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ECOSYSTEM CHARACTERIZATION OF A LOW PRODUCTIVITY BEECH FOREST IN OLTENIA (GALBENUL VALLEY, POLOVRAGI FORESTRY DISTRICT)

BY

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A forestry ecosystem made up under the conditions of a podzolic-brown soil, intensely acid (pH 3.8), is presented with *Fagus sylvatica* as a main producer. The value of primary production is low (6.6 t/ha/year-production and 274.9 t/ha biomass). The diversity of every biocenotic components is reduced (Simpson/Pielou index is 0.2), although stability is high; that is proved by the old age of the forest. The decaying process is slowly realized by some organisms prevailing *Acarinas* from invertebrates and *Fungi* from microorganisms.

The researches have made great strides in the knowledge of the flora and fauna and their relation with the environment. The general laws which govern the groups of organisms at the level of populations, i.e. of biocenosis, as well as the organization forms have been widely studied [1]. What we consider of present interest in the scientific investigation of ecologists, either they are sylviculturists or biologists, is the establishing of a succession in these complex high groups, i.e. ecosystems; just as the classification system of the flora or fauna, of flora communities or of the stand it is necessary to perform an ordering of ecosystems considering not only a component but also the whole complex. In our country such a work is just at the beginning, first as only few data on the structure and functionality have been accumulated so far. They have been obtained interdependently and correlated by the same object of study, i.e. a concrete representative ecosystem.

This paper undertakes to present a minimal model for the characterization of an ecosystem, i.e. a low productivity beech forest we frequently met with in our forests and which raises some problems in forestry practice. This model could be the basis of the knowledge of the ecosystem particularities and not only of a part of the ecosystem [5].

According to the existent classifications in this ecosystem the plants are grouped in the *Luzulo-Fagetum* association (Beldie 51, Morariu et al 68) which, from the phytocenologic standpoint, belongs to the type of beech forest with acidophilous flora, according to forest typology and to the beech forest of hilly type, P_{m-s}, strongly podzolized, moderate edaphic-submoderate edaphic with *Luzula albida* in site typology.

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INVESTIGATION METHODS

The research methods, adequate to the great diversity of the analysed material, are numerous and specific. The measurements were made as follows : soil humidity — gravimetrically by drying at 105°C ; acidity — potentiometrically in aqueous solution ; humus content by Schollenberger's method and total nitrogen content by Kjeldahl's ; tree productivity and biometrical elements on circular areas of 500 m², according to Golden's model [4] and dendrometric methods [3]. The leaf mass was indirectly measured using fresh litter. The structure and productivity of herbs were obtained by numbering the invididuals on surfaces of 0.25 m² (100 repetitions) regularly disposed on a hectare and by the determination of individual biomass. The Simpson/Pielou coefficient $D = 1 - \frac{n_i(n_i - 1)}{N(N - 1)}$, where n is the number of individuals of a population and N is the number of individuals of all populations used for diversity. *The Mammalia* are considered for the whole forest fund. The invertebrate fauna in the forest canopy was collected in a sweep net with a 60 cm diameter (each sample = = 50 shakings up to 4 m height). The mobile fauna on the soil surface was analysed by making captures in Barber traps. *Acarians*, *Nematoda*, *Enchytreidae* and *Collembola* from the invertebrate fauna of soil were collected from the first 10 cm of soil layer, with MacFadyen probes ; sample extraction was made by automatic extractors. *Lumbricidae* were collected from the first 40 cm of soil layer on 4 equal levels. The mass of fresh and decaying litter was gathered on areas of 0.125 m² (15 repetitions) uniformly distributed. Soil dehydrogenase activity was determined according to the method of Casida and Ştefanic. Ecophysiological analyses were made in plants concerning the water content by the gravimetric method, the carbohydrate concentration by the refractometric method, the assimilatory pigments by the method of Comar and Zscheille.

The dynamics of biocenosis has been analysed at two significant moments : vernal phenophase and estival phenophase.

RESULTS AND DISCUSSIONS

ELEMENTS OF THE BIOTOPE

The plot of land studied is on the right bank of the Galbenul river before entering the quay. The Galbenul valley is formed in the south-eastern zone of the Parîng mountains. It is found at 745 m altitude, on an almost plane platform (2—5°) differentiated at the middle of an abrupt slope with W—SW orientation. The canopy of 120 year old trees is incomplete (0.8 covering) but uniform allowing more light to reach at the soil surface, i.e. 8—10 % (a value high enough for beech forests).

The soil is of brown podzol type with continuous litter relatively equally distributed on the whole surface. The upper horizon is very rich in raw humus. Its texture is of sand-clayed type with many great fractions. Soil reaction is highly acid and it varies in depth (table 1) but only within a high acid range. In the summer time the ascendant water flow in the soil depth carries mineral salts which decrease acidity, the values

Table 1

Seasonal dynamics of some edaphic factors in soil active area

Period	Soil depth	Humidity %	Acidity	Humus content %	N_2 content %	C/N ratio
Vernal	0—10	39.04	3.8	—	—	—
	10—20	32.05	3.8	—	—	—
	20—30	29.89	4.0	—	—	—
	30—40	22.25	4.0	—	—	—
Estival	0—10	24.57	4.5	21.90	0.289	43.81
	10—20	21.98	4.3	6.60	0.059	—
	20—30	18.31	3.8	—	—	—
	30—40	17.10	3.8	—	—	—

remaining acid further on. Soil humidity is moderate during vegetation time. A higher drying of the soil in the active area has been noticed in summer but without affecting plant requirements since the coarse particles of soil have a more reduced capacity of keeping water. Soil trophicity does not correspond to the requirements of plants. The total nitrogen content is moderate being in an amount high enough for a good nutrition of plants but under conditions of high acid soils, the combinations under which nitrogen is found can become hardly assimilable. The amount of humus is very high it representing a reserve of organic material whose value is very difficult to be enhanced under poor decaying conditions that has been proved by the high value of C/N ratio. This ratio makes the soil get the characteristics of a peat bog.

ELEMENTS OF BIOCENOSIS

Primary producers

The primary producers are stratified in this biocenosis only at two layers : the tree layer, which is uniform enough, and the herbaceous layer, which has a limited and discontinuous development ; beech seedlings are rare and the shrubs do not participate in making the skeleton of the system.

The tree layer consists of *Fagus sylvatica* in a 100 per cent. The diversity of the stand is 0.00. The beech population has a low density (430 individuals/ha) and its variability (240—540 individuals/ha) has minimum values for the forests in low productivity stations (table 2).

Table 2

Biometrical data of the beech forest

Age (years)	Mean density tree/ha (variable limits)	Height m mean (variable limits)	Diameter cm mean (variable limits)	Basal surface m²		Volume m³	
				Total m²/ha	Mean (variable limits)	Total m³/ha	Mean (variable limits)
120	430 (240—540)	23.2 (17—30)	31.6 (9—63)	16.10 (11.04—1906)	0.0432 (0.02—0.07)	398 (342—463)	1.11 0.081—3.193 (4.037)

The stratification of the stand is between 16—30 m the mean height being around 23 m. The horizontal structure of the forest is assured by trees having 9—63 cm in diameter. Most of the trees have a diameter between 31—32 cm. The beech population in this biocenosis has trunk thicknesses great enough corresponding to heights small enough considering the species growth possibilities. The tree base surface is very small it being first due to the existence of a small number of trees which are not very high. There are few higher trees and they do not exceed 0.07 m². The actual surface occupied in the biocenosis by the tree base surface is under 0.15%. The tree wood volume is of 398 m³/ha on the average, a value characteristic of low productivity forests of the 4th production class. The frequency of better developed individuals (with a 4.3 m³ volume) in the population is reduced. The mean individuals are of 1.1 m³ and their total number is small both explaining the cause of the reduced productivity of the stand. The tree wood density has mean values with respect to species variability it being of 0.69 g/cm³ for the dried wood.

The herbaceous layer is very poor (Table 3). It consists of 4—5 species. Only 10—20 per cent of the soil is covered with a herbaceous

Table 3

Structure data of herbaceous layer

Phenoaspect	Frequency of soil covering %	No. of species		Diversity Simpson-Pielou index	Maximum hetero-geneity	Mean density (ind/m²)	Biomass g/m² (d. wt)	Water content in the bio-mass %
		Herbs	Mosses					
vernal	49	4	3	0.2058	0.7500	21.06	2.030	64
estival	54	5	3	0.2022	0.7500	13.20	1.580	76

layer discontinuously distributed; only in 50 per cent of cases a few herb patches appear. The height of the layer is of 10—15 cm overtopping a little the height of mosses also present in the biocenosis. The vernal and estival phenoaspects are numerically and gravimetrically dominated by *Luzula luzuloides* (Table 4) only. The density of the population decreases much in the summer time although the development cycle typical of the species has the optimum value in that period. We suppose that the decrease of humidity under high acid conditions may be one of the causes. The vernal population of *Anemone nemorosa* is developed during the vernal phenoaspect but with a relatively reduced abundance and with a more reduced biomass; but it represents the greatest water reserve. The mean water reserve is of 64% in spring, it being determined by the most abundant population in the phytocenosis. The participation of the other species is nonsignificant both in spring and in summer. The diversity of the herbaceous layer is very small, the Simpson/Pielou diversity index being of only 0.2058 as a result of a very poor composition and of the numerical and gravimetrical dominance of a single species. The mean density of herbs is moderate to low in this layer. The density values are depending on the nature of the most abundant species — *Luzula* — a species with many tillers even at a reduced frequency.

Table 4

Elements of structure and biomass of herbaceous populations

Species	Frequency %	Density ind/m²	Relative abundance %	Individual biomass g.s.u.	Population biomass g.s.u. 50 m²	Biomass %	Water content %
Vernal phenoaspect							
Luzula luzuloides	40	932	88.51	0.097	90.40	88.87	73
Anemone nemorosa	5	67	6.36	0.043	2.88	2.83	69
Carex verna	4	35	3.32	0.149	5.21	5.12	56
Festuca drymeia	3	19	1.80	0.170	3.23	3.17	60
Estival phenoaspect							
Luzula luzuloides	30	640	96.96	0.110	70.40	88.93	74
Pteridium aquillinum	2	4	0.60	1.590	6.35	8.03	79
Veronica officinalis	1	6	0.90	0.150	0.90	1.14	70
Festuca drymeia	1	8	1.21	1.180	1.44	1.82	59
Epilobium angustifolium	1	2	0.30	0.030	0.06	0.07	84

Moss species: *Polytrichum commune, Dicranum scoparium, Pleurozium schreberi*

Accumulation of the biomass of primary producers

At the tree layer the accumulation of biomass has a low level: tree biomass 274.9 t/ha out of which the leaf mass represents 3.9 t/ha (Fig. 1). The yearly production of wood mass estimated according to the forest mean age is of 2.71 t/ha. This value corresponds to some low productivity stands. The biocenotic parameter responsible for this reduced accumulation of biomass is the density of wood population, as the accumulation of individual biomass is not at the lower limit. This reduced density is possible to show the necessity to increase the minimum individual area of each tree as a result of poor unfavorable edaphic conditions. The leaf mass is great in the year of research as compared to wood production it representing almost 60% of the yearly production. Its value in photosynthesis is also dependent on the quantity of assimilatory pigments contained in the specialized tissues.

The herbaceous biomass is much more reduced in any forestry ecosystem, it being hardly seen in that analysed. It varies between 0.020 t/ha and 0.015 t/ha in spring and summer and represents more than 80% from the biomass amount formed by *Luzula*. The yearly increase is smaller than the total biomass as *Luzula* is a perennial species.

The ratio between the biomass of herbs and that of trees is 1 : 13 700 and that between the yearly production of herbs and trees is 1 : 330, these ratios showing their great difference in productivity. On the other hand, their comparison with those of other stations [5] shows that the production of herbaceous biomass is more strongly affected than the production of wood biomass by the site conditions less favorable to plants.

The energy stock at the level of primary producers is of 1.46×10^9 kcal/ha. The wood mass stores 93% of the energy of primary producers and 7% of the leaf mass of trees. The herbaceous biomass is under 0.001% from the energy stock of producers. Every year the ecosystem assures at its first level an energy flow of 3.2×10^7 kcal/ha to which

about 25 % from this value is added, i.e. the energy consumed in the respiration process. The energy that reaches yearly at the disposal of the organisms that decompose the litter is of 2.4×10^7 kcal/ha.

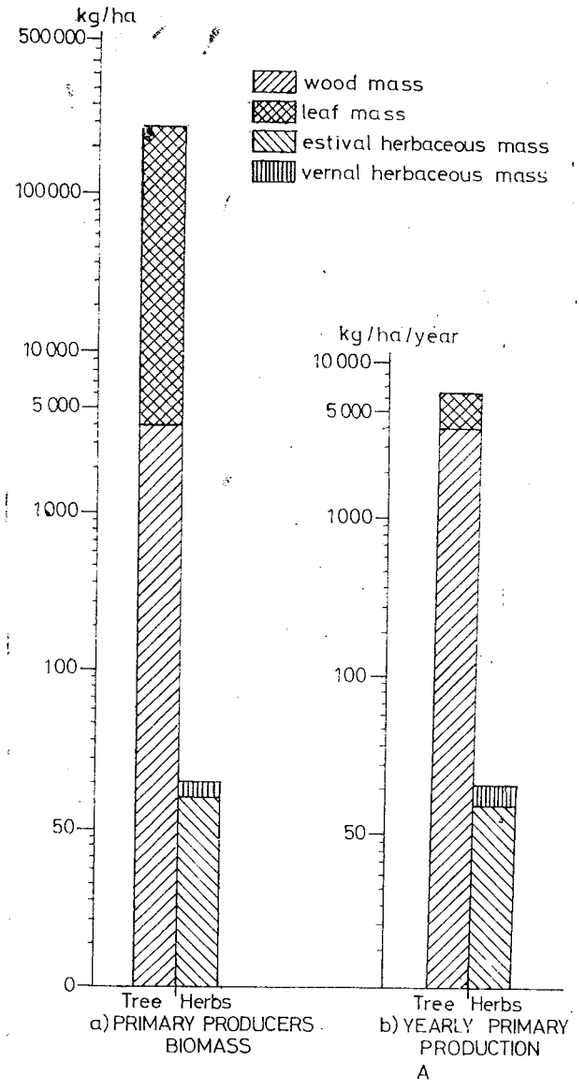

Fig. 1

Indicators of plant metabolic activity

The assimilatory pigments are found in the leaves of dominant plants at a very low level (Table 5). As compared to the leaves of other beech populations placed in optimum productivity stations the chlorophyl

Table 5

Ecophysiologic indicators of the main plant species

Species	Assimilatory pigments $\times 10^{-4}$ g/g.d.w.		The ratio of assimilatory pigments		Cell sap	
	Chlorophyl a+b	Carotene pigment	Chlorophyl a/b	Chlorophyl/carotene	pH	Carbohydrates %
Vernal phenoaspect						
Fagus sylvatica	64	20	2.61	3.21	4.0	9.0
Anemone nemorosa	48	15	2.97	3.18	5.5	10.0
Luzula luzuloides	27	9	3.08	2.86	5.7	6.5
Estival phenoaspect						
Fagus sylvatica	61	19	2.68	3.25	5.8	8.5
Luzula luzuloides	67	22	3.24	3.12	5.8	5.5

content is half or only 1/3 as to the quantity existent in them (under similar light conditions of leaves) [5]. Although the carotenoid pigments are in a more reduced amount as compared to those in the reference populations they have values close one to another.

We consider that the reduced productivity of plants may be affected this way, the assimilatory apparatus being limited not as leaf mass but as possibilities of chlorophyl synthesis. The a/b chlorophyl ratio with very small values under 3 which is their molecular ratio indicates the preferential synthesis of chlorophyl *b*, a typical pigment for shadow or other unfavorable conditions. The equilibrated chlorophyl/carotene ratio indicates a dominance of the respiration process as compared to photosynthesis in Margaleff's interpretation.

Acidity of the cell sap is moderate. The influence of soil pH is shown more by a decrease of the metabolism than by making acid the inner medium of plants. It is well known that the more acid values of the sap cell show generally a higher metabolic activity.

The concentration in carbohydrates is higher in spring it having a more active osmotic and protective role in that period.

Consumers and decomposers of biocenosis

The composition and structure of consumers and decomposers of biocenosis are more difficult to be identified due to their high mobility and to the diversity of participating groups.

The *macroconsumers* are not connected with a single ecosystem and the beech forest investigated is used only in limited periods. As a result of the evaluation of the faunistic-cynegetic density one can notice the presence of deer, roebuck, chamois, wild boars, rabbits, mountain cocks and hazel hens as primary consumers; the secondary consumers are lynxes, wolves, wild cats, otters, common martens and polecats; two species occupy intermediary positions being preponderantly omnivorous, i.e. bear and wild boar (Table 6). A comparison made with the existent situation in the ecological sector in which the investigated zone is included show us that the number of animals is optimum and above it [2]. But at the

Table 6

Pray density on the Oltet hunting fund

Species	Pray density estimated on the hunting fund (1983) 14.000 ha (12.700 ha forest)	Density ind/1000 ha	Ecologic sector (53 9000 ha forest) E_2			
			Estimated density		Optimum density	
			total	ind/1000 ha	total	ind/1000 ha
Roe deer	121	9	523	1	1851	3
Roebuck	104	7	10433	19	16870	31
Chamois	43	25	410	—	725	—
Wild boars	42	3	445	1	1330	2
Rabbits	200	15	28318	—	56980	—
Common badger	11	1	—	—	—	—
Bears	33	2	177	1	320	1
Wolves	11	1	—	—	—	—
Foxes	24	2	—	—	—	—
Wild cats	10	1	—	—	—	—
Lynxes	12	1	—	—	—	—
Tree marten	11	1	—	—	—	—
Stone marten	4	—	—	—	—	—
Polecats (common marten)	14	1	—	—	—	—
Otter	1	—	—	—	—	—
Capercaillie	21	0.2	—	—	—	—
Hazel hens	11	—	—	—	—	—

same time it is established that for the 5 species for which the classification of forest lands was made inferior categories were determined (the third for deer, wild boars and bears and the fourth for chamois). This is also a consequence of the existence in the hunting fund of the stations characterized by few food resources available and a reduced shelter capacity for large vertebrates.

The invertebrate fauna in the forest canopy is the poorest of the beech forests in Romania both as concerns the number of individuals and taxonomic groups. The species *Bourletiella ornasi (Collembola), Haplothrips acanthoscelis (Thysannoptera), Apion sp., Athous sp.* and *Glyptena sp.* (Coleoptera) are presented. In the spring is noticed the abundance of microarthropods with representatives of 5 fauna groups in different proportions and with the variation amplitude ranging between 7.14—32.86% (Table 7). Most are phytophagous rarely zoophagous species.

Table 7

Relative abundance of invertebrate fauna in the canopy (%)

Main groups	Spring	Summer
Collembola	22.22	—
Thysanoptera	11.11	20.00
Homoptera	32.86	20.00
Hymenoptera	7.14	20.00
Coleoptera	15.56	40.00
Aranea	11.11	—

Mobile invertebrate fauna on the soil surface (epigaion). Analysing the components of the food chain in the beech forests studied we find that the zoophagous species have definitely higher values as compared to other link species. Among zoophagous species, *Opilionidae* and *Araneae* are eudominant (43.2%) and (19.93%) and *Carabidae*, which are also recognized as predators, are dominant (Table 8). It is worth noticing that also the

Table 8

Numerical and relative abundance of mobile invertebrate fauna on the soil surface

Main groups	Numerical abundance	Relative abundance
Miriapoda	21	
Julidae	21	6.35
Arachnida	209	
Opilionida	143	43.21
Aranea	66	19.93
Hexapoda	101	
Dermapterae	50	15.11
Carabidae	35	10.57
Scarabacidae	6	1.81
Staphylinidae	10	3.02

Dermaptera which are preeminently phytophagous are eudominant. The decomposers *Julidae, Scarabeidae* and *Staphylinidae (Coleoptera)* are subdominant. Having in view that the taxonomic groups in the epigaion are poorly represented, both as concerns the number of families and individuals, the diversity of the cenoses studied is low.

The decaying process

A 3900 kg/ha new litter is yearly accumulated on the soil surface it being superposed on the litter of the previous years which undergoes a decaying process. The whole amount of litter is of 8800 kg/ha. The decaying rate is slower that the accumulation rate. Litter decaying is not complete its transformation reaching gradually at the raw humus stage only.

Dehydrogenase activity. An indicator of global activity of soil microbiocenosis, dehydrogenase activity is low in both seasons and in all layers (Table 9). The difference between the litter and the first soil layer is small while the differences as compared to the lower layers are somewhat greater. The potential dehydrogenase activity is also low in both seasons and in all layers (Table 9) as a result of the presence of a poor microbian flora represented especially by *Actinomycetales* and *Fungi*. The difference between the litter and the first layer which is somewhat greater shows the existence of a microflora much richer in litter connected with a more abundant organic decaying material.

Invertebrates in the soil. The study of soil fauna included the main groups of invertebrates which play a significant role in the humification process (Table 10).

Lumbricidae are very poorly represented in the soil only by *Dendrobaena* sp. in vernal period and two species *Allolobophora sp.* and *Dendrobaena alpina* in summer. This low density is explained by the soil low pH value and the presence of organic material.

Table 9

Seasonal variation of the present and potential dehydrogenase activity (mg formazan/100 g dried soil)

Soil layer	Spring		Summer	
	Present	Potential	Present	Potential
Litter	10.8	16.3	8.3	13.7
0—3 cm	8.7	12.3	6.2	12.3
3—6 cm	5.3	9.4	4.8	7.4
6—10 cm	4.8	6.9	3.6	6.2

Table 10

Main groups of invertebrates in edaphon

Taxon	Density/m²			Yearly biomass (g)/m²	Energy value (cal/m²)
	Spring	Summer	Yearly mean		
Lumbricidae	1	2	1.5	0.014	103.60
Enchytreidae	3000	5600	4300	0.516	1361.15
Nematoda	64900	86200	75550	0.075	162.83
Acarina	87400	46900	67150	0.671	3105.52
Collembola	15000	7600	11300	0.135	707.43
Total	170301	146302	158301,5	1.411	5440.53

Enchytraeidae are organisms with an important role in soil fertilization. They are found in a high enough number in this beech forest.

Collembola have a density of only 113000 individuals/m² year mean a low value determined by the existence of an extremely reduced amount of organic material which may be incorporated in the decaying processes.

Acarians include Oribatidae, Gamasidae, Uropods, Zerconidae, Acaridae imatures of Oribatidae. Oribatidae are well represented by 65 species among which 9 species are dominant : *Oppiella nova, Oppia ornata, Oppia minus, Op. obsoleta, Suctobelbella subtrigona, Oppia bicarinata, Op. getica, Suctobelbella baloghi, Suctobelba trigona.* These species have been signalled especially in the humus layer and are considered species with a "humigen" role. A great abundance of *Oribatidae* was recorded even in acid pH conditions. They have a maximum density in spring and autumn and a minimum in summer.

Nematoda have a low mean seasonal and annual density (Table 10). *Tylenchidae, Dorylaimidae and Areolaimidae* appear both in spring and in summer. Most *Nematoda* are phytophagous and fungiphagous. In the beech forest analysed the activity of *Nematoda* of breaking up organic matter and of microbian catalysis is much affected by the highly acid, pH ot soil which also diminishes much the *Nematoda* populations.

The biomass of soil fauna is very low, of 1.411 g (Table 10), as compared to the typical values for deciduous forests in the temperate zone of 4 g—30 g [7].

The energy fixed as secondary production as a result of detritus metabolization is also reduced (Table 10), i.e. of 5.44 kcal (22.696 kJ).

The decaying process made by the existing soil fauna and microflora is very slow and justifies the high value of C/N ratio which indicates a poor transformation (of organic substance in CO_2 and water) during the respiration ot organisms in soil. The main reason of their reduced existence and activity is soil high acidity.

There is a microorganisms — mesofauna real complex whose combined action directs the passage from the litter to humus but without lending it superior qualities; the existing humus is due especially to the decaying activity of *Acarians.*

CONCLUSIONS

The ecosystem analysed is at equilibrium at a minimum productivity level (6.6 t/ha/year production and 274.9 t/ha biomass) with a reduced diversity of each biocenotic component (Simpson/Pielou index 0.2), as a result of the exclusive dominance of some populations adapted to live on a strongly acid substrate with a high stability proved by the forest old age. The decaying process is slow it being realized by few organisms somewhat abundant being the *Fungi* among microorganisms and *Acarians* among invertebrates in the soil.

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ELECTRON MICROSCOPIC INVESTIGATIONS ON THE SPORES OF *SPLACHNACEAE (BRYOPHYTA)*. TAXONOMIC, ECOLOGICAL AND BIOLOGICAL CONSIDERATIONS

BY

G. PLOAIE, TR. I. ȘTEFUREAC

The following three species of *Splachnaceae* have been investigated by high resolution electron microscopy: *Tetraplodon angustatus*, *Tetraplodon urceolatus* and *Splachnum sphaericum*. A transmission electron microscope of the JEM-7 type was used and new informations on spore ornamentation, useful in taxonomic studies, were detected. With no further treatment, the informations obtained have indicated that the exines of spores of the three species have similar morphologies. They consist of numerous circular pores distributed on three axes in a compact hexagonal pattern. These discoveries are for the first time revealed for spores of some *Splachnaceae* and are useful in connection with ecological and biological data.

The electron microscopic studies of spores of *Bryophyta* have recently developed both by transmission electron microscopy (TEM) and by scanning electron microscopy (SEM). Using different techniques for the obtaining of preparations, photographs of spores with particularities of sporoderma ornamentations have become known in most *Bryophyta* families [1], [3], [4], [6], [10], [13].

In the case of a few species of *Splachnaceae* family, KOPONEN [5] describes the ornamentations of sporoderma making considerations on the taxonomy, ecology and the origin of this group of *Bryophyta*.

Investigations using an original method [7] on the spores of different groups of *Bryophyta* [8], [9], [11] were also made on the spores of three species of the *Splachnaceae* family.

MATERIALS AND METHODS

The mature spores of native *Bryophyta* from a herbarium of Prof. Tr. I. Ștefureac, which usually grow in some Carpathian massifs (Bucegi, Giumalău, Parîng) were analysed. The spores belong to the following taxonomic units:

Fam. *Splachnaceae*
Tetraplodon angustatus (Hedw.) B. & S.
Tetraplodon urceolatus B. & S.
Splachnum sphaericum Hedw.

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The original method used in the electron microscopic analysis of spores is a direct method for the obtaining of preparations and for visualization, which requires no previous treatment of spores. It has been described in the above mentioned works.

RESULTS AND DISCUSSIONS

The electron microscopic studies on the spores of the analysed species of the *Splachnaceae* family show, from the ultramorphological and ultrastructural point of view, fine particularities of the sporoderma, with characteristic ornamentations, unique for *Bryophyta*.

Tetraplodon angustatus (Hedw.) B. & S.

This species has ovoidal, elongated and relatively small spores of 3—5 × 2—3 μ. They frequently have a larger bulging part and a more pointed, sometimes curved one. Even under dried state, the spores are agglomerated, associated two by two or three by three (Pl. 1 A).

Although the spores have a thick wall, they are penetrated by an electron beam, become relatively transparent allowing to easily distinguish the particularities of the sporoderma. The spore surface has many grooves (pores) of the exine, arranged in a hexagonal pattern, a pore being surrounded by other 6 ones placed on the top of a regular hexagon. This extremely regulated model characterizes almost the whole surface of the spore (Pl. 1 A, B). Here and there one can distinguish more rarely the pentagonal respectively hexagonal models.

When the pores on the one side of the spore are superposed on the pores of the other side (Pl. 1 A, arrow), the well-known aspect called "moiré pattern" is obtained, which is similar with that described by GLAUERT (1966) for the cell wall of bacteria.

The periphery of the spore of *Tetraplodon angustatus*, seen at small magnification, seems to be an ornamentation with electronopaque prominences, but at large magnifications one can clearly notice that these ornamentations represent in fact the space between pores seen under "cross section" (Pl. 1 C).

The pores of the exine have a perfectly cylindrical form with a diameter of 165 nm, and a depth of 45 nm (Table 1). Their basal part is supported on the intine, sometimes visible by transparency as a dark stripe.

Tetraplodon urceolatus B. & S.

Spores are more elongated as compared to those of the previously described species. Their sizes range generally between 5—7 × 3—4 μ. They are less opaque for electrons than those of *T. angustatus*. Sporoderma has the same type of ornamentation as that of the spores of *T. angustatus*, having the exine pores also regularly placed in a hexagonal pattern (Pl. 2 A).

In case of *Tetraplodon urceolatus* one can notice, by comparison, a larger pore space and so the pore sizes are smaller than those of *T. angustatus*, i.e. their diameter is of 128 nm and their depth of 38 nm

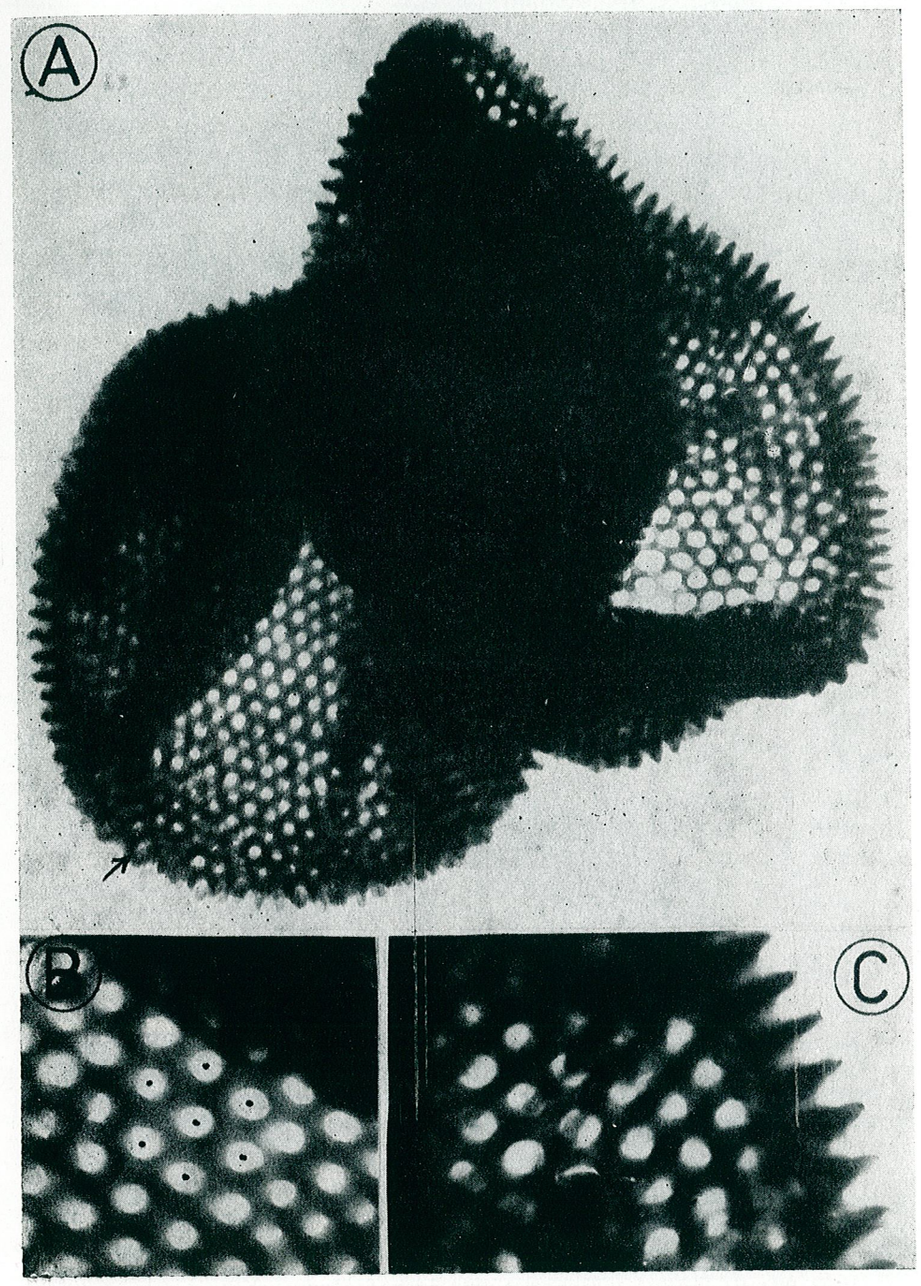

Plate 1 — *Tetraplodon angustatus* (Hedw.) B. & S.

A. Morphological aspect of spores, with ovoidal contour and regular hexagonal ornamentations. × 19,000
B. Detail of spore surface. One can see the ornamentations in a regular pattern. × 57,000
C. Selection of a spore edge where the ornamentation outline can be noticed. × 57,000

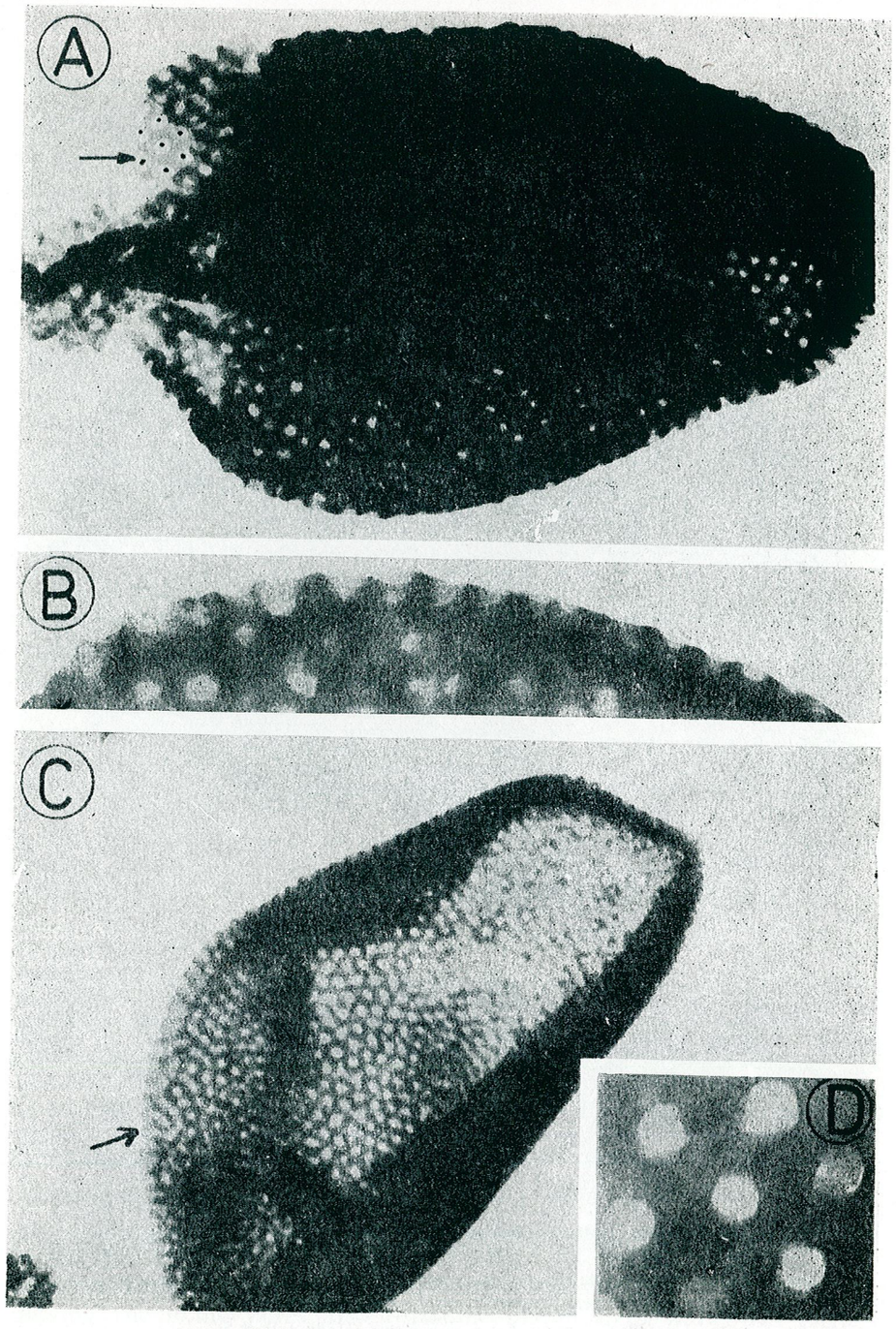

Plate 2 — *Tetraplodon urceolatus* B. & S.

A. General form of a spore and arrangement of ornamentation at *Tetraplodon urceolatus*. × 25,000
B. Section of a spore edge of *Tetraplodon urceolatus*, with cylindrical ornamentations (pores) (profile view). × 55,000
C. Photograph of a spore of *Splachnum sphaericum* Hedw. with cylindrical pores of the exine. × 24.000
D. Detail of sporoderma with ornamentations placed in a regular hexagonal pattern at *Splachnum sphaericum*. × 45.000.

(Table 1). In this case also intine can be noticed as a dark stripe at the spore periphery.

Splachnum sphaericum Hedw. *(S. ovatum* Hedw.)

Spores are elongated, sometimes curved, or with a trapezoidal cross section, having a length of 6—7 μ and a width of 3—4 μ (Pl. 2 C). They are relatively transparent for electrons and one can clearly distinguish the ornamentation type of sporoderma (Pl. 2 C, D), which is similar with that met with the analysed species of *Tetraplodon* genus.

Table 1

Electron microscopic characteristics of sporoderma correlated with some ecological and biological data in some *Splachnaceae*

Genus and species	Sizes of spores (dried) (μ)	Sizes of sporoderma pores (nm)		Ecological and biological considerations		
		Diameter	Depth	Substrate	Altitude	Sexuality
Tetraplodon angustatus	3—5×2—3	165	45	fimicolous cadaveric	mountainous (superior) subalpine alpine	monoic
Tetraplodon urceolatus	5—7×3—4	128	38	fimicolous	subalpine alpine	monoic
Splachnum sphaericum	6—7×3—4	154	42	fimicolous	mountainous subalpine alpine	monoic and dioic

The more opaque space between the exine pores is a little larger than the diameter of a pore which is up to 154 nm. Their depth is of 42 nm as it can be seen from the electron microscopic images. One can also notice the "moiré pattern" in some portions (Pl. 2 C, arrow).

*

From the electron microscopic analysis of spores of the three species of the *Splachnaceae* family it can be seen that, as concerns the sporoderma ornamentations, they belong to one and the same type of ultramorphology of the exine outer layer. Certain variations can be noticed in some species with respect to the sizes of spores and especially of exine pores (their diameter and depth), synthetically given in Table 1, together with some aspects concerning their ecology, biology and spreading. We can consider that these particularities of spores, i.e. the ultramorphological as well as the ecological and biological ones, show to a great extent the adaptative ecologic and biologic character of the *Bryophyta* spores to the substrate.

The images of sporoderma ornamentations for the two species of the *Tetraplodon* genus are similar with those obtained by KOPONEN [5] in the case of *Tetraplodon mnioides*, by the scanning electron microscopy, proving the existence of the same type of organization of sporoderma.

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Only the dense space between pores seems to bulge to the outer layer in KOPONEN's photographs, it being probably due to carbon and golden layers after shadowing.

Similar ultrastructures are also described in case of the spores of *Tayloria octoblepharis* and *Voitia nivalis*, by the same author; in case of the other species analysed of *Tayloria* genus sporoderma has very different ornamentations, some of them being even prominent as those of *Polytrichaceae* [8], [12]. On the other hand, spores of *Funaria hygrometrica* (Ord. *Funariales*) have cylindrical prominent ornamentations ended with conical, sometimes branched, papilae [2], [8].

A somewhat similar organization of exine ultrastructure can be seen in the case of pollen exine of some *Angiospermae (Ipomoea purpurea)* belonging to the *Convolvulaceae* family [14].

Ecologically one can notice that the spores of *Splachnaceae* have some ecological and corological adaptative properties, due to their sporoderma characteristics, having within this *Bryophyta* family leafy mosses, which grow only under special ecological conditions, on a strictly determined substrate (either it is saprophitic, fimicolous or coprophitic, cadaveric or of turfy type) in places with high atmospheric (and edaphic) humidity, especially in the higher mountainous subalpine or alpine layer.

The ecological relations determined by the spreading of *Splachnaceae* spores by insects, especially by *Diptera*, could be also supported by the specific structure of sporoderma. In addition, the presence of spores in an aggregate form shows a biologically adaptative character, the spores being spread together, having more chances of germination and multiplication, no matter the species are monoic, sometimes one and the same species being either monoic or dioic *(Splachnum sphaericum)*.

Having in view all these remarks, data and preliminary interpretations we consider that all ornamentations, with their ultrastructure characteristics are not met by chance in the case of spores of *Splachnaceae* family, which is a taxonomic unity with a relatively small number of species (about 30) and it the only one within the *Bryophyta*, where the spreading of spores is made in a specialized and adapted way by means of insects *(Diptera)* and not by wind as in the case of other *Bryophyta*.

The electron microscopic analysis of spores in case of a great number of *Splachnaceae* can lead to the detailed explanation of the adaptative character of the spores of these *Bryophyta*, characterized by a particular ecology and biology.

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ETHREL-WIRKUNGEN AUF WACHSTUM UND ZELLZYKLUSABLAUF BEI WEIZENKEIMPFLANZEN (*TRITICUM AESTIVUM* L.)

VON

GEORGETA LAZĂR-KEUL, ROZALIA VINTILĂ und M. KEUL

Continnuous treatment of wheat seedlings with ethrel (50 ; 100 and 200 ppm) inhibits the extension growth and the fresh weight of roots and shoots, and lowers the cell division rate in the root meristem, proportionally to the applied concentration. Feulgen-cytophotometric determinations of nuclear DNA content in the root meristem show that ethrel increases the percentage of G_1-cells and reduces that of G_2-cells. These results suggest that ethrel may affect the cell cycle by the prolongation of the G_1-phase duration, preventing the progression of the cells into the S-phase.

Der synthetische Wirkstoff Ethrel (2-Chloräthylphosphonsäure; CEPA; Ethephon) beeinflußt grundlegende wachstums- und entwicklungsphysiologische Prozesse der Pflanzen über die Freisetzung von Äthylen und hat auf dieser Basis breite Anwendungsmöglichkeiten in der landwirtschaftlichen Praxis gefunden [6], [11], [23].

Äthylen ist als weitverbreitetes endogenes Produkt der Pflanzengewebe zusammen mit Auxinen, Gibberellinen und Cytokininen an der Regulation des pflanzlichen Stoffwechsels beteiligt und wird daher von vielen Autoren als Komponente des phytohormonalen Komplexes angesehen [12], [21]. Die pflanzenphysiologischen Reaktionen auf Umwelt- und Stressfaktoren, sowie auf Behandlungen mit Auxinen und Auxin-Herbiziden, wie 2,4-D und Dicamba, sind mit einer Zunahme der endogenen Äthylen-Konzentration korreliert und zeigen im allgemeinen die für exogene Äthylen-Behandlungen charakteristischen Merkmale [9], [10], [21], [27], [29].

Ethrel und exogenes Äthylen verändern das endogene Äthylen-Niveau, den Auxin-Metabolismus und das normale Verhältnis der verschiedenen Phytohormone, die Wachstum und Entwicklung kontrollieren [19], [21]. Beide Substanzen hemmen die Zellteilung und die DNS-Synthese in Meristemen [2], [13], die Zellstreckung bzw. das Längenwachstum der Organe [1], [3], [24] und induzieren mannigfaltige andere morphologische und physiologische Effekte [11], [21]. Dagegen ist hinsichtlich der Wirkung von Äthylen und Ethrel auf den Zellzyklus in pflanzlichen Meristemen relativ wenig bekannt.

In vorliegender Arbeit wurde die Wirksamkeit eines vom Chemischen Zentrum Timişoara synthetisierten Ethrel-Präparates im Vergleich zu einem Standard-Präparat ("Ethrel-Amchem") untersucht. Zur Beurteil-

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ung der Ethrel-Wirkung wurde die Wachstumsdynamik von Weizenkeimpflanzen verfolgt und Feulgen-cytophotometrische Bestimmungen des Kern-DNS-Gehaltes im Wurzelmeristem durchgeführt.

MATERIAL UND ARBEITSMETHODE

Winterweizenkaryopsen [*Triticum aestivum* L., ssp. *vulgare* (Vill., Host.) Mac Kay, cv. "Dacia"] wurden in Linhardt-Schalen auf Filterpapierunterlagen mit destilliertem Wasser (Kontrolle) bzw. mit Ethrel-Lösungen (50; 100 und 200 ppm) zur Keimung angesetzt. Keimung und Anzucht erfolgten bei Zimmertemperatur und im Dunkeln. Die beiden Ethrel-Präparate enthalten ungefähr die gleiche Konzentration an aktiver Substanz (40 %).

Die Wachstumsdynamik des Wurzelsystems und der Sproßachse wurde durch tägliche Längenmessungen an 20 Keimlingen/Schale in 3 Parallelproben pro Variante bis zum 6. Tag nach dem Ansetzen verfolgt. Am Versuchsende wurde das Frischgewicht der Keimpflanzen bestimmt.

Die Ethrel-Wirkung auf den Zellzyklus im Wurzelmeristem wurde nach 72stündiger Behandlungsdauer aufgrund der Feulgen-cytophotometrischen Bestimmung des relativen DNS-Gehaltes der Zellkerne untersucht. Die Wurzelspitzen wurden 2 Stunden in Äthylalkohol-Eisessig (3 : 1) fixiert, anschließend 15 min bei 28°C in 5 N HCl hydrolysiert und 2 Stunden im Schiff-Reagens gefärbt. Die cytophotomestrische Bestimmung des relativen DNS-Gehaltes erfolgte an Quetschpräparaten nach der Zwei-Wellenlängen-Methode unter standardisierten Bedingungen [14], [15]. Die Messungen wurden mit einem Ortholux-Mikroskop mit Photometer-Aufsatz MPE und Geradsicht-Spiegel-Monochromator (Leitz) durchgeführt. Die Arbeitswellenlängen (520 und 487 nm) wurden mikrospektrophotometrisch an 10 Feulgen-gefärbten Interphasenkernen ermittelt. Je Variante wurden mindestens 100 Interphasenkerne gemessen und ihr DNS-Gehalt in Arbeitseinheiten (AE) nach Mendelsohn [20] umgerechnet. Die diploiden (2C) und tetraploiden (4C) Referenzwerte wurden an je 25 Telo- und Metaphasenkernen pro Variante ermittelt. Der prozentuelle Anteil der Zellzyklusphasen wurde anhand der Referenzwerte berechnet [15].

ERGEBNISSE UND DISKUSSION

Die Reaktion der Weizenkeimpflanzen auf die andauernde Behandlung mit Ethrel zeigt die für die Äthylen-Wirkung typischen Wachstumshemmungen und morphologischen Effekte.

Im Bereich der untersuchten Konzentrationen (50; 100 und 200 ppm) wird sowohl das Wachstum des Wurzelsystems (Abb. 1) als auch das der Koleoptile bzw. Primärblätter (Abb. 2) proportional zur Ethrel-Dosis reduziert. Die Keimung der Karyopsen wird durch Ethrel praktisch nicht beeinflußt.

Die Hemmwirkung von Ethrel auf das Längenwachstum der Keimlinge äußert sich schon in den ersten 48 Stunden nach dem Ansetzen während der Ausbildung der embryonalen Wurzeln und der Koleoptile.

Das Ausmaß dieser bereits im Anfangsstadium der Pflanzenentwicklung induzierten Wachstumshemmung bleibt für die jeweilige Konzentration während der gesamten Versuchszeit von 6 Tagen ziemlich konstant. Die Hemmung der mittleren Wurzellänge beträgt je nach Konzentration

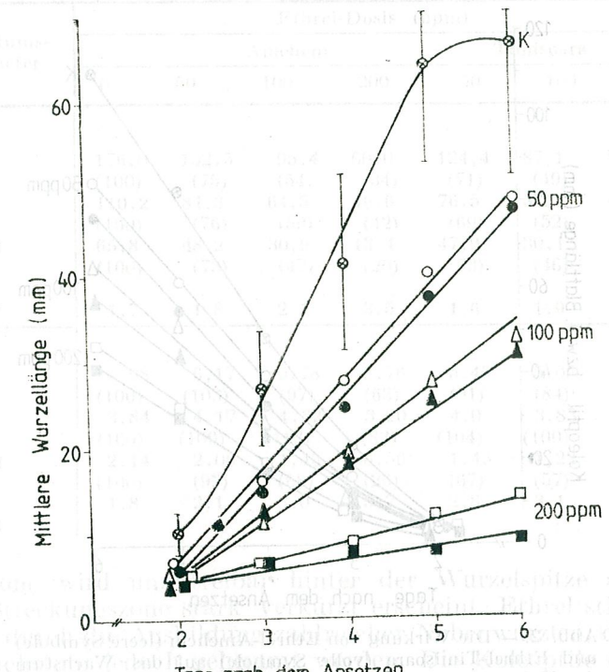

Abb. 1. — Die Wirkung von Ethrel-Amchem (leere Symbole) und Ethrel-Timişoara (volle Symbole) auf das mittlere Wurzelwachstum von Weizenkeimpflanzen (K=Kontrolle).

zwischen ca. 40 (50 ppm) und 80 % (200 ppm), die der Koleptile- und Primärblattlänge zwischen ca. 20 (50 ppm) und 60 % (200 ppm). Die Wirksamkeiten der beiden Ethrel-Präparate verschiedener Herkunft sind annähernd gleich; die konstant etwas betontere Hemmwirkung von Ethrel-Timişoara ist wohl auf einen höheren Gehalt an aktiver Substanz zurückzuführen.

In Tabelle 1 ist der Einfluß von Ethrel auf das Längenwachstum und das Frischgewicht der Keimlinge am Ende der Versuchszeit nach andauernder Behandlung zusammengefaßt. Es ist ersichtlich, daß das mittlere Längenwachstum der Wurzeln allgemein stärker gehemmt wird als das der Koleoptile und Primärblätter. Diese Unterschiede in der Hemmwirkung von Ethrel auf das Sproß- und Wurzelwachstum nehmen mit steigenden Konzentrationen zu und äußern sich in fortschreitend höheren Werten des Sproß/Wurzel-Verhältnisses. Das Frischgewicht der Keimlinge wird ebenfalls proportional zur Ethrel-Dosis herabgesetzt, wobei die Hemmwirkung auf das Wurzelsystem wieder stärker betont ist. Im Ver-

gleich zur drastischen Hemmung des Längenwachstums sind die Ethrel-Wirkungen auf das Frischgewicht schwächer ausgeprägt und im Falle des Sproßes bei 50 und 100 ppm sogar leicht stimulierend. Das auf die Frischmasse bezogene Sproß/Wurzel-Verhältnis nimmt mit höheren Ethrel-

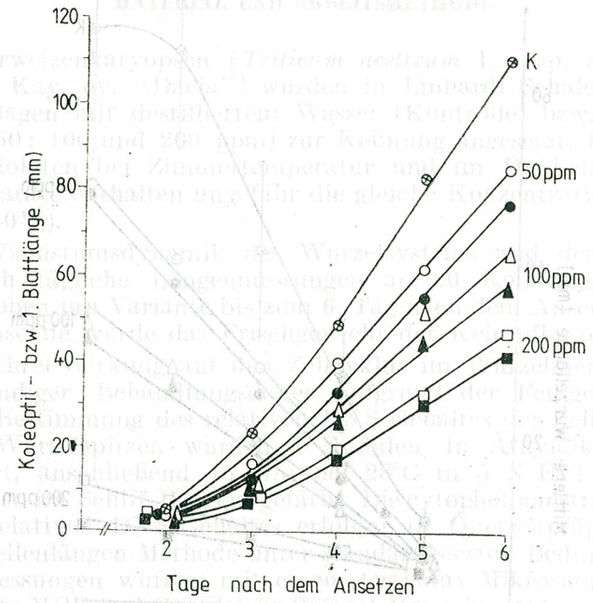

Abb. 2. — Die Wirkung von Ethrel-Amchem (leere Symbole) und Ethrel-Timişoara (volle Symbole) auf das Wachstum der Koleoptile und des 1. Blattes von Weizenkeimpflanzen (K = Kontrolle).

Dosen ebenfalls zu, übertrifft jedoch die für das Längenwachstum berechneten Werte.

Die betontere Hemmung des Wurzelwachstums ist einerseits durch den andauernd direkten Kontakt zwischen Ethrel und Wurzelsystem bedingt; andererseits gibt es Hinweise dafür, daß das Wurzelsystem im Vergleich zum Sproß eine auch für Auxin und Auxin-Herbizide bekannte höhere Empfindlichkeit gegenüber Äthylen und Ethrel aufweist [1], [10]. Die Hemmung des Sproßwachstums durch Ethrel ist wahrscheinlich eine direkte Reaktion auf das von der Wurzel in den Sproß gelangte Äthylen, möglicherweise jedoch auch eine indirekte Folge der Beeinträchtigung der Wurzelfunktionen. In diesem Sinne ist bekannt, daß Ethrel die Wasserabsorption reduziert [19], während Äthylen die Absorption und Translokation der Mineralstoffe, inbesondere der Phosphorionen, herabsetzt [10].

Die Hemmung des Längenwachstums durch Ethrel wird von charakteristischen morphologischen Effekten begleitet. Die Seminalwurzeln sind wellenförmig oder spiralig verkrümmt und ungleichmäßig verdickt. Die

Tabelle 1

Die Wirkung von Ethrel auf die Pflanzenlänge (mm) und die Frischmasse (g/50 Keimlinge) von Weizenkeimpflanzen am 6. Tag nach dem Ansetzen (in den Klammern = %-Werte im Vergleich zur Kontrolle)

Wachstums-parameter	Ethrel-Dosis (ppm)						
	Amchem				Timişoara		
	0	50	100	200	50	100	200
Länge							
gesamt	176,0	132,5	95,4	60,0	124,4	87,1	50,3
	(100)	(75)	(54)	(34)	(71)	(49)	(29)
Sproß	110,2	84,3	64,5	46,6	76,5	57,0	41,7
	(100)	(76)	(59)	(42)	(69)	(52)	(38)
Wulzel	65,8	48,2	30,9	13,4	47,9	30,1	8,6
	(100)	(73)	(47)	(20)	(73)	(46)	(13)
Sproß/ Wurzel	1,7	1,8	2,1	3,5	1,6	1,9	4,9
Masse							
gesamt	5,98	6,17	5,78	3,76	5,43	5,05	3,08
	(100)	(103)	(97)	(63)	(91)	(84)	(52)
Sproß	3,84	4,17	4,32	3,20	4,0	3,83	2,63
	(100)	(109)	(113)	(83)	(104)	(100)	(68)
Wurzel	2,14	2,00	1,46	0,56	1,43	1,22	0,45
	(100)	(93)	(68)	(26)	(67)	(57)	(21)
Sproß/ Wurzel	1,8	2,1	3,0	5,7	2,8	3,1	5,8

Wurzelhaarzone wird unmittelbar hinter der Wurzelspitze ausgebildet, so daß die Streckungszone stark verkürzt erscheint. Ethrel stimuliert die Rhizogenese durch die Ausbildung zahlreicher Nebenwurzlen, die in ihrem weiteren Wachstum jedoch gehemmt werden. Die Koleoptile der Ethrel-behandelten Pflanzen sind verbreitert, während der Durchbruch und die Entfaltung der Primärblätter stark verzögert werden. Ähnliche Symptome der Äthylen- und Ethrel-Wirkung sind wiederholt beschrieben worden [10], [25], [26], [28].

Exogenes Äthylen beeinflußt das Wachstum über eine Erhöhung des endogenen Äthylen-Spiegels und durch die Veränderung des normalen Verhältnisses des wachstumsregulierenden Phytohormone [28]. Die induzierten Symptome zeigen eine auffallende Übereinstimmung mit den Effekten supraoptimaler Dosen von Auxinen und Auxin-Herbiziden [3], [11], [27], [29], die ebenfalls mit einer Erhöhung der endogenen Äthylen-Konzentration verbunden sind [9], [21]. Auf dieser Grundlage wurde vermutet, daß die Auxine ihre wachstumsregulierende Rolle über die endogene Bildung von Äthylen ausüben [21]. Wahrscheinlicher ist die Annahme, daß Auxin und Äthylen komplementär wirken [5].

Äthylen-Behandlungen modifizieren die Wachstumsrichtung und -intensität durch die Hemmung der Zellstreckung und die Stimulierung der radialen Expansion, wodurch die normale Wachstumssymmetrie gestört wird [3]. Diese Wirkungen auf die Zellform und -dimension sind mit Änderungen in der Orientierung der Zellwandfibrillen verbunden [24]. Äthylen beeinflußt die endogene Auxin-Konzentration, möglicher-

weise über eine Wirkung auf das Auxin-Oxydase-System [19], und hemmt den polaren bzw. stimuliert den lateralen Auxin-Transport [3], so daß die normale Auxin-Verteilung verändert wird. Die durch Ethrel verursachten Wachstumsanomalien, die sich in Krümmungen und Schwellungen äußern, sind mit lokalen Erhöhungen der Auxin-Konzentration korreliert [26], [28]. Außerdem hemmt Äthylen die für die Zellstreckung und Zelldifferenzierung notwendige DNS-Synthese [2], [3], [31].

Die Hemmung der DNS-Synthese durch Äthylen ist im Bereich der Meristeme mit einer Reduzierung der mitotischen Aktivität verbunden [2], [13], [18], [21]. Die Hemmung der Zellproliferation ist neben der direkten Einwirkung auf die Zellstreckung [10] ein wichtiger Mechanismus für die wachstumshemmende Äthylen- und Ethrel-Wirkung [2], [13]. Im Einklang mit den obigen Angaben konnte in vorliegender Untersuchung festgestellt werden, daß Ethrel den Mitose-Index im Weizenwurzelmeristem proportional zur angewandten Dosis um 20—65 % herabsetzt. Durch Feulgen-cytophotometrische Bestimmungen des Kern-DNS-Gehaltes wurde versucht, Hinweise über die Ethrel-Wirkung auf den Ablauf des Zellzyklus im Wurzelmeristem zu gewinnen.

Die in Abb. 3 dargestellten DNS-Häufigkeitsverteilungen zeigen, daß Ethrel im Vergleich zum Kontrollmeristem eine Erhöhung des 2C-

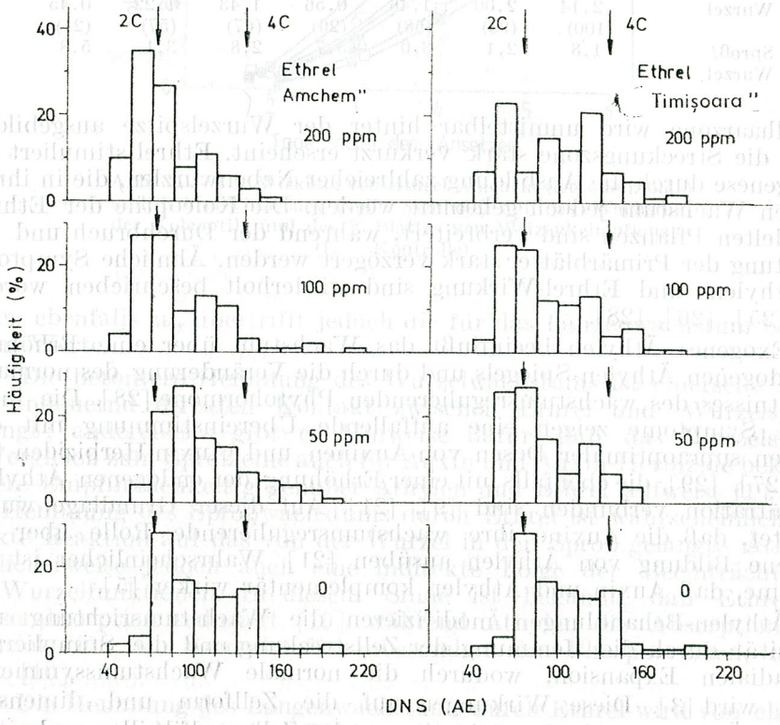

Abb. 3. — Die Wirkung von Ethrel auf die DNS-Häufigkeitsverteilung im Weizenwurzelmeristem im Vergleich zur Kontrolle.

Gipfels und eine Verringerung der 4C-Kerne verursacht. Eine polyploidisierende Wirkung von Ethrel wurde nicht festgestellt. Aus der prozentuellen Verteilung der Zellkerne auf die Phasen G_1, S und G_2 des Zellzyklus ist ersichtlich (Abb. 4), daß beide Ethrel-Präparate im Bereich der untersuchten Konzentrationen annähernd die gleiche Wirkung auf die mitotische Aktivität ausüben, wobei der Anteil der G_1-Phasenkerne erhöht, derjenige der G_2-Kerne dagegen verringert und der der S-Kerne ungefähr konstant bleibt. Diese Befunde lassen auf eine Verlängerung der G_1-Phasendauer schließen, während die Reduzierung der G_2-Kerne im Einklang mit der niedrigen Mitose-Rate auf eine verminderte Proliferationsintensität hinweist.

Über den Einfluß von exogenem Äthylen auf den Zellzyklus ist relativ wenig bekannt. Nach den bisherigen Angaben, ist die Blockierung der Mitoseaktivität durch Äthylen wie im Falle der Auxin-Wirkung eng mit der Hemmung der DNS-Synthese verknüpft und der Zellzyklus wird

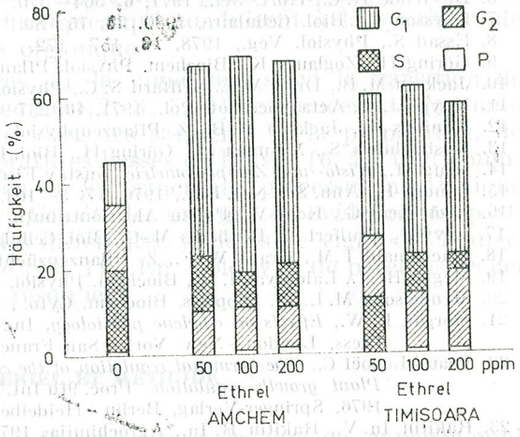

Abb. 4. — Die Wirkung von Ethrel auf die prozentuelle Verteilung der Zellzyklusphasen im Weizenwurzelmeristem im Vergleich zur Kontrolle. G_1 = Posttelophase; S = DNS-Synthesephase; G_2 = prämitotische Phase; P = polyploide Zellkerne.

zwischen S-Phase und Prophase unterbrochen [2], [16], [30]. Demgegenüber deuten unsere Ergebnisse darauf hin, daß der Zellzyklus unter der Einwirkung von Ethrel durch die Verlängerung der G_1-Phase gehemmt wird, so daß der Eintritt in die DNS-Synthese-Phase verzögert wird. Im Wurzelmeristem von *Pisum sativum* und *Zea mays* wurde ebenfalls eine Verlängerung des Zellzyklus durch Äthylen während der G_1-Phase festgestellt [4]. Eine vorübergehende Blockierung des Zellzyklus in der G_1-Phase wird besonders unter der Einwirkung ungünstiger Faktoren induziert [8], und es ist bekannt, daß Stresswirkungen die endogene Äthylen-Produktion erhöhen [9], [10].

Aufgrund der erzielten Ergebnisse ist anzunehmen, daß exogene Äthylen-Behandlungen wahrscheinlich am Ende der G_1-Phase in den Zellzyklusablauf eingreifen und den Eintritt in die DNS-Synthese-Phase verhindern. Über den Mechanismus dieser Wirkung können nur Vermutungen angestellt werden. Wahrscheinlich wirkt Äthylen über die Regulationsmechanismen des Zellzyklus. Der Zellzyklus wird durch spezifische Proteinsynthesen unter hormoneller Kontrolle gesteuert [17], [22] und es wird

vermutet, daß Veränderungen im Verhältnis der Phytohormone für die Hemmung der Proliferation verantwortlich sind [7]. Der Übergang der G_1-Kerne in die S-Phase wird durch eine spezifische Proteinsynthese ausgelöst [17], die durch ein Auxin kontrolliert wird [22]. Hypothetisch könnte man demnach annehmen, daß Äthylen-Behandlungen den Ablauf des Zellzyklus durch die Verzögerung der G_1-S-Transition über eine Veränderung des Hormon-Spiegels hemmen.

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LA DYNAMIQUE DES GLUCIDES PENDANT LA PÉRIODE DE VÉGÉTATION DES DRAGEONS DE VIGNE

PAR

ANCA ANTOHE

The researches carried out on the "Aligoté" and "Feteasca neagră" vine varieties continue a previous experiment conducted in north-eastern Moldova.
The glucide dynamics in the vegetation period shows the physiological differences between varieties and the peculiarities of the ecological adaptation of the native one.

La croissance et le développement de la plante impliquent une série de processus physiologico-biochimiques qui conduisent à la synthèse des différentes formes de matière organique.

Des recherches antérieures sur la vigne ont suivi la variation des glucides des drageons et des feuilles [2, 3, 5, 6], les processus d'accumulation des hydrates de carbone dans les sarments et la préparation des tissus pour résister aux températures basses de l'hiver [6, 7], tout comme l'influence des facteurs de milieu sur les principaux processus métaboliques qui ont lieu dans la plante [8]. Ces études ont mis en évidence les particularités physico-écologiques des différentes variétés de vigne de Transylvanie, d'Olténie et de Munténie [10, 11]. En Moldavie, de pareilles recherches n'ont pas été entreprises jusqu'ici.

MATÉRIEL ET MÉTHODE

La présente étude a été effectuée dans la Station expérimentale horti-viticole Iaşi dans des conditions d'expérimentation identiques à celles mentionnées dans le travail antérieur [1]. C'est une étude plus complexe sur les particularités biologiques des variétés de cru Aligoté et Feteasca neagră.

La dynamique des glucides a été suivie dans les feuilles, les internœuds et les bourgeons avec leurs nœuds d'insertion sur trois catégories de drageons : de la base, du milieu et du bout du sarment. Les échantillons ont été prélevés selon la présence ou l'absence de l'inflorescence et on a homogénéisé 3—4 feuilles, 3—4 internœuds et 3—4 bourgeons avec leurs nœuds. Les échantillons ont été prélevés par phénophases, à savoir : la pousse active de la plante, la grande période d'accroissement en longueur des drageons, la période qui suit la floraison, le mûrissement, la maturation des fruits et le commencement de la chute der feuilles.

Les glucides totaux (saccharoses directement réducteurs, polyoses solubles dans l'eau, polyoses insolubles dans l'eau) ont été déterminés

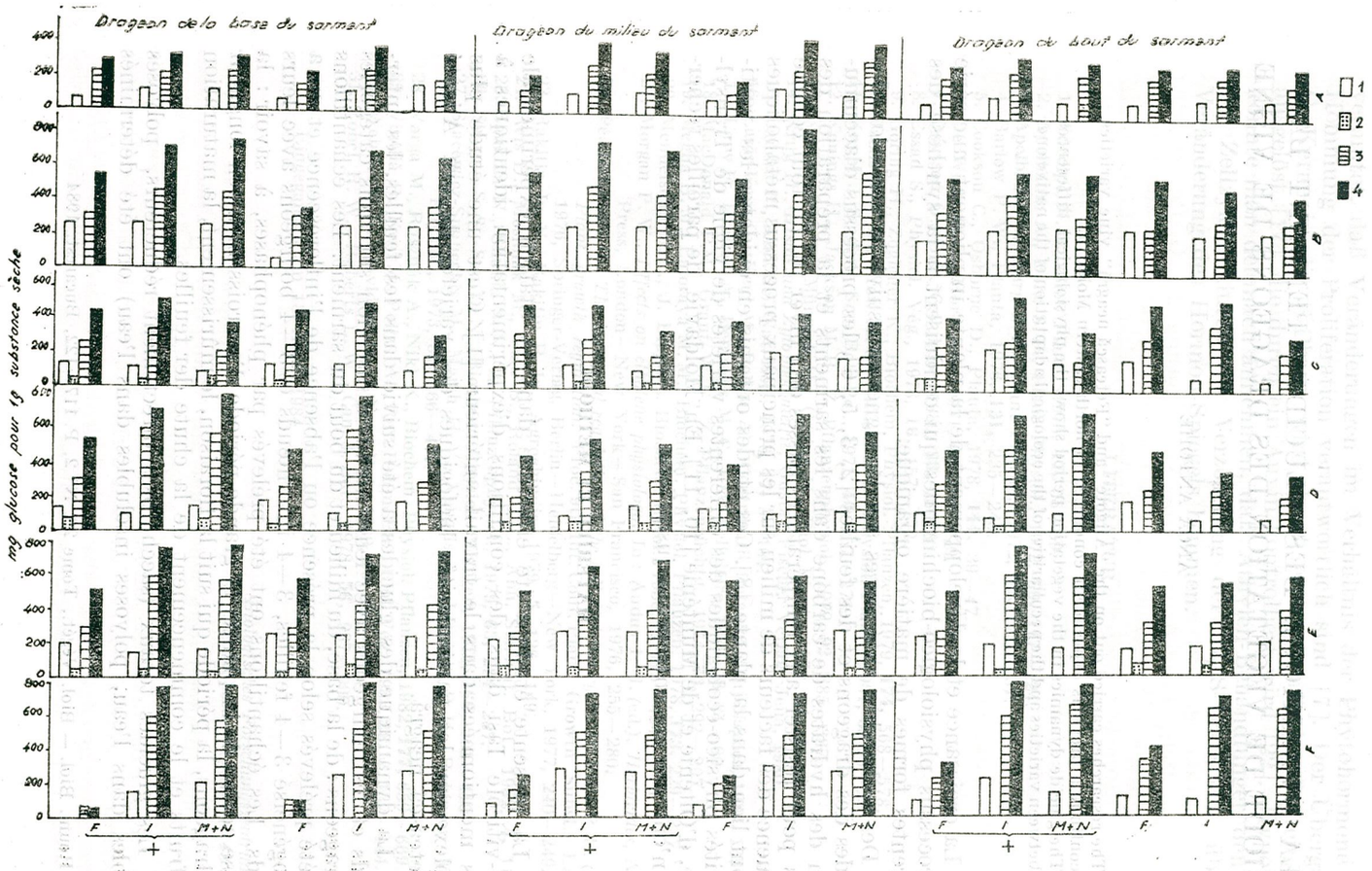

Fig. 1. — La variation quantitative et qualitative des glucides dans la période de végétation de la variété Aligoté : *1*, glucides directement réducteurs ; *2*, polyoses solubles dans l'eau ; *3*, polyoses insolubles dans l'eau ; *4*, glucides totaux. F = feuilles ; I = internœud ; M + N = bourgeon et nœud ; + = inflorescence ; A = croissance active ; B = la grande période de croissance en longueur des drageons ; C = la période qui suit la floraison ; D = le moment où les fruits commencent à mûrir ; E = la maturité des fruits ; F = le début de la chute des feuilles.

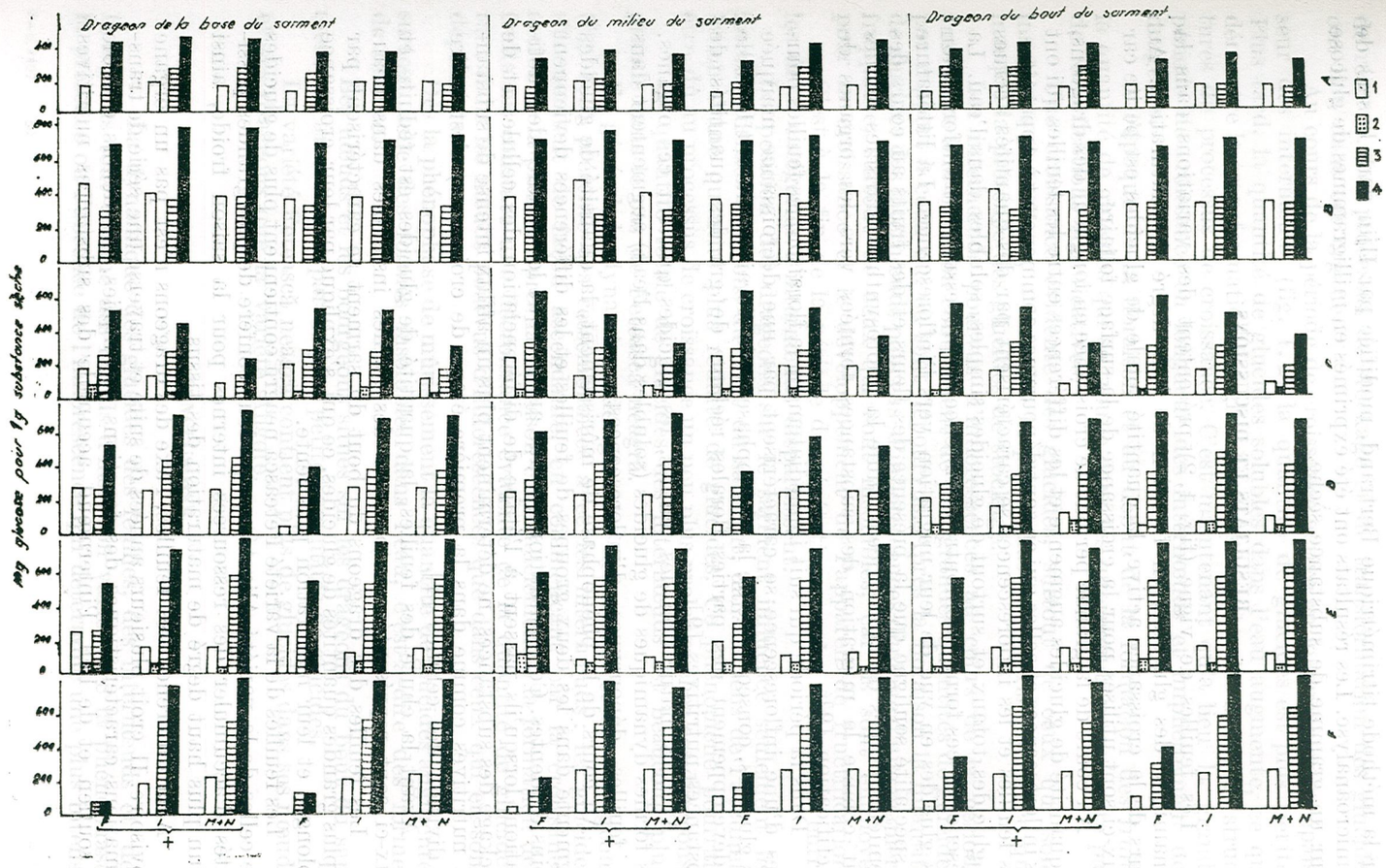

Fig. 2. — La variation quantitative et qualitative des glucides dans la période de végétation de la variété Feteasca neagră. Même légende que pour la figure 1.

par la méthode titrimétrique Bertrand, modifiée par Iljin dans le sens de la microanalyse. Les résultats ont été exprimés en milligrammes de glucose pour un gramme de substance sèche.

RÉSULTATS ET DISCUSSIONS

Les feuilles de vigne (fig. 1, 2) présentent des variations dans la dynamique des glucides, en fonction de leur degré de maturation. Au cours de la croissance active, la quantité totale de glucides est petite car ceux-ci sont utilisés pour la croissance de la surface foliaire.

Au cours de la grande période de croissance en longueur des drageons, la quantité de glucides augmente et les différences entre les feuilles qui ont des inflorescences et celles qui en sont dépourvues commencent à apparaître. Les feuilles et les inflorescences se caractérisent par des quantités accrues de glucides totaux et surtout de glucides simples, solubles dans l'eau. La présence de ces formes démontre l'existence du processus de transformation des glucides en vue de leur migration vers l'inflorescence. La littérature de spécialité souligne que la présence des fleurs et des fruits au cours des premières étapes de croissance stimule la photosynthèse dans les feuilles tout comme la migration des substances formées vers les organes de fructification [9].

Dans la période qui suit immédiatement la floraison, les feuilles pourvues d'une inflorescence se caractérisent par une décroissance marquée des glucides, conséquence de la phénophase de la floraison, durant laquelle le développement des parties florales réclame de grandes quantités de substances nutritives [9].

Avec la maturation des feuilles, les glucides quittent ces organes. Les formes dynamiques de glucides (solubles dans l'eau) augmentent dans les feuilles dépourvues d'inflorescences.

Au cours de la phénophase de mûrissement, la quantité de glucides augmente dans les deux groupes de feuilles et les différences deviennent moins évidentes. Cette situation est due au fait que les organes de fructification, lorsqu'ils passent à l'étape de différenciation des cellules et de stockage des substances, ne déterminent plus un afflux intense de substances nutritives comme dans la première étape de croissance, c'est-à-dire de division des cellules [4].

Avant la chute des feuilles, la quantité de glucides est réduite et ceux-ci sont représentés surtout par des formes insolubles dans l'eau.

Les feuilles des drageons du bout du sarment se caractérisent par de plus grandes quantités de glucides, ce qui s'explique par leur croissance prolongée et leur maturation plus lente.

Les feuilles de la variété Fetească neagră contiennent plus de glucides que celles de la variété Aligoté, ce qui leur confère de plus grandes possibilités d'accumuler des ressources internes pour la saison froide, ainsi qu'un plus haut degré de maturation des tissus.

À la différence des feuilles, l'axe des drageons n'est pas un organe provisoire. Il croît plusieurs années de suite et traverse une série de transformations caractéristiques des fonctions accomplies. En dehors du rôle de soutien et de lien, l'internœud accumule des substances nutritives

de réserve. Les processus de stockage et de transformation des glucides ont lieu dans cet organe.

Au cours de la période de croissance active en longueur et en épaisseur des internœuds (fig. 1, 2) la quantité de glucides est plus petite. Plus tard, la quantité de glucides solubles dans l'eau augmente, ce qui démontre la transformation des glucides dans le cadre des nouvelles structures du processus de croissance. C'est maintenant qu'a lieu la grande période de croissance en longueur des internœuds et implicitement des drageons.

A l'époque de la floraison et au début de la formation des fruits la quantité de glucides qui se trouve dans les internœuds ne présente pas de modifications significatives.

Une croissance évidente des glucides, surtout de ceux qui sont insolubles dans l'eau, a lieu dès la phénophase de mûrissement, ce qui marque la présence des glucides de stockage.

Les internœuds pourvus d'inflorescences au cours de la période antérieure à la floraison contiennent de plus grandes quantités de glucides par rapport à ceux qui en sont dépourvus. Après la floraison la quantité de glucides des internœuds pourvus d'inflorescences diminue d'une manière frappante, surtout de ceux qui sont insolubles, car ils sont employés pour la croissance et le développement de l'appareil de fructification.

C'est à la fin de la période de végétation que les différences entre les deux catégories d'internœuds disparaissent.

La position des drageons sur le sarment influence seulement les internœuds des drageons du bout du sarment, dans le sens de l'accumulation d'une quantité plus réduite de glucides. Ce phénomène pourrait être dû au processus de croissance un peu plus prolongé chez ces drageons.

La quantité totale de glucides des internœuds est beaucoup plus grande dans le cas de Fetească neagră.

En général, la dynamique des glucides des nœuds et des bourgeons (fig. 1, 2) présente des ressemblances avec les internœuds : les glucides s'accumulent de façon ascendante, en commençant par la croissance active de la plante. Après la floraison et parallèlement à la formation des fruits on constate une décroissance évidente de la quantité de glucides tant dans les nœuds pourvus d'inflorescences que dans ceux qui en sont dépourvus.

Dès la phénophase de mûrissement la quantité de glucides augmente, surtout celle des formes insolubles dans l'eau, c'est-à-dire des formes de réserve.

Les nœuds et les bourgeons situés sur les drageons du bout du sarment se caractérisent par de plus petites quantités de glucides.

La variété Fetească neagră contient plus de glucides dans ses nœuds et ses drageons que la variété Aligoté.

CONCLUSIONS

— La dynamique des glucides des feuilles, des internœuds et des bourgeons tout comme des nœuds d'insertion varie avec la phénophase, la présence ou l'absence de l'inflorescence qui détermine une plus intense

photosynthèse de la feuille, un afflux accru de substances nutritives nécessaires à la croissance et au développement de l'appareil de fructification.

— Les drageons situés au bout du sarment se caractérisent par des quantités réduites de glucides, conséquence de leur croissance prolongée due au phénomène de polarité.

— La quantité totale de glucides des feuilles, des internœuds et des bourgeons de la variété Feteasca neagră est beaucoup plus grande ce qui dénote une résistance accrue dans les conditions écologiques locales de cette variété.

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SEASONAL NUTRIENTS FLUCTUATION IN LEAF COMPOSITION IN FOUR SPECIES OF A RAISED SPHAGNUM PEAT BOG

BY

LUCIA STOICOVICI

N, P, K and Ca concentrations were measured in the living leaf material in *Vaccinium myrtillus* L., *V. oxycoccos* L., *Andromeda polifolia* L. and *Eriophorum vaginatum* L. The nutrient content of litter fall in *Eriophorum vaginatum* was also analysed. Seasonal fluctuation was found in all ions examined in *Vaccinium myrtillus*, *Andromeda polifolia* and *Eriophorum vaginatum* as concerns the N, P and K nutrient elements content decline and the increase in Ca concentration as living leaves undergo senescence. Seasonal concentration changes of these ions are inversely related in *Vaccinium oxycoccos*. With reference to changes in substrate ions content the retention capacity for K, Ca and P essential elements by living and dead leaves especially in *Eriophorum vaginatum* is appreciated.

Several investigations [17, 18] have indicated the importance of mineral nutrients for different grades of bog and fen in controlling the characteristic mire species and plant communities. It was also studied [16] the influence of nutrient supply on the amounts of macroelements and trace elements taken up by principal mire species and plant communities. The present paper examines the actual amounts of macroelements N, P,K and Ca taken up by several mire species throughout the growing season from the underlying acid bog peat.

MATERIAL AND METHODS

It would appear from other results [7] that in view of the extremely low nutrient status of raised and blanket bogs, nutrient supply, accumulation and turnover relationships may be at their most critical. Accordingly, we have chosen for the present study two typical bog ericads, *Andromeda polifolia* and *Vaccinium oxycoccos*, as well as *Vaccinium myrtillus* with a wider distribution, and *Eriophorum vaginatum* the most characteristic and abundant species of our raised bogs. These species were obtained from a raised bog located in the village Coşna (Dorna region, altitude 867 m). From each species numerous individuals were collected in three occasions during the growing season and each time dead leaves were separated from living leaf material. The plant material was allowed to dry in the air and then ground prior to analysis. The samples disintegration was made both by dry combustion (in an electric muffle furnace) and by wet digestion (with nitric and perchloric acids). Aliquots of the

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final hydrochloric acid solution (from the residue remained after mineralisation) were analysed directly for potassium, phosphorus and calcium by flame photometry. A method of removing interfering ions has been adopted [14]. Calcium was also measured volumetrically by a titration method using an oxalate-permanganate procedure. Phosphorus was also determined photometrically using the ammonium molybdate method (Duval's procedure) [5] with ascorbic acid as an reduction agent. K, Ca and P were analysed as total forms. The Kjeldahl-Foerster method was applied for the total nitrogen determination [12, 14]. Samples of peat, taken at the rooting level, were routinely analysed for exchangeable phosphorus, potassium, calcium and total nitrogen [5, 11, 14].

RESULTS AND DISCUSSION

In ericaceous species growing on the same substrate, a different behavior may be recognized during the vegetation period. In *Vaccinium myrtillus* and *Andromeda polifolia* a decrease takes place in phosphorus, potassium and nitrogen concentrations towards autumn while calcium content increases in senescing leaves. This tendency is inversely displayed in *Vaccinium oxycoccos* (Fig. 1).

At the beginning of the vegetation period a marked uptake and use of mineral nutrients by young actively growing and metabolizing organs take place, the inorganic ions being almost entirely incorporated in young leaves. Hence, in spring and early in summer great fluctuations occur [9]. As older tissues senesce and before leaf fall the requirements are reduced in leaves, several mineral elements being redistributed or lost through the root system or possibly they are leached in rain, fog or dew [6]. The retention of these elements at the rooting level or in other perennial structures was also discussed.

We suppose that the pattern of essential ions accumulation in *Vaccinium oxycoccos* may be in relation with the vegetation growing type of this species to maintain for a long time the green leaves. The new leaves are being formed late in spring, at the end of May [15], the creeping stems yet bearing the leaves from last year and in autumn a drastic defoliation does not occur. The senescing leaves winter under the snow. The mineral elements cycling and their use seem to be delayed, potassium, phosphorus and nitrogen diminutions being not recorded. The N, P, and K concentrations decline in living and in dead leaves is more striking in *Eriophorum vaginatum* (Fig. 2). In the peat bog the leaf litter forms a thick layer both on the top and on the sides of the tall *Sphagnum* hummocks (above 30 cm). As previously mentioned [7] the needle-like leaves of this species grow from the base and die back from the tip, and both growth and die-back occur on a small scale simultaneously throughout the year in the *Eriophorum* community.

Our graphed results show that potassium accumulates five times, phosphorus three times and nitrogen two times more in the young living leaves collected in June than in the dead material. It is supposed that these elements are translocated away prior to die-back to other plant organs, maybe to the root system and thus not wholly incorporated

into the litter. By contrast, calcium accumulates one time more in dead leaves than in senescing leaves collected in autumn and about the same than in the young living leaves collected in June. A similar phenomenon is described for spruce and oak and it is stated [2] that the absorption

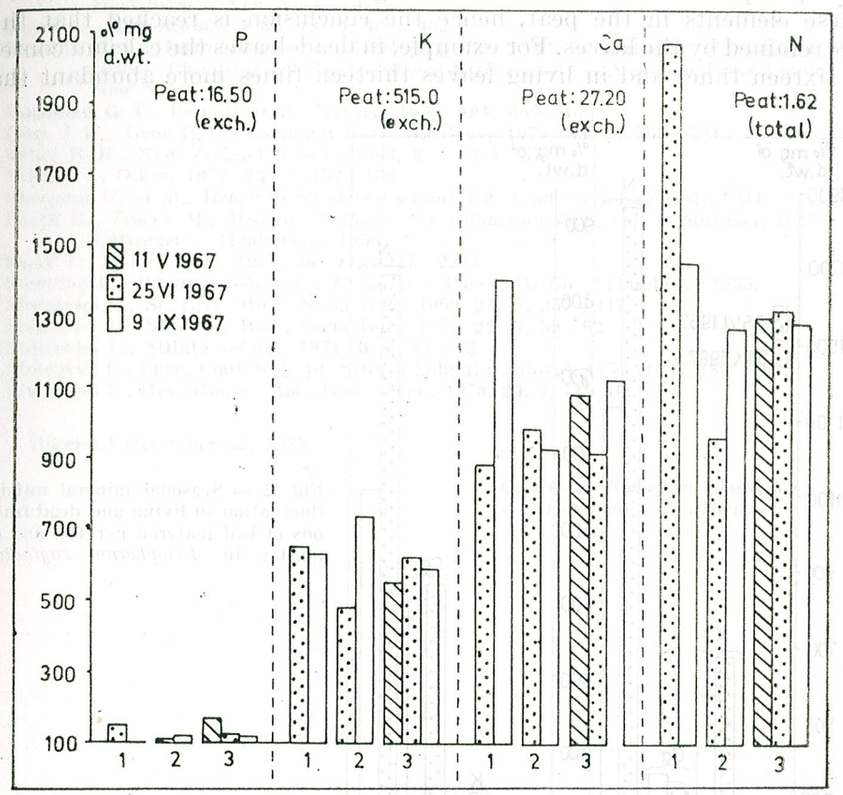

Fig. 1. — Seasonal mineral nutrient fluctuation in living leaves per 100 mg dry matter in three bog ericads. 1. *Vaccinium myrtillus*, 2. *Vaccinium oxycoccos*, 3. *Andromeda polifolia*. Exchangeable (exch.) or total (tot.) P, K, Ca, N nutrient content of peat included on appropriate graph as % mg dry peat except for N which is % g dry peat.

of divalent ions (Ca, Mg) by the organic acids in the litter is stronger than that of monovalent ions (K, Na). On the other hand, the increase in the calcium content of the dead material may be due to a fall in dry weight caused by breakdown of protein prior to die-back [7]. The results of our mineral analyses would be in line with those obtained for herbaceous and wooden species by several workers [1, 3, 4, 8, 9, 10].

From another point of view, it is noticed that potassium is four times and total nitrogen nearly twice more abundant in the acid bog

peat (Fig. 1) than in the dead leaves; but there is an inverse relationship for living leaves. It is noteworthy that exchangeable potassium in the peat reaches the highest concentration among other exchangeable forms of phosphorus and calcium. On the other hand, the amounts of calcium and phosphorus in the dead and living leaves are above the values of these elements in the peat, hence the conclusion is reached that they are retained by the leaves. For example, in dead leaves the calcium content is sixteen times and in living leaves thirteen times more abundant than

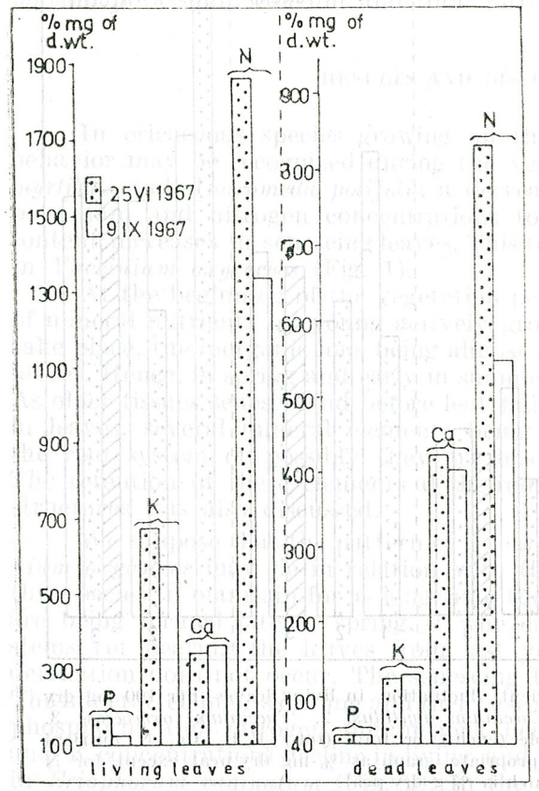

Fig. 2. — Seasonal mineral nutrient fluctuation in living and dead fractions of leaf material per 100 mg dry matter in *Eriophorum vaginatum*.

in the peat. In living leaves the phosphorus content is ten times and in dead leaves three times more abundant than in the peat. It seems that calcium and phosphorus in the living or dead material are not easily washed out by rainfall or other factors. It is also supposed that the living plants of *Eriophorum* which are continuously growing on the peat have the capacity to take up and use phosphorus and calcium in their available form from litter fall or from recently formed peat. In his contribution Small [13] refers to bog species which were found to reabsorb significantly more nitrogen from their foliage preceding leaf fall than non-bog species.

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ASPECTS DE LA VARIABILITÉ PHÉNOTYPIQUE CHEZ LES CULTOVARIÉTÉS ET LES HYBRIDES DE PAVOT

PAR

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The morpho-anatomical features of poppy plants are variable (for the same variety in time, and from one variety to the other, within the same period). The bioproductivity elements registered the best values in the varieties obtained from personal material (seeds).
The morphine content was variable in time, registering values between 0.11% and 0.90%. The lowest amount was usually found in self-pollinated plants.

Les efforts de l'humanité sont dirigés, avec une insistance croissante, vers l'utilisation rationnelle et non destructive des ressources naturelles. Dans ce contexte, on accorde une attention particulière aux plantes médicinales [2]. En même temps que l'exploitation intensive des plantes connues depuis des millénaires, d'autres plantes, pas encore utilisées, sont comprises dans cette sphère d'activité. Les sélectionneurs et les améliorateurs [5, 9, 10] s'occupent de plus en plus fréquemment de toutes les deux catégories d'espèces.

LE BUT DES INVESTIGATIONS

Adeptes de l'idée selon laquelle la variabilité individuelle assure à la sélection (naturelle ou artificielle) le matériel initial, nous avons considéré comme juste la culture de plusieurs cultivars, lignes ou hybrides dans le même champ d'expérimentation. De la sorte, nous avons satisfait à plusieurs desiderata :

— l'établissement du rôle des facteurs génétiques et de milieu dans la transmission de certains caractères ;

— la mise à l'épreuve des capacités combinatoires des différentes espèces ;

— l'obtention d'individus à hétérosis élevé et en même temps extrêmement variables.

Nous avons également estimé que, par la culture en commun de plusieurs cultivars, hybrides et lignes laissés en libre pollinisation, on peut créer une population avec un fonds accru de gènes. On y pourrait séparer ultérieurement des individus qui, par purification et stabilisation, produiraient de nouveaux cultivars, à hautes qualités bioproductives.

Les recherches de ce genre sont de plus en plus nombreuses [1, 3, 10—14, 16—18]. Les données d'observation et d'expérimentation obtenues constituent une aide précieuse dans l'activité de sélection et d'amélioration.

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MATÉRIEL ET MÉTHODES

Les observations et les analyses du présent ouvrage ont visé douze variantes de diverses provenances (dont quatre cultivars, deux hybrides, une ligne et une cultovariété obtenue de semences produites antérieurement au cours d'expérimentations propres). Pour chaque variante on a assuré, au commencement, une seule répétition. Pour les semences provenues de nos propres expérimentations nous avons créé dix-sept répétitions.

Les travaux ont démarré en 1980 à la Station Expérimentale Agricole Secuieni (Roman, département de Neamț). A l'exception de la teneur en morphine, les tableaux ci-joints présentent seulement les résultats de l'année 1982 (y compris les données de climat et de sol).

La multiplication des répétitions de la cultovariété propre a été motivée par sa non-uniformité génétique (les semences provenaient de la culture en commun et la libre pollinisation des cultivars K 103, Hollande 245, De Botoșani et Extase, au cours de cinq ans). Vu la diversité génotypique et phénotypique du matériel propre, et pour surprendre le plus fidèlement possible l'amplitude de variabilité, nous avons effectué un nombre accru de répétitions afin de pouvoir étudier un grand nombre d'individus.

Dès la première année des recherches, une partie des individus de chaque variante ont été autofécondés, les autres étant laissés en libre fécondation. La seconde et la troisième année la perpétuation a été faite selon la teneur en morphine. Chacun des individus obtenus par autofécondation et soumis à l'analyse biochimique pour la détermination de la teneur en morphine a été le point de départ d'un lot de l'année suivante. Par conséquent, après la première année d'expérimentation on a obtenu deux, trois ou plusieurs lots d'individus de chaque cultovariété initiale en fonction du nombre d'exemplaires étudiés pour leur

Tableau 1

Les cultivars ou la provenance	Catalase		Peroxydase		Protéines mg/ml	Morphine		
	Activité spécifique	H_2O_2 mg/ml	Activité spécifique	H_2O_2 mg/ml		1981	Autofécondé 1982	Librement pollinisé 1982
Hongrie	7,254	14,06	4,313	8,36	1,938	—	0,30—0,44	0,20—0,39
Hongrie	7,695	15,63	4,288	8,71	2,031	—	0,30	0,23—0,56
Hongrie	6,942	14,53	4,094	8,57	2,093		0,33	0,24—0,48
URSS	6,841	13,15	4,162	8,00	1,922	—	0,25—0,33	0,26—0,29
Kapeczky	6,867	14,05	3,993	8,17	2,046	0,40	0,28	0,27—0,44
Jemtchouzhnyi	7,501	15,94	3,826	8,14	2,136	0,51	0,23—0,33	0,15—0,60
Eckendorf	6,938	15,14	3,846	8,39	2,182	0,16—0,51	0,17—0,29	0,26—0,38
Modoronécz	6,986	14,35	4,206	8,69	2,054	0,43	0,15—0,48	0,27—0,68
Kapeczky × Jemtchouzhnyi	5,709	10,62	3,930	7,31	1,860	0,16	0,17	0,30
Eckendorf × Jemtchouzhnyi	6,586	15,57	3,684	8,71	2,364	0,43	0,23—0,33	0,23—0,90
De Mureș	6,358	12,71	4,320	8,64	2,000	0,46	0,30	
Matériel propre	5,118—8,228	11,19—15,75	3,351—4,766	7,82—9,14	1,798—2,357	0,11—0,55	0,15—0,76	0,15—0,69

teneur en morphine. Le tableau 1 présente cette situation pour l'individu à la moindre concentration et celui à la concentration maximale de chaque lot.

Nos observations ont visé aussi le comportement d'autres paramètres biochimiques et morphoanatomiques. Les données d'ordre morphoanatomique (tableaux 2 et 3) ont été élaborées selon la méthode statistique [19]. La teneur en morphine a été établie selon la méthode Gyéresi et Rácz [8], la teneur en protéines selon la méthode Lawry et collab. [15], la concentration de la peroxydase selon la méthode Boiarkine [4] et celle de la catalase selon la méthode Georgescu et Păunescu [6].

Tableau 2

Les cultivars ou la provenance	Hauteur des capsules		Diamètre des capsules		Nombre de rayons stigmatiques		Poids de écales	
	$\bar{x}$	s%	$\bar{x}$	s%	$\bar{x}$	s%	$\bar{x}$	s%
Hongrie	54,61	8,75	29,44	14,87	11,51	10,33	1,49	20,80
Hongrie	55,10	7,13	28,19	9,75	11,71	11,35	1,51	22,51
Hongrie	51,38	10,29	31,20	11,63	11,46	8,72	1,46	25,34
URSS	42,64	15,05	25,72	27,91	10,04	18,12	0,97	69,07
Kapeczky	46,67	12,66	32,63	12,84	11,15	10,49	1,53	28,75
Jemtchouzhnyi	46,94	13,33	32,45	16,77	11,37	12,97	1,57	33,23
Eckendorf	51,40	14,13	31,65	13,70	11,05	13,29	1,23	28,58
Modoronécz	45,24	15,72	29,30	17,11	11,31	11,74	1,14	35,23
Kapeczky × Jemtchouzhnyi	39,79	11,58	31,02	13,70	10,95	12,51	1,10	30,90
Eckendorf × Jemtchouzhnyi	51,79	11,58	34,46	12,21	11,07	11,38	1,93	32,12
De Mureș	51,00	10,60	33,55	9,29	11,60	10,51	1,77	20,90
Matériel propre	41,03—61,12	5,04—20,74	29,00—38,94	4,09—21,98	10,15—14,50	8,58—27,15	1,18—2,15	8,95—42,55

LES CONDITIONS PÉDO-CLIMATIQUES DU CHAMP EXPÉRIMENTAL

Pendant l'année agricole 1981—82 l'expérience a été effectuée sur un tchernoziom cambique typique, aux caractéristiques suivantes : pH (en eau) 6, 4, teneur en humus 2,8%, P_2O_5 3,9 mg/100 g sol et K_2O mobile 23,6 mg/100 g sol. Le sol de Secuieni fait partie des sols faiblement acides, faiblement pourvus de phosphore mobile et riches en potassium mobile.

En ce qui concerne le climat, l'année agricole 1981—82 a été caractérisée par un hiver à précipitations et températures normales suivi d'un printemps et un été très secs (tableau 4).

Aux mois de mai, juin et août l'humidité du sol a baissé et s'est maintenu autour du coefficient d'étiolement. Ces conditions ont influencé négativement les productions de plantes médicinales, vu qu'elles ont un enracinement superficiel et qu'elles demandent des conditions optimales, de l'eau et des substances nutritives (surtout dans les phases critiques).

RÉSULTATS ET DISCUSSIONS

Bien que nos investigations eussent porté sur le comportement de plusieurs paramètres morphologiques, comme la hauteur des plantes, le nombre de ramifications, etc., nous avons considéré utile de présenter

Tableau 3

Les cultivars ou la provenance	Hauteur-Diamètre			Hauteur-Nombre de rayons			Hauteur-Poids des écales			Diamètre-Nombre de rayons			Diamètre-Poids des écales		
	r	Ry/x	Rx/y	r	Ry/x	Rx/y	r	Ry/x	Rx/y	r	Ry/x	Rx/y	r	Ry/x	Rx/y
Hongrie	0,47	0,43	0,51	0,07	0,01	0,28	0,41	0,02	6,15	0,53	0,14	1,95	0,57	0,04	8,05
Hongrie	0,37	0,25	0,52	—0,17	0,5	0,05	0,73	0,06	8,54	0,51	0,24	1,05	0,69	0,10	4,42
Hongrie	0,25	0,17	0,36	—0,14	0,02	0,74	0,47	0,03	6,71	0,32	0,08	1,16	0,66	0,06	6,47
URSS	0,81	0,90	0,72	0,54	0,15	1,90	0,71	0,07	6,80	0,77	0,19	3,03	0,82	0,07	8,78
Kapeczky	0,07	0,04	0,09	0,05	0,00	0,25	0,23	0,01	3,37	0,33	0,09	1,18	0,45	0,03	6,54
Jemtchouzhnyi	0,31	0,27	0,34	—0,11	0,02	0,51	0,43	0,03	5,62	0,48	0,12	1,28	0,58	0,06	4,99
Eckendorf	0,34	0,21	0,53	—0,50	0,01	0,55	0,59	0,02	12,14	0,36	0,11	1,08	0,27	0,01	4,18
Modoronéez	0,39	0,28	0,58	0,03	0,00	0,17	0,60	0,03	10,46	0,38	0,10	1,41	0,59	0,05	6,95
Kapeczky × Jemtchouzhnyi	0,04	0,03	0,04	—0,25	3,12	0,84	0,35	0,02	5,16	0,35	0,11	1,08	0,70	0,05	8,95
Eckendorf × Jemtchouzhnyi	0,13	0,09	0,18	0,16	0,03	0,76	0,57	0,05	5,68	0,33	0,09	1,10	0,49	0,06	3,89
De Mureș	0,16	0,09	0,27	—0,12	0,02	0,53	0,54	0,03	7,89	0,36	0,14	0,92	0,60	0,07	5,06
Matériel propre	—0,10—	0,03—	0,07—	—0,45—	0,00—	0,04—	—0,28—	0,00—	1,85	0,14—	0,04—	0,38—	0,11—	0,00—	1,71—
	0,80	0,74	8,62	0,27	0,18	1,49	0,97	1,52	16,40	0,57	0,30	2,83	0,92	0,14	13,75

Tableau 4

Les conditions climatiques de l'année agricole 1981 — 1982

		Les mois												
		X	XI	XII	I	II	III	IV	V	VI	VII	VIII	IX	Annuellement
	Nombre de jours à précipitations	10	13	15	11	10	10	20	7	15	16	12	2	
Précipitations (mm)	Première décade	5,0	7,2	2,3	9,5	34,7	2,1	1,2	2,8	4,3	19,3	9,3	—	
	Deuxième ,,	15,9	10,5	12,1	1,2	0,2	8,8	14,6	—	24,5	49,2	0,3	—	
	Troisième ,,	7,0	20,0	12,4	0,5	9,4	23,2	31,8	17,4	33,5	29,3	27,2	0,6	
	Total	27,9	37,7	26,8	11,2	44,3	34,1	47,6	20,2	62,3	97,8	36,8	0,6	447,3
	Moyenne pour 20 années	33,0	31,5	25,7	25,3	22,1	25,8	49,8	65,9	83,2	83,1	58,4	42,8	546,5
	Déviation	—5,1	+6,2	+1,1	—14,1	+22,2	+8,3	—2,2	—45,7	—20,9	+14,7	—21,6	—42,1	—99,2
Températures	Première décade	14,9	3,6	—0,6	—1,2	—4,6	—0,2	7,7	13,8	18,7	16,7	19,8	18,8	
	Deuxième ,,	9,1	—1,4	—3,1	—7,5	—7,0	1,2	6,5	16,4	16,1	19,4	20,9	17,7	
	Troisième ,,	9,0	1,5	—0,7	—6,7	—4,2	4,7	6,5	18.3	19,3	20,4	19,7	15,8	
	Moyenne mensuelle	10,9	1,2	—1,4	—5,2	—5,3	2,0	6,9	16,2	18,0	18,8	20,1	17,4	8,3
	Moyenne pour 20 années	9,2	4,0	—1,6	—5,2	—2,7	1,8	8,8	14,9	18,4	19,5	18,7	14,7	8,4
	Déviation	+1,7	—2,8	+0,2	0,0	—2,6	+0,2	—1,0	+1,3	—0,4	—0,7	+1,4	+2,7	—0,1

seulement des données relatives à la capsule et rien que pour la dernière année.

Les plus élevées valeurs moyennes des dimensions des capsules (hauteur, diamètre, poids, etc.) en tant qu'éléments de bioproductivité ont été enregistrées pour la cultovariété formée à partir du matériel propre ($\bar{x}$ = 61,2 mm pour la hauteur, 38,94 mm pour le diamètre et 2,15 g pour la quantité d'écales/capsule). Des valeurs similaires ont été enregistrées pour les individus du cultivar provenu de Hongrie, l'hybride Eckendorf × Jemtchouzhnyi, etc. Le cultivar de l'URSS a été caractérisé par les moindres valeurs moyennes en ce qui concerne le diamètre des capsules, le poids des écales et le nombre de rayons stigmatiques.

Le coefficient de variabilité a eu des valeurs réduites (s% = 10), tout au plus moyennes (s% = 10,20) pour toutes les variantes, excepté une seule — un lot de matériel propre où la valeur moyenne a été dépassée de peu (s% = 20,74 pour la hauteur des capsules et s% = 21,98 pour le diamètre). Quant au poids des écales, presque toutes les variantes ont présenté une variabilité accrue (s% = 20).

Les corrélations entre la hauteur et le diamètre, la hauteur et le poids des écales, le diamètre et le nombre des rayons stigmatiques, le diamètre et le poids de l'écale ont eu des valeurs positives.

Pour les autres corrélations on a enregistré aussi des valeurs négatives. En chiffres absolus, les valeurs les plus élevées des corrélations ont été trouvées entre la hauteur des capsules et le poids des écales (r = = 0,97) et entre le diamètre des capsules et le poids des écales (r = 0,92) pour des variantes du matériel propre. Pour la variante constituée de semences provenues de l'URSS on a enregistré les corrélations à valeurs maximales entre la hauteur et le diamètre des capsules, entre la hauteur et le nombre des rayons stigmatiques, ainsi qu'entre le diamètre et le nombre des rayons stigmatiques.

Dans la plupart des cas, les régressions ont reflété avec fidélité la dynamique des corrélations.

Les valeurs maximales (8,228) et minimales (5,118) de l'activité spécifique de la catalase ont été enregistrées chez l'un des lots de la variante propre. La quantité de H_2O_2 consommée a été maximale (15,75 mg/ml) pour la variante de l'espèce Jemtchouzhnyi. La peroxydase a eu tant une activité spécifique maximum (4,766) qu'une activité spécifique minimum (3,381) et une activité relative dans les mêmes lots provenus du matériel propre. La détermination de la tenueur en protéines sur le même matériel végétal qui a servi pour les déterminations de la catalase et de la peroxydase (les feuilles des plantes pendant la phénophase de floraison) a mis en évidence une concentration maximale chez la variante provenue de l'hybride Eckendorf × Jemtchouzhnyi (2,364 mg/ml) et une concentration minimale chez un lot de la variante propre (1,748 mg/ml). D'ailleurs chez l'une des variantes propres on a enregistré une teneur accrue en protéines (2,357 mg/ml), proche de l'hybride mentionné.

Pour la tenueur en morphine les analyses ont commencé en 1981. En 1982, comme nous l'avons déjà mentionné dans « Matériel et méthodes », les analyses ont été effectuées tant sur des individus provenant de l'autofécondation, que sur des individus résultant de la libre pollinisation.

La teneur en morphine a varié entre 0,11% et 0,55%, toutes les deux valeurs étant enregistrées en 1981 chez des variantes provenant du matériel propre. Un bon comportement a été signalée aussi chez le cultivar Jemtchouzhnyi (0,51%). En 1982 on a analysé plusieurs individus de chaque variante, chacun constituant le point de départ pour de nouvelles variantes en 1983. Excepté pour les variantes propres, les individus autofécondé ont présenté une teneur en morphine inférieure à ceux librement pollinisés. L'individu à la teneur en morphine la plus élevée (0,90%) a été dépisté parmi les libre-pollinisés de l'hybride Eckendorf × Jemtchouzhnyi, un exemplaire autofécondé du matériel propre ayant aussi un comportement particulièrement satisfaisant. D'ailleurs, une partie des variantes obtenues du matériel propre ont été autofécondées aussi en 1981. C'est peut-être la raison pour laquelle la teneur en morphine des individus librement pollinisés et de ceux autofécondés a présenté des valeurs similaires. En même temps, compte tenu du fait que l'individu à teneur en morphine de 0,76% a été autopollinisé, on peut espérer une uniformité accrue de la descendance en 1983, contrairement à la descendance née de l'individu à 0,90% morphine, laissé en libre pollinisation. D'autre part, on a constaté une variabilité assez élevée de ce paramètre pour chaque variante autant pour la même année, que d'une année à l'autre.

Nous n'avons pu établir aucune corrélation claire entre la teneur en morphine et n'importe lequel des paramètres morphoanatomiques ou biochimiques analysés.

CONCLUSIONS

Le sol sur lequel on a effectué l'expérimentation pendant trois années a été un tchernoziom du type cambique, du groupe des sols faiblement acides, faiblement pourvus de P et riches en K.

Les caractères morphoanatomiques ont varié tant dans le cadre d'une même variante d'une année à l'autre, qu'entre les différentes variantes.

Les meilleures valeurs moyennes des éléments bioproductifs (dimensions, poids et nombre des rayons stigmatiques des capsules) ont été enregistrées chez les variantes basées sur le matériel propre (semences).

Pour toutes les variantes observées en 1982, le coefficient de variabilité a eu des valeurs réduites, tout au plus moyennes.

Les corrélations entre les divers paramètres morphoanatomiques ont eu en général des valeurs positives et assez élevées.

Les plantes résultées du matériel propre ont manifesté la plus large amplitude de variabilité en ce qui concerne l'activité spécifique et la tener en catalase et en peroxydase.

La valeur maximale de la protéine a été enregistrée chez l'hybride Eckendorf × Jemtchouzhnyi, tandis que la valeur minimale a été trouvée dans un lot de la variante propre.

Pour la teneur en morphine on a enregistré des variations tant d'une variante à l'autre, qu'à l'intérieur de la même variante avec le temps. L'amplitude de la variabilité de ce caractère a été comprise entre 0,11% et 0,55% en 1981 et entre 0,15% et 0,90% en 1982. La teneur en

morphine a été en moyenne plus réduite chez les individus provenant de l'autopollinisation que chez ceux provenant de la libre pollinisation.

On n'a pu établir des corrélations ni entre les caractères morpho-anatomiques et biochimiques étudiés, ni entre les divers caractères biochimiques (l'activité des enzymes, la teneur en protéines, la teneur en morphine).

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SOME CYTOGENETIC, PHYSIOLOGICAL AND BIOCHEMICAL EFFECTS INDUCED BY SINGLE AND COMBINED TREATMENTS WITH GAMMA RAYS AND ETHYLMETHANESULFONATE IN TWO-ROW BARLEY AND WHEAT

BY

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Single gamma ray treatments caused more important disturbances of the genetic, physiological and biochemical processes analyzed in wheat plants in comparison with two-row barley plants. The results are the consequence of the "storage effect", that was more marked in wheat (seed conservation period = 22 months) than in two-row barley (conservation of irradiated seeds = 8 months). The combined treatments with gamma rays and ethylmethanesulfonate had in some cases an additive effect, and in most of the cases a highly additive effect on the aberrant anaphases and telophases induced.
The effects obtained in combined treatments depend on many factors such as: biological material, its physiological state, the irradiation dose, the concentration of the chemical mutagen and the time of administration, the sequence of mutagens, the conditions before, during and after treatments, the storage period of the irradiated seeds, etc.

For theoretical and practical reasons, besides investigations into the effects of single treatments with radiations and chemical mutagens in plants, research workers have been lately interested in the repercussions of the combined treatments with the two mutagens. It has been noticed, as we have already shown in another paper [7], that in some cases the effects induced by combined treatments are reduced in comparison to single treatments and in other cases they proved to be additive or even highly additive. It is hoped that by deep-going and complex studies on the mechanism of action of mutagens and on their multiple repercussions in plants, on the factors influencing their action, etc., a high plant variability and even a certain control of experimental mutagenesis would be obtained.

The absorption of radiation energy, or diffusion of chemical mutagen in vegetable material leads to a "premutational injury" which is eliminated or becomes real depending on the efficiency of repairing and recovering processes [18]. More precisely, if such an injury resists after DNA replication, we can speak about a mutational event. Using physical and chemical mutagens in various phases of the cellular cycle, Yamaguchi [16] showed that mutational events took place with a high frequency when acting in the DNA synthesis period, the mutation range being also different depending on the moment of the mutagen administration. The effects of

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mutagens depend on their accessibility to the active genes, on the degree and extension of chromatin despiralization [17].

Because we have few and sometimes contradictory data on the effects of the treatments with ionizing radiations and chemical mutagens on the isoenzyme pattern in plants, we decided to study the repercussions of single and combined treatments with gamma rays and ethylmethanesulfonate on the range of isoesterases and isoperoxidases in two-row barley and wheat. We also investigated the influence of two mutagens on the growth processes, on the frequency of chromosomal aberrations in root meristem, on some biochemical components, on the activity of catalase and peroxidase, etc. Some of the results of these investigations are presented in this paper.

MATERIAL AND METHODS

The researches on two-row barley plants were done in August 1981, using seeds of *Elgina* cultivar, irradiated on the 23rd of November 1980 with 5, 10 and 15 kR gamma rays (^{60}Co, 260 R/min). On irradiation, the seed moisture was 11.9%. The experiments on wheat were carried out in March 1982 on seeds of *Dacia* cultivar, irradiated on the 14th of May 1980 with 6, 10, 15 and 20 Krad (5 Krad/min). The water content of wheat seeds was 12.8% on irradiation. Hence, at the beginning of experiments, the irradiated two-row barley seeds had been stored for 8 months and wheat seeds — for 22 months.

In the treatments with EMS we used the concentrations of 0.01, 0.03 and 0.05% for two-row barley and 0.05 and 0.1% for wheat. The seeds were soaked in EMS solutions for 6 hours. 16 experimental variants for two-row barley and 15 for wheat were created, which were cultivated in an interval of 3 weeks (4 — 6 variants/week) for the isoenzyme study. The treated seeds were cultivated on filter paper in Engelhardt germinators. The germinators were put in a climatized room with a temperature of 23—25°C, air humidity of 65—70% and a luminosity of 5000 lux. In the experiment with two-row barley the light was continuous, and in the wheat experiment light and darkness alternated every 12 hours. During the first 2 days of the experiment, the germinators were covered. 300 seeds were treated for each variant. For the analysis of aberrant anaphases and telophases, the roots were harvested from 50 individuals in each variant. When plants were 5-days old, we measured the dimensions of their roots and stems. The content of soluble sugars and free amine nitrogen was determined in plant stems. In wheat the catalase and peroxidase activity was also determined in the stems and roots of 5-day-old plants. The analysis methods were described in other papers [4].

The values of the analysed indices are shown in Tables 1—3.

RESULTS AND DISCUSSION

The microscopic observations pointed out reduced cytogenetic disturbances in two-row barley plants (only 6.30% aberrant anaphases and telophases at 15 kR). Consequently the growth of plants was only

Table 1

The values of some cytogenetic and physiological parameters in two-row barley plants after single and combined treatments with gamma rays and ethylmethanesulfonate (EMS)

No.	The treatment *	The aberrant anaphases and telophases (%)				The length of roots (mm) $\bar{x} \pm s_{\bar{x}}$	The length of stems (mm) $\bar{x} \pm s_{\bar{x}}$	Total sugars % d.s.	Reducing sugars % d.s.	Free amine nitrogen % d.s.
		B	M	BM	OA					
		$\bar{x}\% \pm s_{\bar{x}}\%$								
1	Control	2.53	0.38	—	—	38.64 ± 0.86	90.48 ± 2.05	8.99	2.85	1.57
2	5 kR	0.97	3.37	0.59	—	38.32 ± 0.88	84.86 ± 1.51	15.44	3.36	1.39
3	10 kR	1.23	3.25	1.10	—	35.30 ± 0.78	82.35 ± 1.35	11.97	3.40	1.54
4	15 kR	2.00	3.46	0.93	—	36.12 ± 0.75	80.08 ± 1.38	14.47	3.46	1.53
5	0.01 % EMS	3.23	0.64	0.21	0.11	—	—	13.77	3.10	2.24
6	0.03 % EMS	3.01	1.63	0.25	—	—	—	12.34	2.82	1.88
7	0.05 % EMS	4.89	0.93	0.23	—	—	—	12.90	3.34	1.97
8	5 kR + 0.01 % EMS	5.77	1.60	0.20	0.20	—	—	13.80	3.69	2.09
9	5 kR + 0.03% EMS	6.07	2.16	—	—	—	—	12.95	2.40	1.71
10	5 kR + 0.05 % EMS	5.02	2.30	—	0.21	—	—	15.98	2.17	1.61
11	10 kR + 0.01% EMS	11.84	1.83	0.23	0.91	21.80 ± 0.77	37.35 ± 1.66	15.71	0.72	1.89
12	10 kR + 0.03 % EMS	8.41	2.87	0.57	0.76	21.24 ± 1.43	37.68 ± 0.97	15.00	0.52	2.34
13	10 kR + 0.05 % EMS	7.41	3.61	0.54	0.90	21.51 ± 0.46	35.51 ± 0.90	9.21	0.33	2.13
14	15 kR + 0.01 % EMS	10.16	7.34	1.42	2.36	12.91 ± 0.57	17.83 ± 0.92	15.21	1.27	1.98
15	15 kR + 0.03 % EMS	5.82	8.19	2.16	3.65	19.38 ± 0.60	39.76 ± 2.39	12.60	1.07	2.63
16	15 kR + 0.05 % EMS	9.30	11.36	1.86	3.51	18.89 ± 0.48	36.15 ± 0.80		0.77	2.17

* The treatment with EMS lasted for 6 hours.
B = bridges; M = micronuclei; OA = other aberrations.

slightly influenced (Table 1). The frequency of chromosomal aberrations was reduced in the case of treatments with EMS too. The species *Hordeum distichum* is very sensitive to EMS treatments. At the beginning there were chosen for the experiment the concentrations of 0.1, 0.2 and 0.3 % EMS, but very few seeds preserved the germination capacity. Even at concentrations 10 times lower, only few plants germinated and survived. This is why for some variants we do not have sufficient individuals for the statistical analysis of their dimensions. Since the cytogenetic perturbations induced by EMS are reduced, we think that it is its toxic effect on the metabolism that is responsible for the high inhibition of seed germination. It is also possible that we have worked in a stage of reduced seed germination.

Surprisingly, at first sight the cytogenetic disturbances induced by gamma rays in wheat are much more important than those induced in two-row barley, although the "genetic resistance" of wheat is higher (Table 2). As seen in Table 2, the frequency of aberrant anaphases and telophases is rather high, reaching 78.63 % at the 20 Krad dose. We think that this phenomenon is accounted for by the so-called "storage effect" that contributed to the amplification of initial radioinduced perturbations, as known ever since the 3rd decade of our century. Roze and Kavatz [11] showed that conservation for 6 weeks of seeds of two species of *Brassica* irradiated with 50 and 150 kR, intensified the effects of irradiation by 43—88 %. In researches on rice, Bayonove et al. [2] noticed that this effect increases with the conservation period, reaching the maximum a year after irradiation.

Under conditions that accelerate the natural ageing of seeds, the storage effect of irradiation is intensified, while the conditions that diminish this process do not influence the percentage of primary radioinduced injuries [10]. The conservation of the irradiated seeds intensifies the accumulation of toxins such as peroxides, quinones, free polyphenols, etc. and decreases the nutritive substances necessary for recovering [9]. It has also been discovered that the "storage effect" is not characteristic only of X and gamma rays, but also of densely ionizing radiations. Besides the component of chromosomal lesions depending on oxygen, there is also an independent component that manifests itself when maintaining the irradiated seeds in vacuum (Conger, cited after Dishler [3]).

The processes of growth in irradiated wheat plants reflect the cytogenetic disturbances. It seems that even the postirradiation recovering processes in wheat were highly diminished by gamma ray treatment, since the catalase and peroxidase activity was in general lower than in control plants (Table 3). This statement takes into account the fact that in the plants treated with EMS, whose growth processes are almost similar to the control plants, oxidases have a similar activity (variants 6 and 7, Tables 1, 2).

Comparing the results of the growth test, the catalase and peroxidase activity in wheat plants immediately after irradiation [13] with the similar data from this paper, the contribution of the storage effect results clearly. Even if the absolute values of the same variants from the two papers cannot be compared because the time and luminosity conditions were different, we can compare the differences between the values of the inves-

Table 2

The values of some cytogenetic and physiological parameters in wheat plants after single and combined treatments with gamma rays and ethylmethanesulfonate (EMS)

No.	The treatment *	The aberrant anaphases and telophases (%)				The length of stems (mm)		The length of roots (mm)	
		B	M	BM	OA	Total aberrant cells $\bar{x}$ % ± s$\bar{x}$ %	$\bar{x}$ ± s$\bar{x}$	s %	$\bar{x}$ ± s$\bar{x}$	s %
1	Control	1.11	0.84	—	0.56	2.51 0.02	60.60 0.78	20.85	48.51 0.85	28.28
2	5 Krad	9.78	1.63	0.44	0.59	12.44 0.05	51.11 1.51	30.36	38.30 1.22	32.74
3	10 Krad	17.55	12.35	2.47	1.96	34.33 0.10	44.95 1.50	28.74	38.62 1.35	30.01
4	15 Krad	26.35	14.83	4.72	2.44	48.34 0.18	39.79 0.94	29.55	37.92 0.79	25.95
5	20 Krad	17.95	41.31	11.11	7.96	78.63 0.37	26.31 1.32	36.79	22.76 1.04	33.79
6	0.05 % EMS	1.33	3.99	—	0.25	5.57 0.01	60.84 0.89	19.41	46.17 0.83	23.82
7	0.10 % EMS	1.54	5.34	—	0.98	7.86 0.03	60.04 0.77	20.90	44.13 0.77	28.66
8	5 Krad + 0.05 % EMS	15.67	2.77	2.30	0.92	21.66 0.13	53.95 2.02	29.90	41.86 1.42	27.09
9	5 Krad + 0.10 % EMS	14.61	8.35	2.43	1.39	26.78 0.12	49.58 2.06	35.80	37.70 1.52	34.72
10	10 Krad + 0.05 % EMS	14.69	17.50	4.37	2.37	43.75 0.43	40.10 1.32	25.68	23.33 1.02	34.33
11	10 Krad + 0.10 % EMS	26.97	19.77	3.37	7.37	57.75 0.15	49.85 1.11	23.39	31.04 1.01	34.34
12	15 Krad + 0.05 % EMS	18.81	23.90	6.19	2.65	51.55 0.23	43.46 1.28	26.41	24.10 0.83	30.05
13	15 Krad + 0.10 % EMS	22.54	22.02	6.99	8.29	59.84 0.32	41.22 1.09	26.85	27.41 0.88	32.61
14	20 Krad + 0.05 % EMS	40.00	9.50	7.00	2.50	59.00 0.85	27.52 0.81	24.31	15.64 1.60	33.75
15	20 Krad + 0.10 % EMS	14.49	34.79	16.91	13.52	79.71 0.54	25.46 0.75	27.11	15.33 2.03	28.16

* The treatment with EMS lasted for 6 hours.
B = bridges; M = micronuclei; OA = other aberrations

tigated parameters in control and irradiated plants from the same experiment. Right after irradiation, the 5 and 10 Krad doses stimulated the growth of stem by 15 % and 13 % respectively in comparison with the

Table 3

The activity of catalase and peroxidase in 5-day-old plants of wheat treated with gamma rays and ethylmethanesulfonate (EMS)

No.	The treatment *	Catalase		Peroxidase	
		H_2O_2 mg/g s.fr.	Sp. activ.	H_2O_2 mg/g s.fr.	Sp. activ.
	Stems				
1	Control	64.60	4.70	23.75	1.72
2	5 Krad	57.74	4.54	25.83	2.03
3	10 Krad	59.84	4.29	24.16	1.73
4	15 Krad	50.46	3.25	19.83	1.28
5	20 Krad	43.38	2.73	19.83	1.25
6	0.05 % EMS	64.90	4.60	24.16	1.71
7	0.10 % EMS	65.07	4.82	26.08	1.93
8	5 Krad + 0.05 % EMS	59.05	4.36	30.91	1.28
9	5 Krad + 0.10 % EMS	60.06	4.35	27.41	1.99
10	10 Krad + 0.05 % EMS	58.71	4.27	30.83	2.24
11	10 Krad + 0.10 % EMS	61.89	4.64	25.99	1.95
12	15 Krad + 0.05 % EMS	63.43	4.31	31.50	2.14
13	15 Krad + 0.10 % EMS	61.71	4.25	22.91	1.58
14	20 Krad + 0.05 % EMS	48.24	3.54	23.33	1.71
15	20 Krad + 0.10 % EMS	50.10	3.40	25.00	1.69
	Roots				
1	Control	26.18	3.04	14.00	1.63
2	5 Krad	24.94	2.18	13.33	1.25
3	10 Krad	25.35	2.45	19.06	1.84
4	15 Krad	30.29	2.67	17.50	1.55
5	20 Krad	—	—	—	—
6	0.05 % EMS	28.98	3.60	12.66	1.57
7	0.10 % EMS	23.86	2.92	22.33	2.74
8	5 Krad + 0.05 % EMS	22.36	2.62	12.50	1.47
9	5 Krad + 0.10 % EMS	24.12	1.81	11.25	0.83
10	10 Krad + 0.05 % EMS	24.45	2.34	14.16	1.35

* The treatments with EMS lasted for 6 hours.

control plants. The doses of 15 and 20 Krad determined the decrease of the stem growth by only 0.3 and 9.5 % respectively. The dimensions of the roots were by 12, 23, 11 and 22 % smaller in the plants irradiated with 5, 10, 15 and 20 Krad in comparison with the control [13].

22 months later the dimensions of the stems at the same doses were reduced by 16, 26, 35 and 57 % in comparison with the control plants, and the roots by 21, 21, 22 and 53 % respectively. The differences could have been more evident if the control seeds had not suffered from the same "storage effect".

We also notice that the catalase and peroxidase activity in the course of 22 months between the two experiments was reduced to half and even more. This is an argument that supports our statement regarding the diminution of the recovery capacity in the irradiated seeds and plants in proportion with the time elapsed from irradiation.

It is surprising that in two-row barley the "storage effect" was lacking or was very slight although the irradiated seeds had been stored for 8 months, while in wheat this effect was very strong even if the time from irradiation was longer (22 months).

With combined treatments with gamma rays and EMS, the cytogenetic injuries were more intense in two-row barley as well as in wheat plants. In some cases, the effects were additive (variants 8 — 10 in two-row barley — Table 1; variants 12 and 15 in wheat — Table 2), in most cases they were highly additive, contributing to a higher percentage of chromosomal aberrations than that with single treatments with 2 agents (variants 11 — 16 in two-row barley — Table 1; variants 8 — 11 and 13 in wheat — Table 2). Only in one case, the percentage of chromosomal aberrations induced by combination of the 2 agents was inferior to that due to single treatments (variant 14 in wheat — Table 2). From this point of view, our results differ from those obtained by Valeva [14, 15] in barley. The author noticed that gamma ray and ethyleneimine (EI) treatments determined a slighter effect than expected, as appreciated after plant dimensions, fertility, percentage of chromosomal aberrations, etc., although the range of aberrations was modified. In this experiment, EI was administered before irradiation. In our earlier experiments on two-row barley and wheat [5, 6] we noticed that preirradiation treatments with EMS had additive and highly additive effects and only in a few cases they were smaller than in the single treatments with gamma rays and EMS.

Valeva pointed out that in the treatments with EI after irradiation the effects induced depended on the dose used in the combination. The frequency of chlorophyll mutations in the combination of EI with 5 kR dose was lower than in single treatments. The combination of EI with 1.5 kR dose had a more marked effect and when the irradiation dose was even smaller (0.5 kR) the effect was additive with the concentration of 0.06 % and highly additive with 0.015 % EI. The author has reached similar conclusions in another paper [1]. Administered after irradiation, ethyleneimine has a protecting effect at reduced concentrations. At high concentrations, the effect of EI is additive in combination with gamma rays and highly additive with fast neutrons.

In researches conducted on barley, Joshua et al. [8] showed that the treatment with diethylsulfate (0.1 %) immediately or 20 days after irradiation with neutrons (0.5 — 1.5 Krad) had a sinergic effect on fragments and bridges in anaphase. In another paper on the same plant and with the same mutagens [12], the authors pointed out that at small doses of irradiation a sinergic effect is produced on plant height, while at high doses the effect is protective. If the combination comprised gamma rays and diethylsulfate, a sinergic effect of mutagens was obtained on the plant height at all doses of irradiation.

Only from these examples and from our results we draw the conclusion that the effect obtained in combined treatments with radiations and chemical mutagens depends on many factors such as : the biological material, its physiological state, the mutagens used and their succession, the irradiation dose and the concentration of the chemical mutagen, the

tests used, the conditions before and after treatments, the conservation duration of irradiated seeds, etc.

As regards the range of chromosomal aberrations in the single treatments as well as in the combined ones with gamma rays and EMS, the micronuclei and bridges had the highest frequency. However, in the combined treatments the frequency of cells with several simultaneous types of aberrations and cells with rare types of aberrations was much higher.

The amplification of cytogenetic disturbances in combined treatments with gamma rays and EMS had negative effects on the plants growth. In two-row barley plants this process was twice less important (see variants 11 — 16 comparatively with 1 — 4, Table 1). The inhibition of the growth is a consequence of the perturbations induced by mutagens in plant metabolism. For example, whereas the soluble sugars content of 5-day-old control plants was of 9 % d.m., the plants of the variants submitted to a combined treatment exhibited twofold values of the same parameter. If we take into account that of the soluble sugars, the reducing ones (the form in which glucides take part in different metabolic processes) occur in small quantities in the plants of some variants (variants 11 — 16), we have a more exact measure of the major perturbations induced by the combined treatments with the 2 mutagens in two-row barley. It is in this sense that can be interpreted the higher values of free amine nitrogen found in the plants of these variants, as a consequence of the lesions induced by mutagens in the nitrogen metabolism of plants.

If we analyse the dimensions of wheat plants treated with gamma rays and EMS, we notice that the growth processes in the roots reflect the degree of cytogenetic perturbations (Table 2); in the stems the growth registered, surprisingly, an amelioration in comparison with the plants treated only with radiations. How can we explain this phenomenon? We can either admit that the recovering processes are more efficient in stems, or that under these conditions the processes of growth in the two organs are somewhat independent. As the latter hypothesis seems hazardous, we must admit the former. If we take into account that the activity of the two analysed oxidases is more intense in stems (Table 3), we can appreciate that the elimination of some peroxidic toxins and consequently the efficiency of the recovering processes is higher in stems. The results of the growth test in wheat reveals once again the importance of the criterion used in appreciating the effects induced by combined treatments with gamma rays and chemical mutagens. A correct appreciation should take into account as many criteria as possible.

CONCLUSIONS

1. The paper presents some effects induced by single and combined treatments (of the postirradiation type) with gamma rays and ethylmethanesulfonate on chromosomal aberrations in root mitoses, on the plants growth, on some biochemical components in two-row barley *(Elgina* cultivar) and wheat *(Dacia* cultivar).

2. The single gamma ray treatments caused more important disturbances of the genetic, physiological and biochemical processes analysed in wheat, in comparison with barley. In our opinion, the results are the consequence of the "storage effect", that was more marked in wheat.

3. With combined gamma ray and EMS treatments, the cytogenetic, physiological and biochemical perturbations are more intense. The effect of combined treatments on the percentage of aberrant anaphases and telophases was in some cases additive, and in most of the cases highly additive. The range of chromosomal aberrations with combined treatments was not modified, but the frequency of cells with several types of aberrations and the frequency of rare aberrations increased.

4. The results of our previous investigations, as well as the present results reveal that the effects obtained by combined treatments with gamma rays and alkylating agents depend on many factors such as: the specificity of biological material, its physiological state in the moment of treatment, the irradiation dose, the concentration of chemical mutagens and the time of administration, the way the mutagens succeed each other, the conditions before, during and after the treatment, the storage period of the irradiated seeds.

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ULTRASTRUCTURAL STUDIES ON THE FUNGUS *ASPERGILLUS FLAVUS* INFECTING THE TERMITES *(ODONTOTERMES OBESUS)*

BY

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The electronmicroscopic investigations carried out on *Odontotermes obesus*, artificially inoculated with *Aspergillus flavus*, are presented. The development of the fungus inside the termite body, after the penetration of the integument, is described. The hyphae were present throughout the body with the maximum infestation in the hemocoel. Hyphal bodies of various shape and size intermixed with hyphae are found. No fungal infection was observed in the lumen of the digestive tract of the dead termites. The adipose and muscular tissues were invaded by the fungus.

The termite/fungi relationship is an important aspect of termite biology. The fungi can be beneficial or harmful to termites; even strains of the same species react differently at different times. Sand [14] described very comprehensively the relationship between the termites and fungi. He mentioned a number of species having a harmful effect, either as a parasite penetrating the cuticle of the host or due to the toxicity of the ingested mycelium and spores. Blackwell and Kimbrough [3] reported 8 genera and 20 species of fungi parasitic on termites.

Becker and Kerner-Gang [2] tested the influence of 30 species of Ascomycetes and Fungi imperfecti on different species of termites and found *Aspergillus flavus* and other species of *Aspergillus* to be very toxic. *A. flavus* is also reported to be pathogenic to *Reticulitermes virginicus* [1] and *Odontotermes obesus* [15].

Studying the pathology of an *Entomophthora* infection in the eastern subterranean termites, Yendol and Paschke [16] demonstrated that the early fungal growth inside the body usually consisted of nonseptate mycelial filaments which are highly vacuolated and often branching. The penetrating mycelium spreads rapidly throughout the hemocoel lysing muscles and adipose tissue. These studies were based on light microscopy.

Very interesting ultrastructural studies on the fungi in insect body were carried out by Zacharuk [17], [18] on the Elateride larvae infected with *Metarhizium anisopliae*. Similar studies were made by Khan and Kimbrough [8] concerning the development of *Termitaria snyderi* inside the termite body. Khan and Aldrich [9] observed the development of haustoria of this fungus into the termite body.

This study was undertaken to interpret the development of *Aspergillus flavus*, on an ultrastructural level, within the body of the termites *(Odontotermes obesus)*. Penetration of the host integument is described in a further paper (in press).

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MATERIAL AND METHOD

The termites infected with *Aspergillus flavus* by the direct exposure method [6] were used. The fungus strain, isolated from diseased termites, has previously been found to be pathogenic [7].

The infected termites, just after the death, were fixed in 2.5% buffered glutaraldehyde (phosphate buffer pH = 7.35) for 4 hours. The material was post-fixed in buffered osmium tetraoxide (1.5%) overnight in the refrigerator. After several rinses in phosphate buffer, it was dehydrated in a graded series of ethanol. Embedding was made in Epon 812, following the method of Glauert and Hopwood [5] with some modifications [10]. The ultrasections were obtained with a Tesla BS 790 A ultramicrotome. The material was stained by Reynold's method [13] and examined on JEM-7 electromicroscope.

RESULTS AND DISCUSSIONS

Aspergillus flavus infected termites died within a short time (72 hours) after inoculation. The fungus continued to grow inside the body degrading different tissues and occupied the hemocoel gradually.

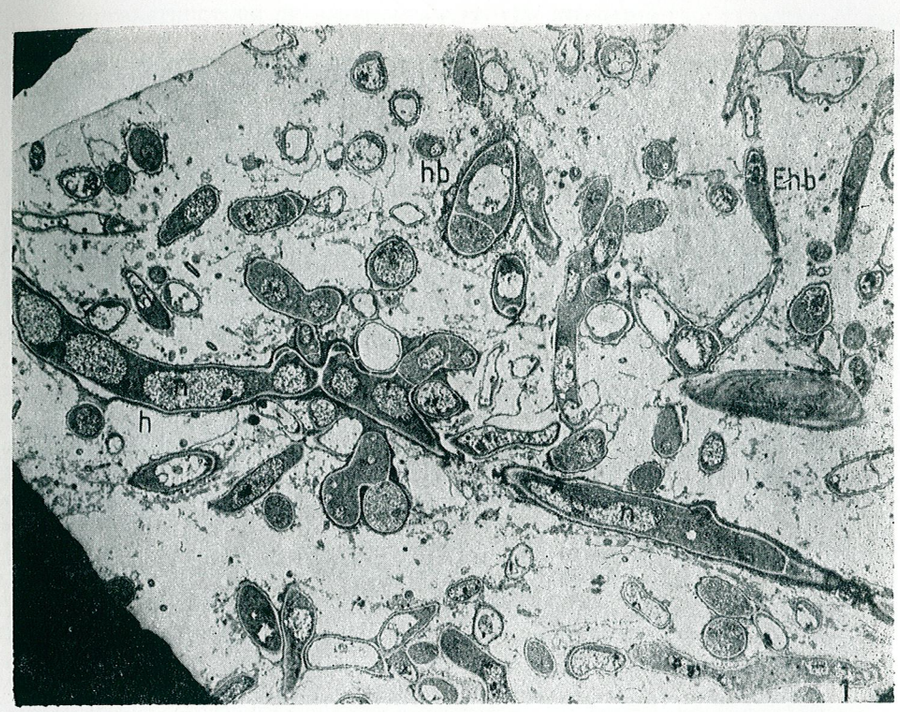
Development of the fungus starts with the germination of conidia which come in contact with the body when termites were allowed to crawl on the fungus sporulated culture. Germinative tubes of conidia penetrate the integument and continue to grow forming young hyphae which are very abundant in the hemocoel (Fig. 1). The penetrating mycelium spread rapidly within the host body. The hyphae varied in size in different parts of the body. Immediately under the cuticle these are thin and not so long (Fig. 5). In the hemocoel long, branched, septate and broad hyphae are observed (Fig. 2). The adipose and muscular tissues are invaded by the fungus (Fig. 6), as was also observed by Yendol and Parschke [16].

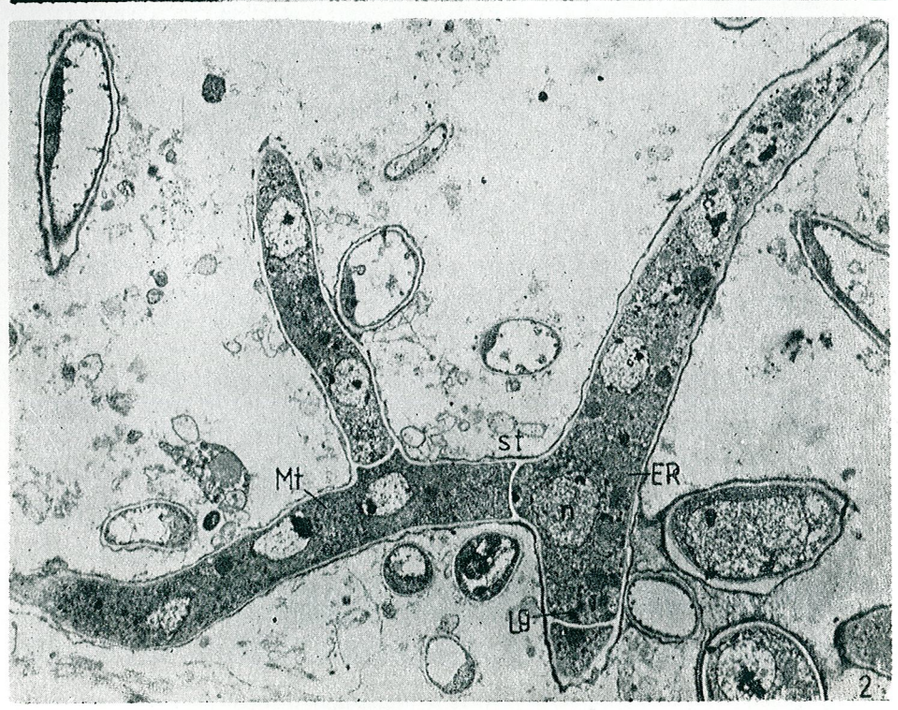
Fungal development continues and the hypha having electron-dense contents due to the cytoplasm rich in ribosomes and appeared intensively coloured (Fig. 2). Similar observations were also reported by Boucias and Pendland [4]. With the development, cytoplasmic content of the hyphae starts becoming transparent and some very electrondense, circular and irregular zones are evident (Figs 1, 2). These types of structures were also observed by Zacharuk [18] while studying the ultrastructure of *Metarhizium anisopliae* and were considered as vacuoles with fine granular contents, only present in hyphae developed in the host body.

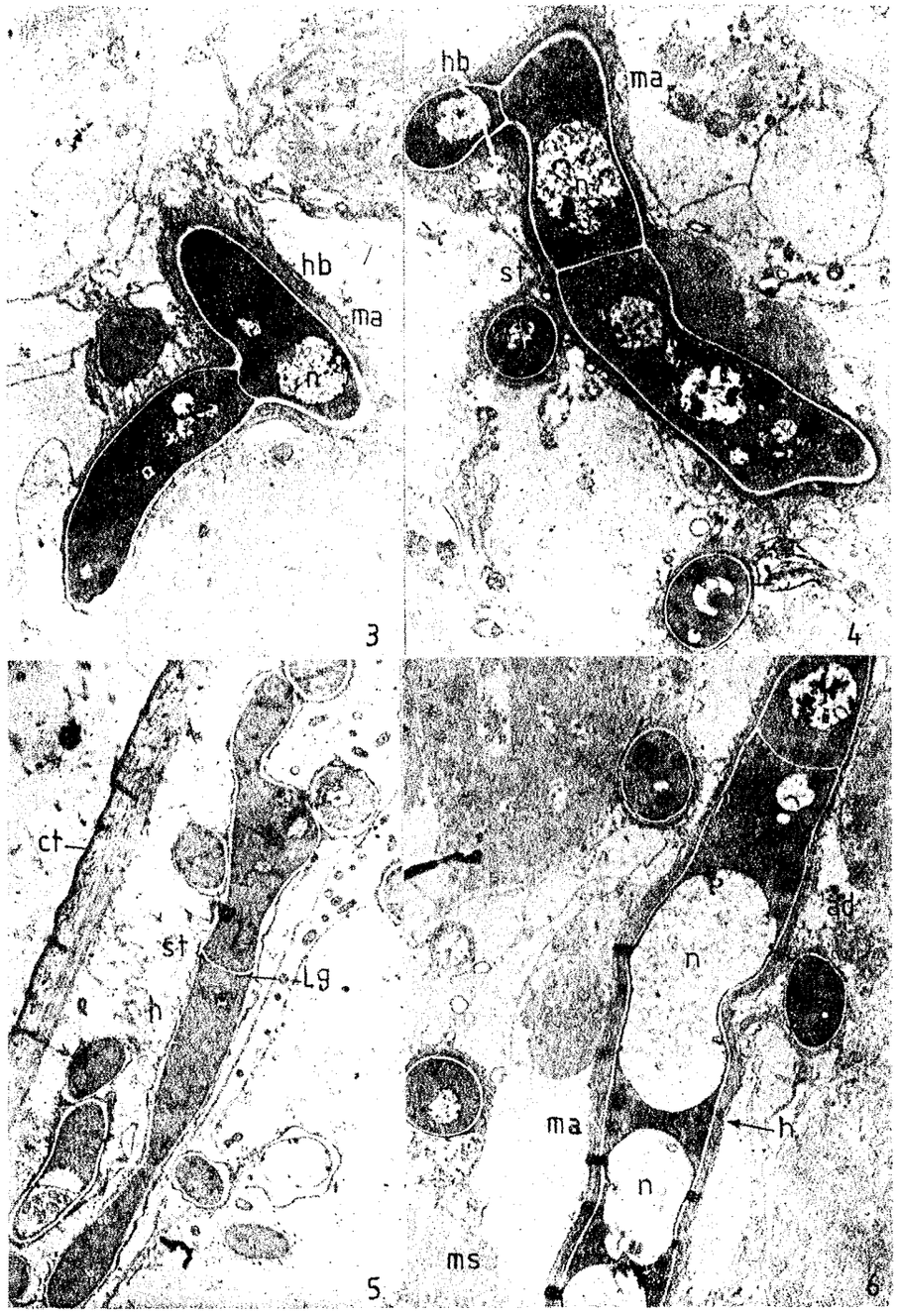
In the developed hyphae some visible cellular organelles, i.e. mitochondria, endoplasmic reticulum, ribosomes are present. Nuclei are in

Plate I

Fig. 1. — An advanced stage of fungus development in the host body characterized by the presence of hypha and hyphal bodies in the hemocoel. × 1,800
Fig. 2. — A branched septated long hypha in hemocoel. × 5,000
h = hyphae; hb = hyphal bodies; Ehb = elongated hyphal body; ER = endoplasmic reticulum; Mt = mitochondria; n = nucleus; st = transverse septum; Lg = Lipoidal granule.







the form of electrontransparent nuclear bodies containing electrondense DNA fibrils (Figs. 2, 6).

Further development of the fungus inside the host body is evident by the presence of hyphal bodies. These are developed initially by the elongation of the hyphae and the transversal septation (Fig. 1) or also by budding (Figs. 1, 4, 5). The formation of hyphal bodies takes place when the penetration hyphae reached the hemocoel as also observed by Boucias and Pendland [4]. Some hyphal bodies were elongated (Fig. 1) similar to the second type hyphal bodies founded by Zacharuk [18] and unicellular hyphal bodies observed by Boucias and Pendland [4].

There is much variation in the size, shape, vacuolation and mode of development of the hyphal bodies. Similar observations were also made by McCauley et al. [11]. These structures are found invading the adipose and muscular tissues (Fig. 3). Prasertphone and Tanada [12] described the formation and circulation of hyphal bodies in the hemolymph of larvae of *Galleria mellonella*. They also mentioned the appearance of hyphal bodies in adipose, muscular and nervous tissues of the host and some of the hyphal bodies were transformed into chlamydospores within the dead larvae.

Formation of new hyphal bodies takes place by the transverse septation of the hyphae or of the hyphal body itself (Fig. 1). Hyphal bodies are also formed by the transverse septation of the lateral extension of the hyphal body (Fig. 3). These observations are in accord with those of Zacharuk [18]. Lipoidal granules are observed on each side of the septum (Figs. 2, 5). Later on the septum becomes double walled and separation gives rise to daughter cells (Fig. 5). The hyphal bodies become elongated and are found inside the whole body along with the other developed hyphae (Fig. 1).

At the periphery of the hyphae (Fig. 2) and hyphal bodies (Figs. 3,4), a zone of amorphous material is observed. Zarnea et al.[19] mentioned that sometimes this zone is very thick. In case of *Anticarsia gemmatalis* infected with *Nomurea rileyi* [4] this zone is also found. Ultrastructural observations have suggested that this is due to the diffusion of the histolytic enzymes by the hypha and hyphal bodies. These enzymes help degrading the host tissues, as also reported by other authors [4].

Development of the fungus continues after the death of the host. The adipose, muscular and other internal tissues are totally degraded. Finally, the fungus forms the conidiophores which break through the integument and come to exterior. Nutrition depletion within host body is supposed to be responsible for the formation of conidiophores [4].

Plate II

Fig. 3. — Elongated hyphal body formed in a very advanced stage of fungus development — a new hyphal body is being formed by lateral extension. × 2,500

Fig. 4. — Hyphae in adipose tissue. Around the hypha an amorphous material is seen — a zone of histolytic enzymes of fungus. × 3,500

Fig. 5. — Hyphacindeveloped immediately under the cuticle. × 3,500

Fig. 6. — Hyphae invading the adipose and muscular tissues. × 3,500
ad = adipose tissue; ms = muscular tissue; ma = amorphous material; ct = cuticle; h = hyphae; hb = hyphal body; n = nucleus; st = transverse septum; Lg = Lipoidal granule.

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Le symposium « Evolution et adaptation », Cluj-Napoca, les 17—18 décembre 1982, Université « Babeș-Bolyai », Cluj-Napoca, 1983, 314 pages

Organisé par la Faculté de biologie, géographie et géologie, en collaboration avec le Centre de recherches biologiques et l'Institut de spéléologie « Emil Racoviță » de Cluj-Napoca, le symposium a permis un fertile échange d'informations et l'approfondissement, avec originalité, de certains aspects d'actualité essentiels pour la connaissance de la vie des organismes (végétaux et animaux) concernant leur évolution, leur différenciation et leur adaptation, dans le temps et l'espace, dans des conditions variées du milieu, y compris au cours des recherches expérimentales.

Le volume récemment paru a réussi à refléter cette remarquable manifestation scientifique de biologie, d'un intérêt tout particulier tant sous l'aspect scientifique fondamental, que sous celui pratique-applicatif.

Les ouvrages présentés dans ce volume (92 comunications et rapports), signés par 40 biologistes de Roumanie (Cluj-Napoca, Bucarest, Pîngărați, Constanța, Hunedoara, Viișoara — département de Mureș —, Sibiu, Miercurea-Ciuc), ainsi que par quelques-uns de l'étranger (Paris, Freiburg), mettent en évidence la variété des problèmes d'actualité débattus au symposium. Le volume comprend aussi la liste des auteurs et des institutions où ils déploient leur activité — universités, centres de recherches, d'enseignement secondaire et autres.

Les communications et les rapports édités dans ce volume, appartenant aux différentes disciplines de la biologie végétale et animale, peuvent être groupés comme suit :

Problèmes de conception en biologie, théories, méthodes et critères: comparaisons entre la phylogénie et l'écogenèse (N. Botnariuc, V. Soran); la théorie de l'évolution des espèces (I. I. Băra); adaptation et polymorphisme (T. Persecă); l'évolution des fonctions (C. Wittenberger); évolution et entropie (V. Bercea); évolution et progrès (T. Lupșe); la structure, la typologie et la dynamique de l'évolution des écosystèmes en général (P. Tudoran) et de ceux marins-côtiers (T. Gomoiu); le rôle de l'agressivité dans l'adaptation (A. Ionescu); mécanismes adaptatifs de thermorégulation (M. Dordea);

Aspects évolutifs de biologie végétale: les archébactéries et la chronologie de l'évolution chimique et organique (Șt. Kiss); différenciation et adaptation chez les algues vertes (Tr. I. Ștefureac); l'effet de la concentration ionique sur la stabilité des communautés de diatomées (L. Șt. Péterfi, L. Momeu, M. Vereș); critères d'appréciation des algues, leur rôle et leur adaptation biologique (F. Nagy-Tóth, A. Barna); l'adaptation des bryophytes aux diverses conditions du milieu (Tr. I. Ștefureac); l'adaptation des plantes — stratégies écophysiologiques dans l'évolution (A. Fabian, V. Soran); implications des cultures d'organes, de tissus et de cellules in vitro dans l'écologie et l'adaptation des plantes (I. I. Băra, E. Wellmann); évolution des explants végétaux in vitro et l'adaptation de néoplantules au milieu septique (D. Cachiță-Cosma, T. Osváth, C. Deliu, A. Andreica); la morphogenèse au niveau des explants caulinaires chez le chrysanthème (M. Lazăr, D. Cachiță-Cosma);

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Travaux à caractère biogéographique: nouvelles directions dans la biogéographie concernant des problèmes de spéciation dans l'évolution (P. Bănărescu); l'évolution géographique de certains poissons cyprins de l'Asie orientale (M. Șerban, P. Bănărescu); un nouveau mécanisme régulateur de l'activité de la péroxydase des plantes, avec des implications adaptatives à diverses niches géographiques (M. Dumitrescu, R. Gorenflot, H. Coudere);

La pensée biologique de quelques prédécesseurs roumains: V. Conta, précurseur de la pensée évolutionniste en Roumanie (I. I. Băra).

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Les discussions qui ont suivi la présentation des ouvrages, avec des citations de la bibliographie de spécialité à l'appui, ont suscité un vif intérêt ; on a également souligné les contributions personnelles des auteurs. Les résultats obtenus, souvent étayés de figures, graphiques schémas, profils, esquisses et tableaux originaux, dénotent une intensification de l'étude des problèmes actuels de la biologie et présentent un réel intérêt scientifique fondamental et pratique-applicatif.

Cette manifestation si bien organisée, à caractère pluridisciplinaire, marque un moment important dans l'évolution même des sciences biologiques à l'étape actuelle en Roumanie. C'est pourquoi les participants au symposium ont fait ressortir la nécessité d'organiser de nouvelles réunions scientifiques de ce genre.

Le volume constitue ainsi un remarquable guide des problèmes d'actualité en biologie aussi bien pour les biologistes roumains que — par les résumés et les titres des ouvrages dans la langue étrangère respective — pour ceux d'autres pays, ce qui confère aux ouvrages une large portée informative.

Du point de vue éditorial, on remarque le soin particulier du Comité de rédaction, sous la direction du professeur dr. Ştefan Kiss et du dr. Nicolae Coman, pour que ce volume paraisse dans des conditions irréprochables, y compris l'aspect technorédactionnel.

Traian I. Ştefureac

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La « Revue roumaine de biologie — Série de biologie végétale » publie de articles originaux d'un haut niveau scientifique, de tous les domaines de la biologie végétale : morphologie, systématique, géobotanique, physiologie, écologie, génétique, microbiologie, phytopathologie. Les sommaires des revues sont complétés par d'autres rubriques, comme : 1. La vie scientifique, qui traite des manifestations scientifiques du domaine de la biologie : symposiums, conférences, etc. ; 2. Comptes rendus des livres de spécialité parus en Roumanie. Les auteurs sont priés d'envoyer leurs articles, notes et comptes rendus dactylographiés en deux exemplaires. Les tableaux et l'explication des figures seront dactylographiés sur pages séparées et les diagrammes seront exécutés à l'encre de Chine noire, sur papier calque.

Les tableaux et les illustrations seront numérotés avec des chiffres arabes. La répétition des mêmes données dans le texte, dans les tableaux ou dans les graphiques sera évitée.

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