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Calea Victoriei 125
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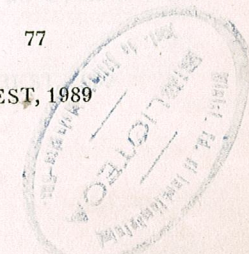
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EFFECTS OF ZINC AND CADMIUM ON THE CELL
STRUCTURE OF THE GREEN ALGA *MONORAPHIDIUM*
CONTORTUM (THURET IN BRÉB.)
KOMÁRKOVÁ-LEGNEROVÁ

NICOLAE DRAGOȘ, VERONICA CRĂCIUN

Exponential cultures of *Monoraphidium* were exposed to Zn and Cd ions (6 mg.l⁻¹) in an appropriate growth medium. Relatively few cell structures were affected after 48h: the nucleus, cytoplasmic vacuoles and mitochondria. The two metals, singly or together, have similar effects on *M. contortum* cells. These effects are mainly the extension of vacuolar electron-dense inclusions and the occurrence of intranuclear ones. No changes could be noticed in chloroplasts, pyrenoids and dictyosomes, as compared to control. Changes were interpreted as the result of cell detoxifying process. Relatively small pyrenoids, of an inner type, not invaginated by thylakoids, occur within the chloroplast of the control. They are devoid of starch sheath and do not have a specific location.

The passive uptake of ions has been proved by many studies to play a leading role in the accumulation of heavy metals by algal cells (7). The mechanism is quick and is mostly due to the binding of metal ions to cell walls and other internal cellular structures. This binding is reversible, at least in the first stage, so that the cations can be desorbed in solutions with chelates (such as EDTA, for instance).

The toxicity of heavy metals is proportional to their intracellular concentration. Over detoxification limit some irreversible changes occur, that end with the death of cells.

Electron microscopy can describe two types of changes occurring at an ultrastructural level. The changes caused by the peculiar affinity of each cellular structure toward heavy metals or by the mechanism of cellular detoxification belong to the first type, while the second could include those changes which express the gradual destroying of cellular structures.

Studies in the first line of research brought about the observation of granular electron-dense inclusions in different types of eukaryotic cells. They contain a large inactivated and non-diffusible quantity of metal.

Studies on the effects of heavy metals on the ultrastructure in eukaryotic algae are relatively few.

MATERIALS AND METHODS

Unialgal cultures of *Monoraphidium contortum* (Thuret in Bréb.) Komárková-Legnerová (strain Mco 3, obtained from the algal collection of the Biological Research Centre) were used for experiments. The cultures were grown in medium 11 (37), at room temperature (20–25°C) in a fluorescent light (9,500 lx).

During exponential growth, the algal suspensions were concentrated and washed by centrifugation and resuspended in a fresh medium chelate free. Heavy metals (as nitrates) were added from stock weakly acid solutions: $6 \text{ mg.l}^{-1} \text{ Zn}$, $6 \text{ mg.l}^{-1} \text{ Cd}$ and $3 \text{ mg.l}^{-1} \text{ Zn} + 3 \text{ mg.l}^{-1} \text{ Cd}$. After a 48 h exposure to metals, samples of algal suspensions were collected and prepared for electron microscopy.

The cells were fixed in 4 % glutaraldehyde for 2 h at 4°C and in 2 % osmic acid for 1 h. This was followed by dehydration in a successive acetone series and embedment in Vestopal W. Both unstained and stained sections were examined in a TESLA BS-500 electron microscope at 80 kV, in the Electron Microscopy Laboratory of the University of Cluj-Napoca.

RESULTS AND DISCUSSION

The cellular wall is made up of two layers differing electronmicroscopically (Fig. 5). The inner layer, with a microfibrillar structure, is thicker and, perhaps, of a cellulosic nature. The outer layer is trilaminar (two electron-dense bands separated by an electron-transparent one). A similar structure can be found in species of the genera: *Chlorella*, *Scenedesmus*, *Coelastrum*, *Pediastrum*, *Ankistrodesmus* and *Kirchneriella* (1, 4, 10, 18, 22, 25, 26, 29). The sporopollenin nature of the trilaminar layer has been shown in *Chlorella fusca* var. *vacuolata* (1).

The single chloroplast (Figs. 1, 2, 4) is parietal and with a characteristic lamellar structure. The plastid lamellae are usually made up of 2–6 thylakoids stacked together. Intraplastidial starch granules can be noted. Relatively small pyrenoids, of an inner type not invaginated by thylakoids, occur in a varying number within the chloroplast. They are devoid of starch sheath and do not have a specific location. Sometimes, a pyrenoid may be derived by plastid lamellae.

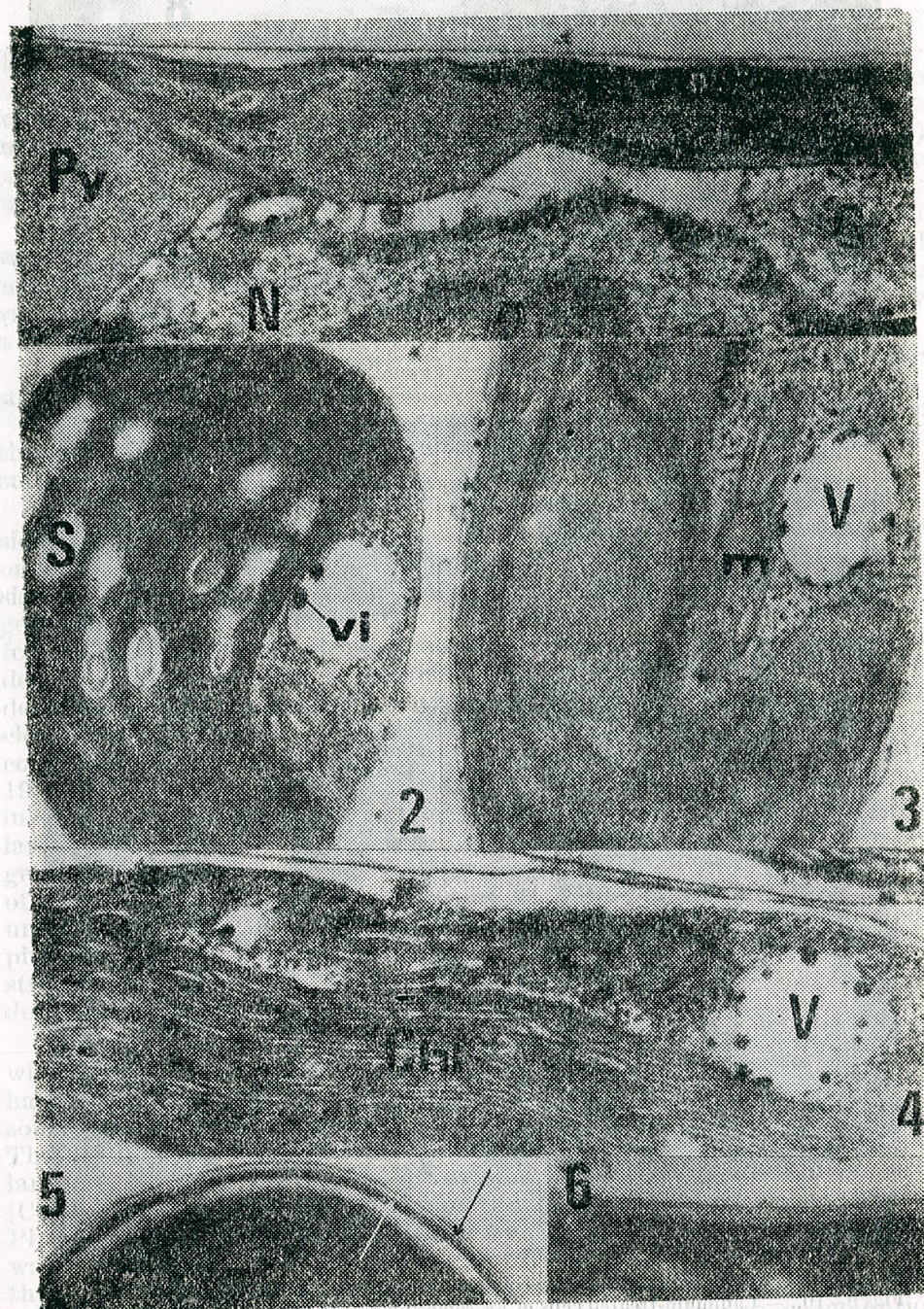
Legnerová (1969) does not record the presence of the pyrenoid in the species diagnosis. It is difficult to observe pyrenoids in the optic microscope because they lack their starch sheath.

The nucleus has a median position, without seemingly differing from the nuclei of other green algae of the same group (Fig. 1).

Relatively few mitochondria and dictyosomes as well as several vacuoles also occur in the cytoplasm (Figs. 1–4). These vacuoles are frequently electron-transparent or contain granular electron-dense inclusions of a varying size and shape. These inclusions have been identified as polyphosphates (5, 26).

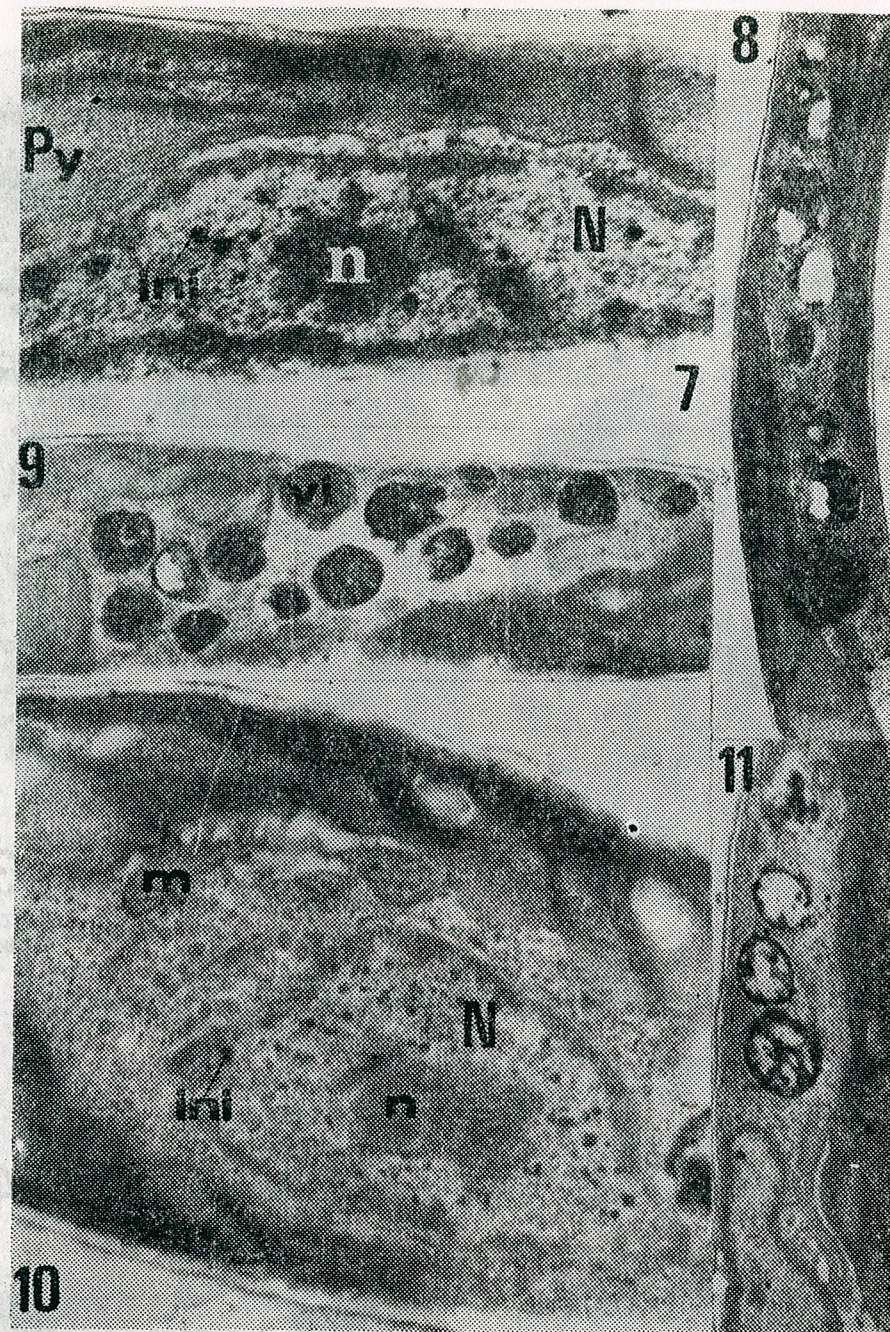
The 48 h exposure of *M. contortum* cells to metals modifies few cellular structures. It is also worth noting that the two metals cause similar ultrastructural changes.

Zn and Cd modify the nucleus. After exposure, several electron-dense inclusions of different sizes and granular appearance showed in the nucleoplasm (Figs. 7, 10, 11). These inclusions do not affect the nucleolus and the outer chromatin areas close to the nuclear envelope, but seem to be attached to its pores (Fig. 7). The larger intranuclear inclusions are not homogeneous and seem to be made up of several spherical units (Fig. 10).



Figs 1–4. — Ultrastructural organization of the normal cell of *M. contortum* (control) ($\times 57,400$).

Figs 5–6. — Cell wall of control cells; white arrow shows the inner cell wall layer and the black arrow, the trilaminar one. Sometimes this structure is doubled by the mother cell wall ($\times 83,000$; $\times 40,000$).



Figs 7-8. — Details from thin sections of zinc-treated cells ($\times 57,000$; $\times 23,000$).
 Figs 9-10. — Cadmium-treated cells of *M. contortum* ($\times 28,000$; $\times 57,000$).
 Fig. 11. — Cell treated with zinc and cadmium mixture ($\times 28,000$); for further explanations see the text.

Abbreviations: chl — chloroplast; G — Golgi bodies; ini — intracellular inclusions;
 m — mitochondria; S — starch granules; V — vacuoles; vi — vacuolar inclusions.

Sometimes, the center of these intranuclear inclusions seems less electron-dense. Very often, cells in different stages of mitosis showing electron-dense inclusion in their nucleus could be noticed. A few inclusions, similar to the intranuclear ones, may also occur very seldom in the mitochondria (Fig. 10) or the cytoplasm. Similar osmiophil inclusions were also observed in the mitochondrial profiles of the control.

Of special interest in Zn- and Cd-treated cells is the extension of vacuolar inclusions in the cytoplasmic vacuolar system (8, 9, 11). In control cells these inclusions were more or less granular, but here they may fill the vacuoles entirely. Sometimes their dense appearance may be replaced by a somehow loose net (Figs. 9, 11).

No changes could be noticed in chloroplasts, pyrenoids and dictyosomes as compared to control (Figs. 7, 10, 11).

The two metals administered together bring about similar effects to those caused by their equivalent concentrations when added singly to the culture medium.

Intranuclear, cytoplasmic or intramitochondrial electron-dense inclusions influenced by heavy metals have been described in many aquatic or terrestrial organisms. It has been established by means of EMMA analysis that they accumulate heavy metals as complex undiffusible formations, generally made up with phosphorus and sulphur. The denomination used for such formations is extremely varied: intranuclear inclusions, electron-dense precipitates, finely granular electron-dense material, electron-dense spheroids, intramitochondrial dense granules, granular deposits, electron-dense deposits, deposits, precipitates, electron-dense particles, concretion bodies, metal containing granules etc. (2, 3, 9, 11, 12, 14, 15, 19, 21, 23, 31-34). In this paper we have chosen the term electron-dense inclusions with an intranuclear, intramitochondrial and cytoplasmic (vacuolar) location. This choice may be supported by the following facts: the granules containing heavy metals are not entirely new structures, some of them (namely the vacuolar and intramitochondrial ones) may also appear under normal conditions (5, 21, 36); the terms, granules, deposits and precipitates are mainly descriptive and emphasize only the most obvious morpho-structural characteristic, the term inclusion is general enough to allow the derivation of collateral terms.

Ultrastructural studies on the changes caused by heavy metals within eukaryotic algal cells are rather few. Silverberg and collab. (31-34) have dealt with the influence of Pb, Cd and Cu on the ultrastructure of some green algae (*Scenedesmus*, *Ankistrodesmus* and *Stigeoclonium* species). Their results evince formation of a large number of granular intranuclear inclusions (Cu and Pb) and the occurrence of electron-dense vacuolar inclusions (Cu and Pb) and the occurrence of granular intranuclear (Cu and Cd) and intramitochondrial (Cd) inclusions. The experiments where Pb was localised with rhodizonate showed its accumulation on the cellular walls. Such changes were also accompanied by the degeneration of the thylakoid membranes, an increase in number of starch granules and plastoglobuli, the enlargement of the endoplasmic reticulum, a vacuolisation of mitochondria etc.

Sicko-Goad and Stoermer (30) have shown that Pb and Cu accumulated within the vacuolar polyphosphate inclusions of *Diatoma tenue* var. *elongatum*.

PARTICULAR INTERACTIONS BETWEEN PROTOPLASTS AND CELLS DURING THE AGGLUTINATION AND FUSION PROCESSES IN YEASTS — ULTRASTRUCTURAL ASPECTS

I. ANGHEL, T. VASSU, A. BREZEANU, A. ROȘU

Our researches concerning protoplast fusion for obtaining recombinants in the yeast strains *Candida robusta* and *Saccharomycopsis lipolytica* pointed out some particular interactions between protoplasts and cells during the agglutination and fusion processes induced by PEG, expressed by close associations not only between protoplasts, but also between protoplasts and cells or protoplasts and ghost cells. Sometimes these processes are associated with morphological modifications of the cell wall and local lyses at the contact areas with plasmalemma. The frequent occurrence of cytoplasts and evaginations of the plasmalemma in these areas suggests their involvement in fusion processes.

In order to use protoplast fusion as a tool for experimental hybridisation applied in improving processes, the industrial yeast species, *Candida robusta* and *Saccharomycopsis lipolytica*, were investigated. The protoplast agglutination and fusion, induced by PEG and Ca^{2+} , were studied by electronmicroscopy.

MATERIALS AND METHODS

The industrial yeasts for proteic biosynthesis, *Candida robusta* and *Saccharomycopsis lipolytica* were investigated.

Protoplast isolation was induced by 2 hours incubation in an enzymatic mixture consisting in crude snail gut juice from *Helix pomatia*, mercaptoethanol and a solution of 1.2 M KCl and 0.6 M $MgSO_4$, as osmotic stabilizer (1).

The agglutination and fusion processes were induced with PEG 6000, 40%(W/v) diluted with Tris HCl 0.05 M, pH-7.5 and 10 mM $CaCl_2$.

For the electronmicroscopical study of the protoplasts and of the fusion products, a standard method, as modified in our laboratory was used: 3% glutaraldehyde fixation over night at 4° C, 2% OsO_4 for 2 hours postfixation, embedding in Cy 212 Araldite. The ultrasections obtained on a Tesla ultramicrotome were stained with uranyl acetate and lead citrate and visualised under a JEM-7 electronmicroscope working at an electron acceleration of 80 kW.

RESULTS AND DISCUSSIONS

PEG in association with Ca^{2+} induce an efficient protoplast agglutination by establishing a very tight contact between them and thus the lipid bilayer of the plasma membrane is perturbed and its fluidity increased (8). Our researches pointed out some particular interactions between protoplasts and cells during these processes, expressed by close associations not

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only of protoplasts but also between protoplasts and cells or protoplasts and ghost cells. For associations the presence of the plasmalemma is absolutely necessary (2, 3).

Sometimes, these processes are associated with morphological modifications at the level of the plasmalemma and of the cell wall. The local lysis of the cell wall at the contact areas with the plasmalemma are also observed. The frequent occurrence of cytoplasts and evaginations of the plasmalemma in these areas suggests their involvement in the fusion process. The connexions established between protoplasts and cells having the surrounding walls and even between protoplasts and ghost cells are mostly intriguing. As the action of the fusion agents is not yet fully understood, it is hard to find an unquestionable explanation for this phenomenon. It may be presumed that the membrane processes partly induced by ATP-dependent reactions may be responsible of this kind of surface interactions between protoplast plasmalemma and some pores existing in cell walls of adjacent unprotoplasticized cells. It is well known that the electrical potential of the plasmalemma is partly established by ATP-fuelled proton release; the H^+ pumps driven by ATP are an integral part of the cell membranes, being deeply involved in the membrane processes, by influencing the pH-gradient and the electrical membrane potential. The protoplasts, being non-polar, initially display a random distribution of ion pumps and channels within the plasma membrane; the variations in the ion composition as well as in the electrical membrane potential in the new environment in which cells and protoplasts were maintained during the fusion process, may trigger these unusual interactions. At the same time, the increased membrane fluidity due to perturbations of the lipid bilayer of the plasmalemma, that is induced by the fusion agents (PEG and Ca^{2+} ions), explains the local disorganization of the plasma membrane at the contact sites, forming cytoplasmic bridges which seem to diffuse through the wall of the adjacent cell in its cytoplasm (4, 5, 7).

Some experimental results support the idea that the cell membrane has pores through which water, non-electrolyte molecules and ions pass simultaneously or independently of one another. It may be presumed that different electrochemical gradients that may exist between the protoplasts and the content of an intact cell, brought together in close proximity by the fusion agents, facilitate the establishing of evident communication channels through which cytoplasmic material passes.

There may also exist some receptor molecules at the plasmalemma level which could recognize and interact with other molecules from the adjacent cell wall and plasmalemma.

CONCLUSION

1. Our studies concerning protoplast fusion in *Candida robusta* and *Saccharomyces lipolytica*, aimed at obtaining some recombinant strains utilisable in protein biosynthesis, pointed out some particular interactions between protoplasts and cells during the agglutination and fusion processes, induced by PEG.

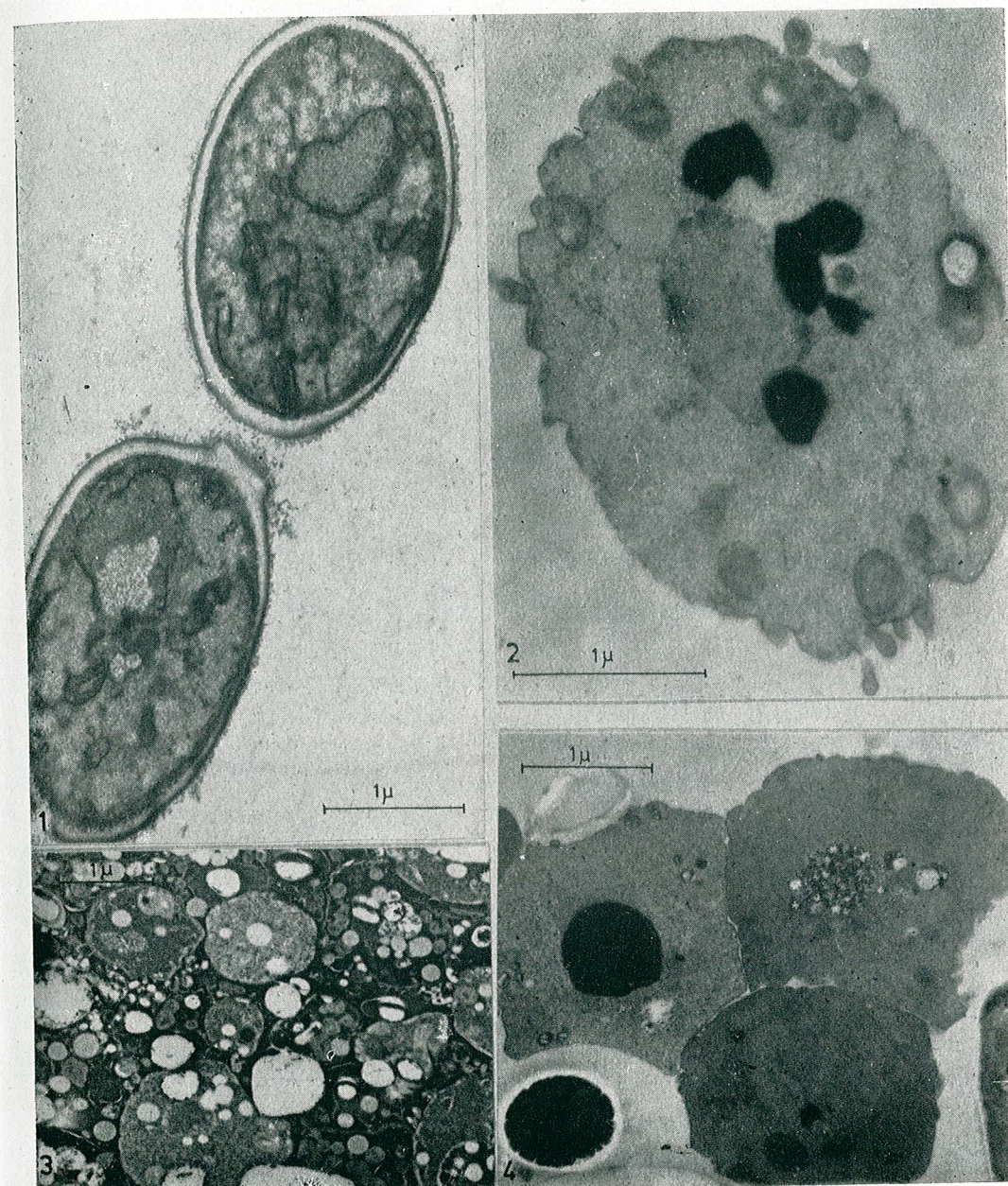


Plate I. — The ultrastructural aspects of the yeast protoplasts agglutination and fusion :

Figs 1—2. — The ultrastructural peculiarities of the yeast cell (Fig. 1) and of the freshly isolated yeast protoplast (Fig. 2);

Fig. 3. — Multiple aggregations of the protoplasts, immediately after the PEG treatment, before its removal.

Fig. 4. — Multiple homocellular aggregations of the protoplasts induced by PEG in the presence of Ca^{2+} ions, after its removal.

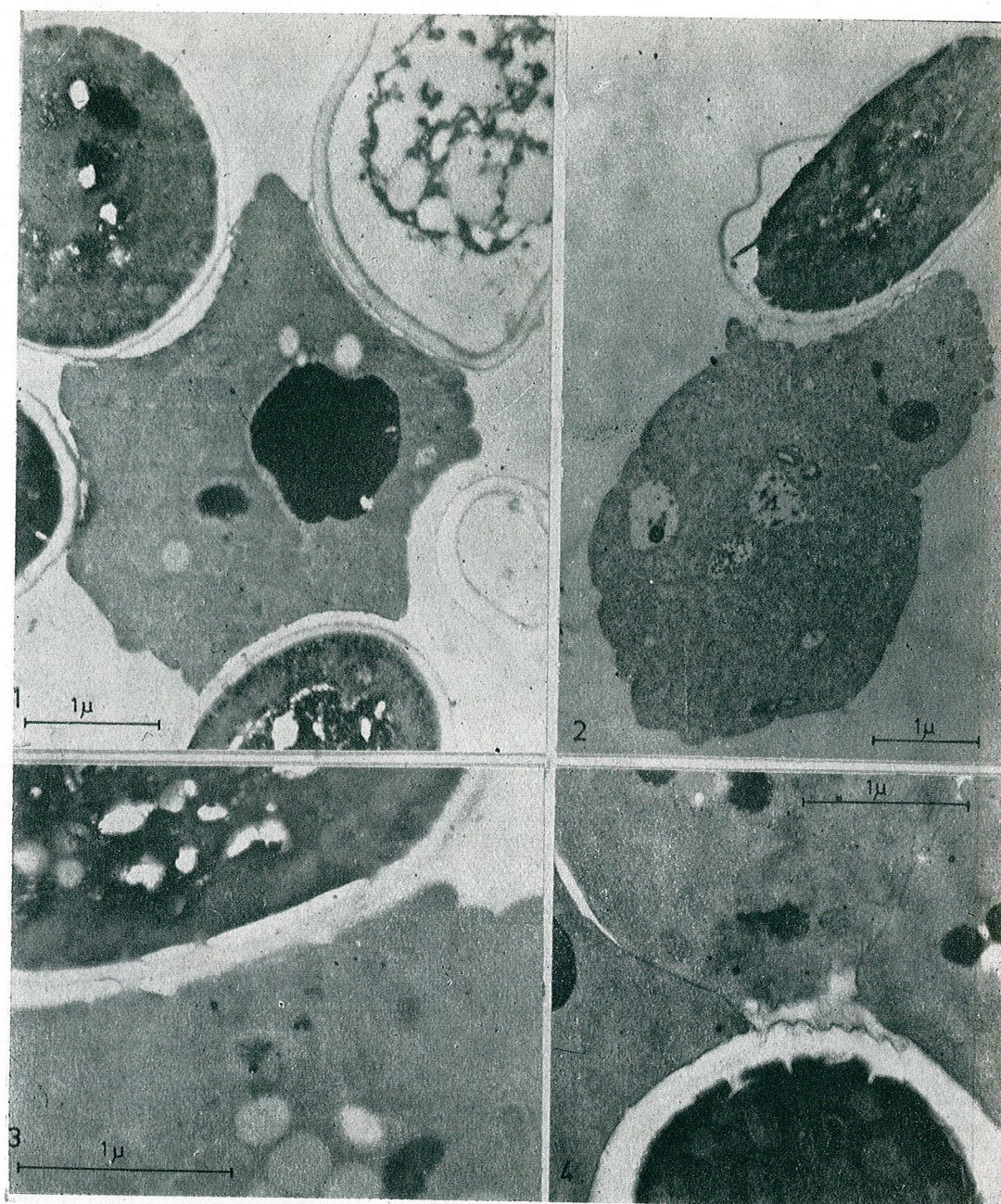


Plate II. — Particular interactions among protoplasts, cells and ghost cells during agglutination and fusion processes induced by PEG and Ca^{2+} ions;

- Fig. 1. — General view;
 Fig. 2. — Detail of the interactions between two fused protoplasts (arrow) and a cell (double arrow);
 Fig. 3. — Close associations between plasma membrane and cell wall by some 'micro-channel' differentiation;
 Fig. 4. — Some morphological modifications of the cell wall and the local lysis at the contact areas with plasma membrane.

2. These interactions are expressed by close associations not only of protoplasts but also between protoplasts and cells and protoplasts and ghost cells;

3. The interactions involved the obligatory presence of one partner having the plasmalemma deprived of cell wall. The cytoplasmic connections between protoplasts and the cytoplasm of the intact cells may be established by complex mechanisms, including the alteration of the electric surface loads of the plasma membrane, the different electrochemical gradients existing between the two cellular environments, increased membrane fluidity by the partly perturbation of the lipid bilayer, as well as the presumed existence of some surface molecules that recognize one another by their reaction affinity.

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University of Bucharest
 Aleea Porțocelor n. 1—3
 București 77
 Romania

COFFEE BY CELL CULTURES—PRELIMINARY RESULTS AND PROSPECTS

ANA ROȘU, DOINA CÂRCIUMĂRESCU, TATIANA VASSU-DIMOV

The biotechnological production of cellular mass in *Coffea* by non-conventional techniques offers potential economic advantages for the temperate areas, where the climatic factors make impractical the culture of this crop in the open environment. The preliminary results regarding the "in vitro" culture of *Coffea* tissues focused on the establishment of the optimal culture conditions for obtaining cellular mass, by inducing active proliferation of somatic cells of various origin.

Coffee beans represent one of the most important agricultural products in the international market; the drink made of roasted and ground seeds of certain species of *Coffea* is adopted almost everywhere throughout the world, due to its stimulating effects and organoleptic qualities.

While the economic importance of coffee crops for many tropical coffee producers is evident, in the countries situated in the temperate areas, where the prevailing climatic conditions make impractical the cultivation of this crop in the natural environment, the consumption of coffee is based entirely on imports, being limited by price fluctuations of the international trade market.

But the recent advances of the non-conventional techniques based on "in vitro" cultivation of cells and tissues may have some unique applications in overcoming these limitations, in terms of the biotechnological production of cellular mass and of natural products, suitable for various technical processes. The "in vitro" systems proved their ability to produce cellular mass by cultivation, on synthetic culture media, under controlled environmental conditions, of the cells pertaining to any plant, tropical or alpine, despite the climate factors or the soil characteristics, enabling the continuous synthesis of secondary metabolites, utilisable in pharmacy, medicine, food industry, etc (8).

As regards coffee, P. M. Townsley (1974) established liquid cultures of coffee cells from friable callus derived from orthotropic shoots of *Coffea arabica* cv. *El Salvador*, with the purpose of obtaining coffee aroma from the "in vitro" cultured cells. The cellular mass, in addition to producing caffeine, when harvested and "roasted", gave a coffee-like substance having the smell and the taste of the coffee derived from roasted coffee beans (3, 5, 6, 7, 9).

These results, suggesting that the coffee cell cultures maintain their ability to synthesize compounds unique to the parent coffee plant, stimulated our interest to establish an experimental protocol for studying the "in vitro" behaviour of various coffee tissues, as a prerequisite for achieving rapidly-growing coffee cellular mass.

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MATERIAL AND METHOD

The plant material used as explant source (Table 1) was harvested from a greenhouse-grown *Coffea arabica* tree. The surface sterilization was firstly performed by soaking in 96° alcohol for a few seconds and in 3% H_2O_2 for 5 minutes, followed by 3 rinses with sterile distilled water, under

Table 1

Material and methods

A. Explant sources

- Mature leaves from orthotropic or plagiotropic branches of greenhouse *Coffea arabica* plant;
- Nodal segments with axillary buds;
- Soft internodes from apical portions of shoots;
- Petioles;
- Fruit pedicles;
- Immature fruits;
- Cotyledonary leaves and mesophyll from aseptically grown plantlets.

B. Surface sterilization

- a) — Soaking in 96° alcohol for a few seconds;
- 5 minutes in 3% H_2O_2 ;
- 3 rinses with sterile distilled water.
- b) — Rapid rinse in 96° ethanol;
- 30 minutes in 0.05% $HgCl_2$;
- 5 rinses in sterile distilled water.

C. Culture conditions

- Darkness, 28–30°C;
- 1000 lux illumination, with a 16-hour-light period, at 25–28°C.

continuous agitation. By using this sterilization procedure, the percent of contaminated explants was relatively high. Therefore, in the subsequent series of experiments, the disinfection of the plant material was accomplished by using a solution of 0.05% $HgCl_2$ for 30 minutes, preceded or not by a rapid rinse in 96° ethanol. This procedure gave fairly good results, though both $HgCl_2$ and ethanol are considered as deleterious or even toxic to coffee cells. In order to eliminate the toxic traces, 5 rinses in sterile distilled water were effective. The explants of various sources were cultivated on media with the composition presented in Tables 2 and 3 (the micro- and macroelements formulations according to Gamborg and Eveleigh, 1968 and the Murashige-Skoog, 1962 basal media supplemented with various combinations and concentrations of hormones).

RESULTS AND DISCUSSIONS

For the initiation of culture, explants were inoculated upon a callus induction medium (variant Cf), consisting of the GE-PRL₄ inorganic salt formulation, supplemented with 2,4-D and kinetin and incubated in darkness. This variant of the culture media, containing minimal quanti-

ties of hormones (0.1 mg/l kinetin and 0.1 mg/l 2,4-D) was not appropriate for callus induction, disregarding the explant type and the physical environment of maintaining the cultures. Slight exemptions were only the petiole, fruit pedicle and green stem explants (Table 4). Although Sharp et al. (1973) obtained callus cultures from a variety of somatic and haploid tissues of *C. arabica*, in the presence of low concentrations of 2,4-D(4), in our tests just limited callus proliferation was recorded.

Table 2

Inorganic salt composition in coffee culture media

Salts	Basal media	
	GE-PRL ₄ * mg/l	MS(1962) mg/l
KNO ₃	1000	1900
NH ₄ NO ₃	—	1650
NaH ₂ PO ₄	90	—
KH ₂ PO ₄	—	170
(NH ₄) ₂ SO ₄	200	—
MgSO ₄ ·7H ₂ O	250	370
CaCl ₂ ·2H ₂ O	150	440
KI	0.75	0.83
KCl	300	—
Na ₂ HPO ₄	30	—
MnSO ₄ ·4H ₂ O	10	22.3
H ₃ BO ₃	3	6.2
ZnSO ₄ ·7H ₂ O	3	8.6
Na ₂ MoO ₄	0.25	0.25
CuSO ₄ ·5H ₂ O	0.25	0.025
CoCl ₂ ·6H ₂ O	0.25	0.025
Na ₂ EDTA	37.3	37.3
FeSO ₄ ·7H ₂ O	27.8	27.8

* In GE-PRL₄ medium iron has been added as sequestreen

Abbreviations

GE-PRL₄ = Gamborg and Eveleigh (1968)
basal medium
MS = Murashige and Skoog (1962)
basal medium

Sondahl et al. (1981) mention that in the coffee leaf explant system no sharp sensitivity exists pertaining to callus growth within a wide range of growth regulator concentrations (6); in our experimental conditions we found the mature leaf explants rather unresponsive.

Working on perisperm tissues of *C. arabica* and *C. stenophylla* Monaco et al. (1977) were able to induce abundant friable callus; perisperm proliferated rapidly in the absence of auxin, having sufficient endogenous auxin for sustaining growth (3). Our data reveal that even if the hormones are added, but in minimal concentrations, the cell proliferation is limited. We concluded that, taking into account the genotypic characteristics

Table 3
Culture media variants used for callus development in *Coffea*

Var.	Basal med.	Vitamins mg/l				Hormones mg/l						L-Cyst. mg/l	Glycine mg/l	Casein hydr. g/l	Malt extr. g/l	Sucrose g/l	Agar g/l
		Myo-inos	Thi-am.	Pyridox.	Nicot. amide	Ascorbic acid	Kin	2,4-D	NAA	IAA	IBA	BAP					
Cf	GE	100	10	1	1	—	0.1	0.1	—	—	—	10	2	0.200	0.5	30	8
C2	MS	100	10	0.5	0.5	—	0.5	—	2	20	—	10	2	0.200	0.5	40	8
C3	MS	100	10	0.5	0.5	—	1	—	4	—	—	10	2	0.200	0.5	40	8
C4	MS	100	10	0.5	0.5	—	2	—	—	—	4	10	2	0.200	0.5	40	8
C5	MS	100	10	0.5	0.5	—	2	1	—	—	—	10	2	0.200	0.5	40	8
C6	MS	100	10	0.5	0.5	—	1	—	1	—	—	10	2	0.200	0.5	40	8
C7	MS	100	10	0.5	0.5	—	4	1	—	—	—	10	2	—	—	40	8
C8	MS	100	1	—	—	—	—	—	—	—	5	15	2	0.100	—	30	8
LS2	LS	100	0.4	—	—	0.2	1	—	2	—	—	—	2	—	—	30	8
G	MS	100	0.1	0.5	0.5	—	1	—	—	1	—	—	2	—	0.1	30	8

Abbreviations

2,4-D = 2,4-Dichlorophenoxyacetic acid
IAA = Indole-3-acetic acid
IBA = Indole-3-butyric acid

NAA = α -Naphthalene-acetic acid
BAP = 6-Benzylaminopurine
Kin = 6-Furfurylaminopurine

and the ontogenetic age of the tissue explanted "in vitro", a lot of testing is necessary to select the parameters which are optimal for coffee cell proliferation.

The 6-week-old cultures were transferred onto a medium consisting of MS salts, with a more complex hormonal supplement (variant C₂), and incubated in the dark, at 28°C. The callus development was poor, in the majority of cases, excepting the explants of immature fruits, which developed abundant masses of white-yellowish callus cells (Fig. 1).

Table 4

Callus development 3 weeks after inoculation

Explant source	Medium variant	Physical parameters	% callus development	Intensity of callus development	Observations
Leaf	Cf	darkness, 27°C	—	—	No callus development occurred; the explants failed to grow and turned brown
	Cf	low light intensity, 20—22°C	—	—	
Petiole	Cf	darkness, 27°C	75	+	Islands of slow growing callus at the cut ends of the explants
	Cf	low light intensity, 20—22°C	70	+	
Green stems	Cf	darkness, 27°C	85	+	Slight hypertrophy at the explant ends, with slow-growing callus
	Cf	low light intensity, 20—22°C	—	—	
Fruit pedicles	Cf	low light intensity, 20—22°C	75	+	Little callus at the cut ends of the explants
Nodal segments	Cf	low light intensity, 20—22°C	85	+	Slight callus development
Green fruits	Cf	low light intensity, 20—22°C	—	—	Pronounced hypertrophy of the pericarp; intense phenolic exudation

Because among the explant sources tested only the green fruits expressed a superior degree of responsiveness regarding the callogenesis competence, our further testings involved explants consisted of entire halves of developing fruits, broadening the range of hormonal types and concentrations (Table 5).

Adding 10 mg/l L-cysteine in the solid culture media in order to prevent the accumulation of the brown exudation containing phenolic compounds, fact that might affect cell growth, was not efficient. Better results were obtained by maintaining the explanted tissues for a few days on a preconditioning medium (6,7), consisting of an agar solidified saline-sucrose formulation, with no supplemental plant growth regulators; this approach proved effective both in reduction browning of the medium and for an efficient screening of the healthy cultures. We also noticed that if the explants were excised from fruits in an advanced stage of maturation,

having a firm parchment, they failed to grow or grew poorly. So, the selection of the explant sources of an appropriate physiological age, collected from plants of well-characterized coffee cultivars, represents an important element to assure the success of callus culture development. After discarding the contaminants and exudations, the explants were inoculated on the inductive culture media, supplemented with kinetin and various auxins (NAA, IBA, 2,4-D, Table 5), according to some culture protocols described in the literature (6, 7). The cultures were maintained in a growth chamber in complete darkness, at 28–30°C.

Table 5

Callus development in green fruit explants

Variant	Hormones mg/l						% Callus develop.	Intensity of callus develop.	Observations
	BAP	Kin	NAA	IBA	2,4-D	IAA			
C ₂	0.1	0.5	2	—	—	20	66	++++	Abundant white callus tissue at the level of the pericarp and of the external layers of endosperm
C ₃	—	1	4	—	—	—	25	+++	Moderate callus growth
C ₄	—	2	—	4	—	—	33	+++	Moderate callus growth
C ₅	—	2	—	—	1	—	75	++++	Fast growing callus
C ₆	—	1	1	—	—	—	30	+++	Medium growing callus

The development of calli becomes visible after two weeks since inoculation, providing that the pericarp had not been damaged during the sterilizing procedure. In all growth regulator interactions tested, the best results were recorded on the variants C₂ and C₆; after four weeks of growing the primary cultures, the massive parenchymatous calli obtained were fragmented and subcultured.

Secondary cultures were thus established under 1000 lux illumination, with a 16-hour-light period, at 25–28°C, by subculturing the 30-day-old primary callus on the medium variants used for callus induction (C₂–C₆). After the subculture, the callus grew poorly on all variants. It has been demonstrated (6,7) that the maintaining of the coffee cell cultures in an illuminated environment appears not to be absolutely necessary for callus induction and development. In our experiments, maintaining the secondary cellus culture under light conditions resulted in limited callus proliferation.

In some instances, where the embryos were not damaged by explant excision, some of them continued to grow into plantlets. The aseptically grown plants are excellent sources of explants, since the surface sterili-



Fig. 1. — Callus development in immature fruit explants.



Fig. 2. — The callogenesis in leaf explant from juvenile tissues (aseptically grown plantlets).

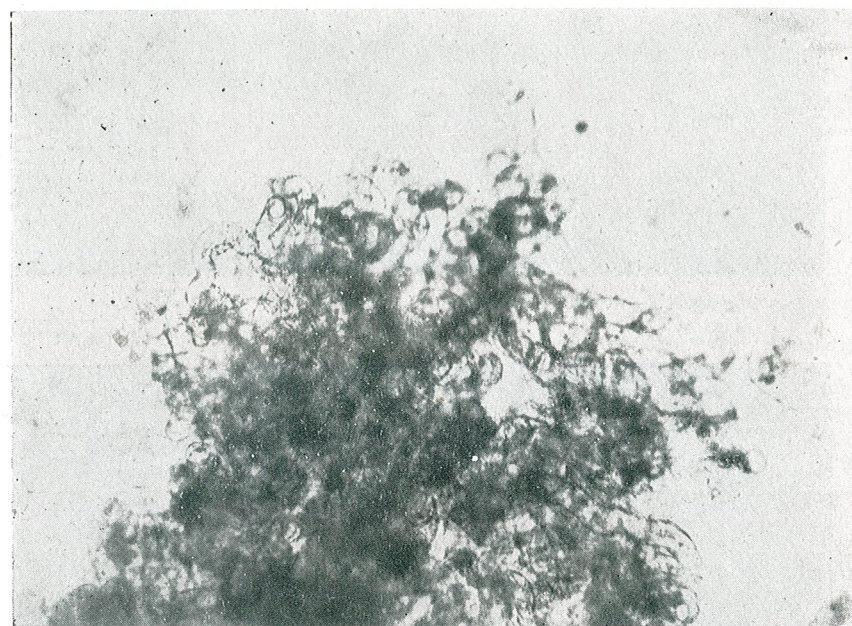


Fig 3. — Cellular aggregates developed in submerged cultures.

zation is not necessary. Therefore, in a manner similar to the culture of various tissues excised from mature greenhouse-grown coffee tree, some juvenile explants from aseptically grown plantlets were also tested for their ability of producing callus (the cotyledonary leaves and the first true leaf blades, trimmed in square pieces of 0.5 mm). Following the culture of these explants onto media containing either kinetin and 2,4-D (variant

Table 6

Media variants for cell suspension cultures in *Coffea*

Var.	Basal med.	Vitamins mg/l				Hormones mg/l						L-Cyst. mg/l	Glycine mg/l	Casein hydr. g/l	Malt extr. g/l	Sucrose g/l
		Myoinos	Thiam.	Pyridox.	Nicot. amide	Kin	2,4-D	NAA	IAA	IBA	BAP					
L1	MS	100	4	0.5	0.5	1	1	—	—	—	—	10	2	0.2	0.5	40
L2	MS	100	4	0.5	0.5	—	1	—	—	—	1	10	2	0.2	0.5	40
L3	MS	100	0.1	0.5	0.5	1	—	1	—	—	—	—	2	—	—	30
L4	MS	100	10	—	—	—	1	—	—	—	—	36	—	1	—	20

C₇) or BAP and IBA (variant C₈), resulted in callus cell proliferation; a yellowish parenchymatous callus mass covered the entire surface of the explant after 3 weeks of culture (Fig. 2), which demonstrated the potential of explants harvested from aseptically grown coffee plantlets for obtaining callus growth.

In order to establish liquid cultures of coffee cells, the MS basal medium was used, testing the effect of various hormone combinations (Table 6). By placing callus pieces in liquid media, good cell growth was recorded. Under the effect of 2,4-D the cells in liquid cultures form often large cellular aggregates (Fig. 3), indicating that 2,4-D represents a major stimulating factor of the coffee cell growth in liquid cultures.

CONCLUSIONS AND PROSPECTS

In summary, our results stress the differential response of coffee explants of various origin in the presence of a common growth medium and in the same physical environment. The experimental data presented reveal the potential of explants from immature coffee fruits and from aseptically grown plantlets to develop dependable "in vitro" cultures that survive regular transfers and allow the obtaining of long-term, continuous callus cultures.

Our preliminary investigation on coffee cell and tissue cultures offers some insights into the feasibility of using this approach as a valid alternative to obtain cellular mass having the same biochemical characteristics as those expressed in the integrity of the whole organism.

Assuming the completion of all aspects regarding the development of culture media and growth conditions appropriate for rapidly growing callus and cell suspensions in *Coffea*, this herein outlined approach, amenable to further optimization, has interesting prospects, by broadening the sources for obtaining good-quality coffee beverages.

Another aspect that may be considered refers to the opportunities offered by "in vitro" techniques of altering the genetic constitution in cultured coffee cells, both by spontaneous somaclonal variation or by mutagenesis, in an attempt to generate cell lines characterized by fast growth, representing at the same time, biochemical mutants expressing desired traits, such as caffeine-free or reduced caffeine content, but preserving the ability to synthesize all the other compounds associated with superior coffee beverages obtained from roasted beans.

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Institute of Biological Sciences,
Splaiul Independenței 296,
Bucharest, Romania

SPECIES OF CORTICIACEAE s.l. (BASIDIOMYCETES) NEW TO THE MYCOFLORA OF ROMANIA (II)

N. HALLENBERG, M. TOMA

Twenty-three species of Corticiaceae (s.l.) from Romania are reported for the first time. This paper is a continuation of that of Hallenberg and Toma (1987), where 35 species new to the country were reported.

Species of Corticiaceae are mainly growing on wood and play an important role in the decomposition of wood in forest ecosystems. A number of species presented here are common elsewhere in Europe, while others are rare or very rare. Still, the knowledge of the wood-fungus flora (Basidiomycetes) in Romania is only fragmentary. However, the existing natural scientific reservations with virgin forests are excellent locations for a rich diversity of such organisms. A majority of the species reported here have been collected in such reservations.

The nomenclature follows Hjortstam (1987). Collected specimens are deposited in BUCM (București) and GB (Göteborg).

Bulbillomyces farinosus (Bres.) Jülich

Fruitbody effused, adnate, ceraceous, whitish-greyish. Hyphae with clamps. Cystidia thick-walled and encrusted when well-developed up to 100 μm long; basidia with 4 sterigmata; spores ellipsoid, $6-9 \times 5-6 \mu\text{m}$, with thickened walls, cyanophilous. In association with the fruitbody there are usually numerous sclerotia, whitish, subglobose, up to 0.4 mm in diameter. The sclerotia may obviously function as floating diaspores. The species is found on wood close to water (ponds, brooks, lake shores, etc.) where it may be rather frequent (Fig. 1).

On rotten branches of *Carpinus betulus* in Codrii Pașcanilor forest, Moțca village and in Voinești village, Iași district.

Cristinia gallica (Pilát) Jülich

(Syn.: *C. mucida* (Bourd. et Galz.) John Erikss. et Ryv.)

Fruitbody effused, loosely adnate, soft, irpicoid to meruloid, pale ochraceous to sulphurous yellow. Hyphae $4-5 \mu\text{m}$ wide, with clamps. No cystidia. Basidia with 4 sterigmata. Spores subglobose, thick-walled, cyanophilous, $5-6 \mu\text{m}$ in diam. *C. gallica* is a very rare species in Europe (Fig. 2).

On rotten branches of *Carpinus betulus* in Repede forest close to Iași town, Iași district.

Crustomyces subabruptus (Bourd. et Galz.) Jülich

On rotten stems of *Fagus sylvatica* in the natural reservation close to Hirlău town, Iași district and in the natural reservation Codrul Secular, Slătioara village, Suceava district.

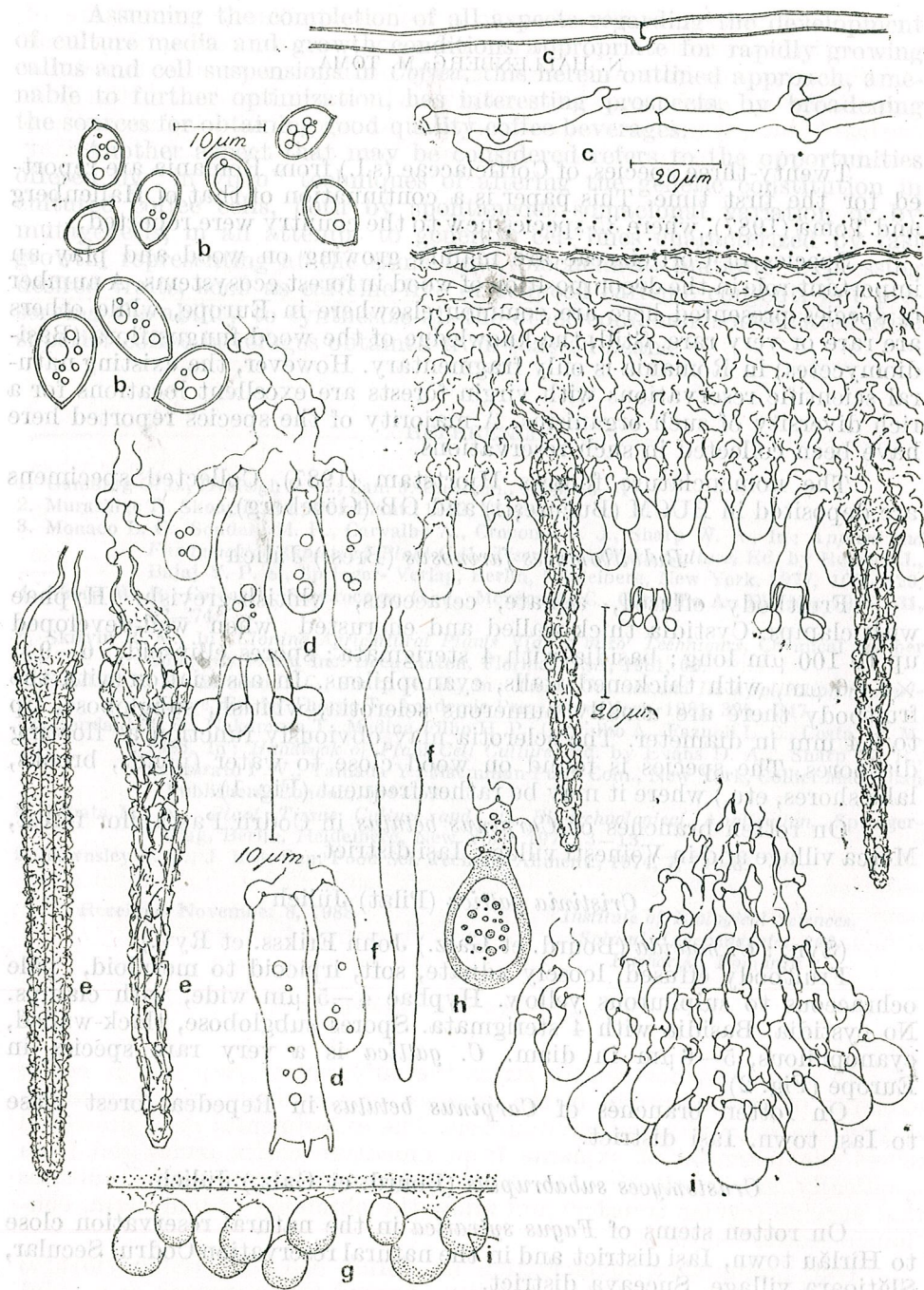


Fig. 1. — *Bulbillomyces farinosus* — a) section through young fruitbody, b) spores, c) hyphae, d) basidia, e) cystidia, f) thin-walled cystidium, g) sclerotial state (*Aegerita candida*) showing the position of section i, h) sclerotial bark cell, showing the grainy central protoplasm in cotton-blue, i) part of sclerotium, showing the rounded bark cells and the radially growing hyphae (Drawings by John Eriksson).

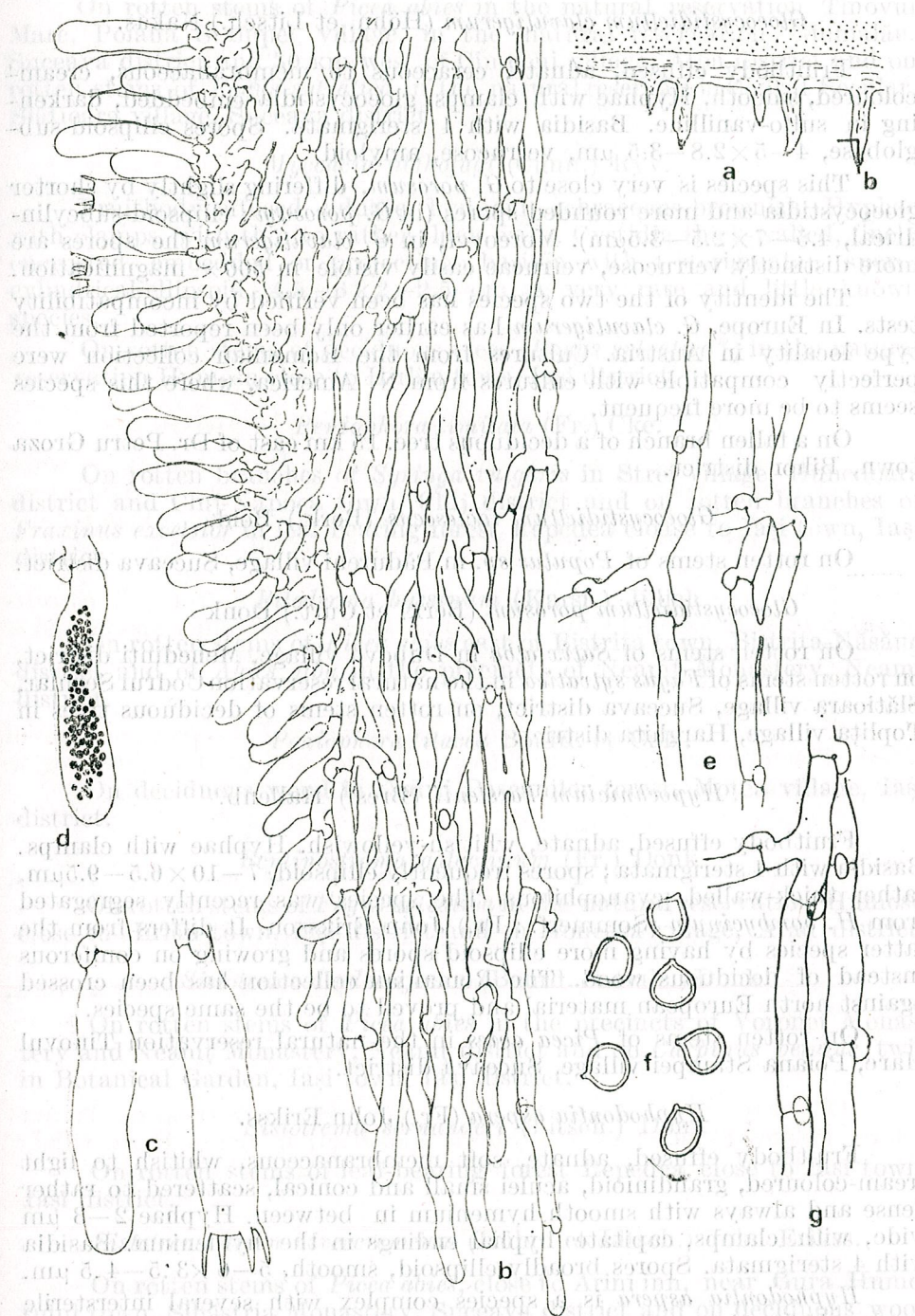


Fig. 2. — *Cristinia gallica* — a) schematic section through fruitbody showing position of b, b) section through distal part of hymenial tooth, c) basidia in KOH, d) basidia in cotton-blue, showing cyanophilous granulation, e) hyphae from apical part of tooth, f) spores, g) hyphae from basal part of subiculum (Drawings by John Eriksson).

Gloeocystidiellum clavuligerum (Höhn. et Litsch.) Nakas.

Fruitbody effused, adnate, ceraceous to membranaceous, cream-coloured, smooth. Hyphae with clamps, gloeocystidia embedded, darkening in sulfo-vanilline. Basidia with 4 sterigmata. Spores ellipsoid-subglobose, $4-5 \times 2.8-3.5 \mu\text{m}$, verrucose, amyloid.

This species is very close to *G. porosum*, differing slightly by shorter gloeocystidia and more rounded spores (in *G. porosum*: ellipsoid-subcylindrical, $4.5-7 \times 2.5-3.5 \mu\text{m}$). Moreover, in *G. clavuligerum* the spores are more distinctly verrucose, verrucae easily visible in $500 \times$ magnification.

The identity of the two species has been verified by incompatibility tests. In Europe, *G. clavuligerum* has earlier only been reported from the type locality in Austria. Cultures from the Romanian collection were perfectly compatible with cultures from N. America, where this species seems to be more frequent.

On a fallen branch of a deciduous tree. 15 km east of Dr. Petru Groza town, Bihor district.

Gloeocystidiellum lactescens (Berk.) Boid.

On rotten stems of *Populus* sp. in Pădureni village, Suceava district.

Gloeocystidiellum porosum (Berk. et Curt.) Donk

On rotten stems of *Salix alba* in Dubova village, Mehedinți district, on rotten stems of *Fagus sylvatica* in the natural reservation Codrul Secular, Slătioara village, Suceava district, on rotten stems of deciduous trees in Toplița village, Harghita district.

Hypochnicium karstenii (Bres.) Hallenb.

Fruitbody effused, adnate, whitish-yellowish. Hyphae with clamps. Basidia with 4 sterigmata; spores frequently ellipsoid, $7-10 \times 6.5-9.5 \mu\text{m}$, rather thick-walled, cyanophilous. The species was recently segregated from *H. bombycinum* (Sommerf.: Fr.) John Eriksson. It differs from the latter species by having more ellipsoid spores and growing on coniferous instead of deciduous wood. The Romanian collection has been crossed against north European material and proved to be the same species.

On rotten stems of *Picea abies* in the natural reservation Tinovul Mare, Poiana Stampei village, Suceava district.

Hyphodontia aspera (Fr.) John Erikss.

Fruitbody effused, adnate, soft membranaceous, whitish to light cream-coloured, grandinioid, aculei small and conical, scattered to rather dense and always with smooth hymenium in between. Hyphae $2-3 \mu\text{m}$ wide, with clamps, capitate hyphal endings in the hymenium. Basidia with 4 sterigmata. Spores broadly ellipsoid, smooth, $5-6 \times 3.5-4.5 \mu\text{m}$.

Hyphodontia aspera is a species complex with several intersterile groups. It has been proved by intercompatibility tests that two of the Romanian collections belong to *H. aspera* in the strict sense.

On rotten stems of *Picea abies* in the natural reservation Tinovul Mare, Poiana Stampei village, in the natural reservation Giumalău, Suceava district and 30 km west of Cimpeni village, Alba district and on rotten stems of *Fagus sylvatica* in the natural reservation Codrul Secular, Slătioara village, Suceava district.

Mycoacia nothofagi (Cunn.) Ryv.

Fruitbody effused, adnate, hydroid, ochraceous-brownish. Hyphae with clamps, with thin to rather thick walls. Cystidia thick-walled, finely encrusted, embedded or projecting; basidia with 4 sterigmata; spores cylindrical-ellipsoid, $4.5-6 \times 2-2.5 \mu\text{m}$. A very rare and little known species.

On rotten stems of deciduous trees (*Fagus sylvatica*?) in the natural reservation Humosu close to Hirău town, Iași district.

Peniophora limitata (Fr.) Cke.

On rotten branches of *Syringa vulgaris* in Strei village, Hunedoara district and Cluj-Napoca town, Cluj district and on rotten branches of *Fraxinus excelsior* in leaf bearing forest Repedea close to Iași town, Iași district.

Piloderma byssinum (Karst.) Jülich

On rotten stems of *Picea abies* east of Bistrița town, Bistrița-Năsăud district and of *Abies alba* in the precincts of Neamț Monastery, Neamț district.

Peniophora lilacea Bourd. et Galz.

On deciduous wood in Codrii Pașcanilor forest, Moțca village, Iași district.

Scytinostroma galactinum (Fr.) Donk

On rotten stems of *Fagus sylvatica* in the natural reservation Humosu close to Hirău town, Iași district and in Moneasa village, Arad district.

Sistotrema diademiferum (Bourd. et Galz.) Donk

On rotten stems of *Picea abies* in the precincts of Voroneț Monastery and Neamț Monastery, Neamț district and on *Carpinus betulus* twig in Botanical Garden, Iași town, Iași district.

Sistotrema sernanderi (Litsch.) Donk

On rotten stems of leaf-bearing forest Repedea close to Iași town, Iași district.

Sistotremastrum niveocremerum (Höhn. et Litsch.) John Erikss.

On rotten stems of *Picea abies*, close to Arini inn, near Gura Humorului town, Sihăstria Monastery, Suceava district and on deciduous wood in Voinești village, Iași district.

Subulicystidium longisporum (Pat.) Parm.

On rotten stems of *Carpinus betulus* of leaf-bearing forest Repedea close to Iași town, Iași district and on deciduous wood in the natural reservation Pădurea Bogății, Brașov district (Fig. 3).

Trechispora cohaerens (Schw.) Jülich

On rotten stems of *Pinus sylvestris* in Lacul Roșu village, Harghita district and on deciduous wood in leaf-bearing forest Repedea close to Iași town, Iași district.

Trechispora vaga (Fr.) Liberta (s.l.)

On fruitbody of *Hymenochaete rubiginosa* in Voinesti village, Iași district and on rotten stems of *Fagus sylvatica* in Moneasa village, Arad district.

Tubulicrinis strangulatus Larss. et Hjortst.

Fruitbody effused, adnate, smooth, whitish. Hyphae 2–3 μm wide, with clamps. Lycocystidia obtuse, 60–80 $\times$ 7–8 μm , amyloid, very thick-walled and with a capillary lumen, except for the apex which is thin-walled. Basidia with 4 sterigmata. Spores allantoid, 5–5.5 $\times$ 2.5 μm , smooth, thin-walled.

This species has been quite recently segregated from *T. borealis* John Erikss. It differs mainly by slightly shorter and wider spores and a non-amyloid basidial layer. Consequently, little is known about its distribution. The Romanian collection has been proved to be compatible with north European ones in mating tests (Fig. 4).

On rotten stems of *Picea abies* in the natural reservation Tinovul Mare, Poiana Stampei village, Suceava district and of *Abies alba* log in the precincts of Neamț Monastery, Neamț district.

Tubulicrinis calothrix (Pat.) Donk

On rotten stems of *Picea abies* in the natural reservation Tinovul Mare, Poiana Stampei village, Suceava district.

Tubulicrinis subulatus (Bourd. et Galz.) Donk

On rotten stems of *Pinus sylvestris*, 7 km west of Bistrița town, Bistrița-Năsăud district and of *Picea abies* in Poiana Brașov, Brașov district.

Tylospora fibrillosa (Burt) Donk

On rotten stems of *Picea abies* in the natural reservation Tinovul Mare, Poiana Stampei village, Suceava district, in Lacul Roșu village, Harghita district and 30 km west of Cîmpeni village, Alba district.

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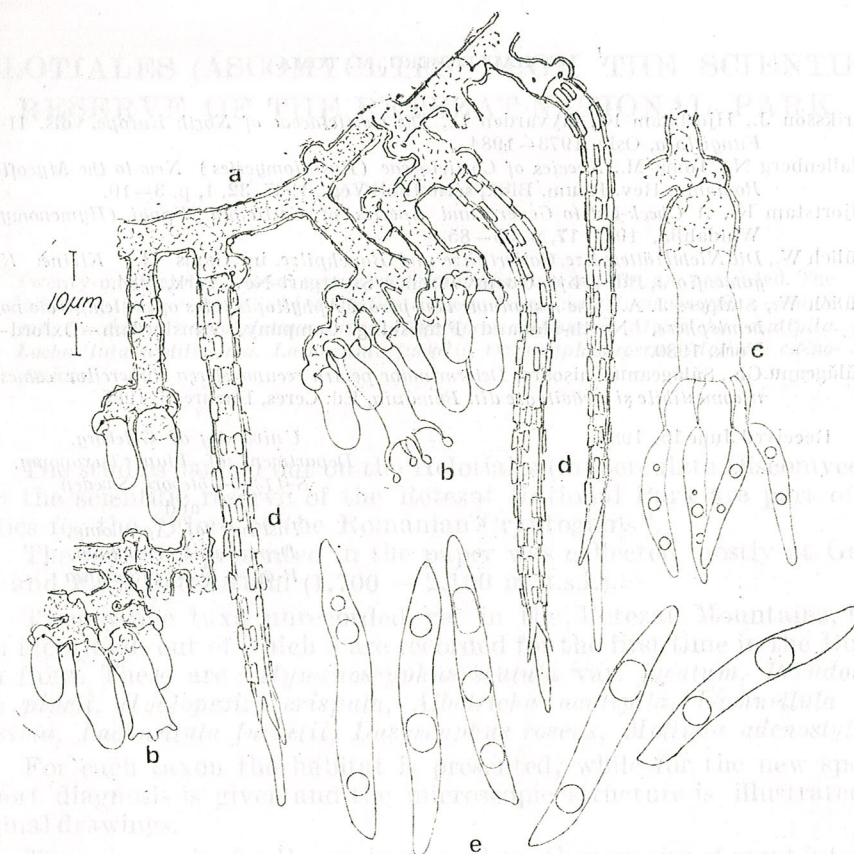


Fig. 3. — *Subulicystidium longisporum* — a) hyphae, b) basidia, c) basidium, d) cystidia, e) spores (Drawings by John Eriksson).

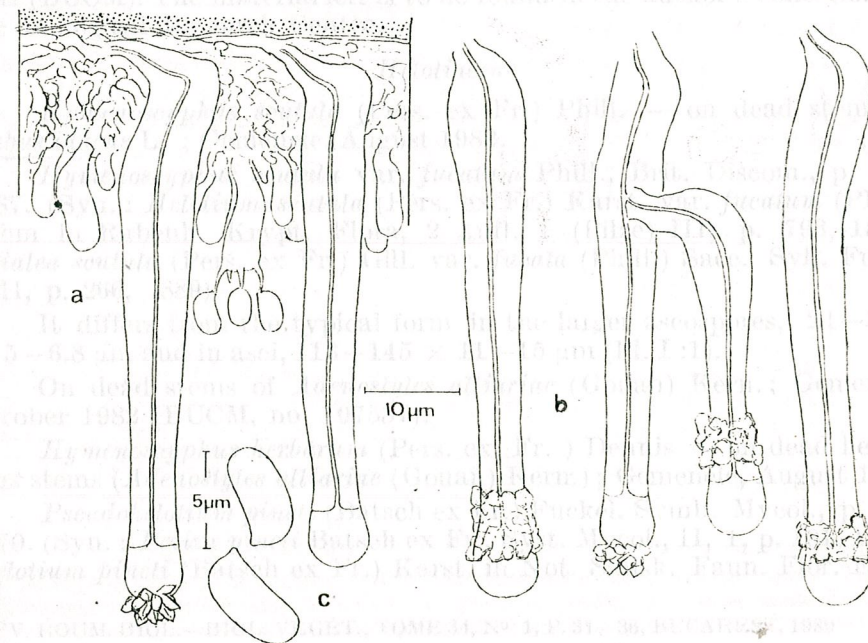


Fig. 4. — *Tubulicrinis strangulatus* — a) section through fruitbody, b) lycocystidia, c) spores (Drawings by John Eriksson).

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University of Göteborg,
Department of Plant Taxonomy,
S-413 19-Göteborg Sweden
and
Institute of Agronomy,
Department of Botany,
R-6600-Iasi-6 Romania

HELOTIALES (ASCOMYCETES) FROM THE SCIENTIFIC RESERVE OF THE RETEZAT NATIONAL PARK

ADRIANA POP

Twenty-one Helotiale taxa, unrecorded yet in the Retezat Mts, are presented. The following taxa are recorded for the first time in Romania: *Hymenoscyphus scutula* var. *fucatum*, *Pseudohelotium pineti*, *Hyalopeziza crispula*, *Albotricha acutipila*, *Lachnellula subtilissima*, *Lachnellula fuckelii*, *Dasysephus roseus*, *Mollisia adenostylidis*.

The studies carried out on the Helotiales (inoperculate Discomycetes) from the scientific reserve of the Retezat National Park are part of the studies for the "Flora of the Romanian Cryptogams".

The material presented in the paper was collected mostly at Gemelele and Fața Retezatului (1,700 — 2,100 m a.s.l.).

Twenty-one taxa unrecorded yet in the Retezat Mountains, have been identified, out of which 8 are recorded for the first time in the Romanian flora. These are: *Hymenoscyphus scutula* var. *fucatum*, *Pseudohelotium pineti*, *Hyalopeziza crispula*, *Albotricha acutipila*, *Lachnellula subtilissima*, *Lachnellula fuckelii*, *Dasysephus roseus*, *Mollisia adenostylidis*.

For each taxon the habitat is presented, while for the new species a short diagnosis is given and the microscopic structure is illustrated by original drawings.

The new species for Romania, as well as other species of great interest, are deposited in the Mycological Herbarium, Institute of Biology, București (BUCM). The material left is to be found in the author's collection, at the Biological Research Centre.

Helotiaceae

Hymenoscyphus scutula (Pers. ex Fr.) Phill. — on dead stems of *Rubus idaeus* L.; Gemelele, August 1982.

Hymenoscyphus scutula var. *fucatum* Phill., Brit. Discom., p. 137, 1887. (Syn.: *Helotium scutula* (Pers. ex Fr.) Karst. var. *fucatum* (Phill.) Rehm in Rabenh. Krypt. Flora, 2 Aufl. I (Pilze) III, p. 793, 1893; *Phialea scutula* (Pers. ex Fr.) Gill. var. *fucata* (Phill.) Sacc., Syll. Fung., VIII, p. 266, 1889).

It differs from the typical form in the larger ascospores, $24-34 \times 5-6.8 \mu\text{m}$ and in asci, $118-145 \times 11-15 \mu\text{m}$ (Pl. I :1).

On dead stems of *Adenostyles alliariae* (Gouan) Kern.; Gemelele, October 1982 (BUCM, no. 107537).

Hymenoscyphus herbarum (Pers. ex Fr.) Dennis — on dead herbaceous stems (*Adenostyles alliariae* (Gouan) Kern.); Gemelele, August 1982.

Pseudohelotium pineti (Batsch ex Fr.) Fuckel, Symb. Mycol., p. 298, 1870. (Syn.: *Peziza pineti* Batsch ex Fr., Syst. Mycol., II, 1, p. 101, 1822; *Helotium pineti* (Batsch ex Fr.) Karst. in Not. Sallsk. Faun. Flor. Fenn.

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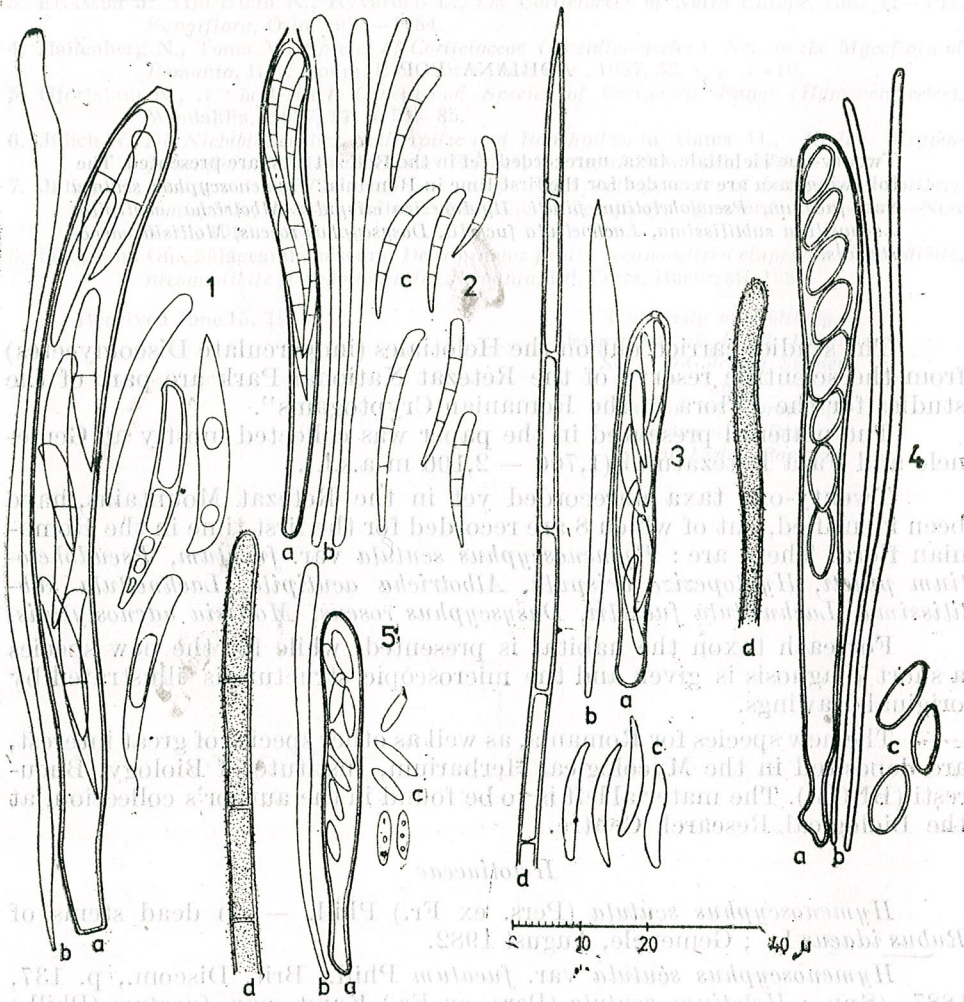


Plate I. — Light microscopy of Helotiales. Fig. 1. *Hymenoscyphus scutula* var. *fucatum*; 2. *Pseudohelotium pineti*; 3. *Albotricha acutipila*; 4. *Lachnellula fockelii*; 5. *Lachnellula subtilissima*. Abbreviations: a—asci; b—paraphyses; c—ascospores; d—hairs.

XI, p. 241, 1870; *Phialea pineti* (Batsch ex Fr.) Quél. in Bull. Soc. bot. Fr., XXVI, p. 234, 1879; *Mollisia pineti* (Batsch ex Fr.) Phill., Brit. Discom., p. 195, 1887; *Belonium pineti* (Batsch ex Fr.) Rehm in Rabenh., Krypt. Flora, 2 Aufl., I (Pilze), III, p. 688, 1892).

Apothecia superficial, disc flat, whitish to buff, up to 1 mm across, exterior smooth, brown below, whitish at the margin. Asci cylindrical-clavate, up to $70 \times 9 \mu\text{m}$ the small pore bluish in iodine, 8-spored; ascospores narrowly cylindrical or slightly clavate, $20-30 \times 1.5-2 \mu\text{m}$, 3-septate, lying more or less parallel in the ascus. Paraphyses cylindrical-clavate, up to $3 \mu\text{m}$ thick (Pl. I: 2).

On fallen cones of *Pinus mugo* Turra; Fața Retezatului, October 1982 (BUCM, no. 107575).

Phialea cyathoidea (Bull. ex Mér.) Gill. — on dead stems of herbaceous plants; Gemele, May to October 1983.

Hyaloscyphaceae

Hyaloscypha curvipila Grelet — on fallen cones of *Pinus mugo* Turra; Gemele, October 1982.

Hyalopeziza crispula (Karst.) Raitv., Scripta Mycol. 1, Tartu, p. 35, 1970. (Syn.: *Peziza crispula* Karst., Not. Salsk. Faun. Flor. Fenn. 10, p. 181, 1869; *Hyalotricha crispula* (Karst.) Dennis, Myc. Pap., 32, p. 77, 1949).

Apothecia sessile, subglobose, white, 0.1–0.3 mm in diameter with hairy margin. Hairs cylindrical, sharply bent near the base, $50-150 \times 5 \mu\text{m}$. Asci cylindrical, $28-40 \times 3.5-5.5 \mu\text{m}$; ascospores fusiform to clavate fusiform, $5-8.5 \times 1.5-2 \mu\text{m}$. Paraphyses cylindrical, $1.5 \mu\text{m}$ in diameter (Pl. II: 1).

On dead stems of *Adenostyles alliariae* (Gouan) Kern.; Gemele, August 1982 (BUCM, no. 107559).

Hyalopeziza millepunctata (Lib.) Raitv., — on dead stems of *Rubus idaeus* L.; Gemele, August 1982.

Albotricha acutipila (Karst.) Raitv., Scripta Mycol. 1, Tartu, p. 42, 1970. (Syn.: *Peziza acutipila* Karst. in Not. Salsk. Faun. Flor. Fenn., X, p. 195, 1869; *Lachnea acutipila* (Karst.) Karst. in Not. Salsk. Faun. Flor. Fenn. XI, 1870; *Lachnum acutipilum* (Karst.) Karst., Myc. fenn., I, p. 173, 1871; *Trichopeziza caduca* Rehm in Ber. Naturh. Ver. Augsburg, XXVI, p. 42, 1881; *Lachnella acutipila* (Karst.) Phill., Brit. Discom., p. 252, 1887; *Dasysecypha acutipila* (Karst.) Sacc., Syll. Fung., VIII, p. 447, 1889; *Lachnum caducum* (Rehm) Rehm, Rabenh. Kryptogamenfl., I (Pilze) III, p. 882, 1893).

Apothecia substipitate, white or ivory, covered with delicate, long, hyaline hairs, with the wall thin and smooth, 6–9 septate, $116-185 \times 3-4 \mu\text{m}$. Asci cylindrical-clavate, $45-65 \times 4-6.5 \mu\text{m}$; ascospores narrowly fusiform, non-septate, $10-15 \times 1.5 \mu\text{m}$. Paraphyses lanceolate, $3.5-6 \mu\text{m}$ in diameter, up to $30 \mu\text{m}$ longer than asci (Pl. I: 3).

On dead grasses of *Calamagrostis villosa* (Chaix) J. F. Gmel; Fața Retezatului, August 1982 (BUCM, nr. 107549).

Belonidium leucostomum (Rehm) Raitv. — on dead stems of *Adenostyles alliariae* (Gouan) Kern.; Gemele, August 1982 (Pl. II: 3; BUCM, no. 107567).

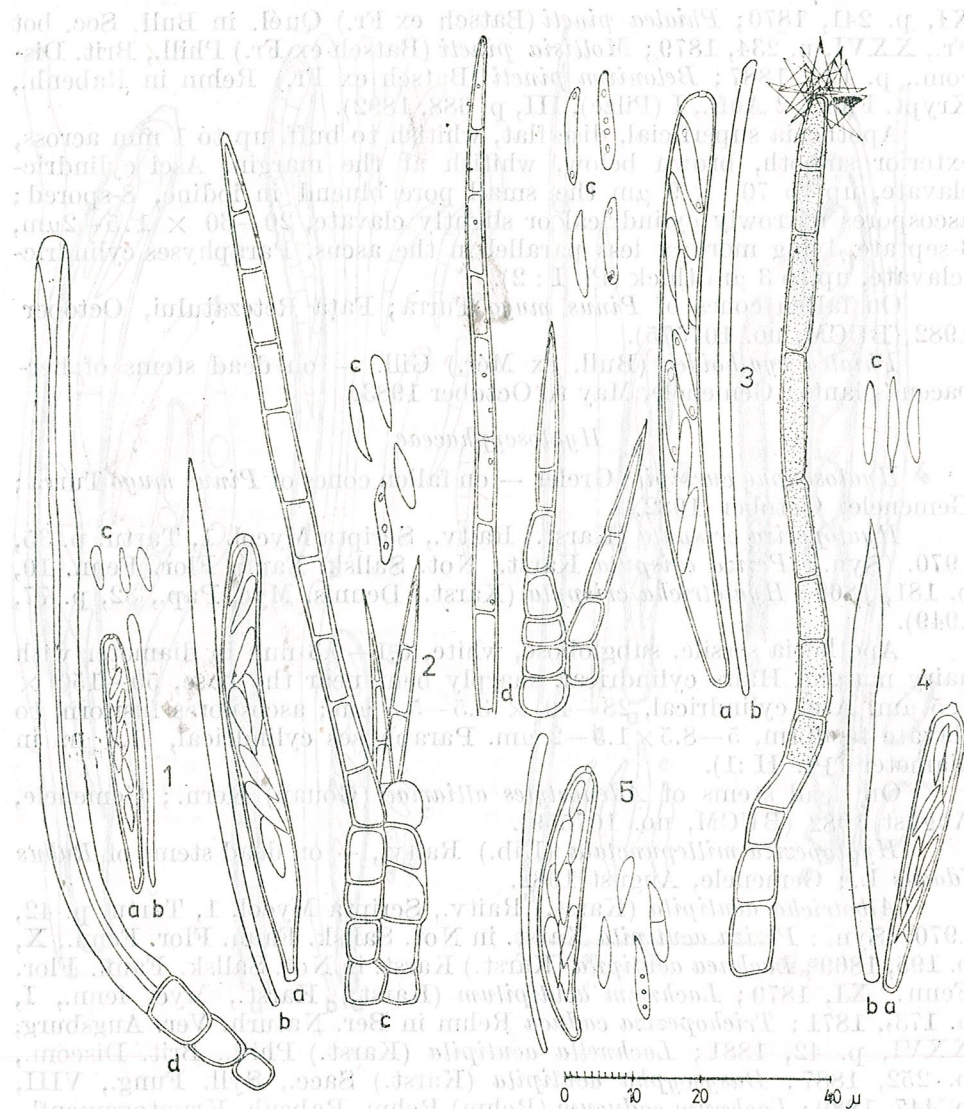


Plate II. — Light microscopy of Helotiales. Fig. 1. *Hyalopeziza crispula*; 2. *Trichopezizella relicina*; 3. *Belonidium leucostomum*; 4. *Dasyscyphus roseus*; 5. *Mollisia adenostylidis*. Abbreviations: a—asci; b—paraphyses; c—ascospores; d—hairs.

Belonidium leucophaeum (Weinm.) Raitv. — on dead stems of *Adenostyles alliariae* (Gouan) Kern.; Gemenele, August 1982 (BUCM, no. 107550).

Trichopezizella relicina (Fr.) Raitv. — on dead stems of *Adenostyles alliariae* (Gouan) Kern.; Gemenele, August 1982 (Pl. II: 2; BUCM, no. 107552).

Lachnellula suecica (De Bary ex Fuckel) Nannf. — on fallen cones of *Pinus mugo* Turra; Fața Retezatului, October 1982 (BUCM, no. 107540).

Lachnellula subtilissima (Cooke) Dennis, Persoonia 2, p. 184, 1962. (Syn.: *Peziza subtilissima* Cooke, Grevillea III, p. 121, 1875; *Lachnella subtilissima* Phill., Man. brit. Discom., p. 244, 1884; *Dasyscypha subtilissima* Sacc., Syll. Discom., p. 438, 1889; *Helotium Abietis* Karst., Myc. fenn. I, p. 154, 1871; *Dasyscypha Abietis* Sacc., Syll. Discom., p. 438, 1889).

Apothecia sessile, cupulate, with orange hymenium, externally covered by white hairs. Asci cylindric-clavate, $45-60 \times 4-6 \mu\text{m}$, ascospores narrowly fusiform, $6-11 \times 2-2.5 \mu\text{m}$. Paraphyses cylindrical, $1.5-2 \mu\text{m}$ in diameter (Pl. I: 5).

On dead stems and branches of *Picea excelsa* (Lam.) Link, *Pinus mugo* Turra; Gemenele, July 1981, August 1982 (BUCM, nr. 107574).

Lachnellula fuckelii (Bres. in Rehm) Dharne, Phytopath. Zeitschr. 53 (2), p. 131, 1965.

Apothecia sessile, cupulate, with orange hymenium, covered by hairs. Hairs cylindrical with firm granulate walls. Asci cylindrical, $90-100 \times 8-10 \mu\text{m}$; ascospores ellipsoid, $10-15 \times 5-6 \mu\text{m}$. Paraphyses cylindrical (Pl. I: 4).

On dead branches of *Pinus mugo* Turra; Fața Retezatului, October 1982 (BUCM, no. 107539).

Cistella acuum (Alb. et Schw. ex Fr.) Svrček — on fallen needles of *Pinus mugo* Turra; Gemenele, October 1982.

Dasyscyphus virgineus S. F. Gray — on fallen cones of *Picea excelsa* (Lam.) Link; Gemenele, August 1982.

Dasyscyphus roseus Rehm, Ber. Nat. Hist. Ver. Augsburg 26, p. 41, 1881. (Syn.: *Lachnum roseum* Rehm, Rabenhorst's Kryptogamen Flora, I (Pilze) III, p. 882, 1896).

Apothecia $0.5-1 \text{ mm}$ in diameter, with short stalk, pale rose-coloured, externally covered by hairs. Hairs multiseptate, finely granulated with apical crystal masses, $100-140 \times 4.5-6 \mu\text{m}$. Asci cylindric-clavate, $50-60 \times 5 \mu\text{m}$; ascospores narrowly fusiform, $11.5-16.5 \times 2 \mu\text{m}$. Paraphyses lanceolate, up to $20 \mu\text{m}$ longer than asci, $4 \mu\text{m}$ in diameter (Pl. II: 4).

On dead grasses of *Calamagrostis villosa* (Chaix) J. F. Gmel; dwarf-pine Fața Retezatului, August 1982.

Dasyscyphus bicolor (Bull. ex Mér.) Fuckel var. *rubi* (Bres.) Dennis — on dead stems of *Rubus idaeus* L.; Gemenele, August 1982 (BUCM, no. 107557).

Dasyscyphus clandestinus (Fr.) Fuckel — on dead stems of *Adenostyles alliariae* (Gouan) Kern.; Gemenele, August 1982.

Dermateaceae

Mollisia adenostylidis Rehm in Rabenhorst's Kryptogamen Flora I (Pilze) III, p. 526, 1896.

Apothecia sessile, 1.5 mm in diameter, disc bluish-gray (violet-brown dry), externally brown, with a minutely downy margin. Asci 40—50 × 5 μm, 8-spored; ascospores fusiform, straight or slightly curved, non-septate, 9—12 × 1.5 μm. Paraphyses cylindrical (Pl. II: 5).

On dead stems of *Adenostyles alliariae* (Gouan) Kern.; Gemelele, August 1982 (BUCM, no. 108103).

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Biological Research Centre
3400 Cluj-Napoca, Str. Republicii no. 48

ECOSYSTEMIC CHARACTERIZATION OF A BEECH FOREST OF OXALIS, DENTARIA, ASPERULA IN THE MOUNTAINOUS AREA OF THE SOUTH CARPATHIANS

MIHAELA PAUCĂ-COMĂNESCU, C. BÎNDIU, AURICA TĂCINĂ, M. FALCĂ,
H. ALMĂȘAN, LILIANA VASILIU-OROMULU, VICTORIA CARACAȘ,
VIORICA HONCIUC, C. ARION, ALEXANDRA VASU

The paper presents elements of cenotic structure of primary producers, macro-consumers and nonvertebrates playing the role of consumers and decomposers in soil and on the soil surface and canopy of a beech forest placed in the premountainous area of the Prahova valley as well as some pedo-climatic characteristics of the biotope. The investigations were carried out at intervals of 10 years after the forest exploitation in "successive" cuttings (extracting 15% of the wooden mass). The accumulated supraterranean biomass is at present of 267.7 t/ha, out of which 3.4t/ha is represented by the leaf mass the annual production of the tree layer being of 6.26 t/ha/year. The herbaceous biomass is much reduced (0.13—0.10 t/ha) and is obtained by a large number of species.

The number of macroconsumers mainly herbivores is over the best density in forests.

The decomposition of litter is intense (Jenny coefficient 0.47).

The gross material is transformed mainly by lumbricides, well represented quantitatively, the more advanced transformation being performed by the very active microbiocenosis in this biotope.

Beech forests occupy about 2 million ha in Romania, representing some 31% of its forest area. They are placed over the whole area of the Carpathians and the Subcarpathian hills, showing a great structural diversity.

Considering the structural and functional complexity of forest ecosystems and the restricted and still unilateral information in science in general and in forestry practice in particular, it has been necessary to point out more intimate connections among ecosystemic components in order to propose a new classification, elaborated on these grounds. According to this classification (Doniță, 1988), the beech forest with *Oxalis*, *Dentaria*, *Asperula*, one of the most frequent types of forest ecosystems is the object of study of this paper. Since in nature only few ecosystems are not influenced by man, except for reservations, we considered interesting to have in view the stage of stability of a mature forest, which started to be exploited according to modern technologies, with little damage, quite a long time after it has recovered by self-regulation processes.

MATERIALS AND METHODS

The soil humidity was determined by gravimetry and drying at 105°C. The acidity was measured potentiometrically in water solution. The humus was determined by Schollenberger's method and the total nitrogen by Kjeldahl's method. Tree productivity and the biometrical elements

were determined on circular surfaces of 500 m², according to Golden, 1979 and the dendrometrical methods (Giurgiu, 1978). The leaf mass was indirectly measured using the mass of fresh litter. The structure and productivity of herbs were obtained by measurements on surfaces of 0.25 m² (100 areas), regularly displayed on one ha and the determination of the individual biomass. The Simpson/Pielou coefficient $(D = 1 - \frac{2n(n-1)}{N(N-1)})$, where n is the number of individuals in the population, and N is the number of populations) is used for assessing diversity. The mammals are evaluated for the whole forest. The canopy fauna of invertebrates was determined by shaking in nets with the diameter of 60 cm (each sample obtained after 50 shakings up to 4 meters height). The mobile fauna on the soil surface was analysed by capturing individuals in Barber traps. The acariens, nematodes, enchytraeids and collembola were collected at 10 cm deep in soil with a Macfadyen soil core-borer; the extraction of animals from samples was performed with a modified Berlese-Tullgren extractor. The lumbricids were collected from the first 40 cm deep, along 4 equal layers. The mass of litter, fresh and decomposing, was collected on the surface of 0.125 m² (30 areas) equally distributed. The decomposition of the litter was calculated according to the Jenny coefficient. The dehydrogenase activity was determined according to Casida (1963) and Ștefanic (1970). From an ecophysiological point of view, the content in sugar was analysed by refractometry, the assimilatory pigments by the methods of Comar and Zscheille, for chlorophyll and Holm for carotenoids and the pH was studied potentiometrically.

The beech forest researched in the forestry district of Sinaia belongs to the geobotanical association of *Fagetum carpaticum* Klika 27 Borza 31, Moor 32, to forest type—*mountainous beech forest with mull flora* and to station type — *mountainous premountainous beech forest Pm brown, middle edaphic, with Asperula, Dentaria* in the subecological region C₃ district 3.

RESULTS AND DISCUSSION

ELEMENTS OF BIOTOPE (STATION)

The beech forest lies at the foot of the Bucegi mountains, Posada district, in a massive forest belonging to the type of fir and beech forests. The area under study is at 790–840 m altitude, on a reduced slope, 5–10°, E–SE exposed.

The cool mountain climate, with heavy rains (meteorological station Sinaia, 879 m altitude) is favourable for the development of beech. Annual rains amount to about 808 mm every year. There is a rich water availability, the quantity in springs and rivers being of 270 mm/year, without periods of drought. The annual average temperature is of 6.1°C, with positive temperatures of 25–24°C. Five months a year the temperature goes over the biologically active level of 10°C, being more than necessary for the beech to grow (100 days of growth).

The substratum was formed during the Lower Cretaceous, with a facies of flysch made of marls, limes and stone, conglomerates and calcarenites (Sinaia strata).

The soil is typically acid brown, clay-sandy, with a moderate humus content of the mull type. It is a moderately acid soil, under the effect of bioaccumulation of nutritive cations, mainly within the first 20 cm and slightly acid in the rest of the profile. The active biogeochemical circuit and the availability of nutritive substances, facilitated by the pH conditions, assure the conditions for the development of trees, affording also a higher productivity.

Table 1

Brief characteristics of soils

Layer cm	pH	Humus (C × 1.72 %)	Nt %	C/N	Wt %
0	1	2	3	4	5
0–10	6.0	7.06	0.263	18	23.13
10–20	6.0	3.11	0.063	—	19.62
20–30	5.5	—	—	—	18.58
30–40	4.9	—	—	—	17.93

ELEMENTS OF BIOGENOSIS

Primary producers

The main plants of the ecosystem that assure its productivity form a well-determined biocenosis, with a normal structure, expanding up to 30 m high, characteristic for the biotope where they develop. The phytocenosis is structured into two layers, trees and herbs. As the forest was subjected to successive cuttings (15%) it presents a sublayer of young trees, regenerated along several belts. The natural structure rebuilt itself on the present structure and the still identifiable stumps, the remaining part of the ecosystem being wholly unmodified.

The tree layer has only one species, *Fagus sylvatica*, 100 %. In the genetic stock of the herbaceous layer, seedlings of *Acer pseudoplatanus* and *Abies alba* were identified. There is no diversity of trees (diversity index Simpson/Pielou equal to 0.31–0.34) the present structure, although modified is not too complex, the forest having rather a plurien aspect. The trees have a vertical distribution of 21–38 m, the passage to the next layer being performed discontinuously. The young beech trees are not uniformly distributed on the whole surface of the phytocenosis and are not higher than 1 m. The canopy cover amounts to 70–80% because of cuttings, but a goodly part of shadowed trees expand their crowns. The horizontal structure of phytocenosis was much more affected by tree cuttings, with an unequal distribution on the surface. In general, the diameters of trees rank within the same limits (11–60 cm) and the frequency of those with a large diameter is reduced.

Table 2

Biometrical characteristics of the tree layer

Age year	Density ind/ha	Height (m)		Diameter (cm)		Basic surface		Volume (m ³)	
		average	limits	average	limits	average cm ²	total m ² /ha	average	total m ³ /ha
110	330	29.5	23—38	33.9	12—60	979	32.33	1.542	509

The present density of trees, of 330 individuals/ha, presents local variations of 200—400. The existing stumps are estimated to be of the same age with the trees in the old generation. The initial density was an average of 380 individuals/ha.

The height of trees was preserved within the same limits of variation, but the present average of 29.5 m denoted a higher value if we consider that the cut trees were higher. Although the trees of 23—24 m high are the most frequent ones, the height of the thick trees ($d=50$ cm) is of 35 m, expressing the high potential of the forest (first class of productivity), if it were included in a completely natural plurien structure. According to the average height there is only an average productivity.

All the trees lie on a basic surface of 32—33 m²/ha. The individual variety is reduced, because of age and the almost similar thickness of trees. Before cutting the trees the total basic surface was of 37.18 m², an average value for the mountain beech forests.

The average diameter of the tree layer is at present of 34 m, being probably more reduced in comparison with the one before cuttings. The tree distribution by categories of diameters indicates a maximum frequency of the trees with diameters between 34—42 cm, recording three categories of diameters, corresponding to three categories of age, the structure of the forest being relatively plurien (Table 3). The volume of wood accumulated

Table 3

Tree distribution by thickness

	Category of diameter (cm)											
	18	22	26	30	34	38	42	46	50	54	58	62
Frequency %	12	3	6	12	15	15	15	12	0	3	3	3

by the trees is of 509 m³/ha. It is uniformly distributed by surface unity, the variations caused by microconditions in the biotope ranging between 505—512 m³/ha. If we add the volume of wood previously extracted from the forest, we come to 618 m³/ha. Both values of the wood volume correspond to a higher category of productivity (second-class productivity for evenage forests).

Beech wood has high density (determined by the method of carrots) yields a conventional value 0.52 g/cm³ and an absolute value of 0.78 g/cm³. The volume of accumulated wood was achieved by a very dense distribution of the material, denoting a growth of quality. The young trees regenerated in the forests, but not belonging to the dominant population of beech, are found in a large number: density = 510000 individuals/ha, height 0.30+1.0 m, with diameters of 1—4 cm. It has a better composition (beech 92%, sucamore maple 5%, fir tree 2%, hornbeam 1%). In this cenosis, all the other categories of age and intermediary sizes of beech population are missing.

The herbaceous layer is rich in species both in spring and summer. Some wooden species are present at this level, first the seedling of trees with potential development in the area (*Fagus sylvatica*, *Abies alba*), but also some bushes like *Sambucus nigra*, *Salix caprea*, *Rubus idaeus*, *Rubus hirtus*.

The large number of component species records a high maximum diversity index, but the Simposn/Pielou diversity index is higher in summer since the development of the herbaceous populations in spring is unequal (Table 4). If in spring only 3 species have a numerical participation higher than 1 %, in summer, their number rises to 13 species.

Table 4

Biometrical characterisation of the herbaceous layer

Fenasept	Frequency of covering with vegetation (%)	Number of species		Diversity index Simpson/Pielou number	Maximum diversity index	Density ind/m ²	Total biomass g.s.u./m ²	Water in content (%)
		total	herbaceous					
vernal	88	28	25	0.2111	0.9615	88.9	14.85	80
estival	95	39	33	0.8977	0.9697	36.7	10.66	82

We may notice that the typically vernal flora is poorly represented (3—4 species out of a total of 25). At the same time, after forest cuttings, some species typical of other associations appear (Table 5). Most of the soil area, more than 90%, is covered with herbaceous vegetation (its distribution being almost uniform). In summer the areas are better covered than in spring, because the vernal species have a small contribution.

The average density of herbs is high in summer, because the population of *Cardamine bulbifera* which is very well developed, reduces its effective gradually till mid-summer. A significant role in the effective of the layer is played especially in summer by the following populations: *Asperula odorata*, *Impatiens noli-tangere*, *Cardamine impatiens*, *Circaea lutetiana* and even by *Viola reichenbachiana* populations.

Biomass accumulation in the primary producers

The accumulation of supraterranean biomass at the tree level is 267.7 t/ha, out of which 264.7 t/ha wooden mass and 3.4 t/ha leaves mass. If we added to it the wooden mass extracted 10 years ago (47.2 t/ha) the total wooden biomass would be of 315 t/ha.

The annual wood production in this beech forest is of 2.8 t/ha together with the leaf mass, which produced annually, falls down and enters the circuit of the ecosystem. We may say that in this beech forest the tree layer has an annual production of 6.26 t/ha. In the annual mass produced, wood amounts to 46 % and the leaf to 54%, the trees being old enough.

The herbaceous biomass is much more reduced quantitatively but much more complex in point of variety, being produced by a lot of species. In spring, 0.13 t/ha accumulated and in summer 0.10 t/ha. The following species are predominant: *Cardamine bulbifera*, *Cardamine impatiens*, *Asperula odorata*, *Atropa bella-dona* the last-less frequent species but with much individual biomass.

The annual herbaceous production is much higher (0.21 t/ha) than the maximum biomass, because the perennial part of the herbs is small (0.019 t/ha).

The tree/herb biomass ratio is 1/2453 in this beech forest while the annual production one is 1/30, layer differentiation being higher in case of both parameters.

The herbaceous biomass in phytocenosis has quite a low nutritive value for the large herbivores because most species are toxic or with a low nutritive value; only about 10% of the total biomass may be used, such as *Mycelis muralis*, *Poa trivialis*, some *Cyperaceae* species. Just as the beech leaf mass, it is a source of nutrition for saprotroph organisms. The water stock in the assimilatory tissue is 7225 kg/ha in the beech leaf mass and 1110 kg/ha in the herbaceous mass, plants with high percentage of water being recorded. The water stocked in tree/herb ratio is of 1/7.

The energy stock at the level of the primary producers

The quantity of stocked energy at the tree level is of 1.41×10^9 kcal/ha, the wooden mass being 1.41×10^9 kcal/ha, representing 87% of the total.

The energy that is annually preserved in the structure of the primary producers is of 4.0×10^7 kcal/ha/year and at least 1×10^7 kcal/ha/year is used for respiration; the wooden mass is 1.33×10^7 kcal/ha/year, the leaf mass 1.76×10^7 kcal/ha/year and the herbs 8.9×10^5 kcal/ha/year.

Indicators of the plants metabolic activity

The quantity of water found in the assimilatory tissue is of 80% for the whole area, throughout the vegetation period.

We may notice the low percentage of averages in the spring period, because the herbaceous vernal species are few, and the estival ones do not have an intense activity, having therefore a lower water level than in summer. The differentiation of species with a low hydric regime is evident (*Fragaria vesca*, *Moehringia trinervia*) and very high in (*Impatiens noli-tangere*, *Mycelis muralis*). In the case of beech, the young leaves have a higher water content than the mature ones, and the mature ones have a higher content of water than those in other stations (Table 6).

The assimilatory pigments are found in quantities high enough for a good photosynthesis activity, but their level is lower in comparison with other stations. In the herbaceous species, even in summer, the quantity is not very high. The relationship between the chlorophyll pigments is variable, around the theoretical value, but the differences are determined by the adaptive response of each species. The physiological reactions of the vernal species are always different from the usual cases. Here is a special case of low quantity of carotenoid pigments in comparison with the chlorophyll one. In plants with a normal life cycle we notice a chlorophyll carotenoid pigment ratio constantly higher than 3 (the respiratory processes being more intense). The chlorophyll synthesis in the tree leaf mass and in herbs reaches in this phytocenosis 2.9 g/m² in spring and 5.6 g/m² in summer.

The acidity of the cellular sap (pH) is variable in the investigated species, belonging to the acid and slightly acid range, being correlated with an intense metabolic activity. The populations with a greater accumulation of biomass have an acidity of more than 5.0 and even more in herbs, 5.5. In the neutral range the population of *Moehringia trinervia* was identified.

Consumers and decomposers

Macroconsumers. The large variety of herbaceous plants and their presence along the whole period of vegetation assures food to permanent primary consumers (*Cervus elaphus*, *Capreolus capreolus*) as well as to the omnivorous ones (*Sus scropha*, *Ursus arctos*) (Table 7). The varied structure of the tree layer and especially the presence of young plants, with a rich leaf mass, completes the trophic source in the vegetation period and permits the survival of these populations even in late autumn and winter.

The data presented below show that the real density exceeds sensibly the optimum one in *Cervus* and *Capreolus*, being double in *Ursus* and three times higher in *Sus scropha*. We also notice that birds of cynegetic interest in the area are also present: *Tetrao urogallus* and *Tetrastes bonasia*. The large variety of the herbaceous vegetation is also important; its buds and seeds are main food for birds in autumn and winter. The secondary consumers (*Canis lupus*, *Felis lynx*) are in a numerical balance with the herbivorous species. The presence of a well-balanced number of *Vulpes vulpes*, *Meles meles*, *Martes martes* controls the number of rodents, as they are dependent on the beech fruit period.

Table 5

Structure and biomass data regarding the main herbaceous populations in the period of their maximum development

Species	Frequ- ency (%)	Den- sity ind./50 m ²	Relative abun- dance (%)	Indivi- dual bio- mass g.d.w.	Popu- lation biomass g.d.w./50 m ²	Rate of biomass (%)	Water con- tent (%)
Vernal fenaspect							
<i>Cardamine bulbifera</i>	82	3704	83.00	1.178	659.31	88.73	84
<i>Cardamine glanduligera</i>	24	98	5.34	0.030	2.94	0.55	85
<i>Anemone nemorosa</i>	1	12	0.27	0.110	1.32	0.18	83
Estival fenaspect							
<i>Impatiens noli-tangere</i>	21	110	5.99	0.126	13.86	2.60	91
<i>Asperula odorata</i>	16	580	31.62	0.156	90.48	16.97	81
<i>Cardamine impatiens</i>	15	154	8.39	0.349	53.74	10.08	81
<i>Myelis muralis</i>	15	48	2.61	0.680	32.64	6.12	89
<i>Geranium robertianum</i>	12	30	1.63	1.157	30.71	5.76	87
<i>Circaea lutetiana</i>	10	88	4.79	0.108	9.50	1.78	84
<i>Urtica dioica</i>	7	28	1.52	0.833	23.32	4.37	84
<i>Moechringia trinervia</i>	7	44	2.39	0.242	10.64	2.00	75
<i>Euphorbia amygdaloides</i>	6	26	1.41	0.326	8.48	1.59	80
<i>Scrophularia nodosa</i>	6	38	2.07	0.594	22.57	4.23	92
<i>Alliaria officinalis</i>	5	40	2.18	0.448	17.92	3.36	75
<i>Viola reichenbachiana</i>	5	70	3.81	0.124	8.68	1.63	81
<i>Atropa bella-dona</i>	4	32	1.74	3.555	113.76	21.34	75
<i>Stachys sylvatica</i>	4	12	0.65	0.376	4.51	0.85	85
<i>Helleborus purpurascens</i>	3	6	0.32	3.410	20.47	3.84	78
<i>Veronica officinalis</i>	3	22	0.19	0.048	1.06	0.20	94

Other species present: *Tussilago farfara*, *Epilobium montanum*, *Ranunculus repens*, *Cirsium oleraceum*, *Rumex* sp., *Solanum dulcamara*, *Hypericum montanum*, *Prunella vulgaris*, *Arum maculatum*, *Poa trivialis*, *Carex remota*, *Cirsium palustre*, *Luzula luzuloides*, *Myosotis sylvestris*.

Table 6

Ecophysiological indices of the main herbaceous species

Species	Fenaspect	Water con- tent (%)	Assimilatory pigments (10 ⁻⁴ g/d.w.)		Rate		pH
			chloro- phyll (a+b)	caro- teno- ids	chloro- phyll a/b	chloro- phyll/ca- rotenoids	
<i>Fagus sylvatica</i>	vernal	73	89	31	3.62	2.82	5.0
	estival	68	159	41	3.19	3.87	5.2
<i>Cardamine bulbifera</i>	vernal	84	78	30	5.02	2.57	5.5
<i>Anemone nemorosa</i>	vernal	83	141	11	3.62	13.09	5.4
<i>Isopyrum thalictroides</i>	vernal	79	79	33	3.83	2.36	5.2
<i>Geranium robertianum</i>	estival	87					4.4
<i>Impatiens noli-tangere</i>	estival	91	100	28	3.01	3.58	5.9
<i>Asperula odorata</i>	estival	81	79	19	2.29	4.18	5.6
<i>Circaea lutetiana</i>	estival	84					4.0
<i>Viola reichenbachiana</i>	estival	81	126	25	2.09	4.92	4.0

Table 7

Effectiveness of macroconsumers (hunting stock, Comarnic)

Species	Effectiveness of macroconsumers for 12610 ha (6000 ha forest)	Density ind/1000 ha	Ecological area C _{3.3}	
			Total	Optimum effectiveness ind/1000 ha
<i>Cervus elaphus</i>	67	11.16	1245	9
<i>Capreolus capreolus</i>	80	5	6445	48
<i>Sus scropha</i>	47	7.83	862	6
<i>Lepus europaeus</i>	159	15.36	19848	151
<i>Ursus arctos</i>	16	2.65	175	1

Other species, in small number: *Canis lupus* (13), *Felis lynx* (4), *Vulpes vulpes* (18), *Meles meles* (21), *Martes martes* (18), *Tetrao urogallus* (22), *Tetrastes bonasia* (19).

The fauna of invertebrates in the canopy is dominated by the affids, primary consumers playing an important role in the trophic webs of the forest ecosystem, Figure 1. Affids represented by the species *Phyllaphis fagi* are eudominant in the biocenosis of these beech forests, presenting a numerical maximum in spring. *Dendrothrips ornatus* is the species of thysanoptera, most largely spread in the beech forest in our country. Characteristic for the secondary consumers is the species *Xysticus kocnii* (Aranea).

The mobile invertebrates on the soil surface (epigeic fauna). The mobile invertebrates on the soil surface, collected in traps, belong to two trophic levels: secondary consumers and decomposers.

Secondary consumers (zoophages and predators) are represented by *Opiliones*, *Carabidae*, *Araneae*, and *Lithobiidae*.

The *Opiliones* and *Carabidae* present values of relatively high density (Table 8), being characterized as eudominant groups, while the *Araneae* are dominant organisms and the *Lithobiidae* recedent ones.

The decomposers are represented by *Isopoda*, *Polydesmidae*, *Scarabidae* and *Staphylinidae*. *Scarabidae* are dominant, while the other groups are subdominant (*Staphylinidae*) and subrecedent.

The amplitude of variation of the relative density in the epigeic fauna ranges between 0.16 % in *Polydesmidae* and 49.29% in *Opiliones*.

In this biotope, there are groups of organisms, with high ecological valency — zoophages — and others with restricted ecological valency — the decomposers (*Polydesmidae*).

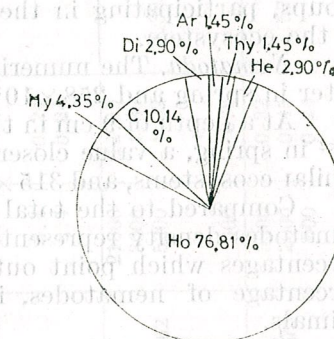


Fig. 1. — Relative abundance of invertebrates in canopy (Ho—Homoptera; Hy—Hymenoptera; C—Coleoptera; Di—Diptera; Thy—Thysanoptera; He—Heteroptera).

The zoophages record 4 groups with a high number of individuals (564) representing 89.38 % from the total number of individuals.

The decomposers, although identified in taxonomical groups, record a much smaller number (67), representing 10.65 % from the total number of individuals.

Table 8
The abundance of mobile invertebrates on the soil surface

Group of organisms	Numerical abundance (number of individuals)	Relative abundance (%)
<i>Isopoda</i>	3	0.48
<i>Miriapoda</i> — <i>Lithobiidae</i>	10	1.58
<i>Polydesmidae</i>	1	0.16
<i>Opiliones</i>	311	49.28
<i>Aranea</i>	49	7.76
<i>Coleoptera</i> — <i>Carabidae</i>	194	30.74
<i>Scarabaeidae</i>	34	5.40
<i>Staphylinidae</i>	29	4.60

The invertebrates in the soil. In the study of the soil fauna, we shall analyse the structure of zoocenose, created by the main taxonomic groups, participating in the activity of decomposing the organic matter in the ecosystem.

Nematoda. The numerical density was estimated at $346 \times 10^3 \text{ m}^{-2}$ in litter in spring and $258 \times 10^3 \text{ m}^{-2}$ in summer (Table 9).

At a depth of 9 cm in the soil, the numerical density was of $596 \times 10^3 \text{ m}^{-2}$ in spring, a value closer to the data mentioned by other authors for similar ecosystems, and $315 \times 10^3 \text{ m}^{-2}$ in summer.

Compared to the total numerical density of the edaphic fauna, the nematodes density represents 91% in spring and 89% in summer, high percentages which point out, like in any other soil, the extremely high percentage of nematodes, in comparison with other groups of edaphic animals.

The weight of biomass was estimated at 29.8 mg d.w./m² in spring and 15.75 mg d.w./m² in summer, representing 1% in winter and 0.4% in summer from the total biomass of the edaphic fauna.

Enchytreidae. The numerical density was put at $2.6 \times 10^3 \text{ m}^{-2}$ in spring and $4.6 \times 10^3 \text{ m}^{-2}$ in summer, representing much lower values than the recorded data for similar ecosystems.

As regards the numerical structure of the edaphic fauna, the enchytreids have a share of 0.4% in spring and 1.3% in summer, indicating that, because of the small number of individuals, their participation in the decomposition and mineralisation of the organic matter processes was quite low. Soil humidity, one of the important factors regulating the number of enchytreids was low, especially in spring, between 17% and 23%, largely explaining the low density in this season.

The biomass was estimated at 83.2 mg d.w./m² in spring and 147.2 mg d.w./m² in summer, representing 2.9% of the total biomass of the edaphic fauna in spring and 3.7% in summer.

Table 9
Numerical density and the biomass of the edaphic fauna

Group of organisms	Level	Numerical density			Biomass		
		Vernal	Estival	Relative abundance %	Vernal	Estival	Relative abundance %
Nematoda	L	346000	67.74	90.956	17.3	12.9	1.034
	S ₁	158000	42.98		7.9	1.85	
	S ₂	80000	32.80		4.0	1.00	
	S ₃	12000	6.72		0.6		
Enchytreidae	0-10	2600	0.40	0.397	83.2	147.2	2.886
	L ^a	23.6	0.81		684.6	281.6	
	S ₁ ^a	19.2	0.49		1091.2	2323.2	
	S ₂ ^a	3.2	0.2	0.010	137.6	409.6	86.902
Acarina (Oribatidae)	S ₃ ^a	3.2	0.2		224.0	275.2	
	S ₄ ^a	3.2	0.2		137.6	377.6	
	S ₅ ^a	6.4	0.24		230.4		
	L ^a	25800	1.83		136.74	113.3	
Collembola	L ^a	3800	3.10		20.14	15.9	
	S ₁ ^a	7600	2.40	6.562	40.28	27.56	7.905
	S ₂ ^a	200	0.2		1.06		
	S ₃ ^a	4600	0.93		24.38		
TOTAL	L	10000	0.71		27.0	3.18	
	S ₁	3200	0.92	2.075	8.64	0.85	
	S ₂	400	0.24		1.08	1.21	
		655258.8			2883.02	4003.21	

a = adults
y = young

Lumbricidae. The numerical density of earthworms recorded at the litter level was of $0.0236 \times 10^3 \text{ m}^{-2}$, in summer. At the soil depth of 30 cm, the numerical density was $0.0588 \times 10^3 \text{ m}^{-2}$ in summer, representing 0.010% out of the total numerical density of the edaphic fauna investigated.

If we consider the structure by ages, the young represent 10% out of the total number of individuals, both in spring and summer, lying in the upper soil in spring and deeper down in summer.

The specific diversity index is characterised by close values, but is reduced both in the litter and in the soil strata. It presents a peak in the 0–10 cm layer (0.37) in spring, with a better stability of the lumbricid fauna at this level.

From the point of view of the biomass, the lumbricids represent 86.9% in spring and 91.6 in summer, out of the total biomass of the edaphic fauna. Within the total biomass produced by the lumbricids, the adults represent 86% in spring and 7% in summer. The decomposition process of the organic matter is performed in best conditions, with the determinant participation of lumbricids and to a lesser extent of the other groups of decomposers.

Acarina-Oribatei. The numerical density was reduced, under the recorded values for similar ecosystems. In the litter, it was estimated at $25.8 \times 10^3 \text{ m}^{-2}$ in spring, and $21 \times 10^3 \text{ m}^{-2}$ in summer. At soil depth of 10 cm the numerical density was estimated at $43.2 \times 10^3 \text{ m}^{-2}$ in spring and $34.8 \times 10^3 \text{ m}^{-2}$ in summer, representing 6.5% and 8.4%, respectively, of the number of individuals belonging to the edaphic fauna.

If the age structure is considered, the adults are dominant mainly in spring, when 89% is recorded, and reduced in summer to 76%.

41 species were recorded, showing a significant diversity index, both at the level of the litter (0.88) and of the soil (0.86).

The index of ecological significance, having high values points to the species *Oppia ornata*, *Quadroppia quadricarinata*, *Suctobelbella baloghi*, which are eudominant, representing 7.31% of the number of species and 44.54% of the total number of individuals.

From the point of view of the biomass, the acarids represent 7.9% in spring and 3.9 in summer, out of the total biomass of the edaphic fauna, pointing out a reduced participation in the processes of matter and energy transfer, compared to lumbricids, but relatively increased in comparison to other groups of investigated edaphic animals.

Collembola. The numerical structure of Collembola is characterised by low numerical densities, recording in the litter a value of $10 \times 10^3 \text{ m}^{-2}$ in spring, and $3 \times 10^3 \text{ m}^{-2}$ in summer.

In a soil block of 6 cm thick, the numerical density was of $13.6 \times 10^3 \text{ m}^{-2}$ in spring, and $3 \times 10^3 \text{ m}^{-2}$ in summer, denoting much lower values than the data reported for similar ecosystems.

The contribution of Collembola to the numerical structure of the edaphic fauna was of 2% in spring and 1.6% in summer, quite close to the structure of the biomass, with values of 1.2% in spring and 0.4% in summer.

The quantity of energy released as secondary production has average values of 69.683 kcal/m^2 (290.35 kJ).

The process of decomposition

The vegetal material on the soil surface is available to decomposers, the herbaceous species entering gradually in the stage of necromass, at the beginning of summer, the others at the end of summer; the whole material is transformed fast, so when leaves fall from the trees, few other vegetal wastes are identified. The litter samples collected from the litter show that there is an average of 7200 kg/ha .

Out of this quantity, 3400 kg/ha is new litter and the rest (3800 kg/ha) litter undergoing decomposition.

Decomposition activity indicators. The dehydrogenase activity is very high, the recorded values being the highest in all the beech forests investigated from this point of view (Table 10).

The seasonal dynamics presents a normal aspect, the dehydrogenase activity being very high in spring and autumn and only moderate in summer. The dehydrogenase potential is also very high, following the dynamics of the present one, demonstrating the existence of a well-developed microbiocenosis, in comparison to other beech forests of the same type; the activity of soil microbiocenosis has the highest values. The same intense activity is noticed in the beech forest investigated by us and compared to all the other beech forests in the south of the country (Muntenia).

Table 10

Level of dehydrogenase activity
(expressed in mg formazan/ 100 g dry soil)

Layer	Spring		Summer		Autumn	
	Present	Potential	Present	Potential	Present	Potential
L	22.40	33.60	14.40	19.20	30.60	43.70
S ₁	19.60	24.80	10.20	14.80	26.70	30.60
S ₂	13.60	16.70	6.40	10.60	18.90	23.40
S ₃	9.80	11.80	4.20	6.70	10.40	15.60

Jenny's decomposition coefficient, that points out the intensity and rate of litter decomposition, records 0.4% in this beech forest. Compared to the average decomposition coefficient for other forests in Romania (0.37) it shows a good value, the litter transformation being carried out quite fast. In comparison to other beech forests, mainly in the hilly region, a more reduced intensity of the process is noticed. It is evident, in this respect, that a good decomposition of the gross organic matter takes place, because of the participation of lumbricids in the soil together with other associated analysed groups, in this ecosystem, with an intense activity of decomposition by means of microorganisms.

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Institute of Biological Sciences,
Splaiul Independenței 296,
Bucharest, Romania

NEW DATA REGARDING THE LAKE SĂRĂTURA 3 IN INDEPENDENȚA (MURIGHIOL), TULCEA COUNTY, ROMANIA

VALENTIN-ALEXANDRU C. BULGĂREANU, STOICA GODEANU,
VICTOR IZVORANU

In this note, new biological and limnological data, achieved in 1985 by complex investigations of lake Sărătura 3, are reported.

The lacustrine area Independența (Murighiol)¹ includes 3 lakes, called, in order from E towards W, Sărăturile 1, 2 and 3. From the limnogenetic point of view (Coteț et al. 1962, Găstescu 1971), the above mentioned salt lakes (Rom., pl. form: "Sărăturile") fill 3 lake depressions of Algerian Sahara "chott"-type, where the formerly high saline concentration was reached by intense evaporation; in fact, the forming processes of "Sărăturile" lakes seem to be mixed ones, namely of flood plain, elastokarstic and aeolian damming origins.

The lakes Sărătura 1 and 2 are both pelogenous (with unctuous, sapropelic muds) but the sole sometimes used for bathing by riparian inhabitants is Sărătura 1. The lake Sărătura 3 (area = 98 ha; maximum depth = 2.23 m) is an ornithological reserve (Papadopol 1965); this lake is not pelogenous. As compared to 1966, when Ștef et al., (1967, p. 659) reported "only in some points" the presence of sapropelic mud, our core drillings found a single area with organic muds having tendency towards unctuousity. According to the early established relationship between the sapropelic muds enzymology and their therapeutical feature (Kiss et al. 1986), we can state that the lake Sărătura 3 sediments, which have only 3 from the total of 7 determined enzymatic and catalytic non-enzymatic activities², are not therapeutical.

Some limnological characteristics of the lake, as determined in 6 stations, are mentioned in Fig. 1. For the eastern area, the lake hydrochemical features are shown in Table 1. Six core drillings carried out in the above-mentioned 6 stations (Fig. 1) made obvious the predominance of non-pelogenous deposits except the eastern zone, where grey-yellowish sometimes sandy silts occur having tendency towards unctuousity; in the central and western zones, the grey, brown and black silts are predominating, while in the south-western part of the lake, sandy, argillized silts occur. The chemical and mineralogical analyses of above-mentioned sediments are shown in Table 2.

¹ Limnogeological studies (1985), I.P.G.G., Bucharest; biological studies (1985), ICEBIOL, Bucharest

² Microbiological and enzymological studies (1985), Faculty of biology, geology and geography, University of Cluj-Napoca

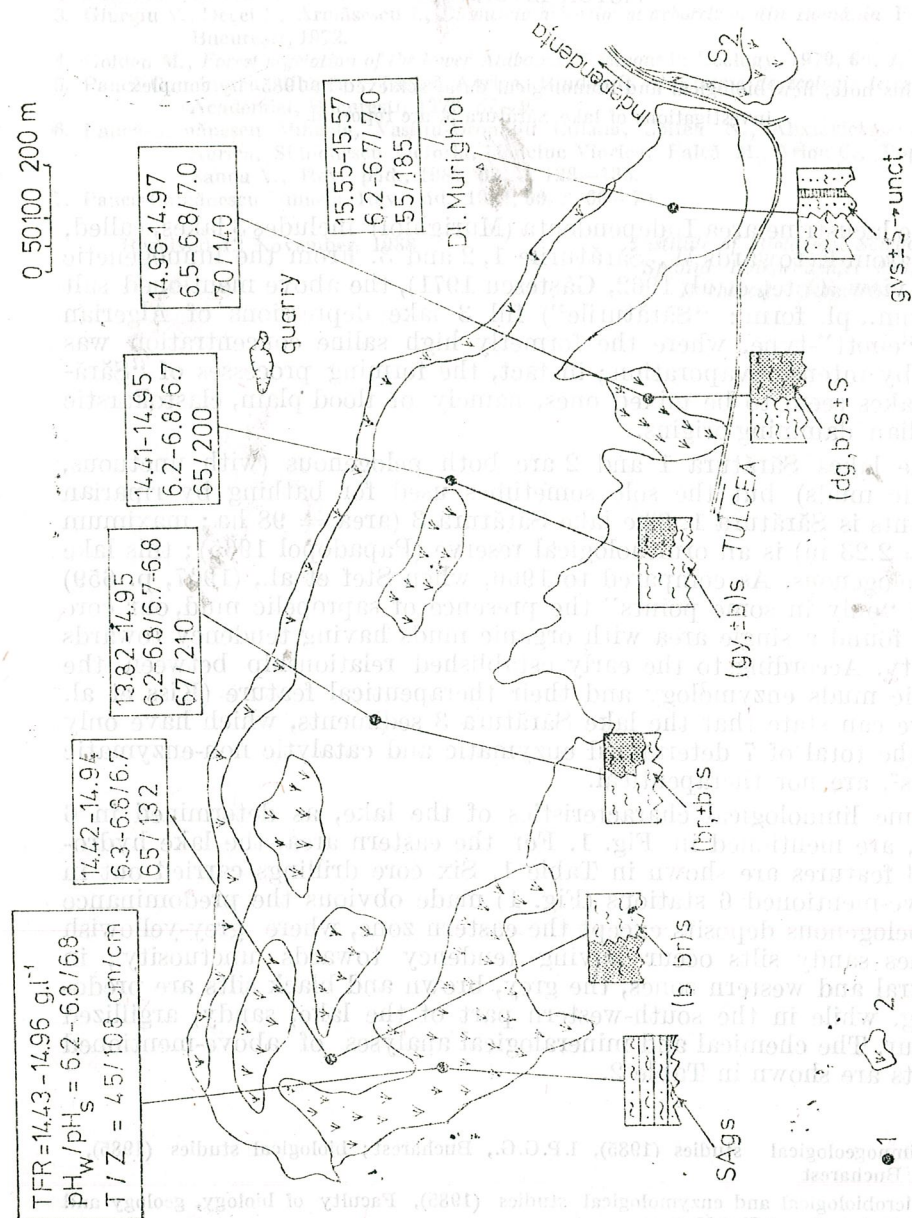


Fig. 1.— The main characteristics of water and sediments within 6 stations (May-Oct. 1985), lake Săratu 3: 1, sampling points; 2, association of *Scirpophragmites*; TFR, total filtrable residue; pH_w, pH_s, water (w) and sediment (s) pH; T, Secchi disk transparency; Z, lake water depth; SAGs, grey, sandy, argillized silts; (b, br)s, black and brown silts; (br, ±b)s, brown, with black zones, silts; (gy, ±b)s, grey-yellow, sometimes black silts; (dg, b)s±S, dark-grey and black, slight sandy silts; gvs ± S → unct., grey-yellow, slight sandy silts, with tendency towards unctuality; S₂, lake Săratu 3; S₁, sediment; S₃, water (w); S₄, total filtrable residue; S₅, pH_w; S₆, pH_s; S₇, water (w); S₈, sediment (s) pH; S₉, T; S₁₀, Z; S₁₁, SAGs; S₁₂, (b, br)s; S₁₃, (br, ±b)s; S₁₄, (gy, ±b)s; S₁₅, (dg, b)s±S; S₁₆, gvs ± S → unct.; S₁₇, S₁₈, S₁₉, S₂₀, S₂₁, S₂₂, S₂₃, S₂₄, S₂₅, S₂₆, S₂₇, S₂₈, S₂₉, S₃₀, S₃₁, S₃₂, S₃₃, S₃₄, S₃₅, S₃₆, S₃₇, S₃₈, S₃₉, S₄₀, S₄₁, S₄₂, S₄₃, S₄₄, S₄₅, S₄₆, S₄₇, S₄₈, S₄₉, S₅₀, S₅₁, S₅₂, S₅₃, S₅₄, S₅₅, S₅₆, S₅₇, S₅₈, S₅₉, S₆₀, S₆₁, S₆₂, S₆₃, S₆₄, S₆₅, S₆₆, S₆₇, S₆₈, S₆₉, S₇₀, S₇₁, S₇₂, S₇₃, S₇₄, S₇₅, S₇₆, S₇₇, S₇₈, S₇₉, S₈₀, S₈₁, S₈₂, S₈₃, S₈₄, S₈₅, S₈₆, S₈₇, S₈₈, S₈₉, S₉₀, S₉₁, S₉₂, S₉₃, S₉₄, S₉₅, S₉₆, S₉₇, S₉₈, S₉₉, S₁₀₀.

Table 1

Hydrochemical characteristics of lake Săratu 3 (eastern part) (analysed by M. Viforeanu, D. Cocic, I. P. G. G., Bucharest)

Parameters	mg.l ⁻¹	Parameters	mg.l ⁻¹
F ⁻	0.6 — 1.5	Na ⁺	4097.2 — 4570.9
Cl ⁻	10070.6 — 11524.5	K ⁺	44.0 — 47.5
Br ⁻	1.5 — 2.0	Ca ⁺	460.9 — 561.1
J ⁻	0.1 — 0.3	Mg ⁺	1133.3 — 1288.9
SO ₄ ⁻	210.3 — 336.0	Mn ⁺	0
NO ₂ ⁻	0	Fe total	0.15 — 0.70
NO ₃ ⁻	traces	NH ₄ ⁺	0 — 1.0
NO ₃ ⁻	0 — traces	Li ⁺	traces — 0.2
HPO ₄ ⁻	268.4 — 280.6	H ₂ SiO ₃	traces — 9.0
HCO ₃ ⁻	16.2 — 30.0	HBO ₂	0 — 16.2
Suspended matter		COD	23.2 — 34.4

Water type: Cl Na

Table 2

Chemical and mineralogical composition of lake Săratu 3 sediments (% dry matter; analysed by El. Opreș, E. Călinescu, I. Prodănescu, G. Cușmirencu, Gh. Enache, I. P. G. G., Bucharest) (P — present)

Parameters	%	Parameters	%
CH ₄	0.348 — 1.400	Na	1.55 — 2.87
C ₂ H ₆ , C ₃ H ₈ , C ₄ H ₁₀	0	K	0.34 — 1.04
CO ₂	15.38 — 82.61	SO ₄	0 — 0.33
C org.	0.94 — 8.38	S total	0.07 — 0.38
C tot.	3.83 — 21.30	Cl	1.33 — 6.69
Extract ethyl ether	0.170 — 1.004	Quartz	4 — 66
E. alcohol + benzene	1.350 — 7.950	Feldspar	0 — 46
Distilled water extract	1.678 — 7.486	Calcite	P — 80
SiO ₂	10.44 — 63.12	Dolomite	0 — 30
Al ₂ O ₃	1.90 — 6.05	Micas*	0 — 11
Fe total	0.64 — 2.05	Kaolinite	0 — 8
Ca	6.44 — 15.60	Chlorite	0
Mg	0.97 — 1.86	Montmorillonite	0 — P
Mn	0.05 — 0.15	Halite	0
P	0.06 — 0.13	Gypsum	0

* include illite, sericite and hydromica

Table 3

Algal and faunal species composition in lake Sărătura 3 waters and sediments
(+ = present; — = absent)

Groups/Taxa	Saprobity	Plankton	Benthos
Cyanophyta			
<i>Lyngbya limnetica</i>		+	+
<i>L. aestuarii</i>		—	+
<i>L. sp.</i>		—	+
<i>Oscillatoria chalybea</i>	beta	—	+
<i>Spirulina sp.</i>		—	+
<i>Chroostipes sp.</i>		+	—
<i>Rivularia planclonica</i>		+	—
Euglenophyta			
<i>Euglena sp.</i>		+	—
Chlorophyta			
<i>Lagerheimia tetraedrica</i>		+	—
<i>Goleminia radiata</i>		+	—
<i>Heleochleris pallida</i>		+	—
<i>Uroglenopsis sp.</i>		+	—
<i>Chlorella vulgaris</i>		+	—
<i>Crucigenia quadrata</i>	alpha-beta	+	—
Bacillariophyta			
<i>Amphora coffeiformis</i>		+	+
<i>Navicula sp.</i>	beta	+	+
<i>Gyrosigma acuminatum</i>		+	—
var. <i>lacustre</i>	beta	+	—
<i>Nitzschia sp.</i>		—	+
<i>Synedra acus</i>	beta	+	—
Ciliata			
<i>Litonotus sp.</i>		+	—
Rotatoria			
<i>Brachionus plicatilis</i>	beta	+	—
<i>Cephalodella catellina</i>		+	—
<i>Filinia brachiata</i>	oligo	+	—
<i>Lecane arcuata</i>	oligo	+	—
Nematoda g. sp.		+	—
Oligochaeta			
<i>Limnodrilus hoffmeisteri</i>	poly-alpha	+	+
Crustacea (Copepoda)		+	—
<i>Arcodiaptomus salinus</i>		+	—
Insecta			
Heteroptera			
<i>Coryxa sp.</i>		+	—
Coleoptera			
<i>Ditiscide g. sp.</i>		+	—
Trichoptera			+
Diptera			+
Ceratopogonidae			+
Chironomidae g. sp.			+
Pisces			
<i>Rodeus sericeus amarus</i> (boerlă)			
<i>Carassius auratus gibelio</i> (caras)			
<i>Proterorhinus marmoratus marmoratus</i> (zimbriș)			

The algal and faunal species composition³ of the lake is listed in Table 3. The phytoplankton, zooplankton and benthos algae are reduced; the fish fauna is small-sized and poor, too.

The aquatic and marsh vegetation (sketched on Fig. 1) is clearly dominated by *Scirpophragmitetum* (especially *Phragmites australis* var. *rivularis*); subordinate forms are: *Typha angustifolia*, *T. laxmanni*, *Schoeno-*

Table 4

Chemical and mineralogical characteristics of substratum with and without plant cover, lake Sărătura 3 (% dry matter)

Components	No plant cover	Scirpophragmitetum plant cover
CH ₄	0.894 — 0.980	0.348 — 1.400
CO ₂	20.00 — 23.53	15.38 — 82.61
C ₂ H ₆ , C ₃ H ₈ , C ₄ H ₁₀	0	0
C org.	2.46 — 8.38	0.94 — 5.15
C inorg.	8.54 — 12.92	2.89 — 12.25
C total	11.00 — 21.30	3.83 — 17.40
Ethyl ether extract	0.360 — 0.502	0.170 — 1.004
Alcohol + benzene extract	1.350 — 7.950	2.648 — 7.368
Distilled water ² extract	3.178 — 7.486	1.678 — 5.788
SiO ₂	10.44 — 26.58	14.07 — 63.12
Al ₂ O ₃	1.90 — 5.93	3.69 — 6.05
Fe total	0.64 — 2.05	1.46 — 1.94
Ca	13.82 — 14.96	6.44 — 15.60
Mg	1.64 — 1.86	0.97 — 1.77
Ti	0.08 — 0.21	0.14 — 0.32
Mn	0.08 — 0.12	0.05 — 0.15
P	0.07 — 0.13	0.06 — 0.08
Na	2.08 — 2.87	1.55 — 2.65
K	0.34 — 1.02	0.59 — 1.04
SO ₄	0 — 0.27	0 — 0.33
S total	0.29 — 0.33	0.07 — 0.38
Cl	3.92 — 6.69	1.33 — 5.95
Quartz	4 — 24	6 — 66
Feldspar	0 — 6	0 — 46
Calcite	57 — 80	0 — 74
Dolomite	0 — 15	0 — 30
Micas (illite + sericite)	0 — 7	0 — 10
Kaolinite	0 — 5	0 — 8

Remark: Analysts are the same as in Table 2.

plectus tabernaemontani, *S. lacustris*, *Scirpus triquetus*, *Heleocharis palustris*, *Potamogeton pectinatus* and *P. trichoides*. The growth environment of *Scirpophragmitetum* association is characterized in Table 4. As compared to the bottom areas without vegetal cover, the reeds grow on a substratum richer in CO₂, SiO₂, Al₂O₃, Ti and respectively poorer in organic,

³ Biological studies (1985), ICEBIOL, Bucharest.

inorganic and total C, distilled water extract, Ca, Mg, P, Na, total S and Cl; from the mineralogical point of view, the substratum with *Scirpophragmitum* is enriched in quartz, feldspar and dolomite and impoverished in calcite.

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Enterprise for Geological
and Geophysical
Prospections, 1, Caransebeș Str.,
Bucharest
Institute of Biological
Sciences,
294, Spl. Independenței
Bucharest

SOME OBSERVATIONS ON THE GLAUCINE CONTENT IN *GLAUCIUM FLAVUM* CR. POPULATIONS TREATED WITH MUTAGENS

ELVIRA V. GILLE, G. I. GHIORGHITĂ, FL. FLORIA

The paper presents the first observations on the biology of yellow poppy, the behaviour of glaucine content after mutagen treatments with gamma rays and alkylating agents.

There was found a decrease of glaucine level along the generations. This tendency was more evident with the plants treated with gamma rays and alkylants. Exception made the plants which received repeated treatments with 10 kR dose, and which realized in the interval 1985—1987 (M_3-M_5) an average glaucine content of 3.58% d.m.

The dormant seeds of yellow poppy do not contain glaucine, but their presence can be detected in the germs. The highest content of glaucine had the shoots with flowers and immature fruits. In the second vegetation year the yellow poppy plants had lower quantities of glaucine as compared to those in the first vegetation year.

The reconsideration of the herbs in therapeutics determined a continuous increase of the vegetal raw material supply for the pharmaceutical industry, on the one hand, and a sustained effort to find new valuable species of medicinal plants and their impact on economy, on the other. In this second category is the place of *Glaucium flavum* Cr. (yellow poppy), an allogamous plant of mediterranean origin (Thanos et al., 1987), to be found all over Europe, West-Asia and North Africa, a plant that contains a series of alkaloids, the most important, with an anti-caughing effect, being glaucine.

Not for a long time in the attention of specialists, the scientific accounts on yellow poppy are not abounding. And yet there have been accumulated, in the last twenty years, a series of interesting and useful data referring to the biology of the species (9, 14, 16, 17), its histo-anatomical plant structure (1, 11, 12), its chemical composition and the pharmacological effects of the basic alkaloid — glaucine (10), (14), the behaviour of plants in culture (9), (14) etc.

In our country, the yellow poppy has raised some interest in recent years, being cultivated and already used to produce a Romanian medicine — Glaucine. Under these circumstances the problem of providing herbs for industrial needs and their quality acquires importance. The obtaining of stable and high production of herbs and glaucine/ha depends on a valuable biological material for growing. As we cannot obtain valuable forms for yellow poppy melioration through selection in local populations of the species, we intend to isolate such forms out of populations treated with chemical and physical agents. This method induced good results in *Papaver somniferum* (2)—(7). Our investigations also have as a goal a thorough knowledge of the genetical, biochemical and eco-physiological peculiarities.

rities of yellow poppy. This paper presents the first observations on the biology of yellow poppy, the behaviour of the glaucine content, in successive generations, depending on the mutagen treatments applied.

MATERIAL AND METHODS

Dormant seeds of *Glaucium flavum* Cr. from SCPMA-Fundulea underwent singular and combined treatments, along one or more generations with chemical and physical mutagens. In spring 1983 lots of yellow poppy were treated (single and combined) with gamma rays (doses between 3 and 25 kR, flow capacity of 1.4 kR/h) and ethylmethanesulfonate-EMS (concentrations of 0.125%—24h). In the same experiment, 4 lots of seeds were treated with solutions of 0.05—0.30% colchicine (for 24h). After the treatments, the seeds were directly sown into the field, inter-row distance: 50 cm, and the distance between plants: 30 cm. The experiment was performed (up to the present) on 3 experimental fields at Piatra Neamț.

In another experiment, lots of *Gl. flavum* seeds were irradiated in the first generation with 3, 6, 10 and 15 kR gamma rays (Co^{60}). In the third generation we performed a new gamma irradiation with 5 and 10 kR. In the M_3 generation we obtained 9 experimental variants. The experiment took place at Cindești (Vrancea) in M_1 and M_2 and at Piatra Neamț in generations M_3 — M_5 .

We aimed to determine the behaviour of the glaucine content depending on the treatments applied. During the flowering period, when the plants presented both flowers and fruits (in different developmental evolution, a period considered optimal for the glaucine level (10), (14), we harvested medium offshoots per variant. For analysis we used 3 grammes of

Table 1

The glaucine content in different organs of the species

Glaucium flavum

The organ analysed	Glaucine content (% d.m.)	
	2.08.1986	22.10.1986
Root	—	0.66
Stem	0.51	0.70
Leaf: — rosette	1.22	1.73
— level I	1.56	1.12
— level II	—	1.42
— level III	—	1.73
— level IV	1.61	2.04
— level V	—	2.29
Flower: — buttons	2.03	1.63
— flower	2.03	1.64
Fruit: — immature	2.04	3.07
— capsule	—	2.04
— mature capsule	—	2.04
The average content in herbs	1.64	1.59

drug extracted in ethyl ether. The extract was finally reduced to 1 ml out of which 0.2 μ l were spotulated on chromatography plates with kieselgel G (Merck). The plates were developed in a solution of benzene/methanol/ammonia 25% (9:2:0.2) and the spots were identified with the Dragendorff reagent. The glaucine content was determined through photocolorimetry. For the standard curve we used glaucine bromhydrate. To establish the results, we took into account the conversion ratio of the glaucine bromhydrate into glaucine of 0.815. The results are given in Tables 2 and 3.

During our investigations we observed the distribution of the glaucine content in different organs of the plant, and the glaucine level in the different developing stages. The results of the analysis are given in Table 1.

We also present the first cytogenetical observations performed on yellow poppy, which finally aim to establish the idiogram and the karyotype of this species. For the time being (using Feulgen's method) after initially treating the roots in 0.2% colchicine solutions (4 hours) — we succeeded to obtain a series of metaphases which helped us to determine exactly the chromosomes.

RESULTS AND DISCUSSIONS

We shall present the results in other order than the one used in the previous chapter. To establish the melioration and selecting action on a species, knowing some of its genetical peculiarities, of its idiogram and karyotype, is absolutely necessary. The cytogenetic analyses showed that the *Glaucium flavum* species has a relatively reduced number of chromosomes, namely $2n=12$ (photo 1), similar to the other species of the *Papaver* family. Thus, *Chelidonium majus* has $2n=12$; *Corydalis cava* $2n=16$, (8); *Fumaria officinalis* $2n=14$; *Papaver rhoeas* and *P. bracteatum* $2n=14$; *P. somniferum* $2n=22$, (13), (15). The reduced chromosome number of yellow poppy may be a favourable chance to obtain some forms with different ploidy degree.

To find the appearance and evolution of the glaucine level (due to our method), along the first ontogenetical stages of yellow poppy we dosed the alkaloids, starting with dormant seeds, germs, plantlets, plants, up to the stage of plant rosette. The dormant seeds do not contain glaucine, but in the yellow-poppy germs its presence can be already detected (traces). Through drying, the initial laticifers, pointed out by Nessler (1982) in the procambium of the radicle, are capable to synthesize glaucine after 48—72 hours from germination, (11). At the same time with the appearance of leaves and with the development of the laticifer system, the alkaloid may already be quantitatively determined, registering values of 0.50% in plantlets and 0.87% in plants (in the rosette stage).

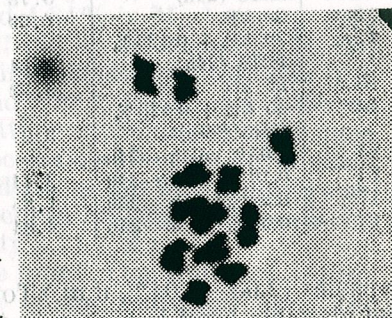


Photo 1. — Metaphase of *Glaucium flavum* Cr. ($2n=12$)

Our investigations, aiming the glaucine presence in different organs of yellow poppy (cultivated at Piatra Neamf), assert the observations made by Lörincz and other authors cited by him (10), in the direction that the roots have the lowest glaucine level, the highest being in the immature capsule (Table 1). We noticed that in the first vegetation year, at the beginning of August, the greatest quantity is in the buds, flowers and immature

Table 2

The glaucine content in successive generations of yellow poppy plants treated with mutagens

The variant	The treatment in M ₁	The glaucine content (% d.m.) in generation:					
		M ₁		M ₂		M ₃	
		first year of veget.	second year of veget.	first year of veget.	second year of veget.	first year of vegetation	
		shoots	shoots	shoots	mature capsules	shoots	mature capsules
1	Control	3.34	2.99	2.99	1.43	2.03	1.04
2	3 kR	2.92	3.82	3.57	1.50	1.29	1.02
3	0.125% EMS (24 h)	4.16	3.32	3.16	1.43	1.53	2.03
4	3 kR + 0.125% EMS (24h)	3.94	4.02	3.57	2.50	2.08	1.73
5	6 kR	3.40	4.02	2.48	1.78	1.27	1.53
6	6 kR + 0.125% EMS (24h)	2.51	3.37	2.99	2.50	2.16	1.02
7	10 kR	2.22	3.62	4.36	1.53	1.53	—
8	10 kR + 0.125% EMS (24h)	2.99	4.02	2.04	2.03	2.03	1.27
9	15 kR	2.18	3.42	2.87	1.43	1.04	0.78
10	15 kR + 0.125% EMS (24h)	3.78	3.42	2.28	1.30	0.50	0.59
11	20 kR	4.69	2.99	2.04	1.43	2.04	1.02
12	20 kR + 0.125% EMS (24h)	3.14	2.32	2.58	1.73	1.27	1.02
13	25 kR	3.72	3.37	2.04	1.43	—	1.02
14	25 kR + 0.125% EMS (24h)	3.27	—	2.99	1.43	2.14	1.53
15	0.05% colch.—24h	—	2.58	—	2.04	1.27	1.74
16	0.10% colch.—24h	2.44	3.93	2.99	1.53	2.58	1.73
17	0.20% colch.—24h	1.85	3.85	3.72	1.22	1.53	2.78
18	0.30% colch.—24h	4.84	2.99	2.14	1.63	1.57	1.04

capsules (2.03–2.04% d.m.). Towards the end of the vegetation (22.10.86) the glaucine level is higher in the immature capsules (3.07% d.m.); the leaves of the higher levels have a higher content than that in the buds and flowers as compared to the previous stage (Table 1). It is to be noticed that the glaucine level maintains rather high in the mature capsules (2.04% d.m.), permitting them to be industrially used after seed harvesting. According to our results, the best harvesting period of *Glaucium flavum* would be towards the end of the flowering and at the beginning of

the capsule maturation, which is in accordance with Astadzov's observations (cited by Păun et al. 1986).

From the data of Tables 2 and 3 one may deduce that the mutagens had the quality to amplify the variability of the glaucine content of yellow poppy plants, which, as noticed in the case of the active principles of other plants investigated by us, is not due to a certain rule. Not even in the case of combined treatments (gamma rays and EMS), or of repeated gamma ray treatments (in M₁ and M₃) such a rule can be detected. One may appreciate that the variability induced to the plants, from the point of view of the glaucine content, is more obvious in the cases that received combined treatments than in the cases that suffered repeated gamma rays treatments (Tables 2 and 3).

Table 3

The glaucine content in yellow poppy shoots originating from gamma rays treatments in M₁ and M₃

The variant	The treatment in generations		Glaucine content (% d.m.) in generation:				
			M ₃		M ₄		M ₅
	M ₁	M ₃	first year (1985)	second year (1986)	first year (1986)	first year (1987)	first year (1987)
1	Control		3.89	2.99	2.99	2.14	2.14
2	3 kR	5 kR	3.01	2.99	2.03	1.04	1.57
3	3 kR	10 kR	3.62	2.99	2.04	1.53	2.99
4	6 kR	5 kR	3.68	3.68	2.69	1.27	2.16
5	6 kR	10 kR	2.84	3.68	2.70	2.14	—
6	10 kR	5 kR	4.14	2.38	2.70	1.75	—
7	10 kR	10 kR	4.14	3.57	3.62	2.99	3.62
8	15 kR	5 kR	3.42	3.62	2.99	—	2.84
9	15 kR	10 kR	2.99	2.85	3.92	1.75	—

In the first experiment (the first vegetation year), the glaucine content of the shoots oscillated between 1.85 and 4.84% in M₁, between 2.04 and 2.99% in M₂ and between 1.04 and 2.58% in M₃ (table 2). In the other experiment, the glaucine level of the first vegetation year was between 2.84 and 4.14% in M₃, between 2.03 and 3.92% in M₄ and between 1.57 and 3.62% in M₅. An interesting and rather surprising phenomenon is proved by these data, namely the ever decreasing glaucine level along the generations. This tendency is more evident in the first experiment. From an average of about 3.25% per experiment in M₁ (the first vegetation year), the plants of M₃ have an average of only 1.71% d.m.

A plausible explanation is difficult to be found. One could, of course, consider the exciting and stimulating effect of the mutagens upon the metabolic ways of glaucine synthesis in the generation following the treatments and its dilution in the succedent generations. Some combinations of the mutagens in the experiment are too powerful and such a hypothesis would not resist. We would rather admit that the obtained phenotype (the glaucine level) is the result of the expression of the genotype treated with mutagens in the offered pedo-climatic conditions (changing from a generation to another). Arguments: the fact that the controls register a

similar curve (in time) of the glaucine level; in the same generation (M_1) but in different years (1986 and 1987) and soils of Piatra Neamț, the glaucine content differs significantly (Table 3). An eventual individual plant selection, started in the generation immediately following the treatment, due to the glaucine content criterion could, more or less, counteract the phenomenon presented.

It is worth noticing that in the case of the plants of some variants this phenomenon is less obvious, the plants having a better stability of glaucine accumulation and synthesis in time. In this situation were the variants 4, 6 and 8 — in the conditions of a more reduced pressure of the mutagens on the biological material (Table 2) — and variants 7 and 8 (Table 3). A special remark for variant 7 (Table 3) the plants of which received repeated treatments of 10 kR (M_1 and M_3) and which realized in the interval 1985–1987 (M_3 – M_5) an average glaucine level of 3.58% d.m. We noticed that this type of treatment, or the one combined with alkylants (postirradiatory) the 10 kR dose (20–30 R/min), also proved very suited in poppy melioration to obtain morphine (4, 5, 7).

Finally, our investigations proved that in the second vegetation year, the yellow poppy plants attained lower quantities of glaucine as compared to the first vegetation year, a fact that proves Astadzhov's observations (cited by Păun et al., 1986). This diminishing of the glaucine content is of a smaller amplitude than the one registered along the generations from the treatment with mutagens (Table 2 and 3).

The data of Table 1 underline once more that glaucine gathers in a greater quantity in shoots with flowers and immature fruits than in mature capsules.

CONCLUSIONS

1. The treatment applied to *Glaucium flavum* Cr. with mutagens (gamma rays, EMS and colchicine) induced a marked variability of the glaucine content in plants. From this point of view, the combined treatments (of the type: gamma rays + EMS) proved more efficient.
2. Along the generations following the treatments, the glaucine level in the plants is lower. The plants of the second vegetation year have a more reduced glaucine level than those of the first year.
3. The glaucine is present in all the organs of the plant, except the mature seeds. Its presence may already be identified in the stage of germ. The greatest glaucine quantity is to be found in the leaves of the higher levels, in the flowers and immature capsules.

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"Stejarul" Research Station,
5600 Piatra Neamț, Romania



SALINITY-HORMONE INTERACTION IN RELATION TO THE CHEMICAL COMPOSITION OF MAIZE AND SAFFLOWER PLANTS

A. F. RADİ*, M. M. HEİKAL*,
A. M. ABDEL-RAHMAN**, B. A. A. EL-DEEP**

The interactive effect of salinity and growth hormones on the chemical composition of maize and safflower plants was investigated.

Pigment contents of maize plants increased with the rise of NaCl salinization and the opposite trend was exhibited by safflower plants. Seed presoaking with GA₃ or IAA generally induced a significant increase in the values of the total pigment contents of the test plants.

The mineral composition of the test plants showed somewhat variable responses to salinization and hormone treatments.

The production of soluble, insoluble as well as total carbohydrates by maize plants was reduced with the rise of salinization level. On the other hand, the pattern of changes in carbohydrate contents of safflower plants were irregular at the different salinity and hormone treatment.

INTRODUCTION

The estimation of the total pigment contents and of the different pigment fractions has been adopted in various studies, particularly in relation to saltstress. Many investigators found that salinization exerts a general reduction in the contents of the photosynthetic pigments (Ashour and Thalooth, 1971; Shimose, 1973; Heikal, 1975; and Ahmed *et al.*, 1980). On the other hand, Margolina (1950) and Dostanova (1966) found that the leaves of salinized sugar beet plants are richer in chlorophylls and carotenoids as compared with those grown under non-salinized conditions.

As regards nitrogen metabolism salinized plants exhibited some disturbances in nitrogen metabolism (Gates *et al.*, 1966; Wilson *et al.*, 1970; El-Shourbagy and Missak, 1975; and Heikal, 1976). In this respect, the protein contents of various plant tissues were found to decline under saline conditions.

Salinity stress seems to affect plant carbohydrate metabolism in several ways (Todd and Basler, 1965). Earlier studies indicated that carbohydrate contents were frequently higher in plants grown under saline conditions, although exceptions were noted (Bernstein and Hayward, 1958 and Barlow *et al.*, 1976).

With respect to uptake, a number of investigators demonstrated that nutrient uptake by certain plant species is curtailed by salinization (Black *et al.*, 1965; Ramzan, 1967; Shimose, 1968; Lashin and Atanasiu, 1972; El-Shourbagy and Missak, 1975; and Heikal *et al.*, 1979). Other investigators showed that salinization treatments resulted in a promotion rather than an inhibition of nutrient uptake (Shimose, 1963 and 1964; Asana and Kale, 1965; Mehrotra, 1971 and Heikal *et al.*, 1980a).

Presoaking seeds with optimal concentration of certain phytohormones have been shown to be beneficial to growth and yield of some crop species grown under saline conditions by increasing nutrient reserves through increased physiological activities and root proliferation (Singh and Darra, 1971; Darra *et al.*, 1973 and Chhipa and Lal, 1978).

The present work was carried out to study the effect of various concentrations of NaCl on chemical composition of maize (*Zea mays*) and safflower (*Carthamus tinctorius*) plants. The role of GA₃ and IAA in modifying the salt-stress induced changes was also investigated.

MATERIALS AND METHODS

The optimum conditions reached from preliminary tests conducted to determine the optimum hormone concentration, the optimum periods of presoaking and drying were exactly the same as described in a previous investigation (in press). Seeds of test plants were presoaked in GA₃ or IAA and after being dried were sown in plastic pots containing 2 kg air-dried soil (sand/clay 1: v/v). Using NaCl and N/10 Pfeffer's nutrient solution, stress levels were chosen at -2, -4, -6, -8 and -10 bar in case of maize plants and -3, -6, -9 and -12 bar in case of safflower plants. The test plants were irrigated with experimental solution every other day for two weeks from the beginning of planting and the soil moisture content was kept near the field capacity. Thereafter, the salinized and non-salinized plants were irrigated every other day with N/10 Pfeffer's solution. The plants were left to grow under the various treatments in a greenhouse for 45 days in case of maize and 60 days in case of safflower.

The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined using the spectrophotometric method recommended by Metzner *et al.*, (1965).

Nitrogen was determined by the microkjeldahl method (Paech and Tracey, 1956).

The anthrone sulphuric acid method (Fales, 1951; Schlegel, 1956 and Badour, 1959) was used for the determination of carbohydrates.

Dry samples were ground into a fine powder by a micromill and assayed for mineral ion determination. Flame photometric method was used for the determination of sodium and potassium (Williams and Twine, 1960). The versene (disodium dihydrogen ethylenediamine tetraacetic acid) titration method (Schwarzenbach and Biederman, 1948) was employed for both calcium and magnesium determinations.

The data were statistically analysed and the least significant differences were obtained (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The biosynthesis of pigments in salinized maize plants was markedly activated by salt-stress (Tables 1 and 2). These results are generally in accordance with those obtained by Ahmed *et al.* (1977, 1978) and Heikal *et al.*, (1980c). In case of safflower plants, the production of pigments was gene-

rally reduced (Table 2). Similar results were also obtained by some other authors using different kinds of plants (Ashour and Thaloorth, 1971; Ahmed *et al.*, 1979; Jashi and Naik, 1980 and Chavan and Karadge, 1980). Seed presoaking with GA₃ or IAA almostly induced a significant increase in the values of the total pigment contents of the test plants (Tables 1 and 2). This promotive effect of seed presoaking obtained in

Table 1

Effect of seed presoaking with GA₃ and IAA on the concentration of pigments in leaves of maize plants stressed for 45 days with different levels of sodium chloride

Treatment	Pigments as mg/g leaf dry weight				
	Chl. a	Chl. b	Carotenoids	Total	Chl. a/Chl. b ratio
Control	3.51	1.72	1.36	6.59	2.05
- 2 bar	7.43**	2.49**	2.35**	12.27**	2.98**
- 4 bar	6.75**	2.49**	2.83**	12.07**	2.71**
- 6 bar	6.46**	1.98*	2.04**	10.48**	3.26**
- 8 bar	5.53**	1.92	2.18**	9.63**	2.88**
- 10 bar	5.36**	1.81	2.34**	9.51**	2.96**
Control + GA ₃	5.37**	1.81	1.81**	8.99**	2.96**
- 2 bar + GA ₃	7.95*	2.77**	3.02**	13.74**	2.87
- 4 bar + GA ₃	9.48**	3.03**	3.27**	15.78**	3.13**
- 6 bar + GA ₃	10.57**	3.18**	2.62**	16.37**	3.32
- 8 bar + GA ₃	6.88**	2.46**	2.29	11.63**	2.79
- 10 bar + GA ₃	6.28**	1.81	2.06	10.15	3.47**
Control + IAA	4.95**	1.51*	1.77**	8.23**	3.28*
- 2 bar + IAA	5.85**	1.81**	1.86**	9.52**	3.23*
- 4 bar + IAA	8.53**	2.51	2.82	13.86**	3.39**
- 6 bar + IAA	8.88**	2.65**	2.65**	14.18**	3.50*
- 8 bar + IAA	8.65**	2.45**	2.59**	13.09**	3.28**
- 10 bar + IAA	7.87**	2.38**	2.57	12.82**	3.31**
L. S. D. at 5%	0.51	0.21	0.30	0.75	0.22
L. S. D. at 1%	0.68	0.28	0.41	1.00	0.29

* Significant differences.

** Highly significant differences as compared with the control.

this experiment is in agreement with the results obtained by Varshney and Baijal (1979) and Shaddad and Heikal (1982) using some grasses and kidney bean plants.

The nitrogen fractions of the test plants exhibited variable values (Tables 3 and 4). These variable changes add more support to the results obtained by Gates *et al.*, (1966); Wilson *et al.*, (1970); El-Shourbagy and Missak (1975) and Heikal (1976) who demonstrated that salinity induces gross disturbances in the nitrogen metabolism of certain plants. However, in this present investigation, irrespective of salinity level, seed presoaking with GA₃ induced a significant increase in soluble, insoluble and consequently the total nitrogen in safflower plants (Table 3).

Table 2

Effect of seed presoaking with GA₃ and IAA on the concentration of pigments in leaves of safflower plants stressed for 60 days with different levels of sodium chloride

Treatment	Pigments as mg/g leaf dry weight				
	Chl. a	Chl. b	Carotenoides	Total	Chl. a/Chl. b ratio
Control	9.01	3.74	3.39	16.14	2.41
- 3 bar	9.02	3.31**	3.14	15.47*	2.73**
- 6 bar	7.21**	2.27**	2.76**	12.24**	3.18**
- 9 bar	4.53**	1.37**	2.17**	8.07**	3.31**
- 12 bar	2.50**	0.86**	1.20**	4.56**	2.91**
Control + GA ₃	10.20**	3.34**	3.82*	17.36**	3.05**
- 3 bar + GA ₃	9.58*	3.35	3.58*	16.51**	2.86
- 6 bar + GA ₃	7.55	2.88**	2.88	12.62	2.62**
- 9 bar + GA ₃	7.25**	2.64**	2.36	12.25**	2.75**
- 12 bar + GA ₃	5.20**	2.22**	1.84**	9.26**	2.34**
Control + IAA	9.21	3.29**	3.30	15.80	2.80**
- 3 bar + IAA	8.90	3.50	3.33	15.73	2.54*
- 6 bar + IAA	8.20**	2.81**	2.96	13.97**	2.92**
- 9 bar + IAA	5.30**	2.41**	1.65**	9.36**	2.20**
- 12 bar + IAA	3.57**	1.38**	1.42	6.37**	2.59**
L. S. D. at 5%	0.47	0.26	0.36	0.57	0.18
L. S. D. at 1%	0.63	0.35	0.49	0.77	0.24

* Significant differences

** Highly significant differences as compared with the control.

Table 3

Effect of seed presoaking with GA₃ and IAA on nitrogen fractions in shoots and roots of maize plants stressed for 45 days with different levels of sodium chloride. Data are expressed as mg/g dry weight

Treatment	Shoots			Roots		
	Soluble	Insoluble	Total	Soluble	Insoluble	Total
Control	11.78	7.87	19.65	16.66	5.51	22.17
- 2 bar	11.08*	9.91	19.99	12.60**	10.59**	23.19
- 4 bar	13.08**	7.37	20.38	12.75**	20.50**	33.25**
- 6 bar	14.71**	8.97	23.68	13.75**	24.56**	38.31**
- 8 bar	12.83**	8.25	21.09	12.50**	6.82	19.32*
- 10 bar	10.33**	4.20**	14.61**	9.17**	3.49	12.66**
Control + GA ₃	13.13**	9.02	22.15	20.67**	4.51	25.18*
- 2 bar + GA ₃	11.04	12.14**	23.18*	22.18**	9.07	31.25**
- 4 bar + GA ₃	14.21**	15.24**	29.45**	16.63**	19.66	36.29*
- 6 bar + GA ₃	15.50*	8.88	24.38	13.94	25.38	39.32
- 8 bar + GA ₃	17.82**	5.37**	23.19	12.60	13.60**	26.20**
- 10 bar + GA ₃	12.09**	5.18	17.27	9.58	6.67*	16.25**
Control + IAA	14.58**	7.50	22.08	17.92	11.32**	29.24**
- 2 bar + IAA	12.08**	9.17	21.25	14.09	22.21**	36.30**
- 4 bar + IAA	13.29	14.58**	27.87**	13.08	21.16	34.24
- 6 bar + IAA	13.61**	12.64**	26.75*	12.50	12.70**	25.20**
- 8 bar + IAA	14.87**	6.78*	21.65	12.92	8.25	21.17
- 10 bar + IAA	13.52**	4.69	18.21*	10.83*	3.33	13.16
L. S. D. at 5%	0.66	1.36	2.80	1.48	2.43	2.63
L. S. D. at 1%	0.89	1.82	3.80	1.98	3.26	3.53

* Significant differences.

** Highly significant differences as compared with the control.

This response would suggest that the deteriorative effect of salt stress on plant growth and other relevant physiological activities can be alleviated and/or modified to some extent by treating the seeds with the proper concentrations of suitable growth promoter.

Table 4

Effect of seed presoaking with GA₃ and IAA on nitrogen fractions in shoots and roots of safflower plants stressed for 60 days with different levels of sodium chloride. Data are expressed as mg/g dry weight

Treatment	Shoot			Root		
	Soluble	Insoluble	Total	Soluble	Insoluble	Total
Control	23.54	10.74	34.28	17.36	18.93	36.29
- 3 bar	20.90**	13.37**	34.27	18.75	23.58**	42.34**
- 6 bar	18.20**	13.54**	31.74**	21.53**	22.83**	44.36**
- 9 bar	17.87**	13.88**	31.75**	13.19**	17.05*	30.24**
- 12 bar	19.33**	12.42*	31.75**	11.81**	5.33**	17.14**
Control + GA ₃	23.96	28.97**	52.93**	30.50**	14.39**	44.89**
- 3 bar + GA ₃	25.86**	16.99**	42.85**	22.92**	17.33**	40.25
- 6 bar + GA ₃	23.47**	16.15**	39.62**	19.36*	18.99**	38.35**
- 9 bar + GA ₃	22.94**	15.52*	38.46**	17.36**	12.88**	30.24
- 12 bar + GA ₃	21.81**	18.01**	39.82**	15.17**	3.99	19.16
Control + IAA	27.08**	17.28**	44.36**	20.83**	24.54**	45.37**
- 3 bar + IAA	23.61**	16.21**	39.82**	28.47**	15.59**	44.06
- 6 bar + IAA	22.29**	8.46**	30.75	36.80**	11.59**	48.39**
- 9 bar + IAA	22.40**	8.87**	31.27	21.83**	6.39**	28.22
- 12 bar + IAA	23.03**	8.73**	31.76	13.33	4.81	18.14
L. S. D. at 5%	1.15	1.63	1.51	2.10	1.40	2.52
L. S. D. at 1%	1.58	2.24	2.07	2.88	1.92	3.45

* Significant differences.

** Highly significant differences as compared with the control.

Salt-stress was found to induce a significant reduction in the carbohydrate contents of maize plants (Table 5). In this respect, Bernstein and Hayward (1958); Cecil and Stewart, (1971); Barlow *et al.*, (1976) and Ismail (1982) reported that salinity affects generally the carbohydrate metabolism. On the other hand, the increase in carbohydrate contents of safflower plants (Table 6) especially at the relatively low and moderate salinization levels could be ascribed to the increased soluble carbohydrate fractions. These results are in agreement with those obtained by Salama *et al.*, (1980) and Ismail (1982) using some glycophytic plants and a mixture of NaCl and other salt as salinizing agents. Seed presoaking with the phytohormones induced an irregular effect on carbohydrates production in salt stressed plants (Tables 5 and 6).

Salinization treatments were found to induce some considerable changes in the concentration of mono (Na⁺, K⁺) and divalent (Ca²⁺, Mg²⁺) cations in the main parts of the test plants. However, the magnitude and the patterns of these changes differed according to the level of salinization, the nutritive element, the plant type and the organ harvested (Tables 7, 8, 9 and 10). The increase in sodium concentration, with the rise of salinization level, is in accordance with the results obtained by

Table 5

Effect of seed presoaking with GA_3 and IAA on carbohydrate production in maize plants, stressed for 45 days with different levels of sodium chloride. Data are expressed as mg/g dry weight of the whole plant

Treatment	Soluble	Insoluble	Total
Control	38.21	116.70	154.91
- 2 bar	44.87**	105.21**	150.08
- 4 bar	33.20**	104.09**	137.29**
- 6 bar	27.20**	80.38**	107.58**
- 8 bar	15.36**	68.97**	84.33**
- 10 bar	10.63**	64.28**	74.91**
Control+ GA_3	36.81	144.21**	181.02**
- 2 bar+ GA_3	44.91	102.95	147.86
- 4 bar+ GA_3	24.04**	105.26	129.30
- 6 bar+ GA_3	23.33**	86.12	109.45
- 8 bar+ GA_3	14.33	84.06**	98.39**
- 10 bar+ GA_3	9.09	82.42**	91.51**
Control+IAA	37.53	109.79**	147.32
- 2 bar+IAA	26.58**	99.18	125.76**
- 4 bar+IAA	23.57**	96.56*	120.13**
- 6 bar+IAA	24.62*	70.23**	94.85***
- 8 bar+IAA	10.30**	71.06	81.36
- 10 bar+IAA	8.85	56.61*	65.46*
L. S. D. at 5%	3.23	9.24	11.69
L. S. D. at 1%	2.41	6.20	8.72

* Significant differences.

** Highly significant differences as compared with the control.

Table 6

Effect of seed presoaking with GA_3 and IAA on carbohydrate production in safflower plants stressed for 60 days with different levels of sodium chloride. Data are expressed as mg/g dry weight of the whole plant

Treatment	Soluble carbohydrate	Insoluble carbohydrate	Total carbohydrate
Control	14.48	64.38	78.86
- 3 bar	15.24	63.59	78.83
- 6 bar	15.31	72.00**	87.31**
- 9 bar	20.67**	63.41	84.08
- 12 bar	17.04	34.76**	51.80**
Control+ GA_3	21.62**	69.89*	91.51**
- 3 bar+ GA_3	20.97**	73.27**	94.24**
- 6 bar+ GA_3	21.62**	73.90	95.52**
- 9 bar+ GA_3	24.54**	65.16	89.70*
- 12 bar+ GA_3	28.12**	35.04	63.16**
Control+IAA	15.18	61.77	76.95
- 3 bar+IAA	15.72	66.24	81.96
- 6 bar+IAA	18.49*	75.82	94.31*
- 9 bar+IAA	25.50**	65.45	90.95*
- 12 bar+IAA	24.52**	28.88*	53.40
L. S. D. at 5%	3.15	4.41	5.25
L. S. D. at 1%	4.32	6.04	7.20

* Significant differences.

** Highly significant differences as compared with the control.

other investigators using some other crop plants (Morani and Fortini, 1963; Yousif *et al.*, 1972; Heikal *et al.*, 1979 and Coughlan and Wyn-jones, 1980). However, the extent of sodium accumulation varied among the main plant parts of the test plants. In this respect, the shoots accumulated generally lower amounts of sodium than the roots. This is in accordance with Jacoby (1965); Lahaye and Epstein (1969) and Heikal *et al.* (1980b).

The reduction in potassium concentration in salinized maize plants is in conformity with the results obtained by Rush and Epstein (1976); Chavan and Karadge (1980) and Heikal *et al.*, (1981). In safflower plants, however, relatively low and moderate levels of NaCl promoted the accumulation of potassium (Tables 9 and 10). This trend was also revealed by Wilson *et al.*, (1970) and Heikal *et al.*, (1980 and b).

Table 7

Changes in the mineral composition of shoots of maize plants as affected by salt stress for 45 days and the role of grain presoaking with GA_3 and IAA in modifying the induced changes. Data are expressed as mg/g dry weight

Treatment	Na	K	Ca	Mg
Control	7.83	45.00	19.16	11.00
- 2 bar	21.33**	43.92	21.33*	12.00
- 4 bar	25.00**	46.43	21.33*	12.00
- 6 bar	28.66**	46.12	22.66**	12.40
- 8 bar	45.00**	41.03**	22.00**	14.75**
- 10 bar	52.00**	39.00**	15.66**	17.36**
Control+ GA_3	6.16	42.35**	21.66*	11.45
- 2 bar+ GA_3	19.00	43.90	24.66**	13.60
- 4 bar+ GA_3	22.00*	49.17**	22.66	16.40**
- 6 bar+ GA_3	28.00	42.66**	24.33	14.60*
- 8 bar+ GA_3	41.00*	40.22	22.33	19.40**
- 10 bar+ GA_3	50.16	37.01*	25.33**	10.56*
Control+IAA	6.50	43.61	16.00**	9.60
- 2 bar+IAA	23.00	43.70	19.50	16.00**
- 4 bar+IAA	27.00	46.12	25.33**	19.60**
- 6 bar+IAA	29.00	45.61	26.66**	19.20**
- 8 bar+IAA	45.00	34.63**	23.33	22.40**
- 10 bar+IAA	50.66	25.80**	19.33**	21.33**
L. S. D. at 5%	2.94	1.89	1.89	1.68
L. S. D. at 1%	4.03	2.59	2.59	2.30

* Significant differences.

** Highly significant differences as compared with the control.

The concentration of calcium in shoots and roots of safflower (Tables 9 and 10) was increased with salinity, while in maize calcium concentration exhibited generally irregular trends (Tables 7 and 8).

Magnesium concentration in the main parts of the salt affected plants showed variable alterations (Tables 7, 8, 9 and 10). This trend was also shown by some authors using other plants (Bierhuizen and Ploegman, 1977 and Helal and Mengel, 1979).

Table 8
Changes in mineral composition of roots of maize plants as affected by salt stress for 45 days and the role of grain presoaking with GA₃ and IAA in modifying the induced changes. Data expressed as mg/g dry weight

Treatment	Na	K	Ca	Mg
Control	10.00	20.74	22.66	21.66
- 2 bar	24.00**	16.09**	23.33	18.26**
- 4 bar	30.00**	14.84**	20.00**	13.60**
- 6 bar	34.00**	14.44**	20.66**	9.80**
- 8 bar	48.00**	9.76**	20.00**	8.16**
- 10 bar	54.00**	7.12**	16.73**	5.77**
Control+GA ₃	9.00	20.74	28.00**	22.00
- 2 bar+GA ₃	22.00	26.03**	21.33*	20.00
- 4 bar+GA ₃	26.00*	26.43**	25.33**	17.60**
- 6 bar+GA ₃	30.00*	18.09**	25.33**	12.00**
- 8 bar+GA ₃	40.00**	10.17	30.00**	9.80
- 10 bar+GA ₃	50.00**	9.96	22.00**	9.60**
Control+IAA	9.00	21.76	18.00**	20.80
- 2 bar+IAA	22.00	23.18**	18.66**	24.00**
- 4 bar+IAA	26.00*	34.16**	40.00**	24.40**
- 6 bar+IAA	31.30	21.96**	43.33**	25.20**
- 8 bar+IAA	40.00**	10.98	36.66**	12.73**
- 10 bar+IAA	49.00*	8.91*	26.00**	9.60**
L. S. D. at 5 %	3.78	1.68	1.73	1.65
L. S. D. at 1 %	5.18	2.30	2.34	2.27

* Significant differences.

** Highly significant differences as compared with the control.

Table 9
Changes in the mineral composition of shoots of safflower plants as affected by salt stress for 60 days and the role of seed presoaking with GA₃ and IAA in modifying the induced changes. Data are expressed as mg/g dry weight

Treatment	Na	K	Ca	Mg
Control	13	41.61	22.33	12.40
- 3 bar	39**	46.98**	27.66**	20.80**
- 6 bar	48**	50.45**	34.00**	22.00**
- 9 bar	55.66**	50.35**	31.33**	24.26**
- 12 bar	62**	38.76**	24.33**	16.80**
Control+GA ₃	12.16	52.69**	32.66**	17.20**
- 3 bar+GA ₃	37.00	54.11**	39.16**	18.20**
- 6 bar+GA ₃	42**	52.72**	36.33**	25.20**
- 9 bar+GA ₃	50**	54.44**	32.33	20.00**
- 12 bar+GA ₃	52**	47.00**	28.66**	23.20**
Control+IAA	12	52.08**	22.00	14.00*
- 3 bar+IAA	38	50.84**	27.00	14.80**
- 6 bar+IAA	44.66*	51.11	37.00**	21.20
- 9 bar+IAA	52*	50.60	27.66**	17.20**
- 12 bar+IAA	56**	38.56	27.16**	12.00**
L. S. D. at 5 %	3.04	0.95	1.38	1.42
L. S. D. at 1 %	4.08	1.27	1.84	1.90

* Significant differences.

** Highly significant differences as compared with the control.

The alteration in distribution and accumulation of mono and divalent cations in the different organs of salt-stressed plants may be one aspect of the role of these cations in regulating the physiological activities of these plants. Evidence to support this suggestion may be obtained from the work of Radi (1964) who studied the effect of other unfavourable environmental factors on the absorption and distribution of mineral elements in maize, sunflower and tomato plants. He concluded that under the influence of unfavourable conditions the cations are distributed and accumulated in the different plant organs in such a way which plays a role in regulating the functional state of these organs.

Table 10

Changes in the mineral composition of roots of safflower plants as affected by salt stress for 60 days and the role of presoaking with GA₃ and IAA in modifying the induced changes. Data are expressed as mg/g dry weight

Treatment	Na	K	Ca	Mg
Control	19	40.79	15.33	20.80
- 3 bar	48**	52.79**	20.66**	16.00**
- 6 bar	50**	54.68**	22.66**	13.60**
- 9 bar	64**	52.39**	21.33**	11.20**
- 12 bar	70**	22.16**	17.33	8.80**
Control+GA ₃	18	52.94**	28.00**	21.20
- 3 bar+GA ₃	40**	55.31**	26.66**	16.80
- 6 bar+GA ₃	45*	57.67**	35.33**	14.40
- 9 bar+GA ₃	52**	54.66**	30.60**	14.80**
- 12 bar+GA ₃	54**	23.14	21.33	12.00**
Control+IAA	17	52.94**	20.00*	20.00
- 3 bar+IAA	40**	54.42*	24.66	16.00
- 6 bar+IAA	48	58.49**	24.66	21.60**
- 9 bar+IAA	56**	54.45**	25.33	23.20**
- 12 bar+IAA	62**	21.55	20.00	10.40
L. S. D. at 5 %	3.85	1.17	4.48	2.13
L. S. D. at 1 %	5.16	1.57	5.32	2.85

* Significant differences.

** Highly significant differences as compared with the control.

On the other hand, seed presoaking with the tested phytohormones was accompanied by impairment of sodium accumulation in shoots and roots. GA₃ or IAA was found to induce a promotion rather than an inhibition in the accumulation of potassium, calcium and magnesium. This response strongly suggests that exogenously applied GA₃ or IAA may be involved in the maintenance of their levels more or less adequate to enhance the growth activities involving metabolism processes in which these nutrients are utilized.

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* Department of Botany, Faculty of
Science, Assiut University, Assiut, Egypt
** Department of Botany, Faculty of
Sciences, Assiut University, Sohag, Egypt

SEASONAL WATER TISSUE BIORHYTHMS IN APPLE TREE LEAVES

SIMONA APOSTOL

The seasonal water rhythms in the leaves of different apple sorts were studied in classical cultivated trees and in intensive apple-orchards. The determinations carried out have established that the normal biorhythms were modified by the pathological agents *Venturia inaequalis* (Cke., Syd.) Wint. and by treatment with pesticides. The results have theoretical and practical importance for *Biorhythmotherapy*.

INTRODUCTION

The distribution of water in leaves is unequal in time, occupying the terminal position in the water circuit, the green leaves are an evidence of the hydrodynamic state of plants.

In previous researches (1,2,3) were revealed the diurnal biorhythms in apple tree leaves and their modifications under the action of the pathological agents — *Venturia inaequalis* (Cke., Syd.) Wint. and *Podosphaera leucotricha* (Ell. et Ev.) Salm. — or of the chemical agents — the treatments with pesticides.

The modifications of the hydric state are rhythmical and symmetrically distributed in time (hours, days, months, years) having many ecological implications.

The study of the seasonal water tissue biorhythms is a new point of view which reveals not only the normal physiological functions development, but also their pathological modifications, having a great theoretical and practical importance for *Biorhythmotherapy*.

MATERIALS AND METHODS

The determinations of the tissular water seasonal rhythms were studied in the years 1985—1986 in classical cultivated trees and in the intensive apple-orchard Miroslava*. At the same time, at the samples collection hours were registered the air temperature, with 0.5°C precision, and the light intensity with PU 150 luxmeter.

To appreciate the actions of the external pathogens (parasites and pesticides), concomitantly, on the same branch sound or parasitised leaves, were collected. As the classical cultivated trees are not treated with pesticides, they served as control.

For correct determinations, in the leaf samples analysed the petioles and nervules were avoid.

* Thanks are due to Chief Eng. V. Cioroiu for permission to effect investigations in this orchard.

RESULTS AND DISCUSSIONS

The results of the determinations effected at different hours (arithmetic mean) obtained for the classical and untreated trees are illustrated in Fig. 1. These data can be considered as normal seasonal tissular water

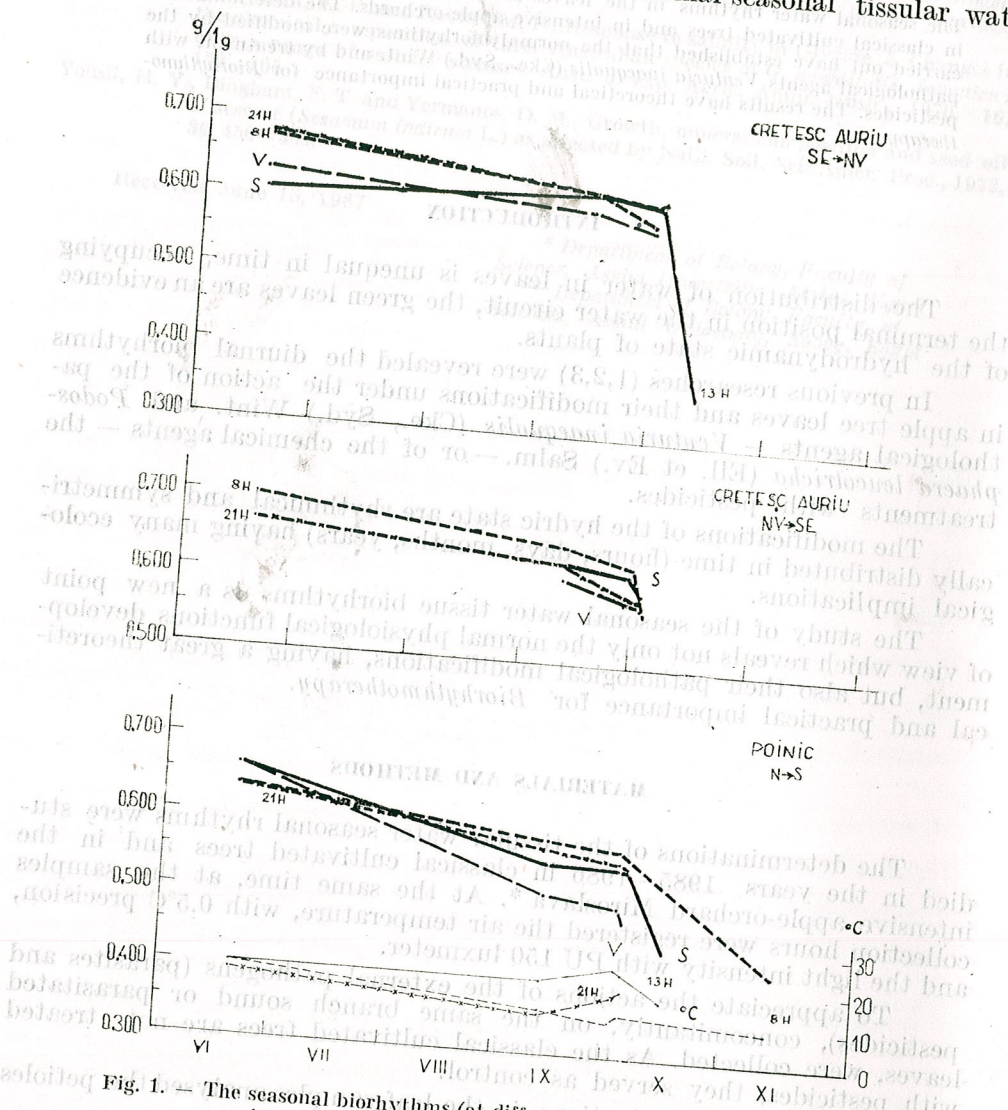


Fig. 1. — The seasonal biorhythms (at different hours) of the tissular water in the leaves of apple-trees classically cultivated. S = sound, V = *Venturia inaequalis* biorhythms in apple trees leaves (S). The amplitude is bigger at 8 hours in the leaves on the branches N → S and NV → SE, oriented, and, at the same time, there are some differences between the branches inversely

placed. Generally, the seasonal rhythms of the tissular water are in continuously descending phases from April to November, but the inclination angles are different due to the internal and external factors, too. The maximum values of water (over 70% of the weight) occur in April, immediately after the opening of the foliage buds, the minimum values (30–40%) being recorded in November, when they turn yellow. The typical bio-

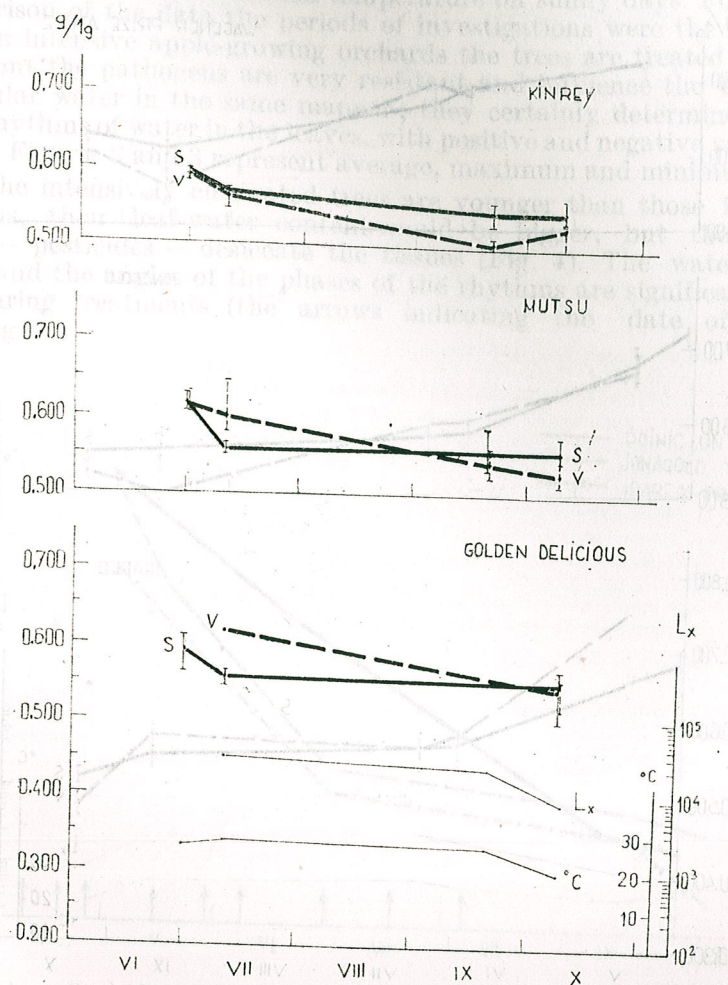


Fig. 2. — The seasonal biorhythms (13³⁰–15⁰⁰Hs) of the tissular water in the leaves of the apple-trees Kinrey, Mutsu, Golden delicious, in the intensively cultivated orchard.

S = sound, V = *Venturia inaequalis*.

rhythms of the tissular water present variations in the phenophases—in the opening of the flower buds periods, during fruit-forming and development, the leaves become matures and at their maximum dimensions the water

content is on the average 60% of the leaves weight. In the periods of fruit maturation the content of the tissular water is almost constant, and immediately after ripping of the fruit, the water again diminishes at 50% of the humid weight of the leaves. These aspects are confirmed by other researches, too (4, 5, 7, 8, 9, 10).

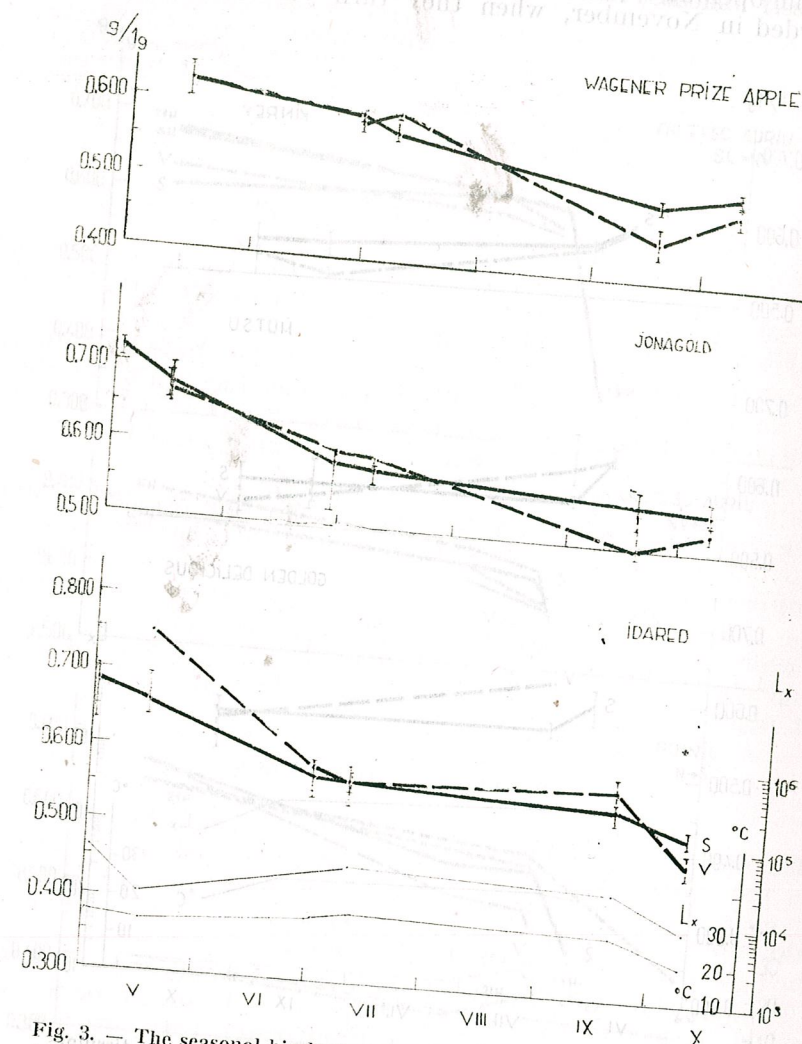


Fig. 3. — The seasonal biorhythms (13³⁰ — 15 Hs) of the tissular water in the leaves of apple-trees Wagener prize apple, Jonagold and Idared in the intensively cultivated orchard.

S = sound, V = *Venturia inaequalis*.

These comparative researches have revealed that the normal seasonal water tissues biorhythms are modified upon the action of the pathological agent *Venturia inaequalis* Wint.(V); mainly the water content in

the leaves is reduced, but there are phases with bigger values, exceeding those found in sound leaves.

The typical seasonal rhythms of the diurnal temperatures in Iași have the peaks in July, when the leaves are mature (2), and the maximum temperature influences the water tissular rhythms. As for determinations of the tissular water seasonal biorhythms, most suitable were considered to be the hours with maximum temperature on sunny days. For a correct comparison of the data the periods of investigations were the same.

In intensive apple-growing orchards the trees are treated with pesticides, but the pathogens are very resistant and influence the biorhythms of tissular water in the same manner; they certainly determine new seasonal rhythms of water in the leaves, with positive and negative values alternating. Figures 2 and 3 represent average, maximum and minimum values.

The intensively cultivated trees are younger than those in classical orchards, their leaf-water content would be bigger, but the chemical agents — pesticides — desiccate the tissues (Fig. 4). The water content values and the angles of the phases of the rhythms are significantly different during treatments (the arrows indicating the date of pesticide praying).

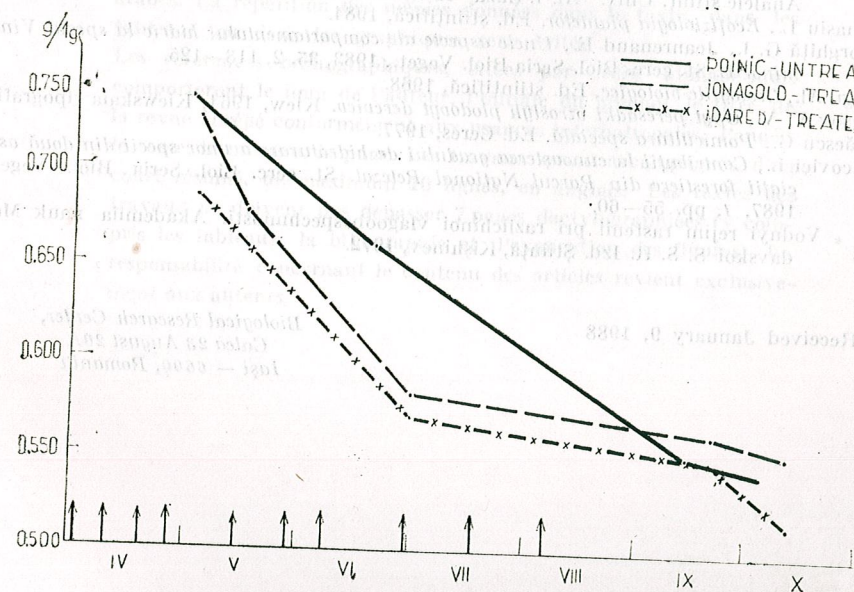


Fig. 4. — Pesticide action upon the development of the seasonal biorhythms (13³⁰ — 15 Hs) of the tissular water in the apple-tree leaves.

Biorhythmology and its laws present a great theoretical and practical importance (1,6). In pommology and phytopathology biorhythmological researches reveal new aspects and its knowledge is useful for the suitable application of pesticides, for the rational and efficient control of the harmful organism, developing even a new science domain — *Biorhythmotherapy*.

CONCLUSIONS

1. The seasonal water tissue biorhythms in apple-tree leaves are generally in a continuous descending phase from spring to autumn, the angles of their inclinations varying owing to the internal and external factors.
2. There are some typical differences between the apple sorts in the seasonal tissular water biorhythms in leaves.
3. The seasonal water biorhythms are modified under the action of the chemical or biological pathological agents.

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Biological Research Center,
Calea 23 August 20A
Iași — 6606, Romania

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