewicht bezogen. Es wurde sowohl die apparente als auch die reelle Photosynthese bestimmt, um die Abtransportgeschwindigkeit der Assimilate feststellen zu können.

ERGEBNISSE UND DISKUSSION

Nachfolgend behandeln wir nur die Ergebnisse der zweiten Versuchsreihe mit den an der gesamten Pflanze durchgeführten Bestimmungen, da die Analysen für die verschiedenen Blätterstufen nicht eindeutige Schlußfolgerungen zulassen.

Die Versuchsergebnisse wurden in den Tabellen 1—9 dargestellt.

Tabelle 1 und 2 geben die Dynamik der reduzierenden Zucker wieder. Es wird festgestellt, daß die Pflanzen in der vegetativen Wachstumsphase allgemein einen ggrößeren Kohlenhydratgehalt aufweisen als die photoinduzierten. Obwohl nach 15 Tagen Behandlung alle Pflanzen Knospen ausgebildet haben, ist die Menge an reduzierendem Zucker (sowohl auf das Frisch- als auch auf das Trockengewicht bezogen) im Falle der behandelten Pflanzen kleiner als bei denen im vegetativen Wachstum. Erst 5 Tage nach der Postinduktion bei Dauerlicht, wenn die

Tabelle 1

Die Dynamik der reduzierenden Zucker in Abhängigkeit von der photoperiodischen Induktionsdauer (g Glukose/100 g Frischgewicht/4 Stunden)

Tageszeit	Induktion (Tage)				Kontrolle (Tage)			
	5	10	15	20	5	10	15	20
8 Uhr 30	0,075	0,075	0,155	0,340	0,099	0,146	0,218	0,334
12 Uhr 30	0,162	0,133	0,227	0,344	0,134	0,142	0,265	0,330
D	0,087	0,058	0,072	0,004	0,035	—	0,047	—
D %	100	100	100	100	40,2	—	65,3	—

D = Differenz

Tabelle 2

Die Dynamik der reduzierenden Zucker in Abhängigkeit von der photoperiodischen Induktionsdauer (g Glukose/100 g Trockengewicht/4 Stunden)

Tageszeit	Induktion (Tage)				Kontrolle (Tage)			
	5	10	15	20	5	10	15	20
8 Uhr 30	0,634	0,599	1,083	1,942	0,680	0,962	1,227	1,830
12 Uhr 30	1,216	0,977	1,535	1,900	0,908	0,853	1,429	1,722
D	0,582	0,378	0,452	—	0,228	—	0,202	—
D %	100	100	100	—	39,2	—	44,7	—

D = Differenz

Pflanzen ein fortgeschrittenes Stadium der Knospung erreichen, wird bei den zur Blüte angeregten Pflanzen tatsächlich ein größerer Gehalt an reduzierendem Zucker gegenüber der Kontrolle festgestellt (Tabelle 2). Hervorzuheben ist die Synthese- und Speicherfähigkeit der induzierten Blätter, die gegenüber der Kontrolle eine bedeutende Zunahme erfährt und auch nach 5 Tagen Postinduktion bei Dauerlicht bestehen bleibt (am 20. Tag bedeckter Himmel).

Tabelle 3

Die Dynamik der löslichen Gesamtzucker in Abhängigkeit von der photoperiodischen Induktionsdauer (g Glukose/100 g Frischgewicht/4 Stunden)

Tageszeit	Induktion (Tage)				Kontrolle (Tage)			
	5	10	15	20	5	10	15	20
8 Uhr 30	0,127	0,190	0,313	0,573	0,202	0,199	0,435	0,583
12 Uhr 30	0,305	0,353	0,455	0,611	0,305	0,393	0,529	0,662
D	0,178	0,163	0,142	0,038	0,103	0,194	0,094	0,079
D %	100	100	100	100	57,7	119,0	66,2	207,9

D = Differenz

Der Gehalt an löslichem Gesamtzucker ist in den Tabellen 3 und 4 dargestellt. Die morgens um 8 Uhr 30 durchgeführten Analysen zeigen bei den photoinduzierten Pflanzen eine stete Zunahme der löslichen Zucker, während dieser Anstieg bei den vegetativen Pflanzen ungleichmäßig verläuft.

Tabelle 4

Die Dynamik der löslichen Gesamtzucker in Abhängigkeit von der photoperiodischen Induktionsdauer (g Glukose/100 g Trockengewicht/4 Stunden)

Tageszeit	Induktion (Tage)				Kontrolle (Tage)			
	5	10	15	20	5	10	15	20
8 Uhr 30	1,067	1,526	2,187	3,170	1,393	1,310	2,452	3,193
12 Uhr 30	2,294	2,591	3,070	3,486	2,063	2,362	2,848	3,457
D	1,227	1,065	0,883	0,316	0,670	1,052	0,396	0,264
D %	100	100	100	100	54,6	98,8	44,8	83,5

D = Differenz

Gleichzeitig ist aber anhand der zu diesem Zeitpunkt—nach dem Abtransport und Verbrauch während der Nacht—durchgeführten Analysen die Schlußfolgerung zu ziehen, daß die Blätter der vegetativen Pflanzen einen größeren Gehalt an löslichem Zucker aufweisen als die Pflanzen, die die photoperiodische Behandlung durchlaufen oder die Induktion abgeschlossen haben. Ähnliche Ergebnisse erzielte Gorbunova bei *Perilla* [7].

Die um 12 Uhr 30 bestimmten Werte zeigen hingegen, daß die der Induktion unterworfenen und Knospen ausgebildeten Pflanzen in den Blättern einen größeren Gehalt an löslichem Gesamtzucker aufweisen als die vegetativen Pflanzen gleichen Alters bei Dauerlicht (Tabelle 4). Auch in diesem Falle stimmen unsere Ergebnisse mit den Angaben von Gorbunova überein [7]. Wir folgern demnach, daß die widersprüchlichen Ergebnisse der Fachliteratur nicht nur auf unterschiedlichen Umgebungsfaktoren (Temperatur, Licht, Feuchtigkeit usw.) beruhen, sondern auch vom Zeitpunkt der Bestimmungen abhängen, denn die induzierten Pflanzen besitzen eine größere Synthese- und Speicherfähigkeit für lösliche Kohlenhydrate als die Pflanzen im vegetativen Wachstumsstadium. Die erwähnten Unterschiede in der Synthese- und Speicherfähigkeit machen sich schon zu Beginn der Induktion bemerkbar, da sie wahrscheinlich mit der physiologischen Umstrukturierung der Blätter zusammenhängen, und bleiben während der gesamten Induktionsperiode sogar unter ungünstigen Bedingungen (z.B. nach 5 Tagen Dauerbelichtung) bestehen.

Tabelle 5

Die Dynamik der Stärke in Abhängigkeit von der photoperiodischen Induktionsdauer (g Stärke/100 g Frischgewicht/4 Stunden)

Tageszeit	Induktion (Tage)				Kontrolle (Tage)			
	5	10	15	20	5	10	15	20
8 Uhr 30	0,524	0,690	1,460	5,071	2,287	2,468	4,784	5,349
12 Uhr 30	1,231	1,901	2,749	5,495	3,216	3,477	5,196	5,730
D	0,707	1,211	1,289	0,424	0,929	1,009	0,412	0,381
D %	100	100	100	100	131,4	83,3	31,9	89,9

D = Differenz

Die Dynamik des Stärkegehaltes zeigt anhand Tabelle 5 und 6, daß die Blätter der Pflanzen während der Induktion oder während der Knospenbildung einen geringeren Stärkegehalt aufweisen als diejenigen der vegetativen Pflanzen. So enthalten z.B. die um 8 Uhr 30 eingebrachten Blatthälften ca. 3mal mehr Stärke als diejenigen der induzierten Pflanzen; bei der 2. Analysenserie um 12 Uhr 30 sind die Differenzen zwar nicht mehr so ausgesprochen hoch, sie bleiben aber noch eindeutig erhalten (Tabelle 6).

Tabelle 6

Die Dynamik der Stärke in Abhängigkeit von der photoperiodischen Induktionsdauer (g Stärke/100 g Trockengewicht/4 Stunden)

Tageszeit	Induktion (Tage)				Kontrolle (Tage)			
	5	10	20	15	5	10	15	20
8 Uhr 30	4,435	5,381	9,942	29,847	15,737	16,208	26,947	29,316
12 Uhr 30	9,225	13,736	18,566	30,391	19,928	20,903	27,960	29,917
D	4,790	8,355	8,624	0,544	4,191	4,695	1,013	0,601
D %	100	100	100	100	87,5	56,2	11,7	110,5

D = Differenz

10 Tage Kurztagsbehandlung (Tabelle 7) stellen die optimale photoperiodische Induktionsdauer dar. Zu diesem Zeitpunkt wurden 2 Analysenserien durchgeführt u.zw. an einem sonnigen bzw. an einem Tag mit geringerer Lichtintensität bei wolkenbedecktem Himmel. Ungeachtet der Lichtverhältnisse zeigten die photoinduzierten Pflanzen eine hohe Synthese- und Speicherfähigkeit für Kohlenhydrate.

Die zur Knospenbildung angeregten Pflanzen (15 Tage Kurztagsbehandlung) weisen im Vergleich zu den vegetativen Pflanzen in den Blättern einen geringeren Stärkegehalt auf. Diese Ergebnisse stimmen mit den Angaben von Gorbunova aus dem Jahre 1951 für *Perilla* und *Nicotiana* überein, widersprechen aber die Bestimmungen die dieselbe Verfasserin im Jahre 1952 durchführte [7].

Wenn die Angaben nach 20 Tagen, also nach 5 Tagen Postinduktion bei Dauerlicht und in einem fortgeschrittenen Stadium der Knospenbildung, analysiert werden, dann gelangt man zur Feststellung, daß die induzierten Pflanzen tatsächlich einen größeren Stärkegehalt der Blätter im Vergleich zur Kontrolle bei Dauerbelichtung aufweisen.

Wie auch im Falle der Anreicherung von löslichem Gesamtzucker muß auch hier die besondere Synthese- und Speicherfähigkeit der physiologisch umstrukturierten (induzierten) Blätter im Vergleich zu denjenigen der vegetativen Pflanzen herausgestrichen werden. Es kann behauptet werden, daß die induzierten Blätter ihren Stärkegehalt im Laufe von 4 Stunden Photosynthese-Aktivität verdoppeln, während die Blätter der Kontrolle kaum 1/4 ihrer Anfangsmenge speichern. Noch mehr, die vegetativen Blätter weisen Schwankungen in ihrer Synthese- und Speicherfähigkeit auf, die entweder mit einem inneren Rhythmus oder mit altersbedingten Veränderungen zusammenhängen.

Von den drei untersuchten Kohlenhydraten ist die größte gespeicherte oder anwesende Menge durch die Stärke vertreten. Sie ist aller-

Tabelle 7

Die Dynamik der Kohlenhydrate nach 10 Tagen Induktion
(g/100 Frisch- bzw. Trockengewicht/4 Stunden)

Tageszeit	Induktion (Tage)				Kontrolle (Tage)			
	reduz. Zucker		lösl. Zucker		Stärke		reduz. Zucker		lösl. Zucker		Stärke	
	Frisch-gewicht	Trocken-gewicht	Frisch-gewicht	Trocken-gewicht	Frisch-gewicht	Trocken-gewicht	Frisch-gewicht	Trocken-gewicht	Frisch-gewicht	Trocken-gewicht	Frisch-gewicht	Trocken-gewicht
8 Uhr 30	0,033	0,250	0,334	2,609	0,690	5,381	0,032	0,206	0,377	2,431	2,468	16,208
12 Uhr 30	0,077	0,556	0,394	2,845	1,901	13,736	0,080	0,487	0,456	2,772	3,477	20,903
D	0,045	0,306	0,060	0,236	1,211	8,355	0,048	0,281	0,079	0,341	1,009	4,695
D%	100	100	100	100	100	100	106,7	91,8	131,7	144,5	83,3	56,2

D = Differenz

dings die stabilste Form, deren Gehalt durch Lichtintensitätsänderungen wenig beeinflußt wird.

Wie ist die mengenmäßig größere Speicherung von Kohlenhydraten in den induzierten Blättern zu erklären? Ist dies die natürliche Folge der Tatsache, daß die gesamte Zuckermenge während der Dunkelheit aus den Blättern abtransportiert wird, so daß die Photosynthese durch die Blattreserven nicht beeinträchtigt wird? Andererseits ist die Lichtintensität während der Nacht gering (um 800 Lux), so daß die bei Dauerlicht wachsenden Kontrollpflanzen nicht so große, die Photosynthese benachteiligende Stärkemengen anreichern können. Um auf diese Fragen eine Antwort zu finden untersuchten wir 1. die Trockensubstanzzunahme in den Blättern und 2, die Transportgeschwindigkeit der Assimilations — produkte.

Tabelle 8

Die Geschwindigkeit der Trockensubstanzspeicherung der Blätter in Abhängigkeit von der Induktionsdauer
(mg./dm²/4 Stunden)

Tageszeit	Induktion (Tage)				Kontrolle (Tage)			
	5	10	15	20	5	10	15	20
8 Uhr 30	11,833	12,469	14,282	17,519	14,531	15,233	17,747	18,247
12 Uhr 30	13,316	13,645	14,807	18,083	14,783	15,623	18,585	19,155
D	1,483	1,176	0,525	0,564	0,252	0,393	0,858	0,908
D%	100	100	100	100	16,99	33,41	159,61	160,99

Die Zunahme der Trockensubstanz (Tabelle 8) ist bedeutender bei den 5 und 10 Tage induzierten Pflanzen bevor seitliche Sprosse ausschlagen und geringer während der Knospenbildung (15 und 20 Tage). Bei den vegetativen Pflanzen ist die Zunahme nach 5 und 10 Tagen gering, obwohl auch diese keine Seitensprosse bilden, und etwas größer nach 15 und 20 Tagen, wahrscheinlich infolge der Einstellung des Blattwachstums.

Tabelle 9

Die Dynamik der Photosynthese in Abhängigkeit von der photoperiodischen Induktionsdauer
(mg./dm²/4 Stunden)

Photo-synthese	Induktion (Tage)			Kontrolle (Tage)		
	5	10	15	5	10	15
apparente	16,80	15,90	16,70	8,80	5,50	9,70
reelle	20,60	21,99	20,40	11,60	10,60	17,50
Transport	3,80	6,09	3,70	2,80	5,00	8,20

Tabelle 9 enthält die Geschwindigkeit der apparenten und reellen Photosynthese sowie den Abtransport der Assimilationsprodukte. Die apparente Photosynthese ist im Laufe bzw. nach Abschluß der Induktion ungefähr gleichwertig, sie ist aber annähernd doppelt so groß gegenüber den vegetativen Pflanzen. [8] [9] [13] [15] [18].

Die reelle Photosynthese (apparente Photosynthese plus Transport und Verbrauch) ist bei den induzierten Pflanzen ebenfalls ungefähr 2mal größer als bei den vegetativen Blättern gleichen Alters. Damit können wir im Einklang mit etlichen Literaturangaben folgern, daß der Transport

und der Verbrauch der Assimilate in den induzierten Pflanzen größer ist [4] [10] [14] [15] [22] [23].

Aufgrund unserer Ergebnisse können wir demnach schließen, daß die stärkere Speicherung der untersuchten Kohlenhydrate in den Blättern der photoinduzierten Pflanzen nicht äußeren Bedingungen zu verdanken ist; sie ist vielmehr die Folge der physiologischen Umstrukturierung der Blätter, die dadurch einer höheren Photosyntheseintensität fähig sind, die Folge der historischen Entwicklung einer Gattung oder Art unter gewissen Umweltbedingungen, die der Ontogenese eigen geworden sind.

SCHLUSSFOLGERUNGEN

1. Die Dynamik des Kohlenhydratgehaltes in den Blättern von *Perilla ocymoides* wird durch die Photoperiode, die Induktionsdauer und die jeweilige ontogenetische Entwicklungsphase beeinflußt.
2. Der Gehalt an reduzierendem bzw. löslichem Zucker und Stärke in den photoperiodisch induzierten Pflanzen übertrifft mengenmäßig denjenigen der vegetativen Pflanzen gleichen Alters.
3. In den knospentreibenden Pflanzen ist der Gesamtzuckergehalt (lösliche reduzierende und nichtreduzierende Zucker, Stärke) größer als bei den Pflanzen im vegetativen Wachstum.
4. Sie Stärke ist die stabilste Zuckerform, deren Dynamik und Gehalt mit der Dynamik der Photosynthese in Bezug auf die Induktion übereinstimmt.

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THE ABSORPTION OF RELATIVELY HIGH CONCENTRATIONS OF MICROELEMENTS BY *SCENEDESMUS QUADRICAUDA* (Turp.) Bréb. AND *CHLORELLA VULGARIS* BEYER

BY

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In 1974, laboratory experiments were carried out in a large number of variants, in order to elucidate the absorption and accumulation of some microelements (Al, Ni, Zn, Cu, and Cr).
The absorption is selective, varying as a function of the administered microelement, of its concentration in the culture medium, of the assimilation time, as well as of the species.
The obtained results, related to the whole algae biomass, in different aquatic ecosystems, show that *Scenedesmus quadricauda* (Turp.) Bréb. has a predominant role, as it achieves the extraction from different mediums of relatively high concentrations of microelements which by their nature have a noxious character.

It is already known that there are some vegetal and animal organisms which have the capacity to absorb a series of chemical components from the surrounding environment.

This capacity of absorption can sometimes be very intense, so that large quantities of substances noxious, by their nature or by the concentration in which they are found in the environment, are accumulated in the organisms. By means of these organisms, one can more or less achieve the biological epuration of some residual or town waters or of those in the process of eutrophization.

Investigations were carried out [1–6], [8–18] on different species of algae and bacteria, noticing that they have the possibility to absorb from the medium relatively high quantities of toxic and noxious substances.

These investigations were carried out by using different nutritive mediums dificient in one or more components by administering increasing concentrations of ^{90}Sr, ^{137}Cs, ^{147}Ce, ^{106}Ru, etc. Thus, one obtains on the one hand the improvement of the water quality and on the other hand the increase of the biomass which can be later used as a fertilizer or as food for other organisms.

In the present paper we present original data referring to the absorption of relatively high concentrations of some microelements, namely Cu, Cr, Ni, Al, Zn, by the algae *Scenedesmus quadricauda* (Turp.) Bréb. and *Chlorella vulgaris* Beyer.

MATERIAL AND METHOD

Laboratory experiments were carried out with two species of algae, namely *Scenedesmus quadricauda* (Turp.) Bréb. and *Chlorella vulgaris* Beyer. The control was lacking the administered microelement. There were performed a great number of variants : 1—115 mg/l in the variants with *Scenedesmus quadricauda* (Turp.) Bréb. and 5—95 mg/l in the variants with *Chlorella vulgaris* Beyer.

The microphyte species grew in 1.5 l. vessels submitted to illumination with fluorescent lamps having the intensity of 5000 lx, the temperature being of about 23°C. The cultures were stirred by air bubbling.

The determination of the respective microelements concentration in the dry substance (1 g) both in the case of the control and of different variants was made by colorimetric methods for Al, Cu, Cr, and Ni [7] [19], while for Zn by identifying the obtained colour and its intensity, which varies directly proportional to the concentration [19].

The results are expressed in mg/g dry substance in the two species of alga. The readings were performed with the FEK-M photometer, green and blue filter.

RESULTS AND DISCUSSIONS

The results are presented in table 1 and figures 1—4.

From table 1, which shows zinc accumulation in algae, it results that this microelement was absorbed during 18 days after the administration in the two species of algae, in relatively high quantities in all variants. In the control, where no zinc was administered in the nutritive medium, the green colour appeared in the dry substance. In the variants of *Scenedesmus quadricauda* (Turp.) Bréb. with increased concentrations (5—115 mg/l) the colours occurred in intensities proportional to the increased quantities of Zn. In *Chlorella vulgaris* Beyer, Zn was also accumulated in high concentrations.

Figure 1 presents the absorption of aluminium in the two species of microphytes. We notice that, in general, in *Scenedesmus quadricauda* (Turp.) Bréb. the absorption is more intense in comparison with that in *Chlorella vulgaris* Beyer. In the first case, there were obtained high absorptions, namely 23.1—26.9 mg Al/g dry substance in variants where 50—60 mg/l of $Al_2(SO_4)_3$ were administered in the nutritive medium. In *Chlorella vulgaris* Beyer the maximum Al absorption from the nutritive medium was remarked in the variants were 35 mg/l of $Al_2(SO_4)_3$ and respectively 90 mg/l of $Al_2(SO_4)_3$ were administered, concentrations to which there corresponded an absorption of 6.1—8.8 mg Al/g dry substance.

Nickel was absorbed in the two species of algae approximately in similar concentrations in some of the variants (Fig. 2). Thus, in *Scenedesmus quadricauda* (Turp.) Bréb. at the end of the experiments there were obtained values of 8.3—9.2 mg Ni/g dry substance, corresponding to the variants where 60 mg/g $NiSO_4$ and respectively 25, 45, 60 mg/l $NiSO_4$ were administered. In *Chlorella vulgaris* Beyer nickel was more intensely absorbed, the recorded values being of 8.1—11.5 mg Ni/g dry

substance, in variants where 70 mg/l $NiSO_4$ and respectively 90 mg/l $NiSO_4$ were administered.

As concerns copper absorption in the two species of microphytes, we may state that it occurred with a reduced intensity (Fig. 3). At the

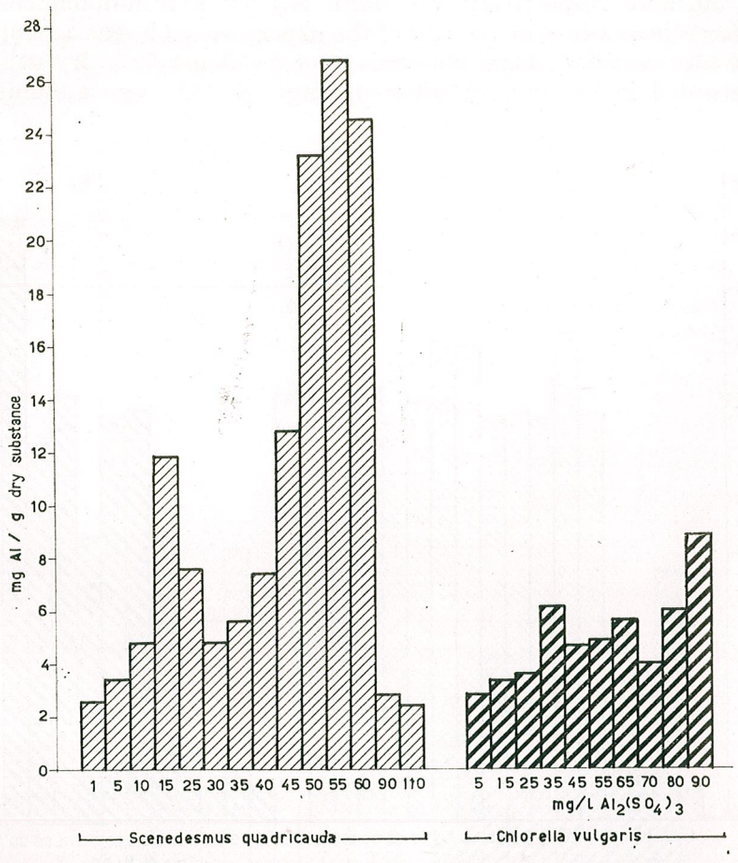

Fig. 1. — Al absorption in *Scenedesmus quadricauda* (Turp.) Bréb. and *Chlorella vulgaris* Beyer, administered in the culture medium as $Al_2(SO_4)_3$.

end of the experiments in *Scenedesmus quadricauda* (Turp.) Bréb. there were recorded values of 1.0—1.6 mg Cu/g dry substance in the variants were 85 mg/l $CuSO_4$ and respectively 75 mg/l $CuSO_4$ were administered. In *Chlorella vulgaris* Beyer copper absorption took place with a reduced intensity as compared to that in *Scenedesmus quadricauda* (Turp.) Bréb. : at the end of the experiments there were obtained values of 0.8—0.9 mg Cu/g dry substance in variants where 75 mg/l $CuSO_4$ and respectively 95 mg/l $CuSO_4$ were administered.

Figure 4 presents the results obtained in the two species of algae as concerns chromium absorption. At the end of the experiments some differences were noticed regarding the absorption intensity of this microelement. Thus, in *Scenedesmus quadricauda* (Turp.) Bréb. there were obtained values of 0.15—0.20 mg/d dry substance in variants where 15, 20, 30, and respectively 35 mg/l K_2CrO_4 were administered. In *Chlorella vulgaris* Beyer at the end of the experiments Cr was accumulated in reduced quantities : maximum values of 0.053 mg Cr/g dry substance were recorded in the variant where 15 mg/l K_2CrO_4 were administered.

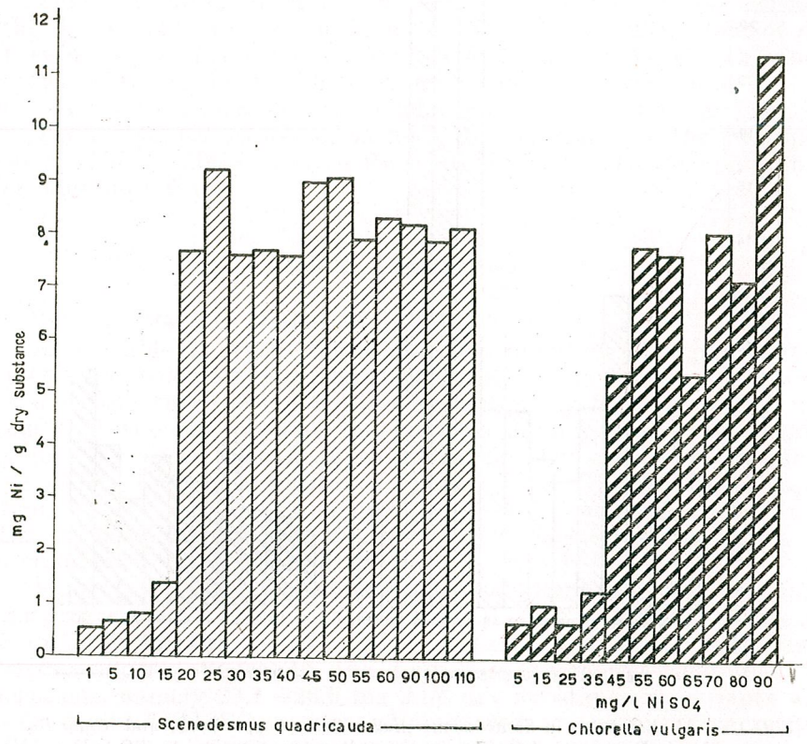

Fig. 2. — Ni absorption in *Scenedemus quadricauda* (Turp.) Bréb. and *Chlorella vulgaris* Beyer, administered in the culture medium as $NiSO_4$.

The data presented, as well as investigations carried out en different species of algae using different nutritive mediums [3] [12] [16], show that in the two species of algae, an absorption of *Z*n, Al, Ni, Cu, and Cr takes place during 12—18 days.

Regarding the capacity of absorption of microelements, the most intense were absorbed Al and Ni, then *Z*n, Cu and Cr.

These results show that microphytes absorb relatively high concentrations of microelements, from different noxious mediums.

CONCLUSIONS

1. The microelements Cu, Cr, Ni, Al, and *Z*n are absorbed in relatively high concentrations by the algae *Scenedesmus quadricauda* (Turp.) Bréb. and *Chorella vulgaris* Beyer.

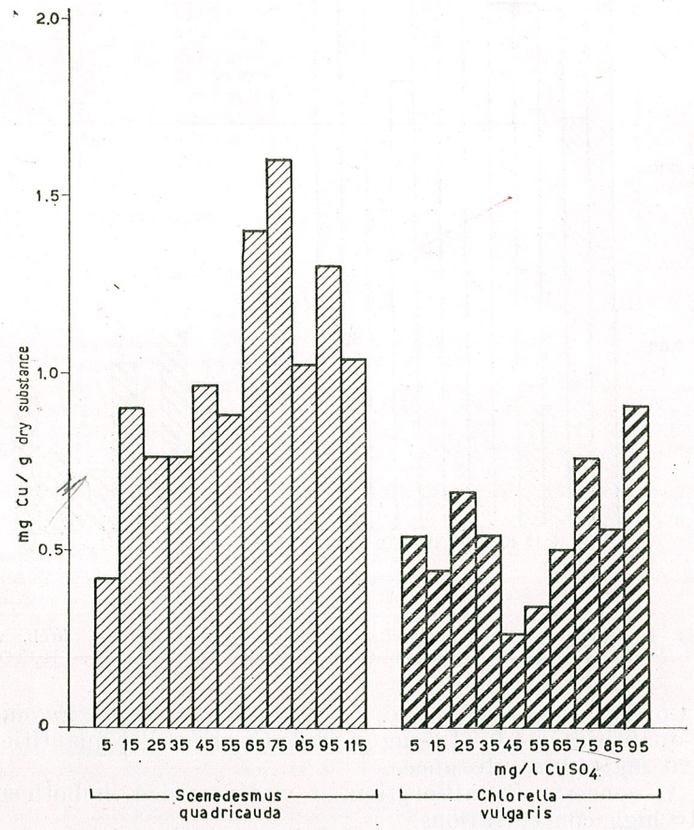

Fig. 3. — Cu absorption in *Scenedesmus quadricauda* (Turp.) Bréb. and *Chlorella vulgaris* Beyer, administered in the culture medium as $CuSO_4$.

2. This absorption is selective, depending on the administered microelement, on its concentration in the culture medium, on the duration of assimilation, and on species.

3. Large quantities were absorbed in *Scenedesmus quadricauda* (Turp.) Bréb. : 23.1—26.9 mg Al/g and 8.3—9.2 mg Ni/g dry substance. In *Chlorella vulgaris* Beyer Ni was more intensely absorbed : 8.1—11.5 mg Ni/g.

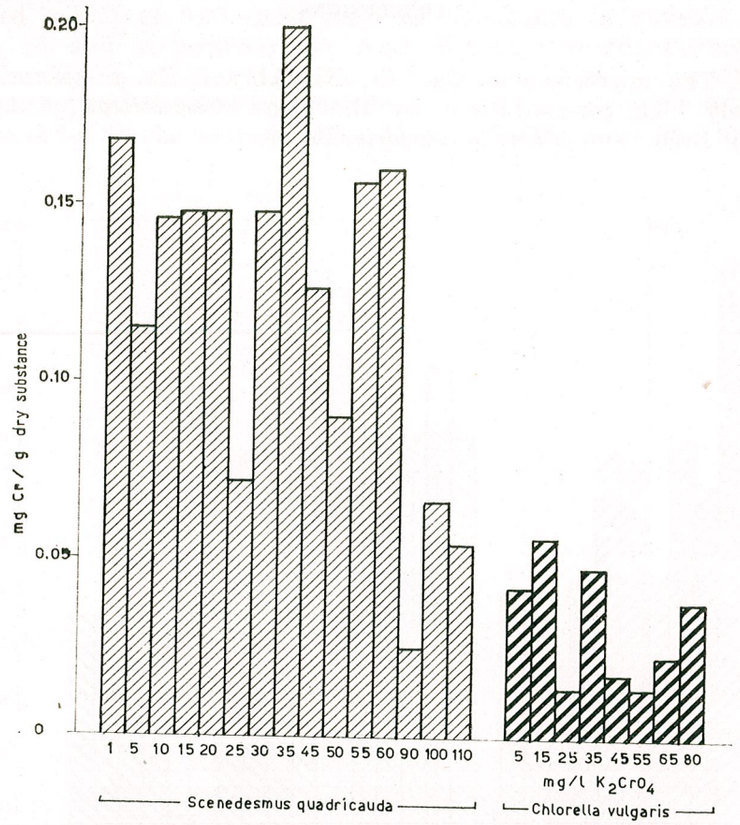

Fig. 4. — Cr absorption in *Scenedesmus quadricauda* (Turp.) Bréb. and *Chlorella vulgaris* Beyer, administered in the culture medium as K_2CrO_4.

4. Copper was assimilated by *Scenedesmus quadricauda* (Turp.) Bréb. in quantities of 1.0—1.6 mg/g, while Cr in smaller quantities namely 0.15—0.20 mg/g dry substance.

5. As concerns Zn absorption, it was recorded in both species in relatively high concentrations.

6. The absorption of Al, Cu, and Cr is more intense in *Scenedesmus quadricauda* (Turp.) Bréb. as compared to *Chlorella vulgaris* Beyer; as for Ni and Zn, no evident differences were registered between the two species of algae.

7. The obtained results, related to the whole biomass from different aquatic ecosystems, show that the algae achieve the biological epuration of waters impurified with large quantities of microelements.

Table 1

Zn accumulation in algae (evolution of results as a function of colour and intensity according to STAS* no. 6327/1967),

Scenedesmus guadricauda		*Chlorella vulgaris*	
Initial concentration of the medium mg/l	Coulour and intensity	Initial concentration of the medium mg/l	Colour and intensity
5	green-blue	5	green-blue
15	blue-violet	15	blue-violet
25	light-violet	25	light-violet
35	light-violet	35	light-violet
45	light-violet	45	light-violet
55	light-violet	55	light-violet
65	dark-violet	65	light-violet
75	dark-violet	75	dark-violet
85	dark-violet	85	violet-red
95	dark-violet	95	red-violet
105	violet-light red		
115	violet-dark red		
control	green	control	green

* State standard

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