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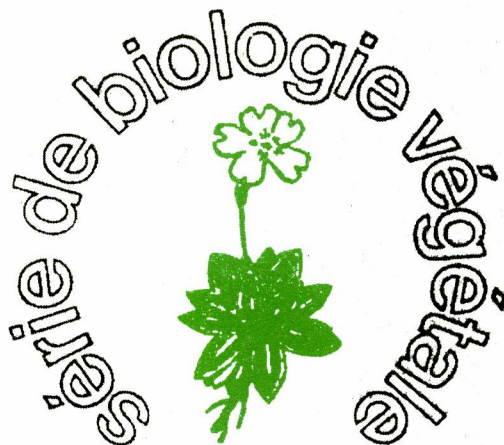
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ULTRASTRUCTURAL CHANGES IN THE GRAPEVINE TISSUES DUE TO VEINBANDING VIRUS

ELENA BUCIUMEANU¹, AURELIA BREZEANU²

Ultrastructural studies of grapevines with veinbanding symptoms showed aggregates formed by polyhedral virus-like particles disposed in 4 - 6 parallel rows, either in the cytoplasm or in the vacuoles. Abnormal shape and size of the organelles, the presence of myelin bodies consisting of many layers that were closepacked in a concentric or irregular fashion, as well as different vesiculated bodies and accumulation of the phenols in the vacuom can be interpreted as a consequence of the infection with a viral complex that changed the metabolism of the cell.

Key words: virus-like particles, veinbanding symptoms, *Vitis vinifera* L., electronmicroscopy.

Grapevine veinbanding is produced by a nepovirus with polyhedral particles of about 30 nm in diameter. Fanleaf, the oldest known virus disease of *V. vinifera* L., is characterised by three distinct syndromes represented by different reactions to the causal agent: fanleaf, yellow mosaic and veinbanding (Pearson and Goheen 1988; Pop, 1988). Observation of symptoms in the field is useful as a first step in selection, but it is not reliable. The ELISA serological test is the quickest and most sensitive method for the plant viruses detection (2). The detection of trabecula or endocellular cordons can provide information on the health of American rootstocks but it is not a specific test (1,7). The ultrastructural research of Matrassa grapevine cultivator with veinbanding symptoms showed crystalloid-like inclusions consisting of icosahedral subunits composed of 6 spherical virus-like particles each (4).

This paper reports the presence of ultrastructural changes in the tissues of veinbanding diseased grapevine.

MATERIAL AND METHODS

V. vinifera L. cv. Timpuriu de Cluj grapevines with veinbanding symptoms have been used for ultrastructural research. Axillary buds, radicle meristems and leaf tissue specimens of 0.5–1.0 mm were taken from *in vitro* grown plants and processed for transmission electron microscopy according to the standard procedure (3). Similar tissues were taken from symptomless viruses for a control.

The tissue samples were fixed overnight at 4°C in 3% glutaraldehyde and postfixed in 2% OsO₄ in 0.1M phosphate buffer pH 7.2 at room temperature for 2 hrs. Dehydration was carried out in an ethanol series and embedding in Epon 812. Thin sections were cut on the ultramicrotome TESLA and stained with uranylacetate and lead citrate by Reynolds's method. The sections were observed using the TESLA BS 500 and Phillips 200 electronmicroscopes.

RESULTS AND DISCUSSION

The most important cytopathic effect observed was the presence of aggregates formed by polyhedral virus-like particles arranged in 4-6 parallel rows. This kind of structure was found either in the vacuoles or in the cytoplasm. The cytoplasm became optically more dense because of the presence of virus particles and the ribosomes spread around these structures; this indicates an intense virus protein synthesis (Plate I, Fig. 1). In many cells, tubular-like formations, parallelly arranged, frequently located near plasma membrane, are observed (Plate I, Fig. 2).

Myelin bodies consisting of many layers that were closepacked in a concentric or irregular fashion have been found in all kinds of the examined tissue, which reflect degenerative changes in the endo membrane system of the cell. Sometimes they include osmophilic globules and vesicles of different size (Plate II).

In the neighbourhood of the myelin bodies different vesiculated bodies (formed by one vesicle or by multivesicles), probably originated from damaged organelles mitochondria, plastides, endoplasmic reticulum profile, etc. (Plate I, Fig. 3) can be observed.

In the foliar tissue there were found very often enlarged rounded chloroplasts with electronodense stroma, 1-5 starch grains and a poor developed thylacoidal system (Plate III, Fig. 1). Golgi bodies with numerous sacules and vesicles, which reflect a hypersecretory activity, were also present (Plate III, Fig. 2). Some cells were vacuolised and the vacuoles were filled with phenols; this kind of big vacuoles had pushed the organelles to the periphery of the cell (Plate III, Fig 1, see arrows).

In many electronmicroscopic images the unusual structures of regular shape and size occupying the centre of the cell (the nucleus was not observed) were revealed. We cannot explain the nature and significance of these structures, so far (Plate III, Fig. 3).

In the axillary bud and leaf tissues (at phloem parenchyma level especially) the modifications produced by the virus infection were more numerous and more clear than in the radicle tissue but were very similar. Such kind of cellular modifications were not found in the control samples.

As we mentioned in previous study regarding the cytopathic effect of leafroll disease on *Vitis vinifera* L. Pinot Gris cultivator, the observed modifications at electronmicroscopical level seem to be non-specific and part of them resemble to the alteration induced by stress factors.

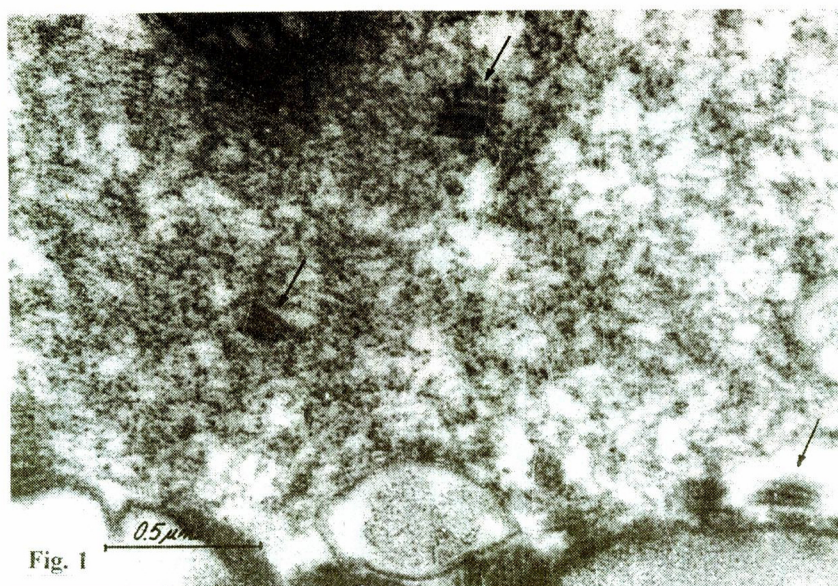


Fig. 1. – Crystalloid-like structures formed by polyhedral virus-like particles (see arrows).

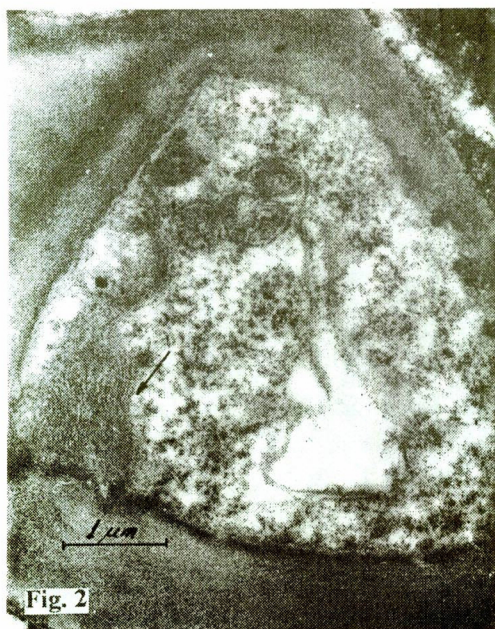


Fig. 2



Fig. 3

Fig. 2. – Tubular like formations, parallelly arranged, located near plasma membrane (see arrows).

Fig. 3. – Multivesiculated bodies in a damaged cell.

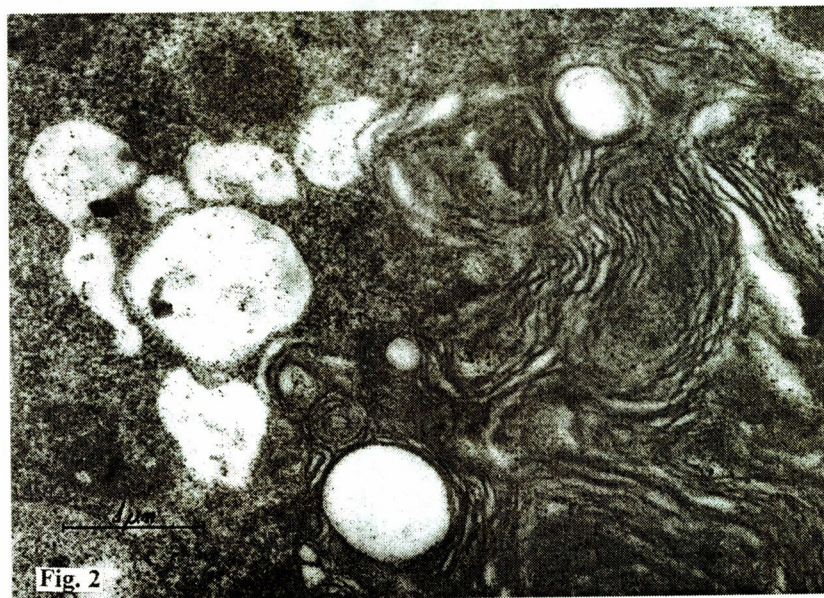
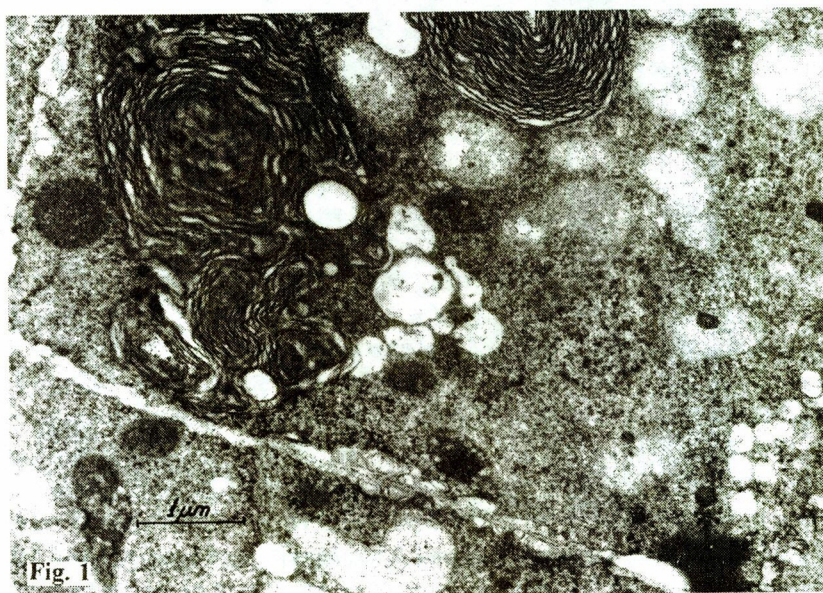


Plate II – Myelin bodies including osmophilic globules and vacuoles of different size.

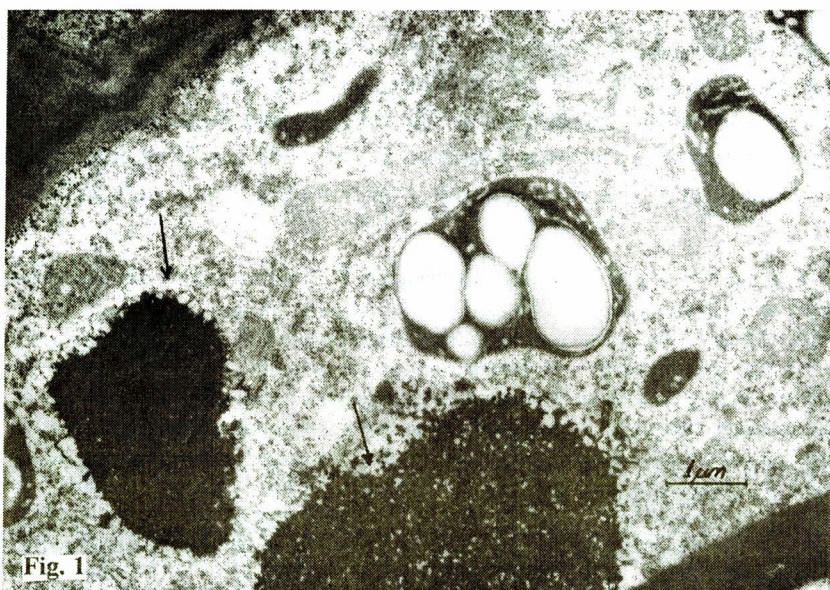


Fig. 1. – The peculiarities of the chloroplast. The poor developed thylacoid system in an electron-dense stroma as well as abundance of starch grains (can be observed).

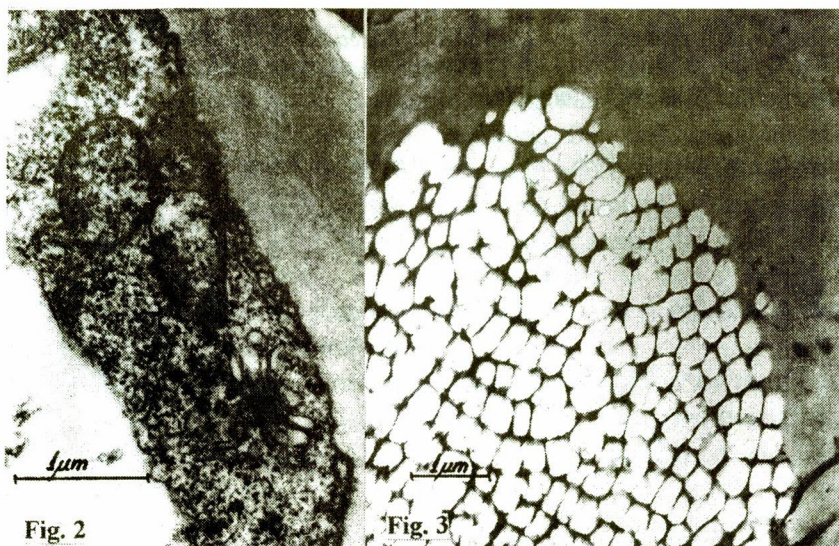


Fig. 2. – Golgi bodies with dilated saccules and numerous vesicles.

Fig. 3. – Unusual structures occupying the centre of the cell.

CONCLUSIONS

1. Polyhedral virus-like particles (the agent of the veinbanding grapevine disease) packed into aggregates of 4 - 6 parallel rows were found either in the vacuoles or in the cytoplasm of the cell, in all the types of examined tissues.
2. The most abnormal structures occurred in the cells could be interpreted as the result of the infection with grapevine viral complex which may act like a stress factor.

Acknowledgements. The authors gratefully thank Dr. P. Ploae for helpful discussion and advice in material preparation.

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PHYSIOLOGICAL AND ULTRASTRUCTURAL MODIFICATIONS INDUCED BY EXCESS OF COPPER IN MAIZE (*ZEA MAYS* L.) AND *CERATOPHYLLUM DEMERSUM* LEAVES

H. TIȚU, GABRIELA VLĂDEANU, NICULINA MITREA

The photosynthesis, chlorophylls *a*, *b* and carotenoids in maize (*Zea mays*) and *Ceratophyllum demersum* leaves were determined in plants treated 7 and 14 days with 0.1 to 1,000 μM Cu^{2+} ; the copper ions were added in nutrient mineral aqueous media as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. The structure of mesophyll chloroplasts was examined by electron microscopy in the same samples but only after 14 days of copper treatment. Lower doses of copper ions decreased synthesis of chlorophyllian and carotenoid pigments and intensity of photosynthesis, especially in *Zea mays*. Copper excess amplified the decrease of the above physiological characteristics and destructive modifications of chloroplast ultrastructure: rupture of stroma lamellae, swelling and disintegration of thylakoids. The degradative changes of chloroplasts were associated with accumulation of osmophilic globules, especially in *Ceratophyllum demersum* plants.

Key words: photosynthesis, photosynthetic apparatus (chloroplast), thylakoid, granum.

Sommer (19), Lipman and McKinney (11) demonstrated for the first time that copper is an essential microelement for plants. Copper content in leaves is ranged between 1-20 mg/kg dry substance. This metal is part of the structure of a large number of vital enzymes and proteins for cell metabolism (9), (22). Chloroplasts are, in normal conditions, the main site of Cu accumulation and play an important role in maintaining the membrane structure of thylakoids (5), (10), (17). Some consistent reviews (8), (15), (18) approached the positive influence of copper on the plants growth. On the other hand, a more recent review (4) approaches the biochemical, physiological and structural effects of excess copper in angiosperms, algae and cyanobacteria.

The purpose of the present paper is the investigation of the photosynthesis, the chlorophyllian pigments and the ultrastructure of the photosynthetic apparatus at two angiosperms distinct by their leaf structure, habitat, a. o.: *Zea mays* and *Ceratophyllum demersum*.

MATERIAL AND METHODS

Maize plants (*Zea mays* L. F 365/93), seedlings with four leaves, resulted from seeds which were obtained by courtesy of The Research Institute of Cereals

and Industrial Crops Fundulea, were grown 7 and 14 days (7d and 14d) on modified Knop medium with Arnon microelements, in laboratory, under natural light conditions.

Ceratophyllum demersum plants were grown 7 and 14 days on Knop-Pringsheim medium with Arnon microelements. The copper ions were supplied as a CuSO_4 in concentrations ranged from 0.1 to 1,000 μM . Photosynthesis was measured by the Ivanov method for maize and by the Winkler method for *Ceratophyllum demersum*.

The chlorophyllian and carotenoid pigments were determined according to Holm (cited in Boldor et al., 1983). Spectrophotometric readings were carried out with Spekol-Jena spectrophotometer at 440.5, 644 and 662 nm.

Electron microscopy. Pieces from the third maize leaf and median zone stem leaves at *Ceratophyllum demersum* after 14 days of treatment with copper were fixed in 4% glutaraldehyde (0.1M phosphate buffer at pH 7.4) for 3 hours at 4°C and postfixed with osmium tetroxide 2% for 2 hours at room temperature. Specimens were dehydrated in ethanol and embedded in Epon 812 (14). Sections were cut on Tesla BS 490A ultramicrotome, stained with 2% aqueous uranyl acetate and lead citrate (16) and examined in a Tesla EM-500 electron microscope.

RESULTS

1. Physiological peculiarities induced by Cu^{2+} at *Zea mays* and *Ceratophyllum demersum* plants

1.1. Influence of copper ions upon assimilatory pigments at *Zea mays*

The data corresponding to chlorophyll *a* biosynthesis (Table 1) following 7 and 14 days (7d, 14d) of growth show a decrease of this pigment at 0.1 μM Cu^{2+} by comparison with the control variant. Chlorophyll *b* and carotenoids content has been increased only after 7 days at 10 μM Cu^{2+} . After 14 days of culture, biosynthesis of chlorophylls and carotenoids was blocked at 1,000 μM Cu^{2+} .

Table 1

The influence of Cu^{2+} upon chlorophyll and carotenoid pigments at *Zea mays* plants.

Pigments mg/dm^2

Variants μM Cu^{2+}	7d			14d		
	chlorophyll	chlorophyll	carotenoids	chlorophyll	chlorophyll	carotenoids
	<i>a</i>	<i>b</i>		<i>a</i>	<i>b</i>	
control	1.39	0.46	0.53	1.71	0.64	0.53
0.1	0.86	0.31	0.39	1.17	0.29	0.31
1.0	1.38	0.46	0.53	1.05	0.51	0.35
10	1.36	0.47	0.54	0.68	0.36	0.25
100	0.71	0.31	0.27	0.68	0.06	0.31
1,000	0.29	0.15	0.25	-	-	-

Photosynthesis was increased at 1.0 - 10 μM doses after 7 days and inhibited after 14 days at 1,000 μM Cu^{2+} (Table 2).

Table 2

The intensity of photosynthesis at *Zea mays* plants cultivated on Knop medium with various Cu^{2+} concentrations

Variables	Intensity of Photosynthesis $\text{cm}^3 \text{CO}_2/\text{h}/\text{dm}^2$	
$\mu\text{M Cu}^{2+}$	7d	14d
control	1.91	1.63
0.1	1.71	1.4
1.0	2.01	1.4
10	1.92	0.9
100	1.6	0.77
1,000	0.76	-

1.2. Influence of copper ions upon assimilatory pigments at *Ceratophyllum demersum*.

The analyses realized following 7 days of treatment for the 0.1 - 10 μM Cu^{2+} variants showed an increase of chlorophyll *a*. After 14 days the chlorophyll *a* gradually decreased for the same above doses; at higher Cu^{2+} concentrations, the biosynthesis of this pigment was blocked (Fig. 1). Photosynthesis was increased at 0 - 1.0 μM Cu^{2+} after 7 days and abruptly decreased between 10 - 100 μM . On the 14th day, the decrease takes place starting with the 1.0 μM dose.

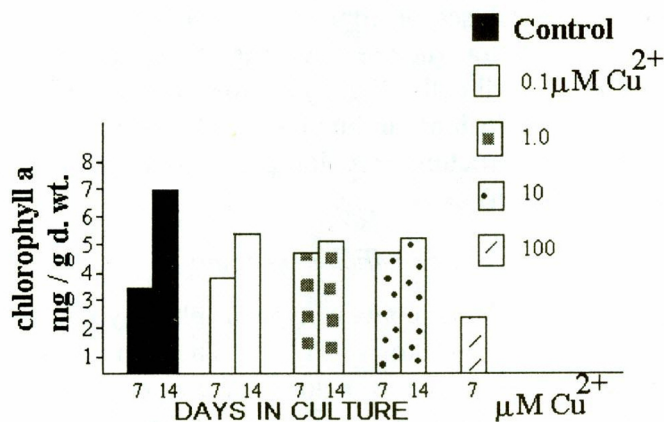


Fig. 1. The influence of Cu^{2+} upon Chlorophyll *a* at *Ceratophyllum demersum* plants cultivated on Knop - Pringsheim medium.

2. The influence of copper upon ultrastructure of the photosynthetic apparatus from the leaves of *Zea mays* and *Ceratophyllum demersum*

2.1. *Zea mays*

The ultrastructure of photosynthetic apparatus in maize leaves is similar with the one of the majority of the C_4 plants: mesophyll cell chloroplasts from control

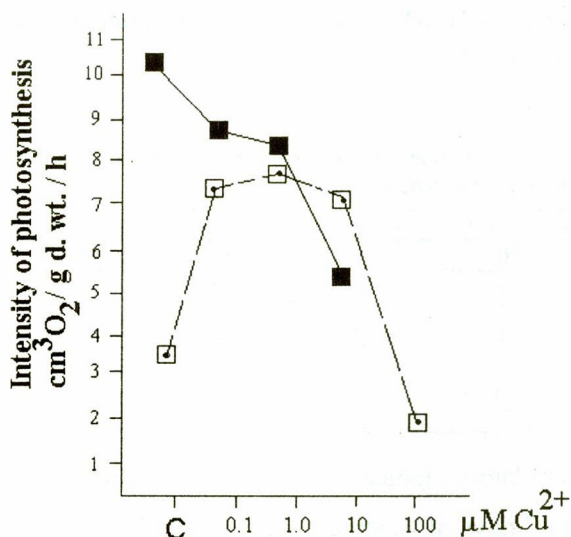


Fig. 2. The intensity of photosynthesis at *Ceratophyllum demersum* plants cultivated on Knop -Pringsheim medium with 0.1 -100 $\mu\text{M Cu}^{2+}$; !: 7 days; 5: 14 days; C: control plants.

plants (Fig. 3) - with well developed grana containing 10-15 thylakoids, and chloroplasts from bundle sheath cells - with rudimentary grana and numerous starch inclusions (Fig. 4). The common feature of mesophyll and bundle sheath chloroplasts is the peripheral reticulum that is seen as a series of isolated or anastomosing tubules. Higher doses of copper (10-100 μM) produce degradative modifications in chloroplast ultrastructure: swelling of thylakoids, stroma lysis, envelope disruption in mesophyll cells (Fig. 5) and diminution of lamellar system and disintegration of starch inclusions in bundle sheath cell chloroplasts (Fig. 6). At 1,000 $\mu\text{M Cu}^{2+}$, the inner structure of chloroplasts was completely destroyed (electron micrographs not shown).

2.2. *Ceratophyllum demersum*

The leaves in control plants contain typical photosynthetic membranes associated in grana with 6 - 14 thylakoids (Fig. 7). The diminution of thylakoid number per granum was observed at lower doses of copper (Fig. 8). At 100 $\mu\text{M Cu}^{2+}$ almost all of the photosynthetic membranes are disintegrated, but large and numerous plastoglobuli are visible (Fig. 9).

DISCUSSIONS

Our investigations concerning the effect of different doses of copper demonstrated the strong toxicity of this metal in concentrations of 100-1,000 μM . Chlorophyllian pigment content and photosynthesis decreased even at lower concentrations than 100 μM in *Zea mays* and *Ceratophyllum demersum* plants after

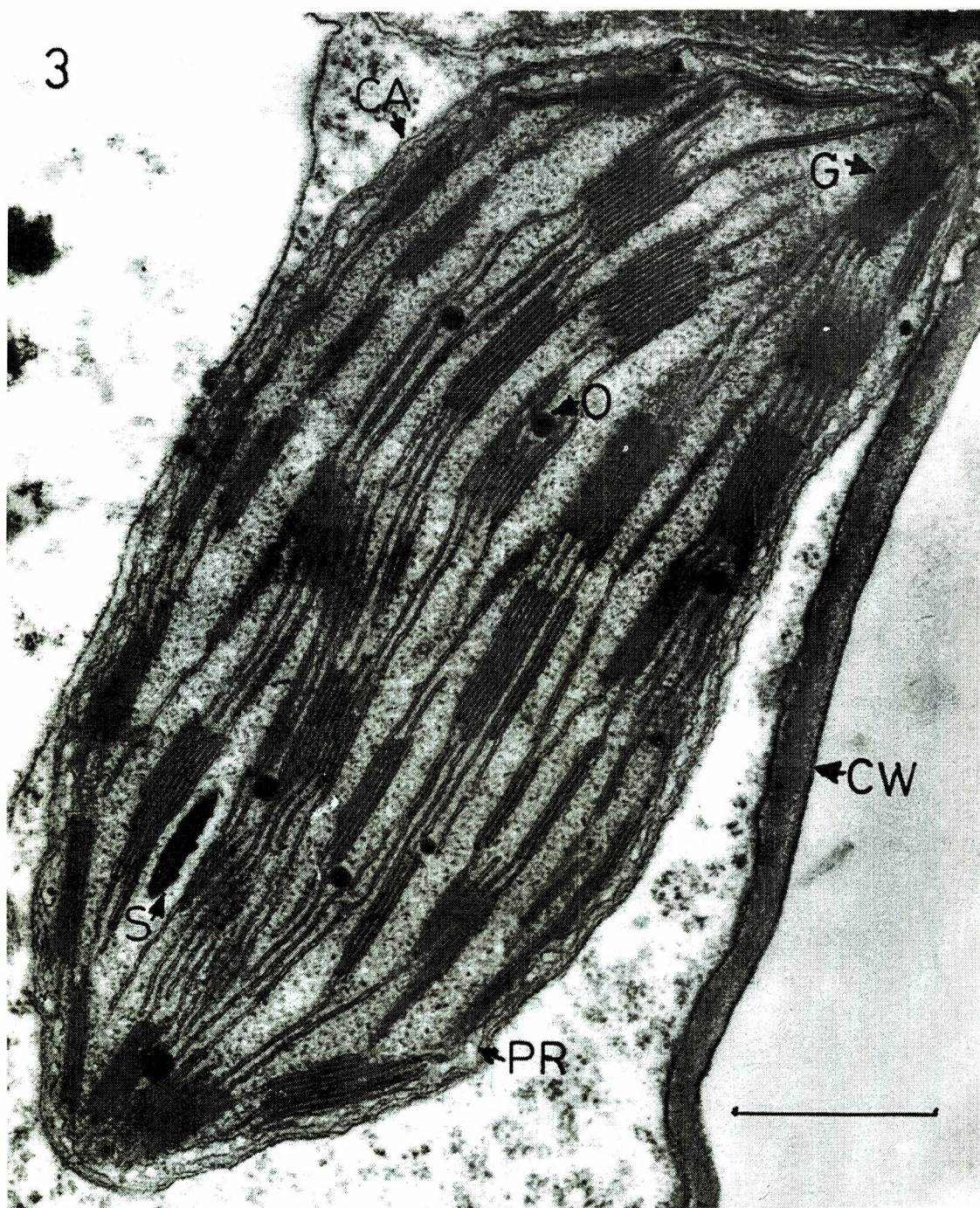


Fig. 3. Ultrastructure of a chloroplast from mesophyll cells in *Zea mays* control plants. CA - chloroplast envelope; O - osmiophilic globules (plastoglobuli); G - granum; S - starch inclusions; PR - peripheral reticulum; CW - cell wall. Bar = 0.5 μ m.

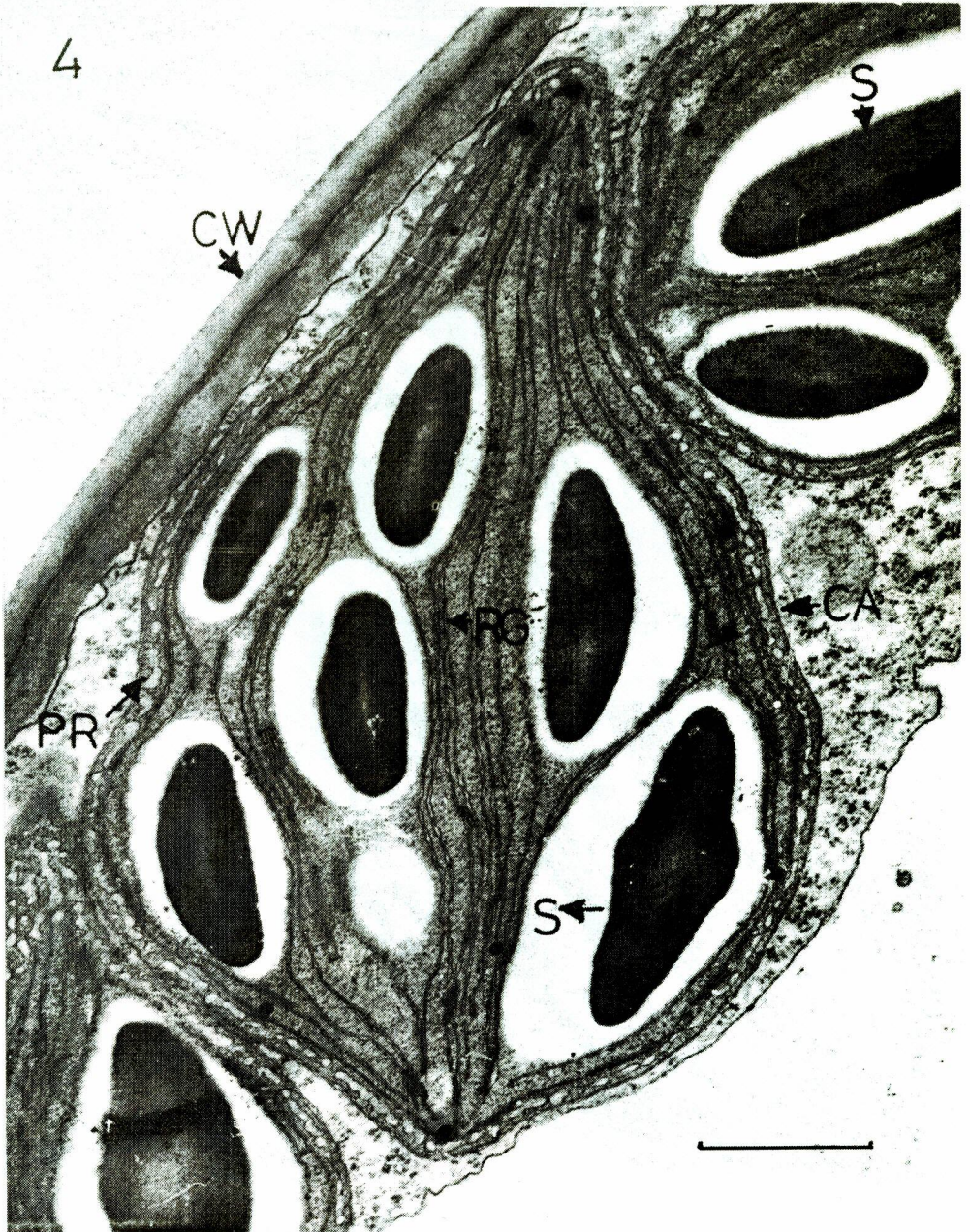


Fig. 4. Ultrastructure of a chloroplast from bundle sheath cell in *Zea mays* control plants. RG-rudimentary grana. Bar = 0.5 μ m.

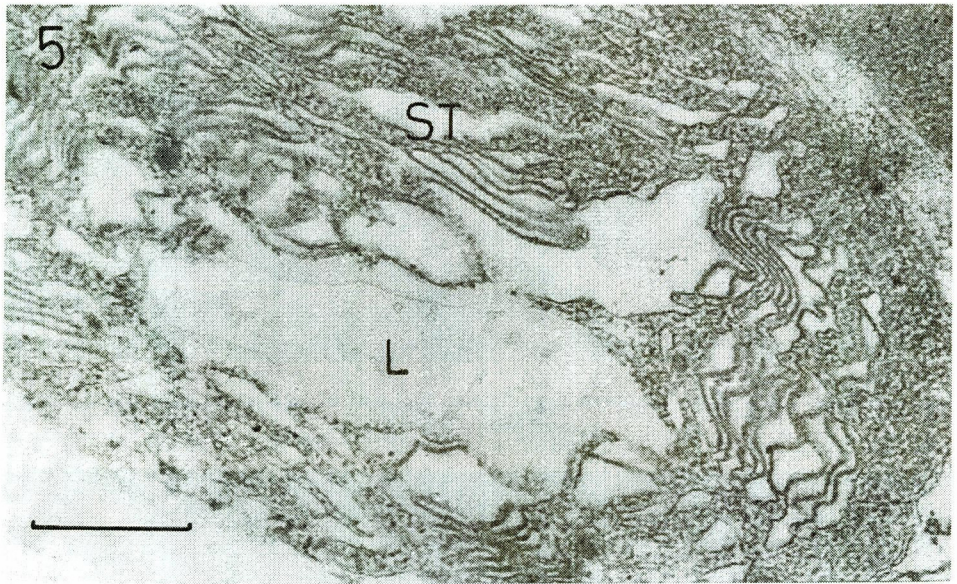


Fig. 5. Ultrastructure of a chloroplast from mesophyll cells in *Zea mays* plants treated with 100 μM Cu^{2+} . ST - swollen thylakoids; L - stroma lysis area. Bar = 0.5 μm .

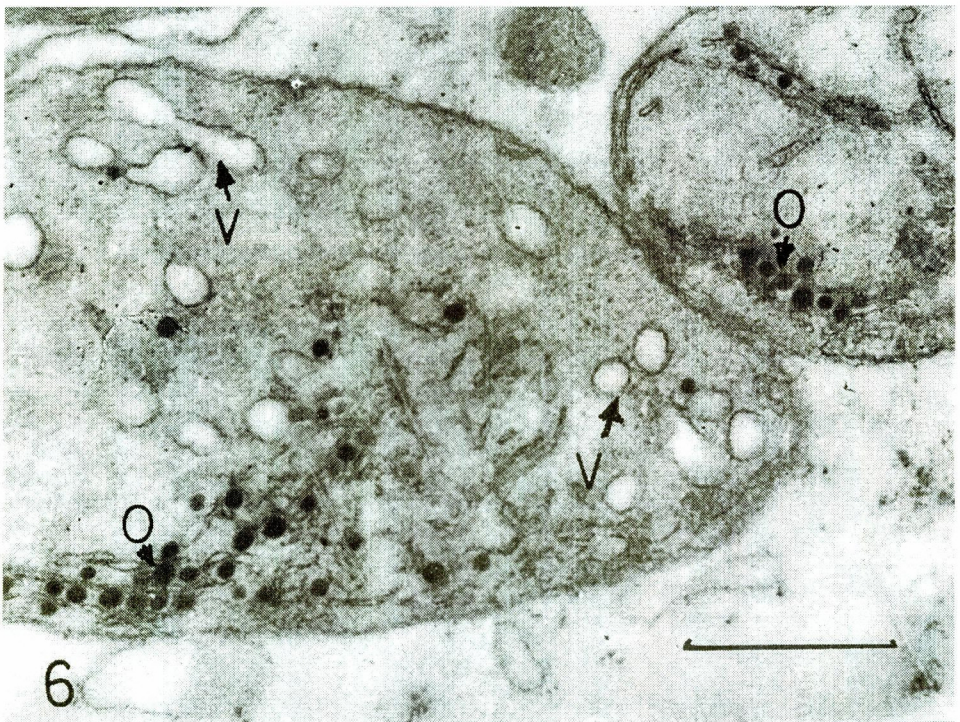


Fig. 6. Ultrastructure of a chloroplast from bundle sheath cell in *Zea mays* plants treated with 100 μM Cu^{2+} . V - vesicles. Bar = 1 μm .

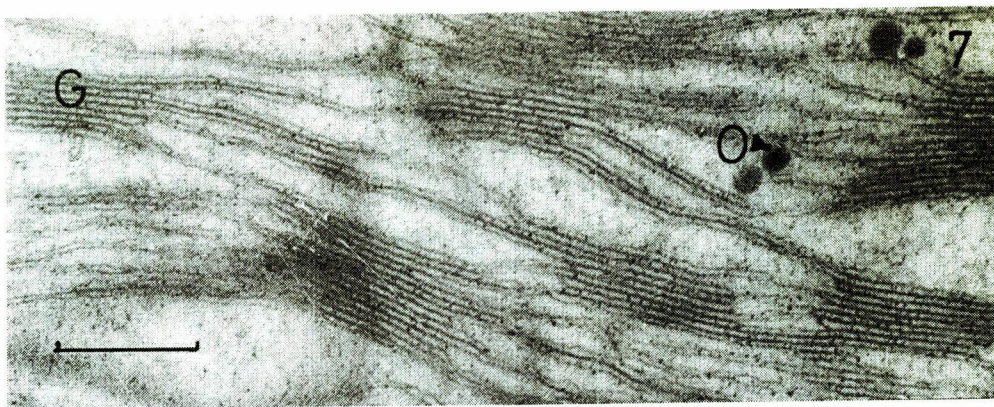


Fig. 7. Ultrastructure of a chloroplast from median leaf part of stem in *Ceratophyllum demersum* control plants. Bar = 0.5 μ m.

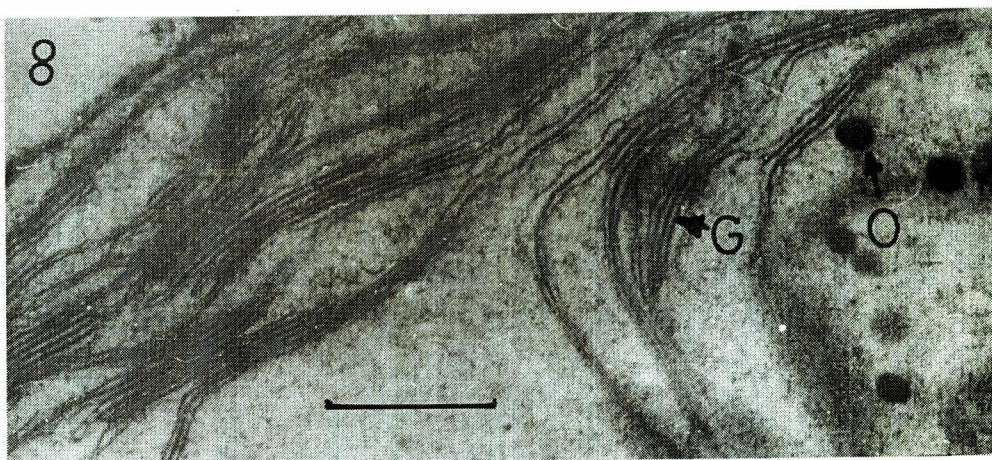


Fig. 8. Ultrastructure of a chloroplast in *Ceratophyllum demersum* plants treated with 0.1 μ M Cu^{2+} . Bar = 0.5 μ m. Explanations in the text.

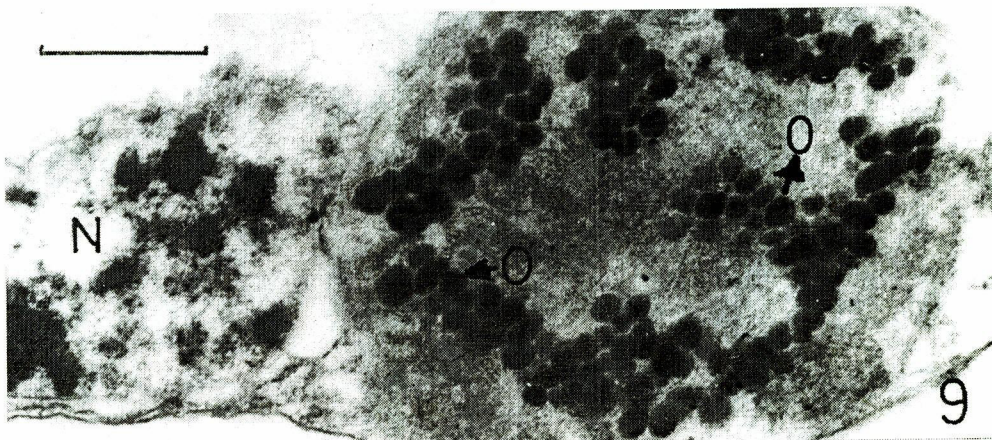


Fig. 9. Ultrastructure of a chloroplast in *Ceratophyllum demersum* plants treated with 100 μ M Cu^{2+} . N - nucleus. Bar = 0.5 μ m.

14 days of treatment. Bishop (2) issued the hypothesis that the excess of some heavy metals in soil and water are responsible for the inhibition of some biochemical processes. After that, Iglesias and Andreo (6), Stiborova and Leblova, 1985 (cited in Fernandes and Henriques, 1991) showed that copper inhibits several enzymes that play a vital role in dark reactions of photosynthesis, namely phosphoenolpyruvate carboxylase and ribulose - 1,5 - biphosphate carboxylase. Sandman and Boger (17) observed chlorophyll degradation and extensive photosynthetic membranes damage in *Scenedesmus* sp. Relative low applied concentrations of copper to spinach plants produced no significant degradative changes in the photosynthetic apparatus, in spite of some deposits, possible of copper, inside the cells and the surface of the chloroplasts (1). In our experiments, the photosynthetic apparatus of *Ceratophyllum demersum* plants, treated with high dose of copper, is more damaged comparatively with the same organelle from maize leaves. Other authors insist upon the role of lower copper doses as a nutrient for numerous cultivated plants (5), (10), (20). Some recent papers also treat of the localization of heavy metals in cell organelles from plant and animal tissues, using more sophisticated methods. Thus, direct localization of lead accumulated by algae was undertaken using Laser Microprobe Analyzer (LAMMA 500). In artificially contaminated cells of the *Phymatodocis nordstedtiana*, lead deposits were identified in the cell wall, the chloroplast as well as in the nucleus (12), (13). Using Energy Dispersive X-Ray Analysis (EDXA), Irmer (7) was able to show that, in *Chlamydomonas reinhardtii*, lead could be localized in the cell wall, the chloroplast and the nucleus and also in vacuoles, where electron-dense deposits were evident. Irmer (7) also found noticeable lead deposits in the eye-spot of the alga; using the same last method (EDXA), Weiss et al. (21) demonstrated the localization of Cu^{2+} within some of the dense bodies that were presumably the large lysosomes in liver cells of killifish *Fundulus heteroclitus*. No Cu^{2+} signal could be generated from either adjacent cytoplasm, presumptive nuclei or from any area of control hepatocytes.

The data that are presented in this paper should be continued using adequate methods for establishing the degree of contamination with copper in both plants treated with the same metal.

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FUNGAL GROWTH IN TECHIRGHIOL LAKE (ROMANIA)

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Although ability to germinate in seawater *in situ* is considered to be a test to separate true marine fungi from terrestrial dormant propagules because a mycostatic principle in seawater inhibits spore germination of some non marine fungi, we found that at main genera isolated from Techirghiol Lake it is not working. This principle is working at the salt concentration more than 1.5M. These are the reasons why we considered these as fungi perfectly adapted to the aquatic habitat including them among the moderate halophilic fungi.

Key words: halotolerant fungi, germination.

Marine fungi could be "obligate" marine fungi growing and sporulating exclusively in the marine or estuarine habitat and "facultative" marine fungi arrived with the fresh water or soil habitat but able to grow and sometimes to sporulate in seawater (12).

Some researches were done on the isolation, cultivation and systematics of marine fungi (1, 3, 4, 8, 9, 10, 11), and others were focused on their physiology (2, 5, 6, 7, 13, 14, 15).

This study contains investigations about the presence and growth of fungi isolated between 1995-1998 and their inclusion in some ecological groups.

MATERIAL AND METHODS

Samples collected from the Techirghiol Lake from 1995 to 1998 and analysed from mycological point of view were: alive *Cladophora* mass, dead *Cladophora* mass, water and therapeutical mud. Pure cultures were obtained on corn meal agar using water from the Techirghiol Lake.

The rate of germination has been done on 1M, 1.5M, 2M and 2.5M NaCl as a test to separate true marine fungi from terrestrial fungi.

RESULTS AND DISCUSSION

From 1995 to 1998 samples were taken from the Techirghiol Lake consisting of *Cladophora* alive mass, *Cladophora* dead mass, water and therapeutical mud.

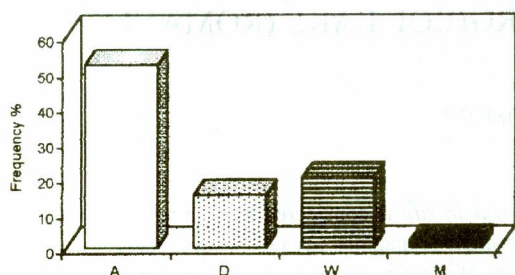


Fig. 1. The occurrence of *Cladosporium* genus in Techirghiol Lake - 1995.

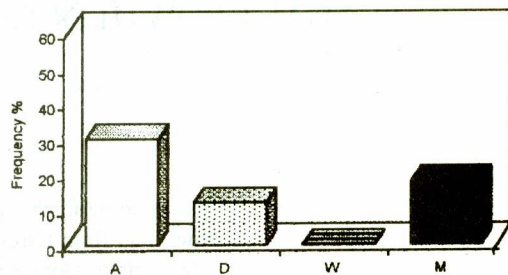


Fig. 2. The occurrence of *Cladosporium* genus in Techirghiol Lake - 1996.

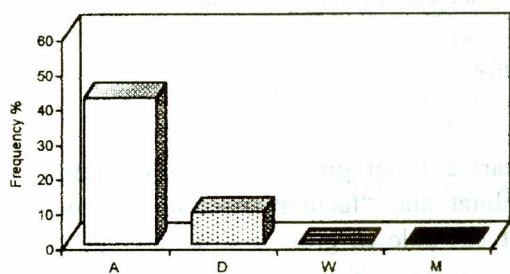


Fig. 3. The occurrence of *Cladosporium* genus in Techirghiol Lake - 1997.

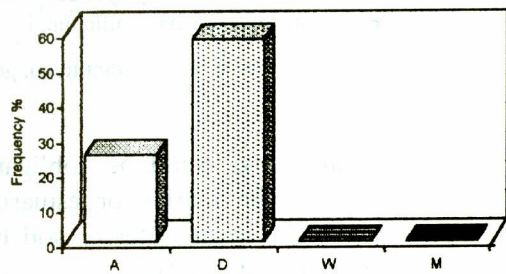


Fig. 4. The occurrence of *Cladosporium* genus in Techirghiol Lake - 1998.

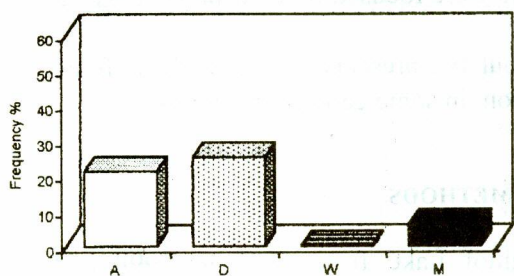


Fig. 5. The occurrence of *Acremonium* genus in Techirghiol Lake - 1995.

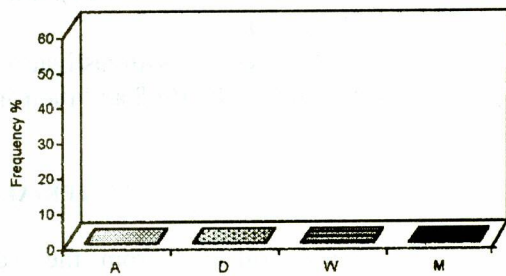


Fig. 6. The occurrence of *Acremonium* genus in Techirghiol Lake - 1996.

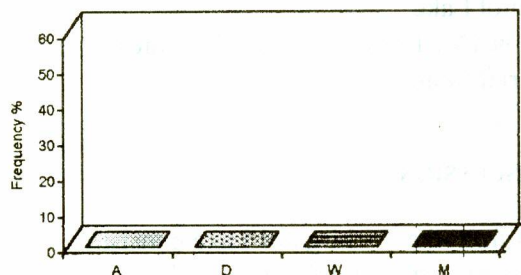


Fig. 7. The occurrence of *Acremonium* genus in Techirghiol Lake - 1997.

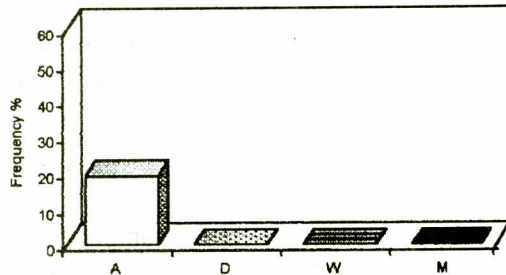


Fig. 8. The occurrence of *Acremonium* genus in Techirghiol Lake - 1998.

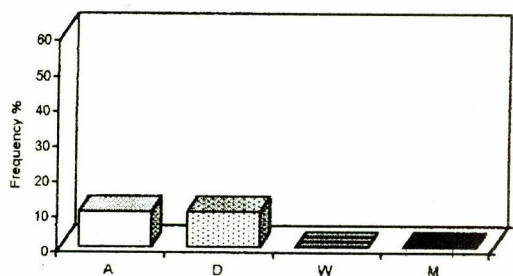


Fig. 9. The occurrence of *Alternaria* genus in Techirghiol Lake - 1995.

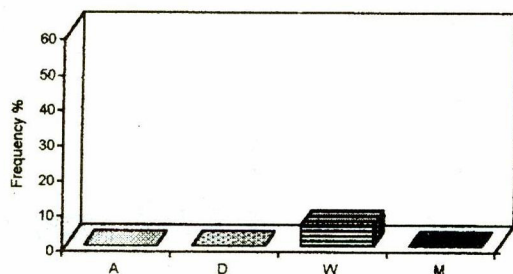


Fig. 10. The occurrence of *Alternaria* genus in Techirghiol Lake - 1996.

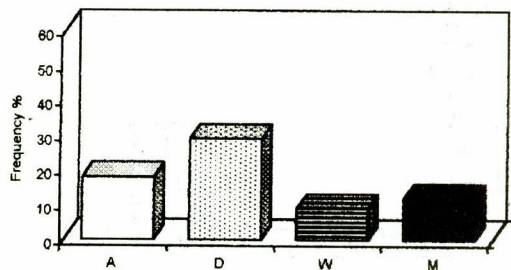


Fig. 11. The occurrence of *Alternaria* genus in Techirghiol Lake - 1997.

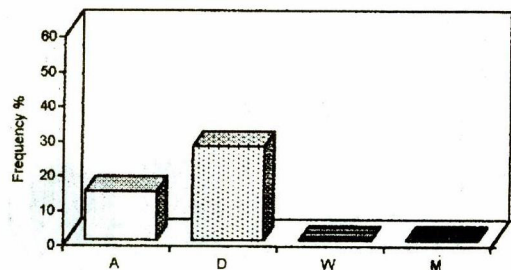


Fig. 12. The occurrence of *Alternaria* genus in Techirghiol Lake - 1998.

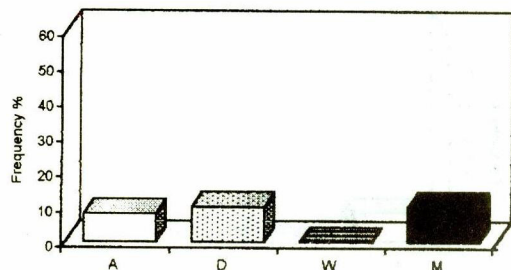


Fig. 13. The occurrence of *Aspergillus* genus in Techirghiol Lake - 1995.

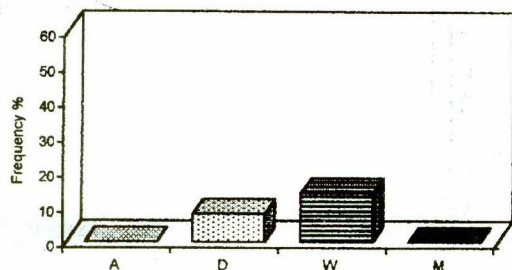


Fig. 14. The occurrence of *Aspergillus* genus in Techirghiol Lake - 1996.

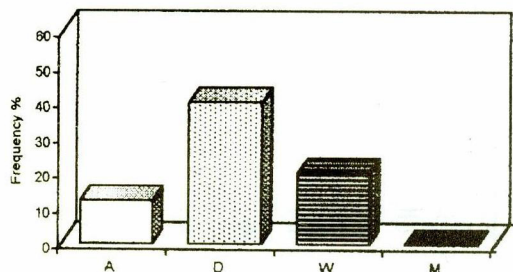


Fig. 15. The occurrence of *Aspergillus* genus in Techirghiol Lake - 1997.

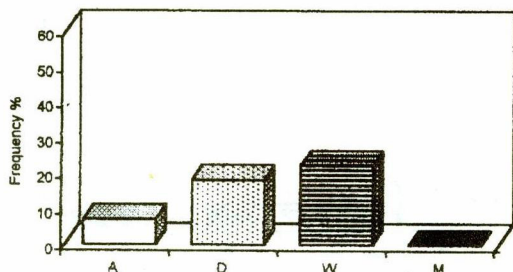


Fig. 16. The occurrence of *Aspergillus* genus in Techirghiol Lake - 1998.

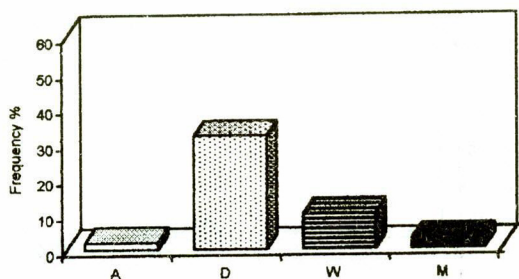


Fig. 17. The occurrence of *Penicillium* genus in Techirghiol Lake - 1995.

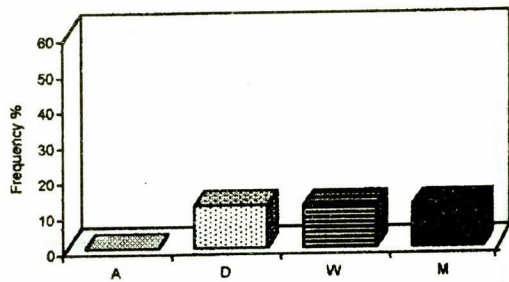


Fig. 18. The occurrence of *Penicillium* genus in Techirghiol Lake - 1996.

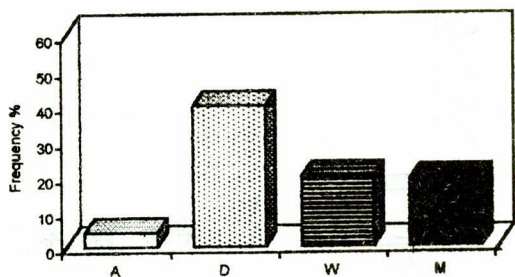


Fig. 19. The occurrence of *Penicillium* genus in Techirghiol Lake - 1997.

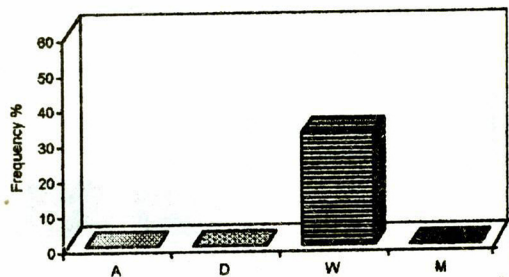


Fig. 20. The occurrence of *Penicillium* genus in Techirghiol Lake - 1998.

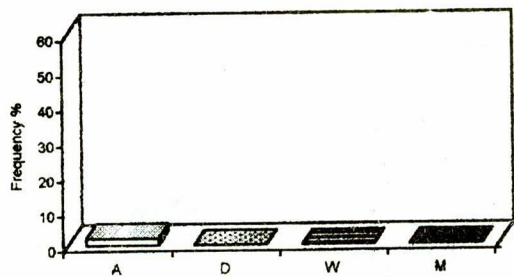


Fig. 21. The occurrence of *Oospora* genus in Techirghiol Lake - 1995.

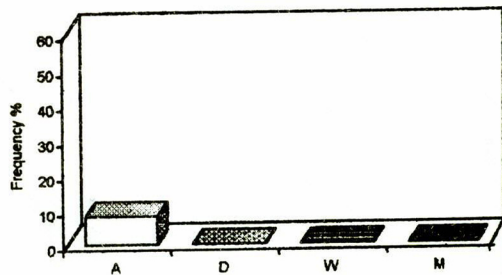


Fig. 22. The occurrence of *Oospora* genus in Techirghiol Lake - 1996.

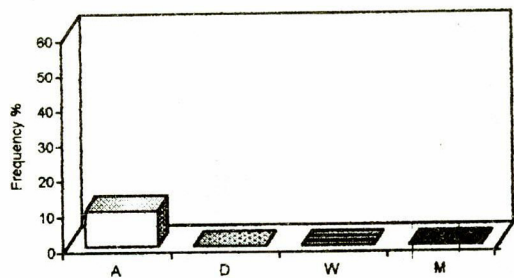


Fig. 23. The occurrence of *Oospora* genus in Techirghiol Lake - 1997.

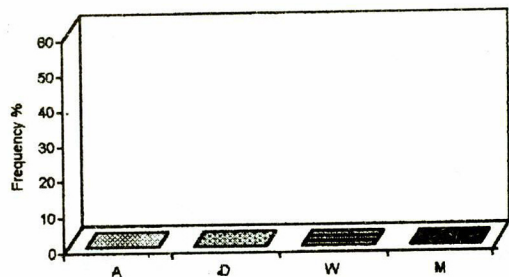


Fig. 24. The occurrence of *Oospora* genus in Techirghiol Lake - 1998.

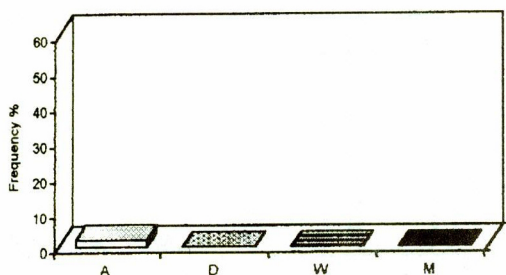


Fig. 25. The occurrence of *Stemphyllium* genus in Techirghiol Lake - 1995.

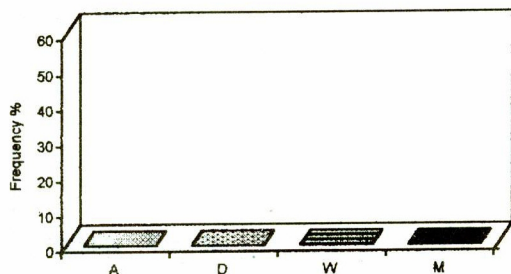


Fig. 26. The occurrence of *Stemphyllium* genus in Techirghiol Lake - 1996.

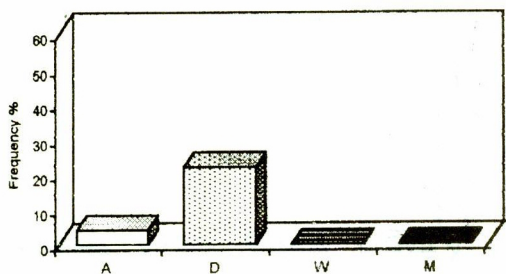


Fig. 27. The occurrence of *Stemphyllium* genus in Techirghiol Lake - 1997.

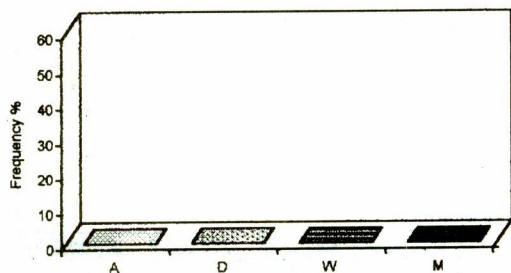


Fig. 28. The occurrence of *Stemphyllium* genus in Techirghiol Lake - 1998.

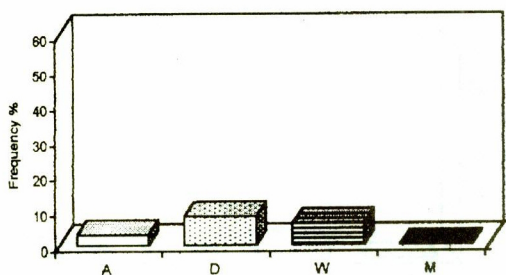


Fig. 29. The occurrence of *Fusarium* genus in Techirghiol Lake - 1995.

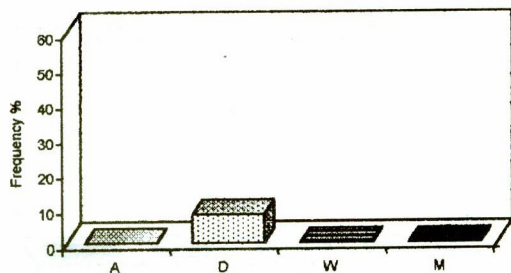


Fig. 30. The occurrence of *Fusarium* genus in Techirghiol Lake - 1996.

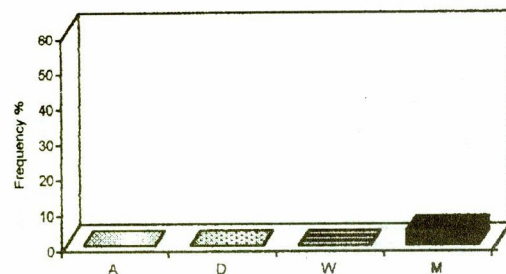


Fig. 31. The occurrence of *Fusarium* genus in Techirghiol Lake - 1997.

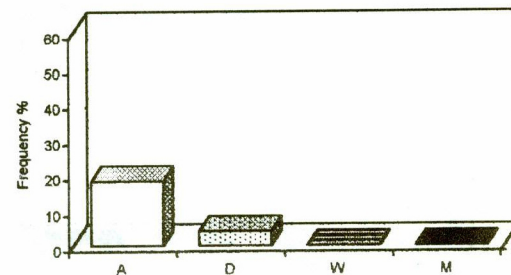


Fig. 32. The occurrence of *Fusarium* genus in Techirghiol Lake - 1998.

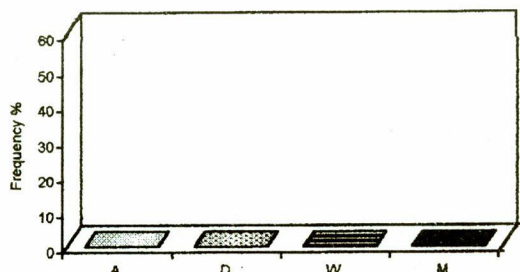


Fig. 33. The occurrence of *Macrosporium* genus in Techirghiol Lake - 1995.

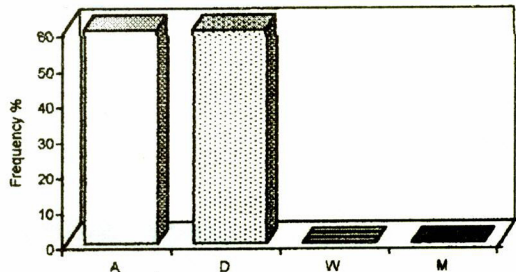


Fig. 34. The occurrence of *Macrosporium* genus in Techirghiol Lake - 1996.

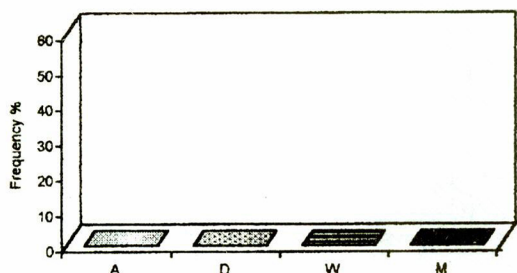


Fig. 35. The occurrence of *Macrosporium* genus in Techirghiol Lake - 1997.

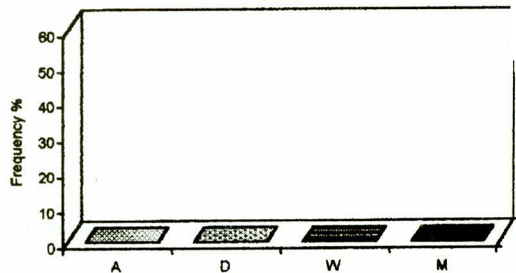


Fig. 36. The occurrence of *Macrosporium* genus in Techirghiol Lake - 1998.

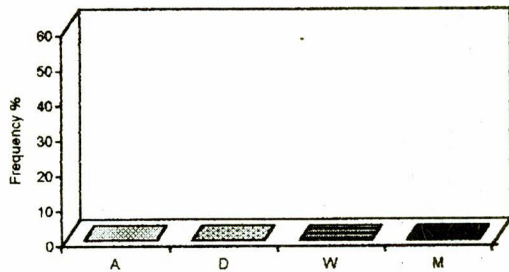


Fig. 37. The occurrence of *Fusidium* genus in Techirghiol Lake - 1995.

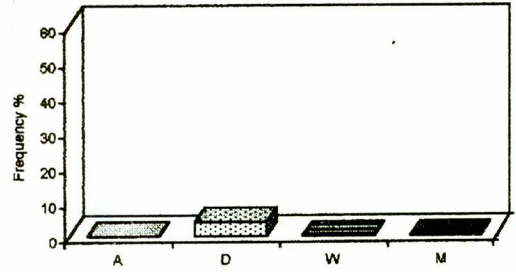


Fig. 38. The occurrence of *Fusidium* genus in Techirghiol Lake - 1996.

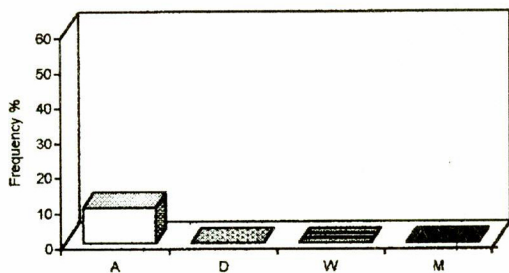


Fig. 39. The occurrence of *Fusidium* genus in Techirghiol Lake - 1997.

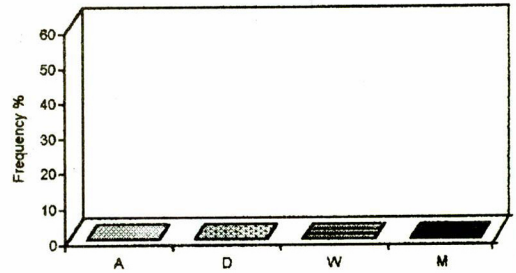


Fig. 40. The occurrence of *Fusidium* genus in Techirghiol Lake - 1998.

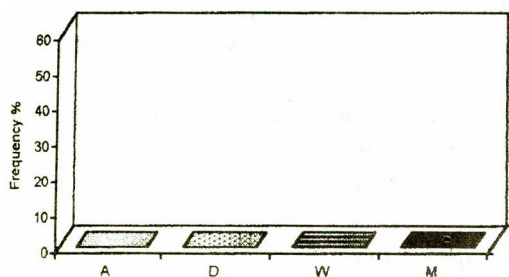


Fig. 41. The occurrence of *Helminthosporium* genus in Techirghiol Lake - 1995.

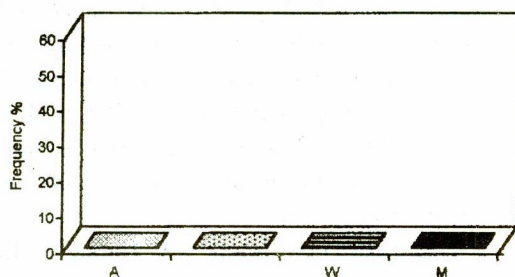


Fig. 42. The occurrence of *Helminthosporium* genus in Techirghiol Lake - 1996.

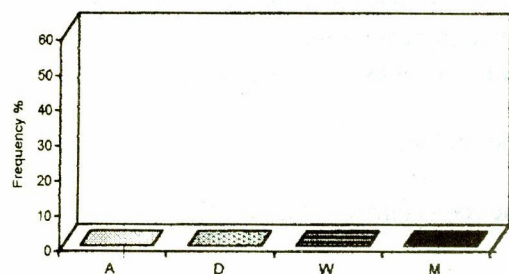


Fig. 43. The occurrence of *Helminthosporium* genus in Techirghiol Lake - 1997.

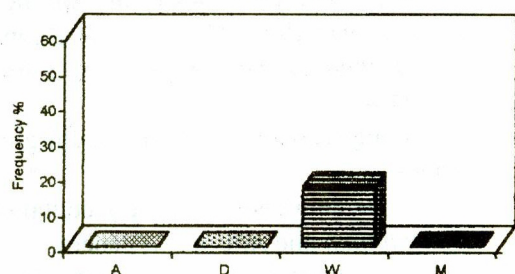


Fig. 44. The occurrence of *Helminthosporium* genus in Techirghiol Lake - 1998.

A = alive *Cladophora* mass
M = therapeutic mud

W = water
D = dead *Cladophora* mass

Fungi were most frequently found on the *Cladophora* mass both alive and dead, over the entire period. They were also found in water, but only in 1995 and 1996. This could be due to the place samples were collected from up to 2 m distance from the algal mass.

Cladosporium genus was found on the *Cladophora* mass over the entire period. In 1995 and 1996, it was found both in water and therapeutic mud, but in a low ratio (Fig. 1-4).

Penicillium genus grows on the *Cladophora* mass and in water (Fig. 17-20). It was also found on therapeutic mud in 1996 and 1997, when the water level of the lake was higher and the shore was flooded by water, and grass area covered by water. In those conditions, the content in cellulose was improved.

Aspergillus genus was found all the time on the *Cladophora* mass and water but most frequently in 1997 (Fig. 13-16). In a low ratio, *Aspergillus* strains were found in the therapeutic mud in 1995 and 1996. Our supposition about their air origin was confirmed when we attempted to grow them on therapeutic mud.

Alternaria genus grew on the *Cladophora* mass all the time but in 1997 it was found both in water and therapeutical mud (Fig. 9-12). The explanation is the same as in the case of *Aspergillus* and *Penicillium* genera.

Oospora genus was found in all samples, most frequently on the *Cladophora* mass, but in 1998 this genus was found only in water (Fig. 21-24).

Acremonium and *Stemphylium* genera grew only on the *Cladophora* mass (Fig. 5-8, 25-28).

Fusarium genus was found on the *Cladophora* mass in a low ratio only in 1995 (Fig. 29-32).

Macrosporium is a genus isolated in a high ratio (60%) but only in 1996 on the *Cladophora* mass (Fig. 33-36).

Fusidium genus grew only on the *Cladophora* mass in 1996 and 1997, when the shore was flooded by water as a consequence of heavy rain (Fig. 37-40).

Helminthosporium genus was found only in 1998, in water, in a low ratio (Fig. 41-44).

Fungi isolated from the Techirghiol Lake belong to the following ecological groups:

- the cellulosolytic group, containing: the *Aspergillus*, *Penicillium*, *Fusarium*, *Acremonium* and *Alternaria* genera;
- the proteolytic group, containing: the *Cladosporium*, *Oospora* and *Fusidium* genera;
- the pectinolytic group, containing: *Stemphylium* and *Macrosporium* genera.

All mentioned fungal genera are known to belong to soil. We considered that all of them are adapted now to decompose organic material near the shore. All strains were tested in media containing 1M, 1.5M, 2M and 2.5M NaCl for the germination of spores (Table 1).

Table 1

Germination of spores (%) in media with different concentration of NaCl

Strain	Germinated spores (%)											
	5 g% NaCl			7.5 g% NaCl			10 g% NaCl			12.5 g% NaCl		
	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
<i>Oospora</i> sp.	8	100	M	31	100	M	0	20	52	0	5	8.6
<i>Fusarium</i> sp.	100	M	M	100	M	M	50	M	M	0	M	M
<i>Stemphylium</i> sp.	0	50	M	0	M	M	0	0	0	0	0	0
<i>Aspergillus</i> sp.	M	M	M	50	M	M	74	M	M	0	36	56
<i>Penicillium</i> sp.	61.6	M	M	0	M	M	0	15	53	0	0	34
<i>Alternaria</i> sp.	0	M	M	0	M	M	0	0	M	0	0	0
<i>Paecilomyces</i> sp.	0	8.5	30	0	0	M	0	0	0	0	0	0
<i>Cladosporium</i> sp.	0	11	28.4	0	0	14	0	0	0	0	0	0

Legend:

M = mycelium

The highest ratio of germination was found for *Fusarium* (100%) at 1M and 1.5M NaCl and for *Penicillium* at 1M NaCl (61.6%) after 24h incubation. *Oospora*, *Aspergillus* and *Alternaria* developed mycelia after 48h of incubation and *Stemphylium* after 72h on 1M NaCl.

The ratio of *Oospora* spores increased four times in the first 24h and developed mycelium after 48h of incubation on 1.5 M NaCl. The same situation was observed for the rest of the fungi, except for *Cladosporium*, which has a low rate of growth.

The germination time was longer for *Oospora*, *Fusarium*, *Aspergillus*, *Alternaria* and *Penicillium* incubated on 1.5M NaCl but *Stemphylium* and *Cladosporium* did not germinate.

On 2.5M NaCl, only 8.6% *Oospora* spores, 56% *Aspergillus* spores and *Penicillium* spores germinated but *Fusarium* developed mycelium after 72h of incubation. *Stemphylium*, *Alternaria* and *Cladosporium* cannot germinate.

All strains developing mycelia grow on the media surface but the *Alternaria* mycelium was anchored strongly to the tub walls, which explains its adhesion on biological and mineral substrate.

The very quick germination of these fungal spores in media with 1M and 1.5M NaCl could explain their multiplication and growth on the organic material from the Techirghiol Lake, where the NaCl concentration in the water is about 5.0-6.0g%. In the case of the *Oospora* genus, 1.5M NaCl is an optimum for spores germination.

CONCLUSIONS

1. Fungi isolated from the Techirghiol Lake, being moderate halophilic, belong to soil but are adapted to the new conditions.
2. Fungi were found on the *Cladophora* mass, water and therapeutical mud, less in the last one.
3. The main ecological groups are cellulosolytic, proteolytic and pectinolytic.
4. The studied fungi multiplying in the Techirghiol Lake and producing cellulase, protease and pectinase are involved in the decomposition of organic materials.

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STUDIES ON THE BACTERIA FROM THERMAL HABITATS IN THE SOUTHERN PART OF ROMANIA

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In the thermal water samples provided by Băile Herculane and Căciulata were found thermophilic aerobic heterotrophic bacteria, sulphur oxidizing bacteria and sulphate-reducing bacteria. There is a connection among numerical distribution of bacteria, temperature and chemical composition of water. As a consequence of their morphological and physiological characteristics, strains isolated belong to moderately thermophilic bacteria like *Thiobacillus tepidarius* (sulphur oxidizing, chemolithotrophic), like *Desulfotomaculum* (sulphate-reducing), and extremely thermophilic bacteria like *Thermus* (aerobic heterotrophic), like *Thermothrix* (sulphur oxidizing) and like *Thermodesulfobacterium* (sulphate-reducing).

Key words: moderately and extreme thermophiles, sulphur oxidation, growth, cultivation.

Thermal natural habitats have a high diversity of temperature, pH and chemical composition. Research in this field has been done both in thermal aquatic habitats in order to investigate the physiology, biochemistry and biodiversity of microorganisms. Thermal habitats contain moderately and extremely thermophilic bacteria involved in sulphur cycle (sulphur oxidizing, sulphate-reducing) and in nitrogen cycle involved in the decomposition of biomass.

This paper contains studies about isolation and morpho-physiological characteristics of moderately and extremely thermophilic, aerobic and anaerobic bacteria.

MATERIALS AND METHODS

Thermophilic bacteria were studied in water samples with different temperature, chemical composition and way of coming out to the surface. Thermal springs from Băile Herculane area are situated on both sides of Cerna river. They come out (from about 500 m depth) freely to surface like 3 Springs or are collected like Scorilo Spring. At Căciulata geothermal water is collected from 3000 m depth by well (1006 well).

Thermal water samples were taken in September 1997, from Scorilo Spring, 3 Springs (Băile Herculane) and 1006 well (Căciulata), and were analysed from the microbiological point of view.

Chemical tests were done for content of NH_4^+ , Ca^{2+} , NO_2^- , NO_3^- , Cl^- , PO_4^{3-} , SO_4^{2-} , total hardness and that given by carbonates.

From the microbiological point of view there were isolated moderately and extremely thermophilic bacteria involved in sulphur cycle (sulphur oxidizing bacteria and sulphate-reducing bacteria) and extremely thermophilic heterotrophic bacteria, using enrichment methods and specific culture media for each group and incubation at 45⁰ and 70⁰C.

Moderately thermophilic bacteria

Moderately thermophilic sulphur oxidizing bacteria like *Thiobacillus tepidarius* were isolated on mineral media with thiosulphate (by reducing pH from 6.0 - under 5.0) or with sulphur (reducing pH from 4.5 to 3.0). The isolates were passed into media with sulphur (as energy source) and yeast extract having in view the growth after 21 days incubation at 45⁰C by microscopic examination and pH value (8).

Sulphate-reducing bacteria like *Desulfotomaculum* were cultivated in Postgate liquid medium and incubated at 45⁰C (6). For their counting it was used MPN method taking into account the black colouring of media due to iron sulphide. Pure cultures obtained on Postgate agar medium in anaerobic conditions were investigated to establish optimum pH and temperature. To establish optimum temperature all strains were cultivated in liquid Postgate medium (pH 7.0), measuring the growth at 30, 40, 50, 60, 70, 80 and 90⁰C as a result of reducing of sulphate (gravimetric method) during 7 days. To establish optimum pH we used 77 liquid medium ironless, different pH (5.0, 6.0, 7.0, 8.0), incubation was done at 50⁰C for 9 days in stationary conditions (7). The absence of iron in culture medium gave us the possibility to measure optical density at 660 nm.

Extremely thermophilic bacteria

To isolate aerobic heterotrophic bacteria, the thermal water was enriched with yeast extract and triptone (0.1%), incubated at 70⁰C for 3-7 days and after culture medium became unclear it was spread onto 162-agar medium (2, 3). The main heterotrophic aerobic thermophilic bacteria isolated had the following codes: HBS-15 (Scorilo Spring), HBS-21 (3 Springs), HB-63 (pit), HB-65 (3 Springs - 54⁰C), HC-67 (spring in river) and CT-31 (well 1006).

To establish optimum conditions for growth and the affiliation isolates to *Thermus* genus physiological tests were done:

- growth of isolates in 162 liquid medium, incubation at 40, 50, 60, 70, 80 and 90⁰C for 96 h in stationary conditions;

- growth of isolates in 162 liquid medium with different pH (2.9, 5.1, 6.0, 6.6, 7.0, 7.45, 8.1), incubation at 70°C for 96 h in stationary conditions. The growth was measured as optical density (660 nm);

- sensitivity to antibiotics: ampicillin (10 µg), penicillin (10 UI), kanamycin (30 µg), chloramphenicol (50 µg), erythromycin (15 µg), streptomycin (50 µg), tetracycline (50 µg) tested on 162-agar medium after 24 h of incubation at 70°C;

- starch hydrolyse on 162-agar medium with starch (1%);

- caseine hydrolyse on 162-agar medium with caseine (1%) (5).

Sulphate-reducing bacteria belonging to *Thermodesulfobacterium* were counted in thermal water due to their growth on Postgate medium, incubated at 70°C in anaerobic conditions correlated with black colouring of medium in 10 days (6). Growth at different temperatures and pH had been done similarly as for moderately thermophilic sulphate-reducing bacteria.

Sulphur oxidizing bacteria grew on Brannan and Caldwell medium by incubation at 70°C for 21 days, following appearance of turbidity and sediment into the medium (4). Optical density was measured at 460 nm as a result of growth on medium with tetrathionate and incubation at 50°C and 70°C for 11 days.

RESULTS AND DISCUSSIONS

Thermal water provided by Scorilo Spring has 52-53°C, pH 6.4-6.6 and that from 3 Springs has 43-54°C, pH 8.0-8.4. These characteristics are constant in time. Occasionally, depending on weather conditions in Cerna River are visible scarce springs with 47-53°C and pH 7.8-7.9. From chemical point of view it is a sulphurous water having 225-275 mg SO₄²⁻/l.

Geothermal water from 1006 well has 88-94°C, pH 6.86-6.90 and it is a chlorinated water being rich in CaCl₂ (8.520 mg Cl/l and 1380 mg Ca/l), having a high total hardness.

As a result of the high depth and the geological strata which are crossed by thermal waters, these have different temperatures and chemical composition (Table 1). All samples contain a small amount of NH₄⁺, NO₂⁻, NO₃⁻ and PO₄³⁻.

Table 1

Physical and chemical characteristics of thermal waters from Băile Herculane and Căciulata

Place	Temperature (°C)	pH	SO ₄ ²⁻ (mg/l)	Cl ⁻ (mg/l)	Ca ²⁺ (mg/l)	Total hardness (°d)
Băile Herculane						
Scorilo Spring	52-53	6.4-6.6	250	319.5	26	4.0
3 Springs	38-54	8.0-8.4	275	220.1	20	2.8
spring in river	47-53	7.9	225	170.4	20	4.0
water in a pit	34-47	7.8	nd	nd	nd	nd
Căciulata- well 1006	88-94	6.9	225	8520	1380	240

nd - not determined

Moderately thermophilic sulphur oxidizing bacteria are more numerous in samples from Băile Herculane than in those from 1006 well (Table 2). The same distribution on mineral media (content of sulphur or thiosulphate decreasing pH to 3.5 and 4.5-5.0 respectively) and absence of growth on organic medium (contains yeast extract and thiosulphate) show that they are strictly chemoautotrophic. According to the morphology of isolated bacilli, mobile, no evidence of spores, Gram negative and autotrophic characteristics, we consider them to belong to *Thiobacillus tepidarius* (1).

Table 2

Quantitative estimation of thermophilic bacteria involved in the sulphur cycle

Place	Moderately thermophilic bacteria		Extremely thermophilic bacteria		
	sulphur oxidizing bacteria		sulphate-reducing bact/ml.	sulphur oxidizing bact / ml	sulphate-reducing bact/ml.
	m.Starkey	/ ml m. Waksman			
Băile Herculane					
Scorilo Spring	25 x 10	11 x 10 ²	0.7	45 x 10	0
3 Springs	45	20	4.0	15	0.9
pit	nd	nd	25 x 10	11 x 10	0
spring in river	4.5	3	7.5	3 x 10	9.5
Căciulata -well 1006	2.5	0	0	45	0.9

() sample of water with sediment; nd - not determined

Moderately thermophilic sulphate-reducing bacteria were found only in samples from Scorilo and 3 Springs, more abundant being in sediment from 3 Springs (Table 2). Moderately thermophilic sulphate-reducing bacteria are anaerobic and sporulated bacilli, Gram positive. They reduce sulphate to H₂S at 40-70°C but the higher ratio was at 50°C (Fig.1). At extreme temperature 30, 80 and 90°C they are unable to grow. From the experiment in which we tested the growth of 3 strains on 77 medium at different pH (50°C) it results that they have a good growth on 77 medium with pH 6.0 (Fig. 2).

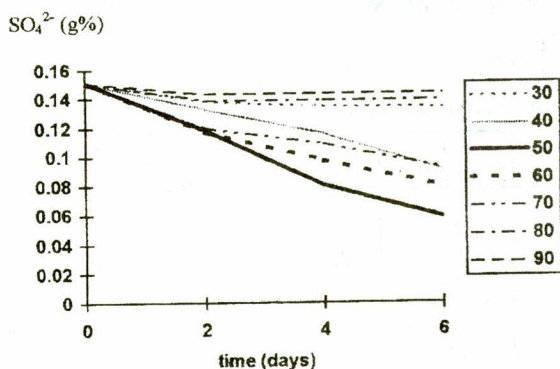
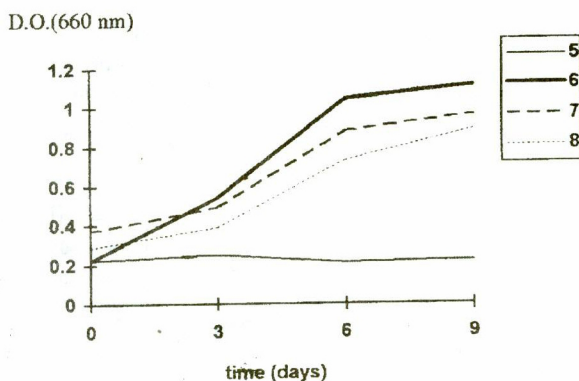


Fig. 1 – Dynamics of sulphate reducing by DC 6 strain at different temperatures on Postgate medium.

Fig. 2 – Dynamics of growth of DC 6 strain on 77 medium at different pH and 50°C.



Extremely thermophilic heterotrophic bacteria were found in all samples but only 6 strains were studied. They are bacilli, Gram negative, 0.37-0.62/0.93-2.97 μm , nonmobile, nonspore-forming, disposed in short chains. On 162 medium at 60-70°C we obtained the highest optical density (Fig. 3, 4). At 70°C and acid pH (2.9 and 5.1) a poor growth took place, at pH 6.0 the growth was good, at pH 7.0-7.45 being optimum (Fig. 5).

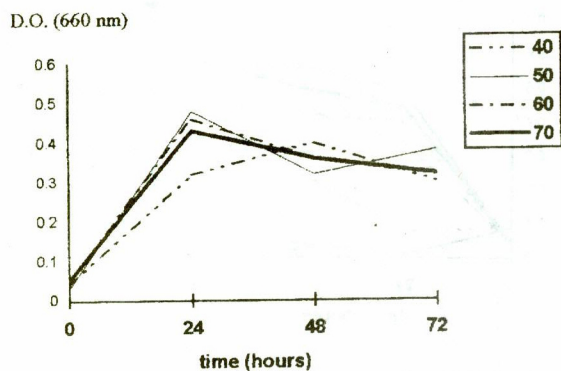


Fig. 3 – Dynamics of growth of HB-65 in 162 medium at different temperatures.

Table 3

Sensitivity of thermophilic heterotrophic aerobic bacteria to antibiotics

Strains	Ampicillin (10 μg)	Penicillin (10 UI)	Kanamycin (30 μg)	Chloramphenicol (50 μg)	Erythromycin (15 μg)	Streptomycin (50 μg)	Tetracycline (50 μg)
HBS-15	±	-	-	-	-	-	-
HBS-21	-	-	-	-	-	-	-
HB-63	-	-	-	-	-	-	-
HB-65	-	-	-	-	-	-	-
HC-67	±	-	-	-	-	-	-
CT-31	±	-	-	-	-	-	±

(-) no growth; (±) scarce colonies in the inhibition area

All strains were sensitive to penicillin (inhibition area of growth = 2.4 3.1 mm). Less sensitive they were to kanamycin, chloramphenicol, erythromycin, streptomycin, tetracycline. Strains HBS-21, HB-63 and HB-65 had a clear inhibition area to ampicillin but HBS-15, CH-31 and HC-67 had an uncertain one (Table 3).

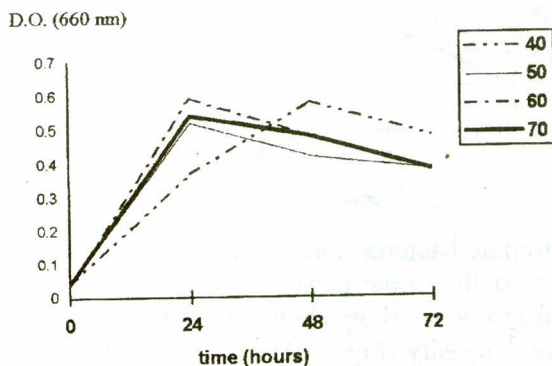


Fig. 4 – Dynamics of growth of HC-67 in 162 medium at different temperatures.

Fig. 5 – Dynamics of growth of HB-65 at different pH (incubation at 70°C).

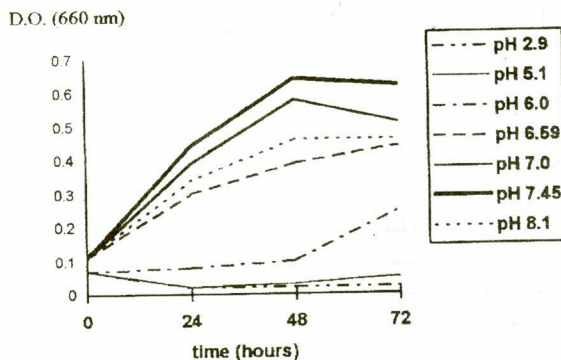
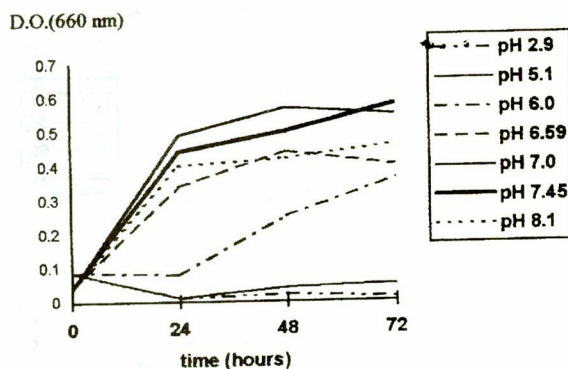
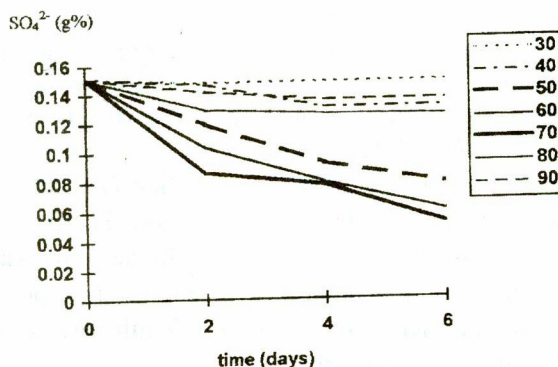


Fig. 6 – Dynamics of growth of HC-67 at different pH (incubation at 70°C).

Starch and caseine were hydrolysed by all strains in 48-72 h, using medium 162 (5).

Fig. 7 – Dynamics of sulphate-reduction in medium Postgate by Td-11 strain at different temperatures.



D.O. (660 nm)

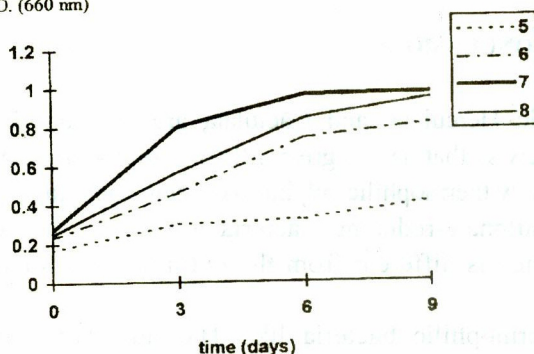
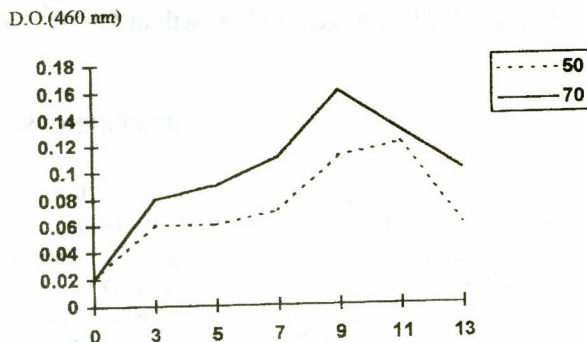


Fig. 8 – Dynamics of Td-11 strain growth in 77 medium at different pH and 70°C.

Fig. 9 – Dynamics of 16 strain growth onto medium with tetrathionate at 50 and 70°C.



Extremely thermophilic sulphate-reducing bacteria were present (Table 2) in low number in all samples because of the inadequate temperature of both thermal habitats (Băile Herculane 45-53°C; Căciulata 90-94°C) for this kind of bacteria. Our experiments demonstrated that these bacteria did not grow at 90°C. Strains Td-11 and Td-12 reduced sulphate at 50 - 80°C but optimum was at 70°C

(Fig.7). They had a good growth at pH 6.0 - 8.0 but optimum was 7.0 (Fig.8). According to their morphological characteristics (Gram negative and nonsporulated bacilli) and physiological characteristics (reduction of sulphate and optimum temperature of 70°C) we consider them to belong to *Thermodesulfobacterium*.

Sulphur oxidizing bacteria like *Thermothrix* are more abundant in sulphurous samples from Băile Herculane (table 2). 13 extremely thermophilic bacteria had a good growth at 50 and 70°C but the best one was at 70°C (Fig. 9). Because they are isolated bacilli sometimes in chains, nonspore-forming, Gram negative and can grow in a medium containing thiosulphate at 70°C we consider them to belong to *Thermothrix thiopara*.

CONCLUSIONS

1. Thermal waters from Băile Herculane and Căciulata are different from temperature and chemical point of view so that have a great influence on the numerical distribution of moderately and extremely thermophilic sulphur oxidizing bacteria.

2. Extremely thermophilic sulphate-reducing bacteria are scarce in both habitats due to the temperature which is different from the optimum one for this physiological group.

3. Aerobic heterotrophic thermophilic bacteria like *Thermus* were often found in thermal water samples from Scorilo Spring, 3 Springs and well 1006. They are bacilli Gram negative, sensitive at penicillin and able to hydrolyse starch and caseine. They have good growth at 60-70°C and 7.0-7.45 pH.

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MICROMORPHOLOGICAL CONSIDERATIONS OF THE SEEDS SURFACE OF SOME *CAMPANULA* SPECIES IN ROMANIA

ANGELA TONIUC

In this paper we describe the micromorphology of the seeds surface of 11 *Campanula* species using the scanner electron microscope (SEM). The features of the seed coats are valuable for distinguishing the *Campanula* seeds from the seeds of the other genera in the family and for the delimitation of the sections and the subsections of the genus.

Key words: *Campanula*, seed micromorphology, SEM, Romania.

This paper continues our previous researches on the structure of the seeds (6) and fruits (7), made with the optical (OM) or/and electron microscope (EM).

Campanula, a genus in the *Campanulaceae* family, contains more than 300 species. This genus is of great horticultural value due to its wide range of flower size and colour.

In order to identify the species of the genus, we took into consideration the morphological characters of the root, the stem, the leaves, and those of the flowers and fruits. The seeds, usually numerous and small, are alike with little macroscopical differences and their features are rarely matching the determination keys. Lately, the researches made with the electron microscope are more often used to complete the diagnosis on the basis of which the species of a genus are grouped in sections or subsections. Only limited information has been published on the morphological structure of the *Campanula* seeds using the electron microscope (2, 4). The species we took into study are either missing or only mentioned in the papers mentioned above.

MATERIAL AND METHODS

Our research is about the seeds of 11 species of the *Campanula* genus which grow in Romania (3,8). The seeds were collected from the wild plants in the following localities: *C. alpina* Jacq. from the Ceahlău Mountains, *C. bononiensis* L. from Mârzești (Iași district), *C. carpatica* Jacq. and *C. persicifolia* L. from the Rarău Mountains, *C. grossekii* Heuff. and *C. lingulata* W. et K. from Băile Herculane (Caraș-Severin district), *C. latifolia* L. and *C. rapunculoides* L. from Bârnova (Iași district), *C. macrostachya* W. et K. from Hanu Conachi (Galați

district), *C. romanica* Săvul. from Culmea Pricopanului (Tulcea district), and *C. rotundifolia* L. from Piatra Fântânele (Bistrița-Năsăud district).

Four of them are considered to be rare plants in Romania (*C. carpatica*, *C. grossekii*, *C. lingulata*, and *C. macrostachya*) and one is an endemit in Romania (*C. romanica*) (5).

The dried mature seeds were mounted on aluminium stubs, then coated with platinum and observed in a TESLA BS 340 scanning electron microscope. Photographs were taken with a Super Rollex camera, using AZOMUREȘ photomaterials.

In this study, in order to describe the seed surface we used the characteristics proposed by N. Barthlott (1): the shape and dimensions of the epidermal cells, the wave of the external periclinal wall, the thickness degree of the anticlinal walls, as well as the aspect of the cuticle. The lengthening direction of the cells is taken into account with the long axis of the seed (the long axis means the distance between the micropylar and chalazal end).

RESULTS

Campanula seeds are small and flattened blade-like. Our preliminary measurements suggest that the majority have less than 2 mm long and 1 mm width. The shape appears oval for *C. carpatica* (Fig. 1), *C. lingulata*, *C. macrostachya* and *C. romanica*, elliptical for *C. bononiensis* (Fig. 2), *C. grossekii*, *C. latifolia*, *C. persicifolia* (Fig. 3), *C. rotundifolia* (Fig. 4), and elongated-elliptical for *C. alpina* and *C. rapunculoides*.

SEM shows that the seed surface is striped-reticulated for all species analyzed. This common pattern is given by the peculiar aspect of the epidermal cell wall.

In surface view, the epidermal cells are elongated in the axis direction. These cells have anticlinal walls strongly thickened. They outline a $\pm$ narrow lumen. The thickness of the anticlinal walls is much higher than the periclinal external wall. The latter remains thin and with a concave position in the cell lumen.

C. alpina (Fig. 5) and *C. bononiensis* (Fig. 6) have over 200 μ m elongated epidermal cells. The anticlinal wall evenly thickened has the width equal to the lumen.

The elongated epidermal cells of over 150 μ m length appear at *C. grossekii* (Fig. 8), *C. latifolia* (Fig. 9) and *C. macrostachya* (Fig. 11). The thickness of the wall is moderate.

In *C. romanica* (Fig. 14), *C. rapunculoides* (Fig. 13) and *C. rotundifolia* (Fig. 16) the dimensions of the cell length are between 50-100 μ m. The anticlinal thick walls are almost totally closing the lumen. At *C. romanica* (Fig. 15), these thickenings are accentuated towards the hilum and the surface seems furrowed.

This is a special condition in *C. rotundifolia* (Fig. 16), because the external periclinal wall is missing and the lumen is very large.

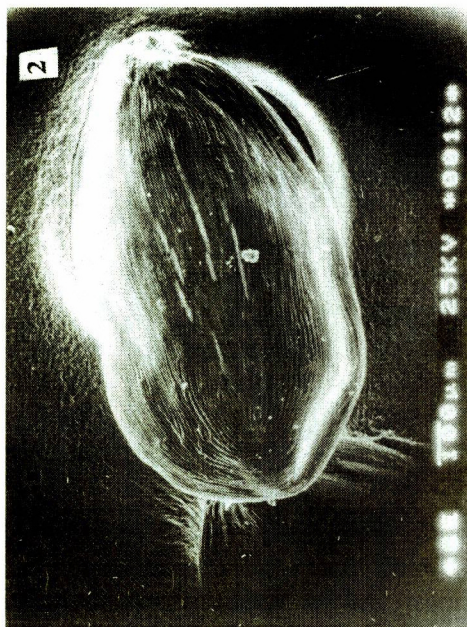


Fig. 2. The seed form of *Campanula bononiensis*.

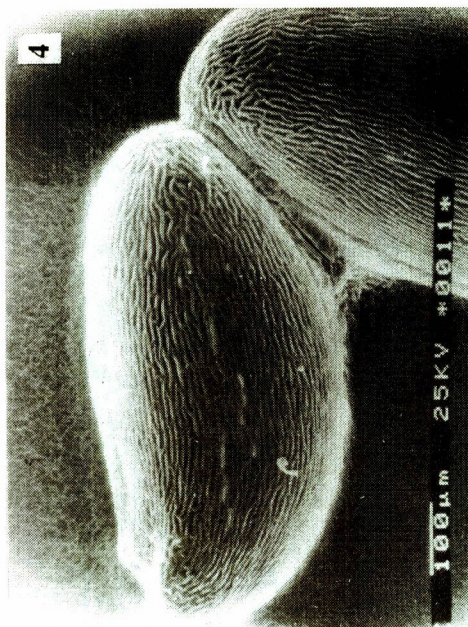


Fig. 4. The seed form of *Campanula rotundifolia*.

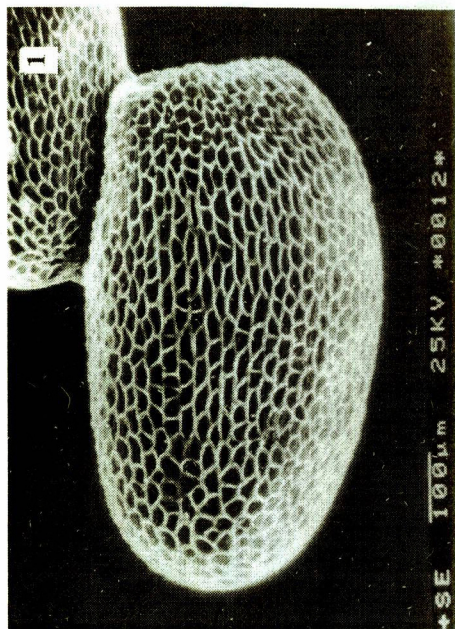


Fig. 1. The seed form of *Campanula carpatica*.



Fig. 3. The seed form of *Campanula persicifolia*.

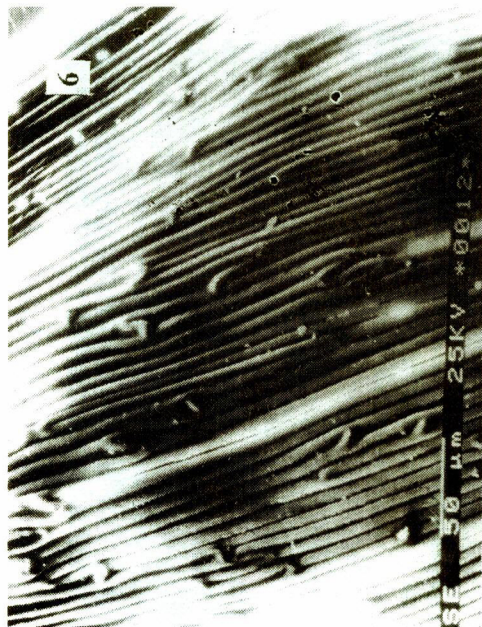


Fig. 6. Details of the seed surface of *Campanula bononiensis*.

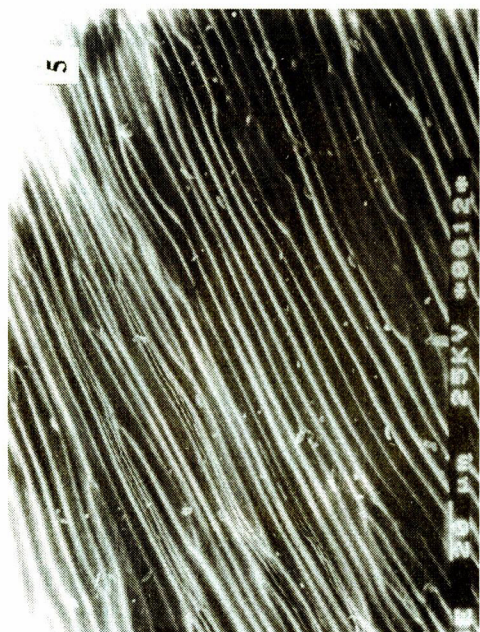


Fig. 5. Details of the seed surface of *Campanula alpina*.



Fig. 8. Details of the seed surface of *Campanula groszekii*.

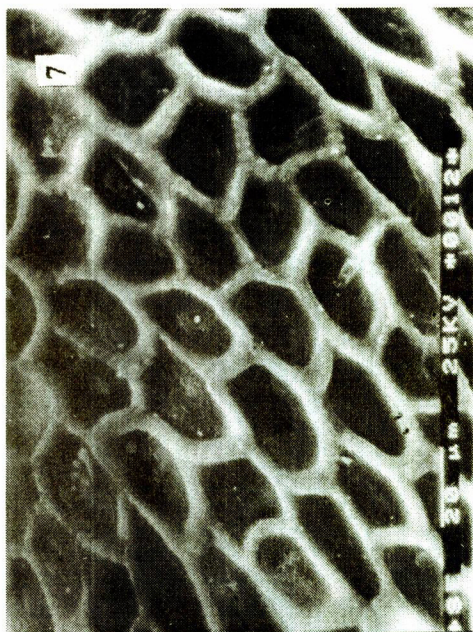


Fig. 7. Details of the seed surface of *Campanula carpatica*.

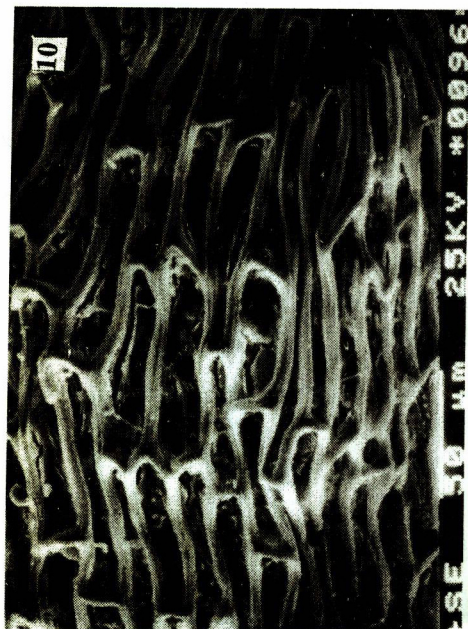


Fig. 10. Details of the seed surface of *Campanula lingulata*.

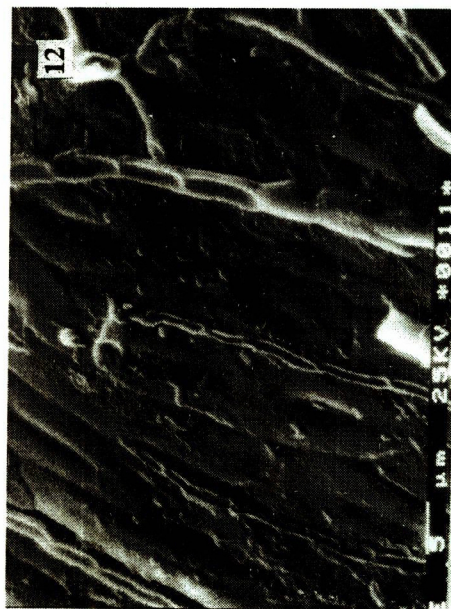


Fig. 12. Details of the seed surface of *Campanula persicifolia*.

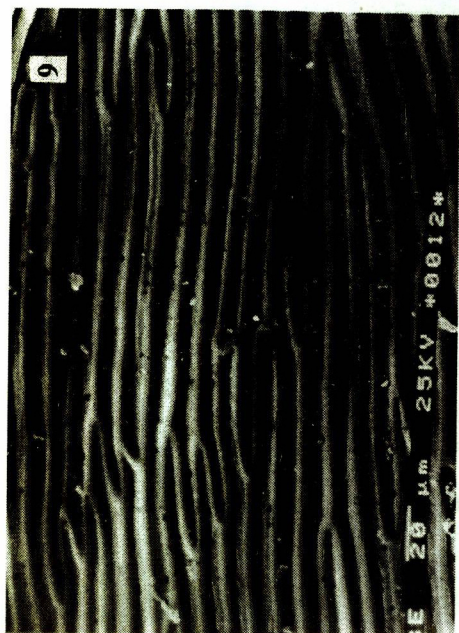


Fig. 9. Details of the seed surface of *Campanula latifolia*.



Fig. 11. Details of the seed surface of *Campanula macrostachya*.

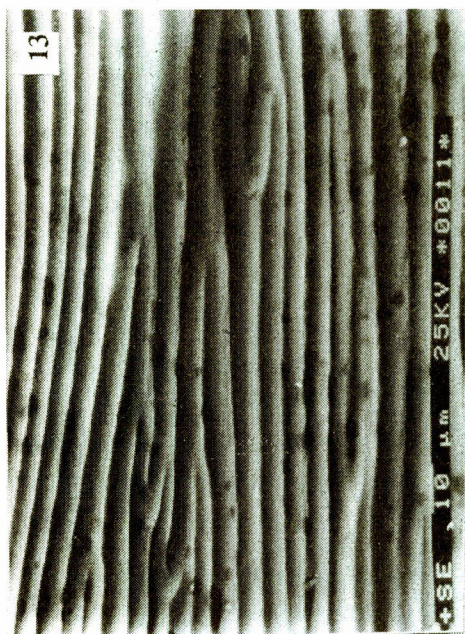


Fig. 13. Details of the seed surface of *Campanula rapunculoides*.

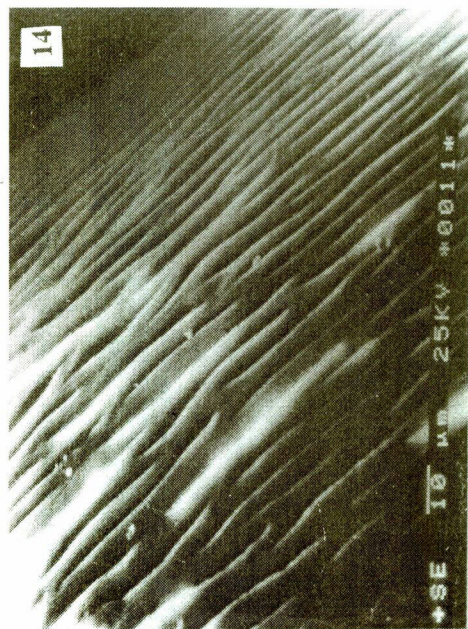


Fig. 14. Details of the seed surface of *Campanula romanica*.

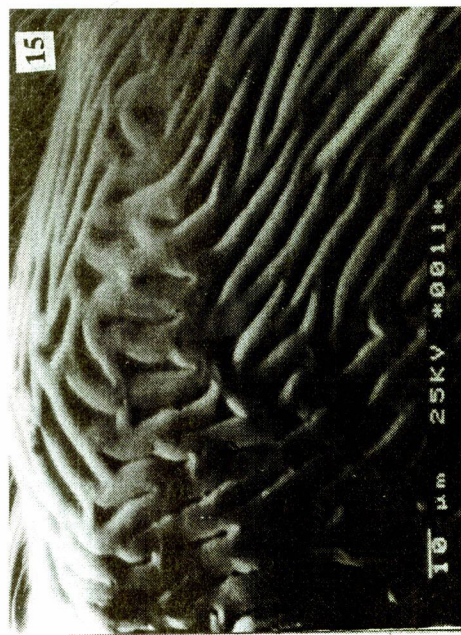


Fig. 15. Details of the seed surface of *Campanula romanica*.



Fig. 16. Details of the seed surface of *Campanula rotundifolia*.

In *C. lingulata* (Fig. 10), the periclinal external wall is broken, and we may observe visible remnants, only.

The epidermal cells are $\pm$ isodiametrical in two species only: *C. carpatica* (Fig. 7) and *C. persicifolia* (Fig. 12). For the first one, the anticlinal walls are not so thick. In *C. persicifolia* (Fig. 12) the wall thickness appears very strong and low, the periclinal wall has a small intrusion in the lumen and the seed seems smooth.

The cuticular layer is continuous but the cuticle is of variable thickness. In two species, there are relatively few micropapillae present (*C. rotundifolia* and *C. carpatica*), caused by folds of the cuticle.

This study will contribute to the knowledge of the morphology of the mature seeds of some *Campanula* species. A lot of microcharacters have a diagnose value because the ultrastructural features of the seed surface are very distinct.

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THE SEASONAL DYNAMICS OF THE PHOTOSYNTHETIC PIGMENTS IN SEVERAL EVERGREEN PLANTS

V. ARTENIE, ANCA GODJA, E. UNGUREANU, MIHAELA CIURCĂ

The paper presents the results obtained in the study of the dynamics of the photosynthetic pigments in the evergreen plants *Picea pungens* and *Thuja occidentalis* during the four seasons of the year.

The concentration of the photosynthetic pigments in the two studied plants is different in each season. The biosynthesis of the chlorophyll *a* in the *Picea pungens* and *Thuja occidentalis* leaves generally presents a similar seasonal rhythm, having maximum values in June and October and a minimum value in April.

The concentration of the chlorophyll *b* varies during the four seasons and the differences between the two species are small. Thus, in the *Picea pungens* leaves, the chlorophyll *b* has a maximum concentration in August and October, while in the *Thuja occidentalis* leaves the maximum concentration of chlorophyll *b* occurs in September. The lowest concentration of the chlorophyll *b* was obtained in April and November for both species.

The quantity of the carotenes presents a more intense fluctuation in the *Picea pungens* leaves than in the *Thuja occidentalis* during the four seasons.

Key words: photosynthetic pigments, chlorophyll *a*, chlorophyll *b*, carotenes, evergreen plants, *Picea pungens*, *Thuja occidentalis*.

INTRODUCTION

The photosynthetic pigments are characterized through their structural and functional variety (12). The chlorophylls and the carotenes play a great role among the photosynthetic pigments existing in the green plant (6). The chlorophylls are magnesium-porphyrins, and the carotenes represent polyisoprenic combinations (1). Both chlorophylls and carotenes participate in the chemical reactions involved in the light phase of the photosynthetic process in green plants, leading to the reduced nicotinamide adenine dinucleotide phosphate and adenosine triphosphate synthesis (4).

The chlorophyll and the carotene biosynthesis in the leaves of green plants vary depending on different endogenous and exogenous factors. Thus, the chlorophyll and the carotene content from green plants depend on the systematic position of these organisms (7, 13). The chlorophyll resynthesis during the life of the green leaf represents one of the essential features of its photosynthetic device. The chlorophyll molecule formation has different intensities in the green plants

ontogenesis and is influenced by temperature, light (5) and several chemical substances. Taking into account the special biochemical role of the chlorophylls and carotenes in plant life, in the present paper we decided to study the dynamics of photosynthetic pigments for two species of evergreen plants: *Picea pungens* and *Thuja occidentalis*, during the four seasons of the year.

MATERIAL AND METHODS

The dynamics of the photosynthetic pigment content was studied on the leaves of *Picea pungens* and *Thuja occidentalis* species in "A. Fătu" Botanical Garden of "Al. I. Cuza" University of Iași, from March 1996 to March 1997.

The leaves for the tests of the photosynthetic pigments were monthly drawn, every first three days of the month, at the same morning hour (8 o'clock). The pigments content was studied on the very drawn biological material. We used for extraction of the photosynthetic pigments 90% acetone. In the same extract there were measured the chlorophyll *a*, the chlorophyll *b* and the carotene sum using "Spekol" spectrophotometer. The chlorophyll *a* was determined at 662 nm, the chlorophyll *b* at 644 nm and the carotene sum at 440.5 nm, given the used extraction (10, 14). In all cases there were taken for determination of the pigments 5 leaf samples from every species of the studied plants.

The experimental data obtained have been processed statistically according to the Student test (9).

RESULTS AND DISCUSSION

Our researches emphasized the fact that in the leaves of the two species of evergreen plants studied (*Picea pungens* and *Thuja occidentalis*), the concentrations of the chlorophyll *a* and *b* and the carotenes too, register fluctuating values during the seasons of the year.

Thus, two maximums and two minimums (fig. 1) characterize the quantity of chlorophyll *a* in *Picea pungens*. During the winter months (December, January and February) and March, the concentration of the chlorophyll *a* for this species has a value of the same order of dimension. In April, we found the lowest value of the chlorophyll *a* content in the *Picea pungens* leaves. The gradual rise of temperature in May, June, but especially in July is associated with an intensification of the chlorophyll *a* biosynthesis that reaches a maximum at the beginning of July. In August this process is also pronounced, the quantity of chlorophyll *a* being smaller than in July but larger than in every other month of the year. At the beginning of September, the quantity of chlorophyll *a* in *Picea pungens* diminishes to a value that is almost the same in May, June and November. The second maximum of chlorophyll *a* was recorded at the beginning of October.

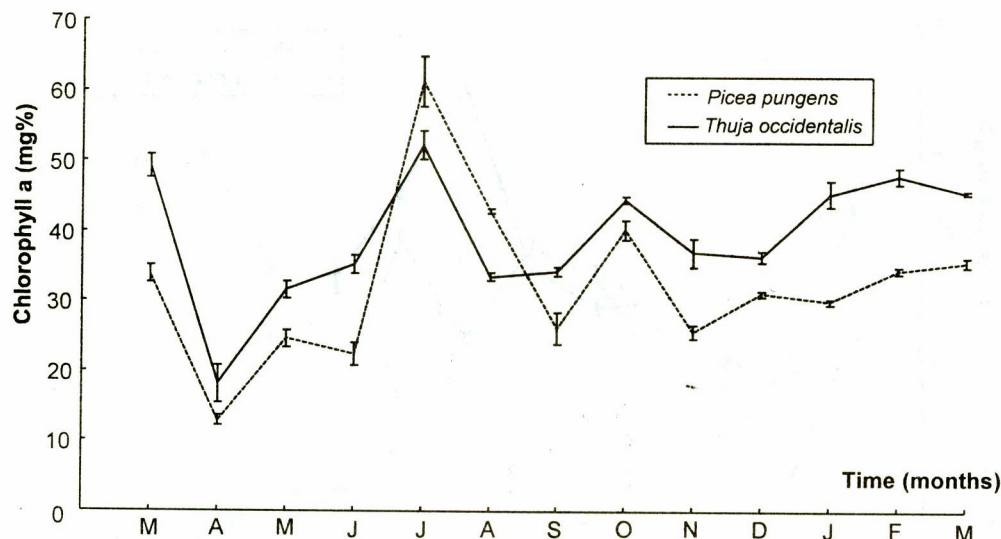


Fig. 1. – The seasonal dynamics of chlorophyll *a* (mg%) in the species *Picea pungens* and *Thuja occidentalis*.

The dynamics of chlorophyll *a* content in the *Thuja occidentalis* leaves (fig. 1) has a similar profile, but with a more clear outlining of the maximum and minimum values. The highest value of chlorophyll *a* was found in July, followed by the one in February-March and the maximum in October. The chlorophyll *a* concentration in *Thuja occidentalis* has the lowest value in April-May. In May, June, August, September, November and December, this indicator has close and intermediate values between the maximum and the minimum limits.

In figure 2 it is seen that the chlorophyll *b* biosynthesis in *Picea pungens* and *Thuja occidentalis* species unfold likewise in spring and summer months, differentiating partly in the autumn and winter months. The chlorophyll *b* concentration in *Picea pungens* has the highest values in July-August and October and the lowest values in April and November.

In *Thuja occidentalis* the chlorophyll *b* reaches a higher value in September, then a more diminished value in January. From April to May and November to December, the quantity of chlorophyll *b* is minimum in this species.

In figure 3 is presented the seasonal dynamics of the carotene content in the leaves of the studied plant species. The data obtained by us show an intense fluctuation of the carotene content in the *Picea pungens* leaves during the four seasons, having a maximum value in March and a minimum in January. In the case of the *Thuja occidentalis* species for the carotene concentration three maximums are revealed localized in February-March, May-June, October-November, and three minimums in April, August and January too.

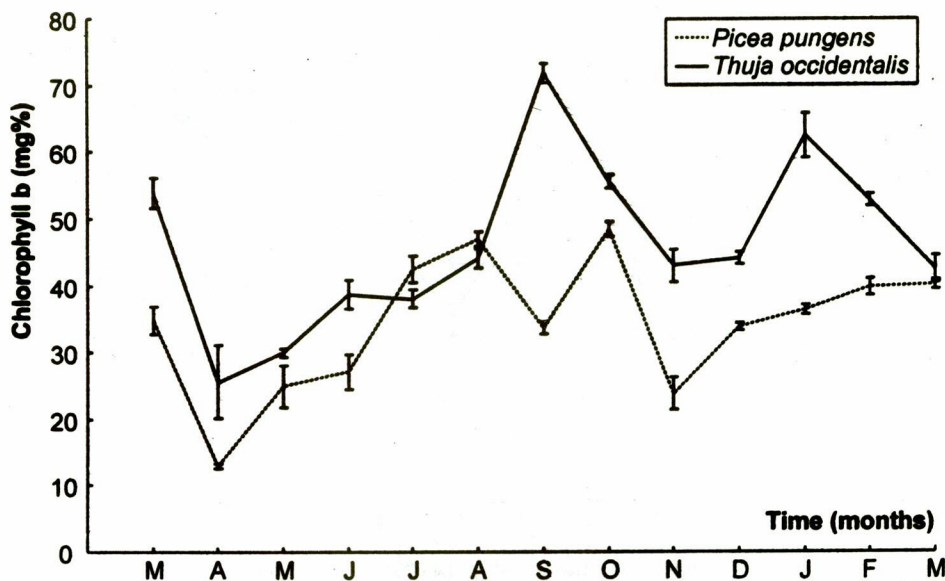


Fig. 2. – The seasonal dynamics of chlorophyll *b* (mg%) in the species *Picea pungens* and *Thuja occidentalis*.

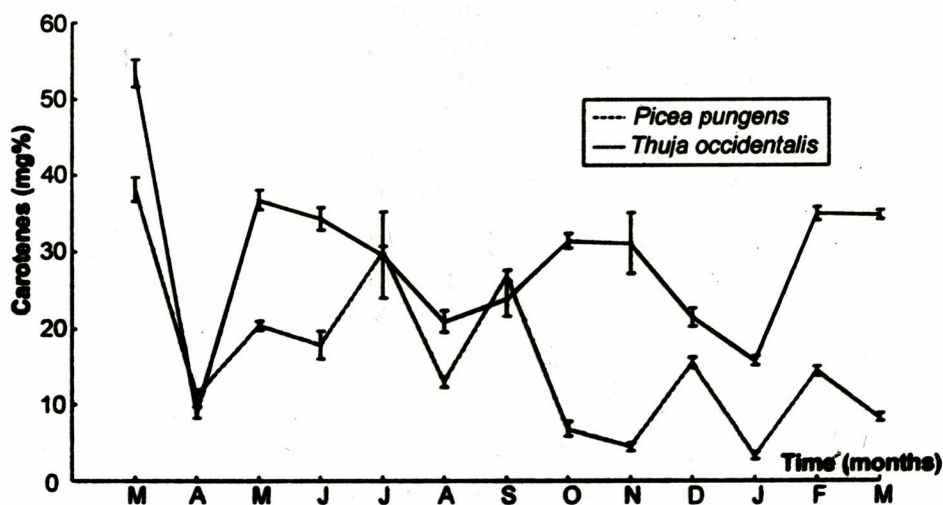


Fig. 3. – The seasonal dynamics of carotenes (mg%) in the species *Picea pungens* and *Thuja occidentalis*.

The seasonal rhythm differences of the chlorophyll and carotene biosynthesis in the *Picea pungens* and *Thuja occidentalis* leaves can be explained by the genetic specific features of the two studied species.

Similar data were obtained by the study of the photosynthetic pigment dynamics in the leaves of several *Basella* species – *Basella alba* L. and *Basella rubra* L. – grown in hot house and field conditions (3). Also researches were down concerning regularities of changing the photosynthetic pigment content in several coniferous-tree species. Some of the results of these researches are contradictory, and others do not match our data. Thus, in study (11) the progress of the photosynthetic pigment accumulation in *Pinus silvestris* has the aspect of a curve with maximums and minimums. According to the other authors (8), the photosynthetic pigments found in the *Picea pungens* leaves of different ages increases slowly from spring to summer, without registering statistically significant oscillations in the other seasons. These data (8) do not match our results regarding the seasonal dynamics of the photosynthetic pigments in *Picea pungens*.

CONCLUSIONS

1. The chlorophyll *a* biosynthesis in the *Picea pungens* and *Thuja occidentalis* leaves presents a similar seasonal rhythm, having the highest value in July and the lowest in April.

2. The chlorophyll *b* concentration in *Picea pungens* and *Thuja occidentalis* is modified according to a similar dynamics in spring and summer months, differentiating partly in autumn and winter months. In the *Picea pungens* leaves, chlorophyll *b* has the maximum concentration in October, then in August and the minimum concentration in April and November. In the *Thuja occidentalis* leaves the highest chlorophyll *b* content was found in September and the lowest in April.

3. The carotene quantity in the *Picea pungens* leaves changes more than in the *Thuja occidentalis* leaves during the four seasons.

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ÉTUDE DE L'EFFET DE PERENCOMBI ET DICOFUSIL SUR LA STRUCTURE DE CERTAINES ESPÈCES DE MAUVAISES HERBES POUSSANT DANS LES VIGNOBLES

MIHAELA NIȚĂ

As far as the responses of three species, *Convolvulus arvensis* L., *Atriplex tatarica* L. and *Aristolochia clematitis* L., to the treatment with two herbicides – Perencombi and Dicofusil – are concerned, differences have been registered; their effects on different tissues explain some modifications produced by the action of herbicide substances. Many of the plants from the investigated species were destroyed by herbicides action early enough, from the seedling stage. Our research refers to plants which could grow, their structural modifications being induced by herbicides having a small amplitude. Among the three investigated species, *Atriplex tatarica* L. was the most sensible weed to the action of the used herbicides; in this case we registered the most profound cyto-histological modifications.

Key words: weeds, herbicides, anatomy.

RÉFÉRENCES HISTORIQUES

La littérature consacrée à l'action des herbicides sur la morphologie, l'anatomie et la physiologie des plantes est très vaste; les investigations visent, le plus souvent, les mêmes espèces de mauvaises herbes ou de plantes cultivées et diffèrent seulement par les substances utilisées, dont le 2,4-D est le plus fréquent.

Les caractéristiques générales de la structure des trois espèces que l'on s'est proposé d'étudier se retrouvent dans les ouvrages de synthèse publiés par Korsmo (1954), Metcalfe et Chalk (1972), Napp-Zinn (1973, 1974, 1984, 1988), ainsi que dans les articles publiés par Burduja et collab. (1966), Gasanov (1962), Toma et collab. (1994) et Vögl (1963); tous ces ouvrages et articles décrivent en bref la structure normale des organes végétatifs chez les espèces de mauvaises herbes analysées.

Dans la littérature consultée on a trouvé des informations concernant l'influence des herbicides, en général, et du 2,4-D, en particulier, sur d'autres espèces de mauvaises herbes que celles qui nous intéressaient (Burduja et Rugină, 1976, 1977, 1979; David et Guillot, 1956; Dyansagar et Khosla, 1969; Rugină, 1977, 1982; Rugină et collab., 1984; Toma et collab., 1984).

De tous ces ouvrages nous avons retenu les espèces de mauvaises herbes chez lesquelles on avait étudié l'influence des herbicides sur la structure des organes végétatifs et reproductifs, les malformations subies par ces organes, les phénomènes d'hypertrophie et d'hyperplasie enregistrés chez les différentes mauvaises herbes dicotylédonées.

On mentionne, tout particulièrement, la thèse de doctorat de Regli, de 1964, qui parle largement de l'influence de l'herbicide 2,4-D sur l'ontogénèse vasculaire.

Les données d'anatomie expérimentale sur les espèces que nous avons étudiées sont laconiques et disparates dans la littérature (Badea et Chirilă, 1961; Hamner et Tukey, 1944; Tukey et collab., 1945-1946); celles qui concernent l'action des herbicides que nous avons utilisés y sont tout à fait absentes.

Les résultats de nos études viennent confirmer et compléter les observations que d'autres chercheurs roumains et étrangers ont faites sur d'autres espèces végétales traitées par d'autres herbicides.

MATÉRIEL ET MÉTHODE DE TRAVAIL

Le matériel d'étude (*Convolvulus arvensis* L., *Atriplex iatarica* L., *Aristolochia clematitis* L.) provient des vignobles de l'IAS - Husi (district de Vaslui) et a été prélevé le 6 septembre 1986.

Les échantillons analysés appartiennent à trois variantes: V_1 - témoin; V_2 - traitement pré-émergent par Perencombi - 10 Kg/ha; V_3 - traitement post-émergent par Dicofusil - 10 Kg/ha.

Le matériel a été préparé par les méthodes couramment utilisées dans les recherches de morphologie et d'anatomie végétale.

L'étude histo-anatomique comparative, témoin-(variante) plante traitée, a été effectuée sur des sections transversales dans la tige et dans la feuille (pétiole et limbe) à différents niveaux; on a également procédé à des sections superficielles au niveau du limbe foliaire pour mettre en évidence les épidermes supérieur et inférieur.

RÉSULTATS ET DISCUSSIONS

Convolvulus arvensis L.

La tige

Témoin (V_1) (Pl. I: Fig.1, 2, 5)

Au niveau de l'entrenœud terminal, le contour de la section transversale est circulaire-costiforme, à côtes très proéminentes. Les cellules épidermiques sont grandes et couvertes d'une cuticule crénelée et, parmi elles, il y a des stomates. L'écorce est mince et différenciée en une zone externe de chlorenchyme et une autre, interne, incolore, de parenchyme incolore.

Les tissus conducteurs forment deux anneaux concentriques: l'un, de liber, plus mince à l'extérieur et l'autre, de bois, plus épais à l'intérieur. Certaines cellules de la zone périphloémique et de la zone périmédullaire contiennent du latex.

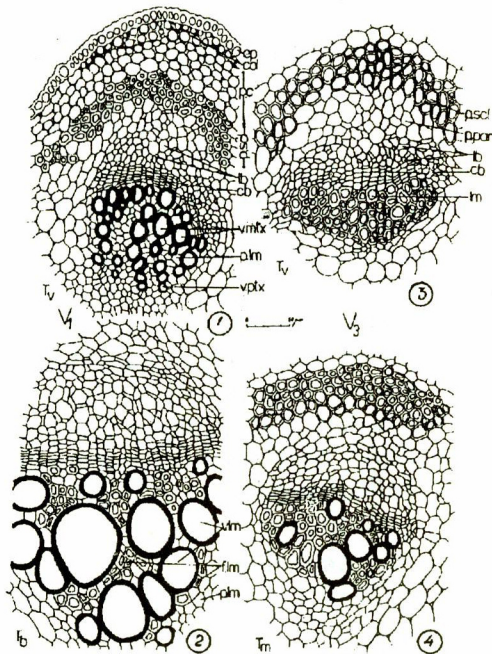


Planche I – **c.scl** - cellules de sclérenchyme; **ep** - épiderme; **f.lm** - fibres libriforme; **i.lb.c** - îles de liber conducteur; **lb** - liber (**e** - externe, **i** - interne); **lt** - laticifères; **lm** - bois (xylème); **p.c** - parenchyme cortical; **p.lb** - parenchyme libérien; **p.dzg** - parenchyme désorganisé; **sb** - suber; **v.lm** - vaisseaux ligneux.

Vers la base de l'organe, l'épaisseur de l'anneau de bois varie, la section transversale mettant en évidence trois secteurs plus épais comportant des vaisseaux à très grand diamètre, entourés de libriforme. Le liber externe et le liber interne présentent des éléments à parois légèrement collenchymatisées et le bois est très sclérifié et lignifié, tout comme les rayons médullaires unisériés qui le traversent.

Variantes expérimentales (V₂, V₃) (Pl. I: Fig. 3, 4, 6, 7, 8)

L'épiderme des V₂ et V₃ présente de rares poils protecteurs tricellulaires; par endroits on distingue le phéllogène, qui produit 2-3 couches de suber juste au niveau de l'entrécœud terminal; l'anneau de bois secondaire présente, par endroits, de petites îles de parenchyme cellulosique; la moelle est désorganisée dans sa région centrale.

Vers la base de l'organe, bien des cellules épidermiques sont en forme de papille; l'écorce primaire est épaisse; en position périphloémique on observe des fibres sclérenchymatiques; dans la moelle on distingue des cellules oxalifères; à la périphérie de l'organe on distingue deux zones discontinues de tissu subéreux entre lesquelles il y a du tissu phellodermique.

La feuille

Témoin (V₁)

Le pétiole présente, en section transversale, un contour semi-circulaire, sa face adaxiale étant creusée d'un large sillon. Au niveau de l'épiderme on observe des stomates proéminents, qui dépassent visiblement le niveau du tissu protecteur, et des poils protecteurs tricellulaires.

Sous le parenchyme hypodermique du type collenchymatique il y a une couche mince de collenchyme qui s'étend jusqu'à l'épiderme en positions adaxiale et abaxiale. Dans le parenchyme fondamental on distingue 3 faisceaux conducteurs et certaines cellules contiennent du latex ou des oursins d'oxalate de calcium.

Le limbe. Au niveau des sections superficielles, l'épiderme présente des cellules dont le contour est irrégulier et dont les parois latérales sont ondulées particulièrement vers la face inférieure du limbe. Les stomates, du type paracytique, sont localisés dans les deux épidermes. Par endroits il y a des poils protecteurs tricellulaires, peu nombreux et dont la cellule terminale est très longue.

En section transversale (Pl. IV: Fig. 11), la nervure médiane est très proéminente vers la face inférieure du limbe; elle présente un seul faisceau conducteur, appuyé contre les deux épidermes à l'aide des piliers du collenchyme.

Le mésophylle est parfois peu différencié en tissus palissadiques et lacuneux; le plus souvent il est homogène, formé de 5-6 couches de cellules palissadiques basses; le limbe a donc une structure bifaciale équifaciale (isofaciale).

Variantes expérimentales (V₂, V₃)

Le pétiole. Chez V₂ et V₃ on signale les suivantes modifications de structure: les cellules de l'épiderme sont plus petites et leurs parois externes sont plus

minces; il y en a quelques vues en forme de papille; les poils protecteurs sont absents; le chlorenchyme hypodermique est représenté seulement par 1-2 couches de cellules allongées tangentiellement; le collenchyme est à peine représenté, décelable grâce à l'épaisseur des parois des cellules composantes.

Le limbe (Pl. IV: Fig. 12). Chez V_2 et V_3 on constate que les cellules épidermiques présentent des parois latérales moins ondulées sur la face supérieure du limbe; les poils protecteurs sont extrêmement rares ou absents; le collenchyme hypodermique situé en face de la nervure médiane est à peine visible; le mésophylle est plus mince, avec des cellules plus basses et le tissu du type plus ou moins palissadique ne se distingue que vers la face supérieure du limbe.

Atriplex tatarica L.

La tige

Témoin (Pl. II: Fig. 1)

Les cellules épidermiques ont la paroi externe très épaisse, couverte de cuticule. Le cortex est presque entièrement du type collenchymatique, les cellules composantes ayant des parois extrêmement épaisses. Parmi les cellules de l'écorce interne (parenchymatiques) il y en a plusieurs qui contiennent des oursins d'oxalate de calcium.

Le cylindre central contient de nombreux faisceaux conducteurs, de tailles différentes, disposés sur un anneau sinueux, ayant tous une structure secondaire, au moins au niveau du bois; celui-ci forme avec les rayons médullaires, sclérifiés et lignifiés, un anneau épais à la périphérie duquel il y a un autre anneau, de liber, très mince; celui-ci vient à l'extérieur en contact avec des cordons minces de fibres sclérenchymatiques, séparées par les cellules de l'endoderme primaire.

Dans l'épaisseur de l'anneau de bois secondaire on distingue un anneau de liber intraxylémique.

Certains auteurs considèrent que ce liber se trouve à l'extérieur du bois fasciculé et les faisceaux initiaux viendraient donc en contact direct avec l'anneau du bois secondaire.

Dans la moelle parenchymatique, certaines cellules sont oxalifères.

Vers la base de la tige, la structure est plus complexe et comporte: une zone de suber (2-4 couches de cellules qui ont provoqué l'exfoliation de l'écorce primaire), un anneau externe, mince de liber, un anneau interne, épais, de bois et de la moelle; dans l'anneau de bois on distingue plusieurs zones de liber intraxylémique, tangentielles et presque parallèles. La face interne de l'anneau de bois présente des faisceaux conducteurs initiaux, détachés de l'anneau, bien enfoncés dans la moelle et dont la structure secondaire est à peine formée.

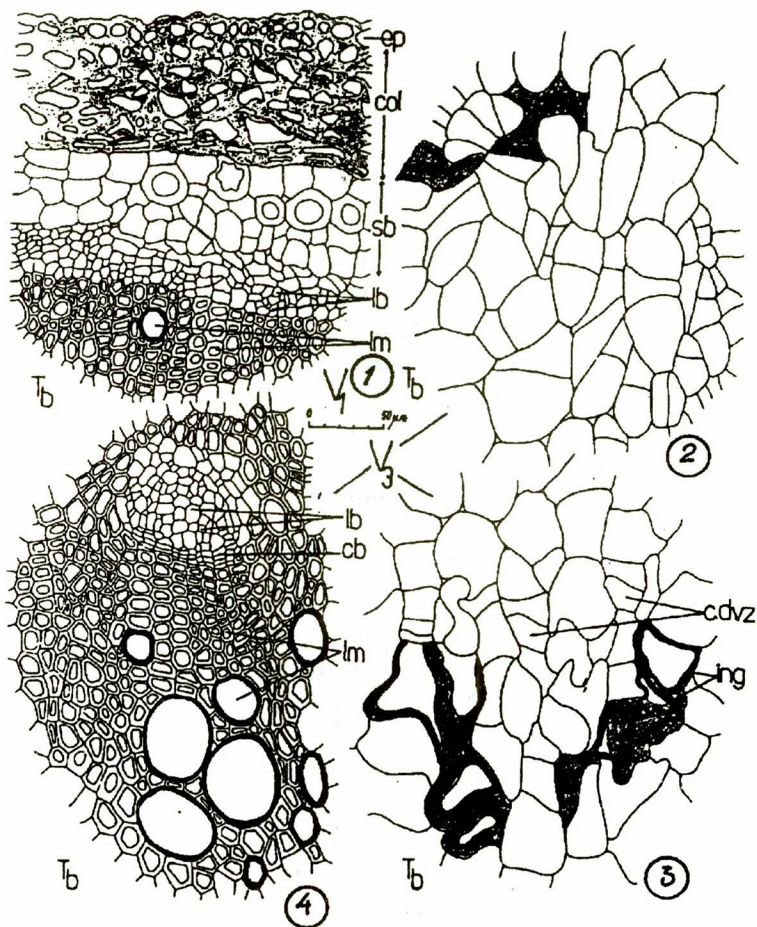


Planche II. Structure de la tige (T) de *Atriplex tatarica* à la base (b), chez le témoin (V₁) et chez une variante (V₃); détails des sections transversales. **Fig. 1** - épiderme, écorce et éléments conducteurs de liber et de bois secondaire; **Fig. 2, 3** - cellules parenchymatiques à intense activité de division et aux épaisissements anormaux; **Fig. 4** - faisceau conducteur interne.

Planche II – cb - cambium; c.dvz - cellules à intense activité de division; col - colenchyme; ep - épiderme; îng - épaisissement; lb - liber; lm - bois; sb - suber.

Dans la moelle on distingue des zones locales de phellogène produisant un suber à structure anormale.

Toute la structure de la tige chez *Atriplex tatarica* confirme les données trouvées dans la littérature consultée (Metcalfé et Chalk, 1972; Toma et collab., 1994) selon lesquelles chez les représentants des Chenopodiaceés et des Amaranthaceés, la structure de la tige, ainsi que celle de la racine, est en majeure partie anormale, déterminée par l'activité des cambiums surnuméraires. De

l'activité du méristème procambial il apparaît un anneau de faisceaux libéro-ligneux qui présente une structure secondaire partiellement formée. Ensuite, certains éléments du péricycle se différencient successivement en zones cambiales concentriques, générant un parenchyme abondant qui se sclérifie et se lignifie et qui englobe peu de vaisseaux ligneux et beaucoup d'îles de liber cellulosique dispersés.

Variantes expérimentales (V₂, V₃) (Pl. II: Fig. 2-4)

Chez V₂, les modifications produites par l'herbicide sont encore plus évidentes que celles observées chez le témoin (V₁) et elles provoquent plus rapidement la mort de la plante étudiée.

Chez V₃, les poils protecteurs sont plus fréquents, la zone de collenchyme hypodermique est plus mince et discontinue, tant que l'écorce primaire se maintient (dans l'entrenœud terminal), le nombre des cellules oxalifères est très grand; tous les faisceaux conducteurs présentent seulement une structure primaire (il en résulte, donc, que l'herbicide inhibe ou retarde le processus de différenciation cellulaire); dans l'écorce interne et dans la moelle apparaissent de larges cavités aérifères, les rayons médullaires sont peu sclérifiés et faiblement lignifiés. Dans le tiers moyen de la tige, la moelle se désorganise complètement en position centrale, déterminant l'apparition d'un large canal aérifère.

Au niveau de l'entrenœud basal, sous l'effet de l'herbicide utilisé, la structure est fortement altérée surtout à la périphérie de la tige:

- l'épiderme et l'écorce collenchymatique sont complètement exfoliées;
- à la périphérie de l'anneau de xylème il y a une zone phloémique qui comporte une alternance de paquets de fibres sclérenchymatiques et de paquets de libers mous (tubes criblés, cellules annexes, cellules de parenchyme);
- à la périphérie de l'anneau de phloème il y a une zone irrégulière de tissu subéreux, fragmentée par des protubérances méristématiques enflées aux extrémités, très proéminentes à l'extérieur, qui traversent les restes de parenchyme cortical; elles représentent une sorte de primordes radiculaires anormaux dont la formation est stimulée par le traitement avec des herbicides (des cas similaires ont été décrits par David et Guillot (1950) chez *Chenopodium album* et chez *Amaranthus retroflexus* ainsi que par Toma et collab. (1976-1977) chez *Helianthus annuus*); l'apparition de ces <<racines>> nuit à la plante, car elles poussent vers l'extérieur en perçant tous les tissus situés à la périphérie du xylème et contribuent à la destruction de la mauvaise herbe.

La feuille

Témoin (V₁)

Le pétiole a un contour semi-circulaire en section transversale, avec deux ailes adaxiales qui délimitent un sillon large et profond. Sous l'épiderme il y a une zone de collenchyme angulaire fragmentée au niveau des ailes. Dans le

parenchyme fondamental il y a de nombreuses cellules contenant des oursins d'oxalate de calcium et 7-9 faisceaux conducteurs de dimensions différentes, dont les plus petits sont englobés dans un étui parenchymatique, caractéristique propre à plusieurs représentants des Chénopodiaceés.

Le limbe. Les sections superficielles mettent en évidence un épiderme formé de cellules à contour polygonal, à parois latérales planes. Dans les deux épidermes il y a, par endroits, des stomates du type anomocytique.

En section transversale (Pl. IV: Fig. 13) la nervure médiane est proéminente sur les deux faces du limbe et contient 4 faisceaux conducteurs; le mésophylle est homogène, formé seulement de quelques couches de cellules isodiamétriques ou légèrement allongées perpendiculairement sur l'épiderme; il y en a quelques-unes qui contiennent des oursins d'oxalate de calcium.

L'une des caractéristiques propres aux espèces des Chénopodiaceés est la présence, autour des petites nervures, d'un étui parenchymatique, complet ou incomplet, formé de très grandes cellules.

Variantes expérimentales (V_2 , V_3)

Le pétiole. Chez V_2 et V_3 , le collenchyme hypodermique est moins développé, le nombre des faisceaux conducteurs est plus grand, les petits faisceaux, enveloppés par un étui parenchymatique, sont absents; dans les faisceaux plus grands, les fibres sclérenchymatiques de la partie périphloémique sont plus nombreuses.

Le limbe (Pl. IV: Fig. 14, 15). Chez V_2 et V_3 , le limbe présente plusieurs faisceaux conducteurs, la fréquence des cellules oxalifères et des poils protecteurs est plus grande, le mésophylle est plus épais, l'épiderme peut s'exfolier à l'extrémité du limbe et, en-dessous, il apparaît du suber de cicatrisation.

Aristolochia clematitis L.

La tige

Témoin (V_1) (Pl. III: Fig. 1, 2)

L'épiderme présente des cellules à paroi externe très épaisse, couverte d'une cuticule mince. Les stomates sont faiblement localisés au-dessous du niveau de l'épiderme, ayant donc la chambre substomatique étroite et peu profonde. L'écorce est mince, chlorenchymatique, peu collenchymatisée dans les côtes. Le cylindre central commence par un péricycle épais, différencié en un anneau externe, sinueux, de sclérenchyme et en un anneau interne, très mince, de parenchyme. Les tissus conducteurs forment plusieurs (9-10) faisceaux du type colatéral ouvert, séparés par des rayons médulaires assez larges, dont quelques-uns ont des cellules légèrement allongées radialement. La moelle est partiellement désorganisée, ce qui fait apparaître une cavité aérifère centrale.

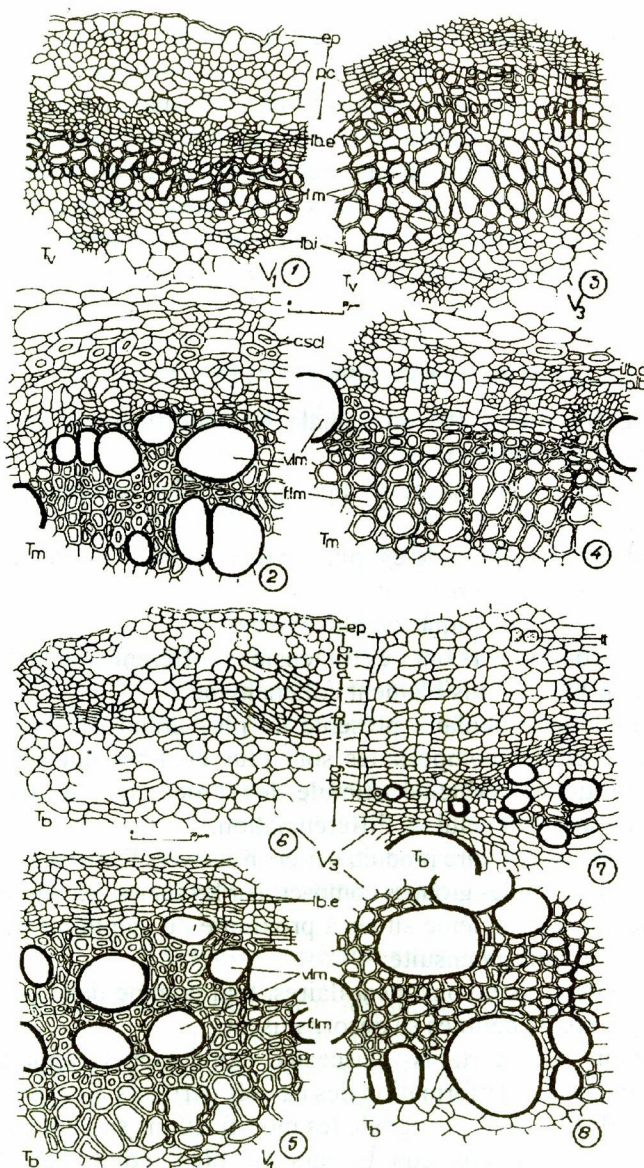


Planche III. Structure de la tige (T) de *Aristolochia clematitis* chez le témoin (V₁) et chez une variante (V₃), aux différents niveaux (v - apical, m - médian; b - basal): détails des sections transversales. Fig. 1 - épiderme, écorce et faisceau vasculaire à éléments conducteurs primaires; Fig. 2 - fragment du cylindre central; faisceau vasculaire à éléments conducteurs secondaires; Fig. 3, 4 - faisceau conducteur; Fig. 5 - fragment de l'écorce et cambium au niveau duquel la division cellulaire est stimulée; Fig. 6 - bois secondaire traversé par des rayons médullaires; Fig. 7 - éléments atypiques de liber et de bois; Fig. 8 - rayon médullaire entre deux faisceaux conducteurs; Fig. 9 - cambium à activité atypique; Fig. 10 - rayon médullaire désorganisé.

Planche III - cb - cambium; col - collenchyme; ep - épiderme; flm - libriforme; fscl - fibre sclérenchymatique; ing - épaississement; lb - liber; lm - bois; p.c - parenchyme cortical; p.lm - parenchyme xylémique; p.par - péricycle parenchymatique; p.scl - péricycle sclérenchymatique; rf.med - rayon médullaire; t.dzg - tissu désorganisé; v.lm - vaisseau ligneux (mtx - métaxylème; ptx - protoxylème).

Vers la base de l'organe, l'épiderme est discontinu à cause de la formation de lenticelles riches en suber et pauvres en phelloderme. Le collenchyme hypodermique est peu visible et le péricycle parenchymatique devient beaucoup plus épais. La structure des faisceaux conducteurs est typiquement secondaire, résultée de l'activité du cambium intrafasciculaire; celui-ci est en contact avec le cambium interfasciculaire (ayant des cellules visiblement plus grandes) et tous les deux forment un anneau dont l'épaisseur varie sur la circonférence de l'organe. Les faisceaux conducteurs sont plus nombreux (13-14), les plus grands alternant avec les plus petits.

Variantes expérimentales (V₂, V₃)

Chez V₂ (Pl. III: Fig. 5-10) on signale les modifications suivantes de la structure décrite chez le témoin (V₁):

- le collenchyme est moins développé, les cellules composantes ayant les parois plus minces;
- le sclérenchyme du péricycle est plus épais (jusqu'à 15 couches de cellules à parois très épaissies et fort lignifiées);
- le péricycle parenchymatique est plus mince;
- les faisceaux conducteurs ont des dimensions différentes; leur nombre varie de 6-8 à 11-12 et leur liber est visiblement collenchymé;
- le cambium intrafasciculaire présente un plus grand nombre de couches, mais les parois des cellules composantes sont plus épaisses que chez le témoin (V₁); il en résulte que l'herbicide stimule les processus de division et de sclérification tout en inhibant celui de différenciation;
- le cambium interfasciculaire produit, par endroits, vers l'extérieur, des éléments de sclérenchyme qui forment des groupes compacts sur les flancs des faisceaux;
- certains éléments du xylème situés à proximité du cambium se délignifient et se gélifient, se désorganisant ensuite;
- au niveau de certains rayons médullaires, tout comme dans la partie axiale de l'organe, bon nombre de cellules se désorganisent;
- à l'intérieur de certains faisceaux conducteurs apparaissent des discontinuités qui entraînent la formation des cavités aérifères à contour irrégulier.

Chez V₃ (Pl. III: Fig. 3, 4) on signale les modifications suivantes:

- le nombre des faisceaux conducteurs est plus réduit dans l'entrenœud terminal (6-7) ainsi que dans l'entrenœud basal (10-11); leur structure reste, en grande partie, d'origine primaire tout au long de l'organe; il en résulte que l'herbicide inhibe le processus de différenciation bien qu'il stimule la division cellulaire au niveau du tissu méristématique;
- les cellules du péricycle sclérénchymatique ont des parois plus épaisses, l'herbicide stimule donc le processus de sclérification;
- le péricycle parenchymatique est visiblement plus mince en position interfasciculaire.

La feuille

Témoin (V₁)

Le pétiole. La section transversale a un contour pentagonal-costiforme. L'épiderme est couvert d'une cuticule épaisse. En position hypodermique on localise le tissu collenchymatique du type tangentiel, plus développé dans les côtes. Dans le parenchyme fondamental il y a 5 faisceaux conducteurs disposés sur un arc, ayant tous, une structure primaire. À la périphérie de tous les faisceaux conducteurs il y a un anneau continu et sinueux, formé d'éléments sclérénchymatiques à parois épaissies et lignifiées.

Le limbe. Les sections superficielles mettent en évidence un épiderme dont les cellules présentent un contour irrégulier et des parois latérales plus ou moins ondulées. Les stomates, du type anomocytique, ne se trouvent que dans l'épiderme inférieur.

En section transversale (Pl. IV: Fig. 16, 17), la nervure médiane contient du collenchyme tangentiel en position hypodermique et un seul faisceau conducteur dans le parenchyme fondamental; certaines cellules de ce dernier, ainsi que bien des cellules de parenchyme libérien contiennent du latex (Napp-Zinn, 1973, 1974).

Les deux épidermes ont, en face de la nervure médiane, de petites cellules isodiamétriques et, ailleurs, de grandes cellules allongées tangentiellement, à paroi externe plus épaisse et couverte d'une cuticule mince. Les chambres sustomatiques sont petites dans l'épiderme inférieur.

Le mésophylle est différencié, vers la face supérieure en tissu palissadique unistratifié, à cellules assez basses, et vers la face inférieure, en tissu lacuneux pluristratifié; le limbe a, donc, une structure bifaciale hétérofaciale (dorsiventrale). Bien des cellules du mésophylle contiennent du latex.

Variantes expérimentales (V₂, V₃)

Le pétiole. L'influence de l'herbicide sur sa structure est insignifiante.

Le limbe. (Pl. IV Fig. 18, 19). Chez V₂, la paroi externe des cellules épidermiques (dont la plupart sont papilliformes) est très épaisse, le tissu palissadique présente des cellules très hautes et, par endroits, il est bistratifié.

Chez V₃, bien des cellules de l'épiderme inférieur sont papilliformes, les cellules palissadiques sont plus hautes et le tissu palissadique occupe 60 % de l'épaisseur du mésophylle.

CONCLUSIONS

Bien que les deux herbicides – Perencombi et Dicofusil – provoquent des réactions différentes chez les trois espèces, *Convolvulus arvensis* L., *Atriplex tatarica* L. et *Aristolochia clematidis* L., leur action générale sur les différents tissus:

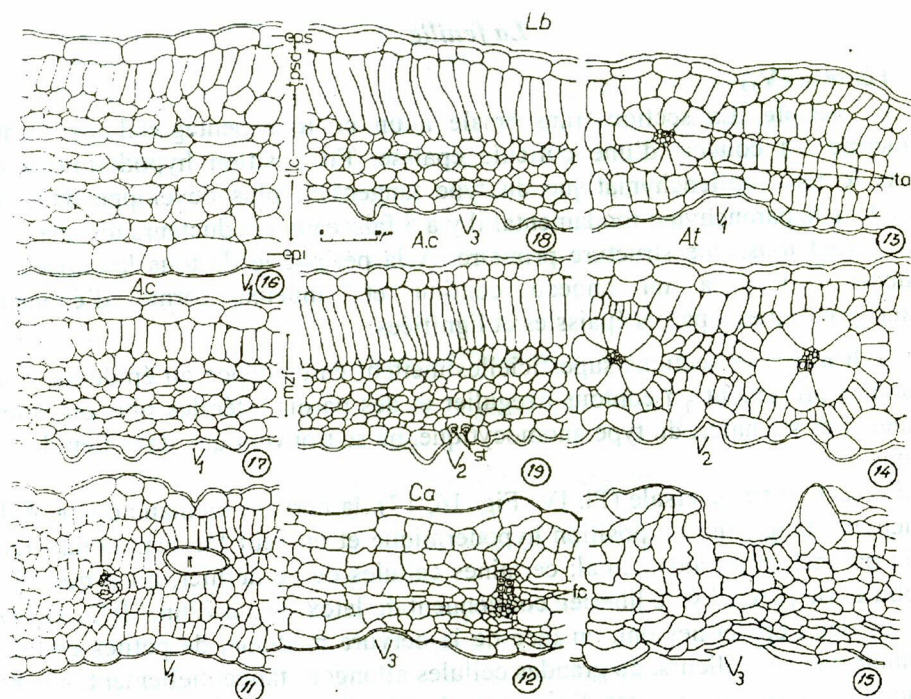


Planche IV. Structure du limbe foliaire (Lb) des *Convolvulus arvensis*, *Atriplex tatarica*, *Aristolochia clematitis* chez le témoin (V_1) et chez les variantes (V_2 , V_3): détails des sections transversales: **Fig. 11, 12** - *Convolvulus arvensis* (C.a.); **Fig. 13-15** - *Atriplex tatarica* (A.t.); **Fig. 16-19** - *Aristolochia clematitis* (A.c.).

Planche IV – ep - épiderme (i - inférieur; s - supérieur); f.c - faisceau conducteur; lt - laticifères; mzf - mésophylle; st - stomate; t.a - étui assimilateur; t.lc - tissu lacuneux; t.psd - tissu palissadique.

- inhibe le processus de différenciation cellulaire, bien qu'elle stimule la division au niveau du cambium; les cellules qui en résultent peuvent rester non-différenciées ou elles subissent un épaississement rapide des parois sans évoluer vers un type tissulaire normal (c'est ce qui arrive surtout au liber, à la suite du traitement par Perencombi);

- stimule le processus de sclérification au niveau des éléments composant le péri-cycle sclérénchymatique et le libriforme et au niveau du liber qui se transforme très tôt en collenchyme;

- détermine la transformation des cellules de l'épiderme foliaire en éléments papillifères;

- stimule la formation des protubérances méristématiques au niveau du cambium péri-cyclique (c'est le cas de l'arroche sauvage);

- détermine la différenciation précoce du phéllogène de la tige, ce qui provoque l'exfoliation de l'épiderme primaire;

• stimule la croissance des cellules palissadiques qui deviennent, ainsi, plus hautes que celles du témoin.

On précise encore une fois que la plupart des sujets appartenant aux espèces étudiées ont été détruits, dès leur stade de plantule, par l'action des herbicides. Nos recherches concernent les sujets qui ont continué à pousser ayant subi des transformations moins importantes.

Des trois espèces de mauvaises herbes étudiées, l'arroche sauvage s'est avérée être la plus sensible aux herbicides utilisés, étalant les modifications cyto-histologiques les plus profondes.

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THE COMBINED EFFECT OF THE GAMMA RADIATIONS OF Co^{60} AND DIFFERENT CHEMICALS ON THE CELLS OF THE RADICULAR VEGETATIVE TIP OF *ALLIUM SATIVUM* L.

RODICA DUMITRESCU,* D. POPESCU,* M. ANDREI**

The gamma ionizing radiation used alone in treatment affected differently the cells belonging to the two zones of the root, causing modifications of the cellular parameters, which have been previously estimated (5). Data in literature established that the damage of the interphasic nucleus of the cells determines the DNA synthesis obstructions and the delay of the cells in mitosis. Sparrow A.H.(13), Miller M.W. (1964), Al-Rubeai, (1) affirmed the existence of a relationship between the nuclear volumes and the radiosensitivity of the different types of cells. The aim of this experiment was to counteract the harmful effect of the gamma radiations at cellular level with the help of combined treatments: gamma radiations associated with different chemicals having radioprotective effects. The biological significance of the obtained values was established by statistical calculation with the help of Student (t) test. The values of the estimated cellular parameters: NV, ICV, CV, NCR showed that although there were certain fluctuations, their values were not always significant.

Key words: gamma radiations, chemical treatment, meristematic cells, *Allium sativum* L.

MATERIALS AND METHODS

The bulbils of *Allium sativum* L. have been submitted to pre-treatment for 24 h with the following chemicals: 1‰ procaine, 1‰ tiastime, and 1‰ combination (P+T+Cy); after that they have been irradiated with 10 Gy/min. flow rate and more gamma radiation doses: 10 Gy, 30 Gy, and 50 Gy. After 4 days from irradiation the 3-6 mm roots were fixed for 24 h in Carnoy bath (3:1), then put in 70° alcohol and kept at 4 °C. At the processing moment double hydrolysis and 45% acetic carmine stain were used thus obtaining something like the Squash type. With the help of a micrometric lamella the big and the small cellular and nuclear axes have been measured at each one hundred cells in each root's zone: the meristemal and increasing zone.

The estimated cellular parameters are: NV, ICV, CV, NCR. The biological significance of the values was established by Student (t) test, according to P. Mureşan (9).

RESULTS AND DISCUSSIONS

In this paper the relationship between the interphasic nuclear volume (NV) and the nuclear radiosensitivity was estimated, as well as the relationship between nucleocytoplasmic ratio (NCR) and the cellular radiosensitivity.

Studies showed that modifications of certain biological parameters confirmed the living organism sensitivity damage. The most accepted theory is about the relationship between ICV and nuclear sensitivity. ICV represents the chromosome volume and other nuclear materials named "nuclear shape" (7,8). The nuclear volume differences between the different types of cells of the same species are due to the different quantities of nucleoplasm and they can influence the radiosensitivity (2,11).

The amplitude of the disturbances caused by gamma radiations, used alone in treatment, was proportional to the radiation doses and it manifested specifically for each zone of the root (5).

1. The average nuclear volumes (NV)

1.1. In the apical meristematic zone, regarding some samples of treatment such as: 1‰ procaine + 30 Gy ($633.18 \mu\text{m}^3$), 1‰ tiastime + 30 Gy ($831.52 \mu\text{m}^3$), 1‰ tiastime + 50 Gy ($562.14 \mu\text{m}^3$), and 1‰ (P+T+Cy) combination associated with all gamma radiations doses, the nuclear volume decreases under control ($1185 \mu\text{m}^3$) were noticed. The statistical calculation showed that these decreases are very significant meaning serious modifications of the interphasic nucleus, and the chemical radioprotection does not exist. (Table 1, Fig. 1).

1.2. In the increasing root's zone the average nuclear volumes at the samples treated with procaine and tiastime increased over control corresponding zone ($1069 \mu\text{m}^3$), except for the sample of treatment: 1‰ tiastime + 30 Gy ($1053 \mu\text{m}^3$). The increases had a very high biological significance ($p < 0.001$). 1‰ (P+T+Cy) combination used alone in treatment due to the synergical effect of its chemicals caused a higher increase of the nuclear volumes ($2950.26 \mu\text{m}^3$), but in association with gamma radiations they determined gradual decreases of the nuclear volumes, proportional to irradiation dose increase. This fact indicates a nuclear radiosensitivity increase. (Table 2, Fig. 1).

2. The interphasic chromosome volume (ICV)

ICV represents values correlated to those of the nuclear volumes.

2.1. At the cells in apical meristematic zone, 1‰ procaine, 1‰ tiastime and 1‰ (P+T+Cy) associated with gamma radiations caused an ICV decrease to the control. (Table 1, Fig. 2).

2.2. At the cells in the root's increasing zone there were noticed high values of ICV to the control. The increase of gamma radiation doses used in treatment caused a gradual decrease of ICV values, still overtaking the control value. (Table 2, Fig. 2).

Table 1

Combined effect of gamma radiations on the meristematic area cell of the root in *Allium sativum* L.

Treatment	NV μm^3	Biological significance	CV μm^3	Biological significance	ICV μm^3	NCR
Control	1185± 107	NS	3066± 282	NS	74	0.39
Procaine 1‰	1472± 83	X	6828± 651	XXXX	92	0.21
Procaine 1‰+10Gy	1086± 110	NS	5581± 552	XXXX	68	0.19
Procaine 1‰+30Gy	633± 53	XXXX	6164± 561	XXXX	40	0.10
Procaine 1‰+50Gy	1021± 75	NS	7567± 1010	XXXX	64	0.13
Tiastime 1‰	1047± 123	NS	3962± 381	X	65	0.26
Tiastime 1‰+10Gy	546± 24	XXXX	5789± 514	XXXX	34	0.09
Tiastime 1‰+30Gy	832± 112	X	3672± 436	NS	52	0.23
Tiastime 1‰+50Gy	568± 38	XXXX	3316± 254	NS	36	0.16
P+T+Cy 1‰	1153± 282	NS	7141± 537	XXXX	10	0.16
P+T+Cy 1‰+10Gy	916± 85	X	4690± 667	X	57	0.19
P+T+Cy 1‰+30Gy	331± 29	XXXX	3576± 315	NS	21	0.09
P+T+Cy 1‰+30Gy	508± 44	XXXX	3695± 309	NS	32	0.13

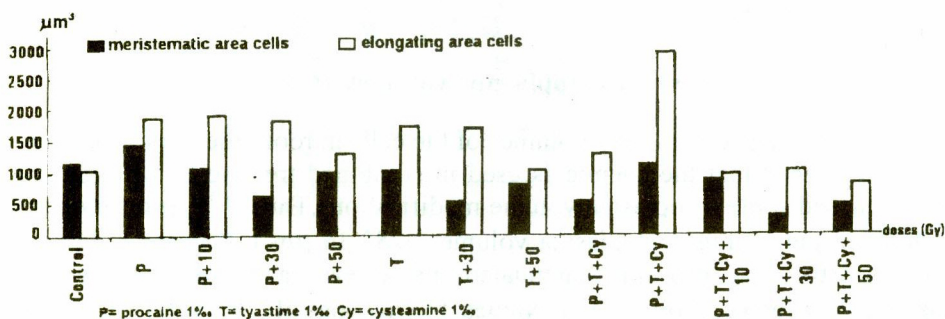


Fig. 1. – Nuclear volume(combined effect)-*Allium sativum* L.
P=procaine 1‰, T=tiastime 1‰, Cy=cysteamine 1‰.

Table 2
Combined effect of gamma radiations on the elongating area cell of the root in *Allium sativum* L.

Treatment	NV μm^3	Biological significance	CV μm^3	Biological significance	ICV μm^3	NCR
Control	1069± 110	NS	7954± 1031	NS	67	0.13
Procaine $1^0_{/00}$	1871± 172	XXXX	36509± 1205	XX	117	0.05
Procaine $1^0_{/00}+10\text{Gy}$	1939± 254	XXX	26204± 3078	XXXX	121	0.07
Procaine $1^0_{/00}+30\text{Gy}$	1856± 177	XXXX	17765± 1977	XXXX	116	0.10
Procaine $1^0_{/00}+50\text{Gy}$	1296± 110	NS	16840± 1583	XXXX	81	0.07
Tiastime $1^0_{/00}$	1788± 199	XXX	16604± 1945	XXXX	112	0.10
Tiastime $1^0_{/00}+10\text{Gy}$	1735± 111	XXXX	15027± 1403	XXXX	108	0.11
Tiastime $1^0_{/00}+30\text{Gy}$	1053± 74	NS	13371± 1381	XXX	66	0.08
Tiastime $1^0_{/00}+50\text{Gy}$	1258± 424	NS	13454± 1168	XXXX	80	0.09
P+T+Cy $1^0_{/00}$	2950± 210	XXXX	42084± 4395	XXXX	184	0.05
P+T+Cy $1^0_{/00}+10\text{Gy}$	1015± 94	NS	11780± 915	XXX	63	0.09
P+T+Cy $1^0_{/00}+30\text{Gy}$	1052± 460	NS	20159± 1102	XXXX	66	0.05
P+T+Cy $1^0_{/00}+30\text{Gy}$	825± 89	NS	9012± 1187	NS	52	0.10

Significance: X ($p < 0.05$)
 XX ($p < 0.02$)
 XXX ($p < 0.01$)
 XXXX ($p < 0.001$)

P = procaine
 T = tiastime
 Cy = cysteamine

NS = non significant difference

3. Average cytoplasmic volumes (CV)

3.1. The average cytoplasmic volumes of the cells in root's meristematic zone.

It was noticed that the chemicals used in combined treatments with gamma radiations caused some cytoplasmic volume modifications. Thus, $1^0_{/00}$ procaine used alone doubled the average cytoplasmic volume ($6282.36 \mu\text{m}^3$). Gamma radiations associated with $1^0_{/00}$ procaine attenuated its effect, although the average cytoplasmic volumes showed high values to the control ($3065.77 \mu\text{m}^3$). The statistical calculation demonstrated that the effects caused by procaine at cytoplasmic level have a high biological significance ($p < 0.001$).

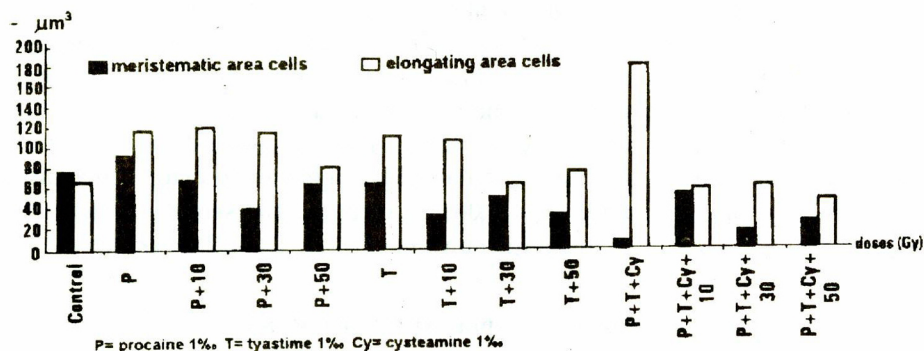


Fig. 2.— ICV (combined effect)-*Allium sativum* L.
P=procaine 1 %,T=tiastime 1 %,Cy=cysteamine 1%.

In comparison with procaine, the effect caused by 1% tiastime on cytoplasmatic volumes is cooler close to the control. The values of the samples treated with 1% tiastime and 1% tiastime + 10 Gy showed biological significance. P+T+Cy combination caused a double of the cytoplasmatic volumes ($7111.09 \mu\text{m}^3$) to the control ($3065.77 \mu\text{m}^3$). By association of the chemical combination with gamma radiations decreases of the cytoplasmatic volumes have been noticed. 1% (P+T+Cy) + 30 Gy ($3575.51 \mu\text{m}^3$) and 1% (P+T+Cy) + 50 Gy ($3695.07 \mu\text{m}^3$) showed the lowest values. From biological point of view these values are insignificant (NS). (Table 1, Fig. 3).

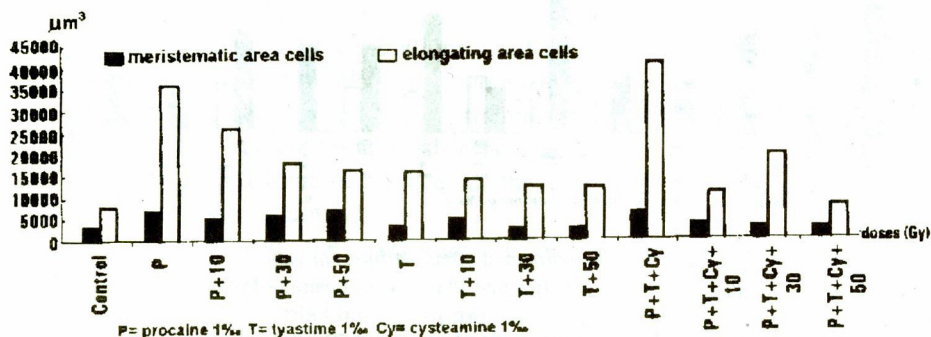


Fig. 3. — Cytoplasmic volume (combined effect)-*Allium sativum* L.
P=procaine1%, T=tiastime 1%,Cy=cysteamine 1%.

3.2. The average cytoplasmatic volumes of the cells in the increasing root's zone.

At the level of the cells in this root's zone, the synthesis of the main factors which influence the plant's growth takes place. All chemicals used alone in treatment facilitated the increase of the cytoplasmatic volumes. Thus, 1% procaine

determined an increase of the cytoplasmatic volumes five times ($36.509 \mu\text{m}^3$) to the control ($7954.39 \mu\text{m}^3$), $1^{0/00}$ tiastime ($16.607 \mu\text{m}^3$) two times and $1^{0/00}$ (P+T+Cy) chemical combination six times ($42.084 \mu\text{m}^3$). In association with gamma radiations, the chemical combination caused a decrease of the radiation doses, although the values are higher to the control. The statistical calculation demonstrated that the effects caused by combined treatments on cytoplasm had a very high biological significance ($p < 0.001$), except for the sample treated with $1^{0/00}$ (P+T+Cy) +50 Gy having insignificant values (NS). (Table 2, Fig. 3).

4. The nucleo-cytoplasmic ratio (NCR)

The fluctuations of nuclear and cytoplasmic volumes caused modifications of the NCR.

4.1. Regarding the cells in root's meristematic zone the nucleus is 40% per cell for control; the rest is occupied by hyaloplasm. These cells have a strong mitotical activity and NCR is between 0.09-0.39.

Between the different treatment samples, as a result of the high decrease of the nuclear volumes, as well as the increase of the cytoplasmic volumes, NCR differences showed up (Table 1, Fig.4).

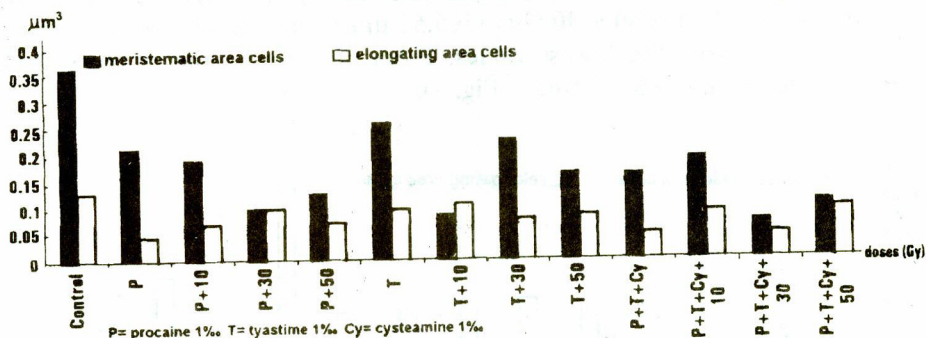


Fig.4. – RNC (combined effect)-*Allium sativum* L.
P=procaine 1%, T=tyastime 1%, Cy=cysteamine 1%.

4.2. In cells in the zone of root's increase have their NCR between 0.05-0.13. Their mitotical activity is reduced. The electronmicroscopic studies (5) showed an increase of the vacuolisation process, which led to the increase of the cytoplasmic volumes. The NCR decreases to the control showed without doubt the increase of the irradiated cells radiosensitivity, expressed by cellular proliferation modification. (Table 2, Fig. 4).

Many scientists tried to explain the way in which gamma radiations interfere and modify the cellular parameters, as well as their implications. The biological

activity of the nucleus is connected to its structure characteristics, both in mitosis and in interphase. The highest nucleus modifications in interphase take place between telophase-G₁ and G₂-prophase and they are associated with chromosome structure modifications.

In phase "S", DNA, RNA, and proteins synthesis takes place.(6,12,14,15) specified that gamma radiations affect DNA molecule, chemically modifying its bases. Nasazzi, N. and his collaborators (10) showed that the DNA catenes break, the adjacent bases recombinations cause alterations of the chromosomes in mitosis. These modify the chromosomes dispersion in nucleus influencing the nuclear volume.

The chemicals used in treatment before irradiation contain in their molecule sulphur under the form of -SH groups and they have a radioprotective role. They interfered differently depending on the radiation dose and they were associated preventing the release of hydrolytic enzymes from lysosomes through their membrane stabilization.

In the case of *Allium sativum* L. 1⁰/₀₀ concentration of chemicals was very strong so that it was not possible a proper radioprotection of cellular level. The cells of the radicular vegetative tip have been seriously affected. It was noticed a decrease of their proliferation capacity, and regarding the samples treated with high doses of radiations it was noticed the cellular death and the disappearance of the plants in time.

CONCLUSIONS

1. Gamma radiations affected differently the two root's zones, the amplitude of the disturbances caused at cellular level being proportional to the gamma radiation doses used in treatment.

2. Although the gamma radiations used alone in treatment caused nuclear volumes fluctuations of the cells in the two root's zones, their values were close to each other. In the case of combined treatments with chemicals, high value differences between nuclear volumes showed up.

3. The nuclear volume differences of the cells of the same tissue due to the different nucleoplasma quantity.

4. It was proved the existence of one correlation between nuclear radiosensitivity and ICV.

5. The average cytoplasmatic volumes showed fluctuations depending on the root's zone and treatment. In the meristematic zone of the root their increase to the control was due to the big cellular axes increase while the cellular small axes showed values close to the control. In increase root's zone the cytoplasmatic volume increases were due to the big and small cellular axes.

6. The chemicals associated with gamma radiations decreased gradually the cytoplasmatic volumes, proportionally to the increase of the irradiation dose, their values being still high to the control.

7. The results showed that *Allium sativum* L. is very sensitive to gamma radiations, and 1⁰/₀₀ concentration was very strong and did not supply a proper radioprotection.

8. By Student (t) test it was proved that the fluctuations of the estimated cellular parameters have always a biological significance.

9. NCR showed decreased values to the control for all treatment samples, which indicates a high radiosensitivity at the cellular level.

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THE INFLUENCE OF THE SIMAZINE HERBICIDE ON GROWTH, GASEOUS EXCHANGES AND ASSIMILATORY PIGMENT CONTENT IN *BOTRYOCOCCUS BRAUNII*

DANIELA ANCA LAZĂR

The reactions of *Botryococcus braunii* cells treated with a triazine derivative, simazine, were investigated. The effects on cell numbers, growth, assimilatory pigment content and gas exchanges were recorded.

Simazine herbicide is a triazine derivative and it was added in Zehnder-Gorham nutritive medium of algal suspension in increasing concentrations from 0.02 to 0.6 μM .

LD 50% after 2 days was 0.364 μM and LD 50% after 14 days dropped at 0.125 μM simazine. Compared with the control, the 0.2 μM simazine concentration reduced the growth, photosynthesis and respiration to 44.5, 78.2 and 41.7% respectively. For the 0.2 μM treatment, the assimilatory pigment content showed a decrease below 50% of control value after 14 days.

Key words: *Botryococcus braunii*, simazine herbicide, growth, photosynthesis, respiration, assimilatory pigments.

Herbicides play an important role in agriculture and demand for them is increasing.

For the chemical weed control one should apply herbicides which interfere only with the plant specific processes, like photosynthesis or biosynthesis of pigments. Accordingly, a special attention in herbicide development is paid to chloroplast as a typical plant organelle (3).

Very often the specific herbicidal effects are still unknown and only become evident generally as growth inhibition (10).

Research on mode of action of herbicides has made remarkable progress in recent years. There is a growing trend to corroborate the green-house screening by studies on model systems, like unicellular algae, cell cultures and cell-free enzymatic assays contributing to physiological and biochemical parameters (1, 2, 11).

Unicellular algae are frequently used in studies of photosynthesis (5) and represent a useful model for the study of the herbicide impact on plant physiology (1). The unicellular algae can multiply very quick and provide an enough experimental material for the study of the herbicide influence on some physiological processes. The results obtained by studying the herbicide action on the unicellular algae can be used for the understanding of the herbicide effects on higher plants.

The purpose of the present investigation was to study the physiological reactions to a triazine derivative, simazine (2-chloro-4,6 bis (ethyl-amino)-S-triazine), in *Botryococcus braunii* Kuetz* cells grown on Zehnder-Gorham medium.

The herbicides which contain simazine are used in maize, sorghum crops, as well as in vineyards and in apple and pear-tree orchards. Simazine controls a great variety of mono- and dicotyledonous annual grasses, like *Atriplex*, *Chenopodium*, *Xanthium*, *Avena*, *Digitaria*. This herbicide acts by inhibition of photosynthesis, but it is possible to have some secondary actions, too (12).

For one culture cycle, the effects on cell numbers, growth, gas exchanges and assimilatory pigment contents were recorded.

MATERIALS AND METHODS

Botryococcus braunii alga was cultivated on Zehnder-Gorham nutritive medium (13). The experiments were carried out in a chamber with artificial illumination of 8000 lux. To avoid mutual shadowing, the algal suspensions, in cylindrical glass recipients of 1000 ml, were bubbled with steady stream of air produced by vibrator devices. The culture medium was inoculated with an amount of algal biomass producing a 100 000 cells/ml suspension in all experiment variants. The ambient temperature varied between 24-25°C. The results represent average values of three repetitions for each variant.

The simazine herbicide was introduced in the culture medium before inoculation and the concentrations of the tested herbicide solutions varied from 0.02µM to 0.6µM.

The commercial product Simadon was used as a source of simazine. The Simadon herbicide has 50% active substance. The simazine belongs to Triazines chemical family and its molecular formula is C₁₅H₁₉N₃O₃. The solubility in water at 20°C for this herbicide is 5 mg/l (12).

The growth of algal was observed at each two days within the culture cycle (established at 16 days) with a "Cecil 1020" spectrophotometer at $\lambda = 676$ nm. A linear regression was performed in order to study the relationship between the extinction of the algal culture and the number of cells counted with Thoma haemocytometric mount for 24 samples from different treatments. The R squared was significant at a P value = 0.001 (Fig. 1). This relationship was used to convert the extinctions in number of cells.

The areas (A) encompassed under the graphs for the number of cells/ml (X) from each concentration (c) at different days (t) were calculated with the following formula:

* isolated at the Biology Research Center of Cluj-Napoca from marshes of Sălicea (13).

$$A_{(c, t_n)} = \sum_{i=0}^n (t_{i+1} - t_i) \times \frac{(x(c, t_i) + x(c, t_{i+1}))}{2}$$

for $t = 2$ and 14 days.

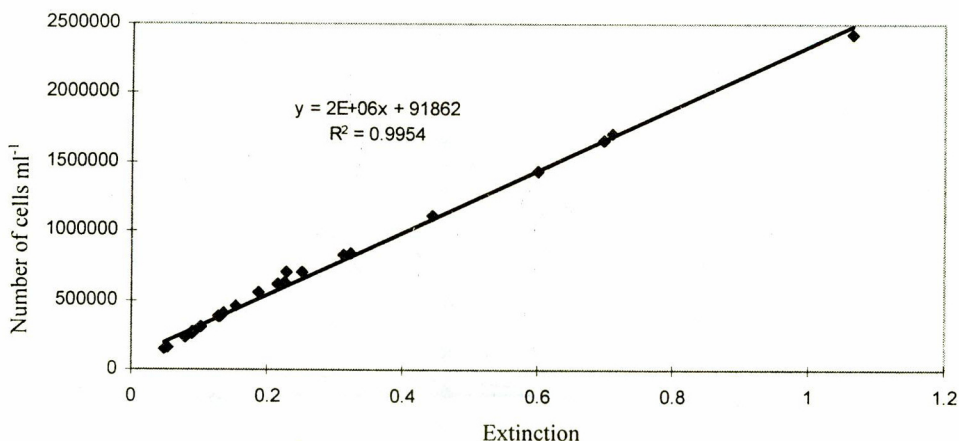


Fig. 1. – The relationship between extinction and the cells number/ml.

For each replicate, several simply non-linear regressions were performed between the reductions (% from control) of these areas (X values) and the doses of herbicide as Y values. The equation: $Y = a + bX^3$ was preferred because the confidence intervals were narrower than those obtained by other types of equations which produced better values for R^2 . The equations for each replicate were used to interpolate the LD 0, 25, 50, 75, 100% for these replications in order to calculate the standard deviations. The regressions for all the replicates from a given day were used to calculate a common R^2 . The interpolated values with these equations were similar to those obtained from replicates.

The photosynthesis rate was investigated with Warburg method using the treated algal suspension and the Warburg buffer solution number 9 (15 ml Na_2CO_3 0.1M + 85 ml NaHCO_3 0.1M) (4).

The respiration rate was established with Warburg method using the treated algal suspension and a solution of KOH 30% to capture the CO_2 resulted in the respiration process (4).

The absorbance of the solution extracted with 100% acetone was measured at 661.6, 644.8 and 470 nm and the calculation of the content in a,b chlorophylls and carotenoids was performed according to Lichtenthaler (1987) (9); the values were expressed in mg/g dry matter (DM).

RESULTS AND DISCUSSIONS

The effects of the different concentrations of the simazine herbicide on the growth of *Botryococcus braunii* alga were recorded (Fig. 2).

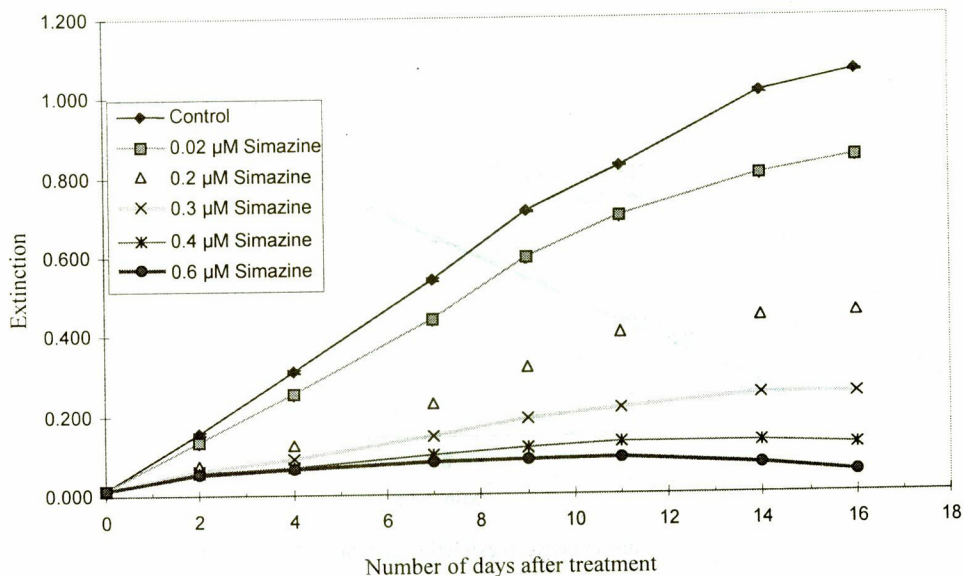


Fig. 2. – The influence of simazine on extinction of culture of *Botryococcus braunii*.

In general, for all concentrations, in the first growth period, relative low values from control were noticed. The concentration of 0.2μM simazine reduced the growth to 44.5%. The extinctions for the 0.02μM concentration were relatively close to control in the first four days of the culture cycle. Of course, the cell numbers from the volume unit of the algal suspension showed the same evolution.

Table 1

Simazine lethal doses (μM) for *Botryococcus braunii*

Death rate (%)	2 days		14 days	
	Average	SD*	Average	SD*
0	-0.016	0.010	0.027	0.011
25	0.032	0.011	0.039	0.011
50	0.364	0.015	0.125	0.010
75	1.267	0.033	0.359	0.008
100	3.026	0.070	0.814	0.004
R2 = 0.862			R2 = 0.937	

*) SD = standard deviation

The averages for the simazine lethal doses for 2 and 14 days, calculated for each replicate as described in "Materials and methods" are presented in Table 1. The calculated concentration for killing all the *Botryococcus braunii* cells in 2 days is somehow at the lower limit of the concentration of 2.4 - 4.8 μM reported in literature to control the unicellular algae in 3 - 14 days (7).

The photosynthesis intensity (established after the amount of oxygen released) showed a decrease from control at all concentrations (Fig. 3). The 0.02 μM concentration had no significant inhibitory activity on photosynthesis intensity, excepting the final observation (14 days after the beginning of the experiment).

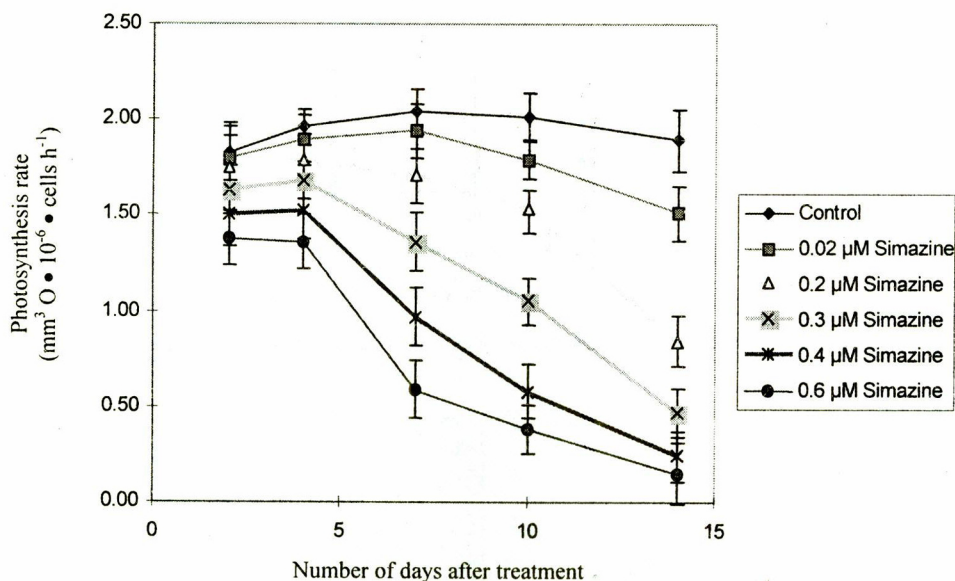


Fig. 3. – The influence of simazine on photosynthesis rate of *Botryococcus braunii*.

The simazine herbicide in concentrations of 0.2 μM to 0.6 μM produced an inhibitory effect on the oxygen absorption and concentration of 0.02 μM showed values relatively close to control in the first 10 days of the culture cycle. On an average the concentration of 0.2 μM simazine reduced the respiration to 41.7%. The effect of 0.6 μM simazine concentration on *Botryococcus braunii* respiration rate did not differ significantly from that of 0.4 μM concentration, but it was greater than the effect of 0.2 μM with more than two standard deviations (Fig. 4). A survey of mechanisms and modes of action of herbicides reveals that only few compounds are thought to interfere strongly with respiration (6, 8), but a hampering of another process may be indirectly reflected in respiration rate.

The assimilatory pigments content showed a decrease from control in all used concentrations, especially in the fourteenth day of the culture cycle (Fig. 5 a,

b, c). For the 0.2 μ M treatment, the assimilatory pigments content showed a decrease below 50% of control value after 14 days.

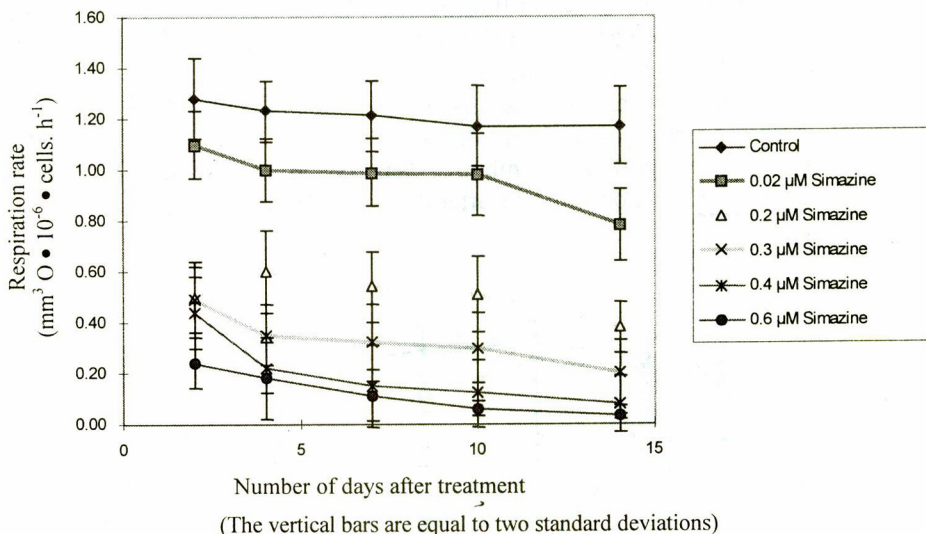


Fig. 4. – The influence of simazine on respiration rate of *Botryococcus braunii*.

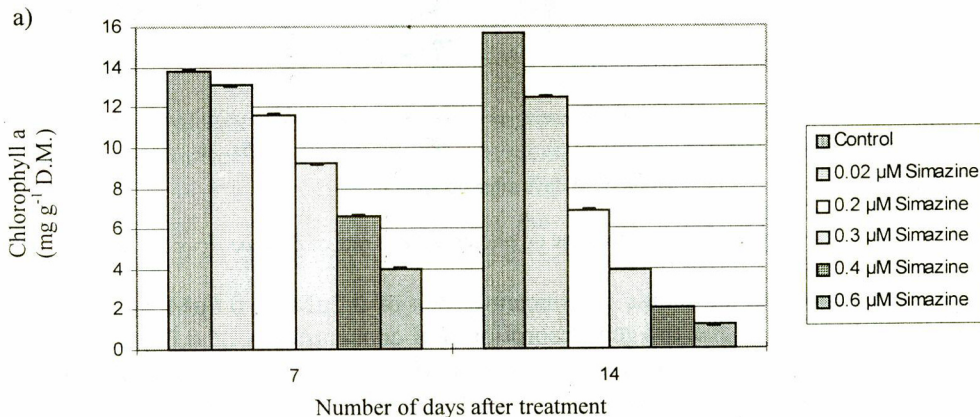


Fig. 5 a. – The influence of simazine on chlorophyll a content on *Botryococcus braunii*.

The simazine herbicide used in this experiment showed that for low concentrations (0.02 μ M) in the first growth period values relatively close to control were noticed. But soon, an obvious depression appeared in the evolution of the growth and of the cell numbers from the algal suspension at all used concentrations.

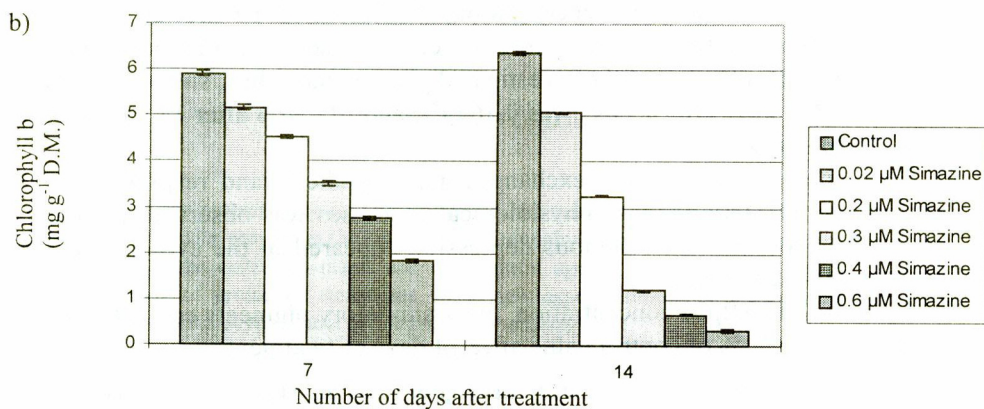


Fig. 5 b. - The influence of simazine on chlorophyll *b* content on *Botryococcus braunii*.

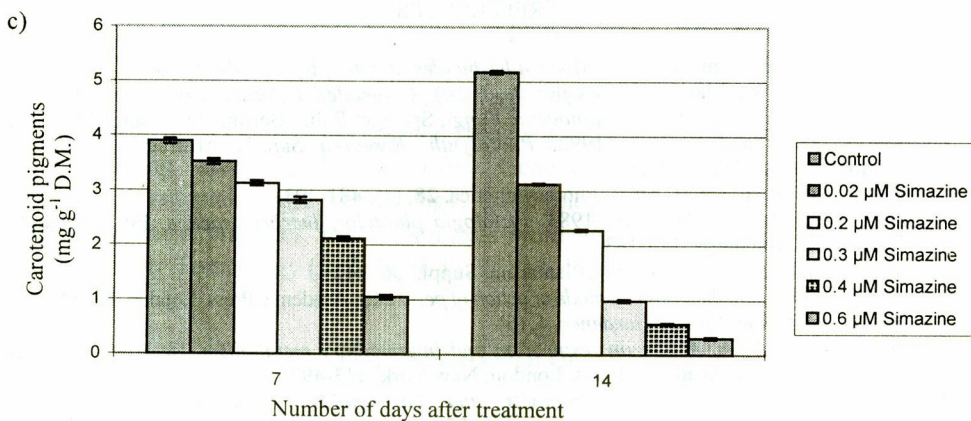


Fig. 5 c. - The influence of simazine on carotenoid pigments content on *Botryococcus braunii*.

The simazine herbicide in all used concentrations produced an inhibitory effect on the photosynthesis, oxygen absorption and assimilatory pigments content.

The *Botryococcus braunii* proved to be a suitable model for the study of the effect of simazine on the discussed physiological processes.

CONCLUSIONS

The following can be noted based on these investigation results regarding simazine herbicide influence on some physiological processes of *Botryococcus braunii* alga, in Zehnder-Gorham culture medium:

- in general, for low concentrations ($0.02\mu\text{M}$), in the first growth period, values relatively close to control were noticed. But soon, an obvious depression appeared in the evolution of the investigated phenomena. Thus, simazine is a toxic herbicide. (LD 50% after 2 days was $0.364\mu\text{M}$ and LD 50% after 14 days dropped at $0.125\mu\text{M}$ simazine);
- in regard of the gas exchanges, photosynthesis and respiration, a low level of the intensity of these physiological processes was observed. On the 14th day of the experiment, an obvious depression appeared in the evolution of both investigated phenomena;
- for the $0.2\mu\text{M}$ concentration, the assimilatory pigments content showed a decrease below 50% of control value after 14 days of treatment.

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CHRONOPHYTOPATHOLOGY

SIMONA APOSTOL

Crop protection being now integrated, the research is multidisciplinary but, as experimentally proved, at the same time Chronophysiology must be considered with regard to the cultivated plants and the harmful agents as well. For increasing the efficiency of protection treatments, these new aspects should be also known and applied for the development of *Chronophytopathology*.

Key words: chronophytopathology, cultivated plants, harmful agents.

In order to assure the humanity's food, the means to combat harmful organisms are almost as important as the agricultural ones: in the last years, researches in Phytopathology developed extended investigations on diseases and pests, by integrated management.

The problems of crop protection and pests combat are complex, and require a holistic treatment. As a consequence, the studies are multidisciplinary, many scientific branches being involved: Biochemistry, Genetics, Immunology, Microbiology, Mycology, Plant pathology, Nematology, Entomology, Biotechnology. To improve crop management practices, more chemicals were used, and thus the investigations must also imply Toxicology (5, 7, 8, 10, 11, 13, 14, 15, 16, 18, 19, 20, 21, 22, 24).

CHRONOPHYSIOLOGY

In order to develop the defence mechanisms against harmful organism or agricultural chemicals, the physiology of cultivated plants must be well known, but according to recent studies, Physiology should be regarded as Chronophysiology (1, 2, 4, 5).

Chronophysiology of plants

A phenomenon that presents great importance in Physiology is the "biological clock", which programs circadian oscillations of functions in organism, according to the light-temperature cycles of the environment. In plants it orders the abundance of certain photosynthetic components to be accumulated before the end of each dark phase, in anticipation of the imminent light phase. The fundamental

nature of this clock is not yet known (9). The molecular studies in the plant circadian system will be essential in elucidating the molecular architecture and its interaction with other receptors, notably the phytochrome, also in the metabolism and development in plants (17). The Biorhythmogenetics (6) development is also very important, as bioperiodicity has a genetic character, corresponding to the temporal organisation of species.

Chronophysiology must be understood not only as time importance in the succession of processes, but also as the "biological time" for each function, mainly as Rhythmophysiology. The rhythmicity of physiological functions was evidenced in the field and in laboratory experiments too.

As is known, water is an important component of all plant organs. Some authors (10) consider that the sensibility to maladies is increasing with the plant water content. Also, the water stress, the water status of plants, especially the leaf water content are mostly studied and the importance of tissular water rhythms in plants culture was proved. Generally, it is appreciated that the water potential of the leaf and the relative water content decrease with maturity, but, for example, in different sorts of apples and in varied orchards, cultivated "classically" or "intensively", diurnal and seasonal water tissue biorhythms in apple tree leaves were recorded (1, 2, 4). As to the photosynthetic rate, this is maximum before noon and decreases towards evening, diurnal changes in photosynthesis at cultivated plants being evidenced (24).

Chronophysiology of harmful organisms

Like other organisms, harmful bacteria, fungi or insects have certain biorhythms of their physiological functions, and the knowledge about their display can be useful for combating the plants diseases, for interventions in the most sensible phases.

As an example, for one of the most frequent cryptogamic agents, *Venturia inaequalis* (Cke.) Wint. Syn *Endostigme inaequalis* (Cke.) Syd, also known under the name of "apple scab", the vital cycle is obviously seasonally differenced – winter form is the perithecium, in March is the vegetation phase (dicaryophase), the diplophase begins in April, the haplophase occurring in August. Plasmogamy and karyogamy are distanced in time and space. Perithecium differentiates in autumn, following the sexual process in the attacked leaves and arriving at maturity in the next spring; while the projection of ascospores begins in March. Thus, in the evolutive cycle, the parasite form (asexuate) alternates with the saprophyte form (in the hibernal pause of the trees) (8, 10, 13, 19, 21). Moreover, recently (3) it was discovered the existence of the circadian biorhythms at the same time. For example, it was recorded the diurnal biorhythm of the delivery of ascospores from perithecium at different sorts of apples, the top of the diurnal rhythm being placed at 6 p.m. (following the top of temperature's diurnal rhythms). In the seasonal biorhythms the maximum values were recorded on April 23-24 (3, 13, 19). These aspects have a

great practical importance, because the number of ascospores is a main bioindicator for advertisements in opening the pesticide application campaign.

Regarding the insects, different biorhythms were also recorded, photoperiod being the primary environmental regulator of diapause induction. For example, *Cydia pomonella* is a typical long-day insect, it reproduces and develops in long days and enters diapause in response to short days. Such information can be used in a decision-making model for timing the latest chemical treatment against the pest, taking into account that larva which enters diapause, leaves the agroecosystem for the current season (23). The many-years biorhythms must be also considered.

CHRONOPATHOLOGY

In Phytopathology, the knowledge of the developmental rhythms of parasites is very important, correlated with those of the host-organisms, their modifications resulting from their interactions in different phases of the cycles. Thus, researches should include the pathogenesis, the disease cycle and the epidemiology cycle. The developing parasite rhythm must agree with the host plant rhythms – if the plants exceed their receptive phase being in a resisting one, the results are different (19).

For apple leaves, the presence of *Venturia* is manifested primarily in the reduced amplitude of the tissular water rhythm, but in the early morning hours the amplitude is increased, exceeding the normal levels. The water content biorhythm of plants reflects the state of health, being modified in the diseased plants. Similar aspects were registered for the diurnal normal biorhythms of the photosynthetic pigments and their modifications caused by *Venturia inaequalis* Wint. Analysing the situation from the pathological physiology point of view, we understand that the water content in the leaves tissue is modified upon the action of the parasites, that is, of their vital cycle (micro and macrosporogenesis) (2).

The virulence and aggressiveness of the parasites are due to their property to secrete enzymes and toxins, but it is known at present that every biochemical indicator has an established circadian rhythm. The parasites can be synchronical or asynchronical with the phases in the host plants biorhythms, the results being thus different. As was determined (2, 12), in the parasited leaves, the different biorhythms modify their amplitude and even a phase desynchronization occurs. Studies regarding the molecular interactions can state the plant resistance mechanisms (22).

CHEMICAL CONTROL AND CHRONOTOXICOLOGY

The intensive use of chemicals in agriculture determined noxious secondary effects, also the harmful organisms became resistant to the insecticides applied during the past three and a half decades (14).

Also, the chemical agents (pesticides) like biological pathogen agents exert clear influences, modifying the normal biochemical and physiological biorhythms in plant organs (4, 7, 11, 16).

The application of pesticides at certain hours, that is at certain biological sensible phases of the harmful organisms, can assure the efficiency of treatments. The investigations regarding insect's chronotoxicology state the changes of the resistance degree, as a significant percentage of mortality in larval, pupal or adult stages, due to their specific "biological time", in view to apply the pesticides at most suitable time. In experiments with aphids, I evidenced these new concepts, and thus Biorhythmotherapy in Pomiculture developed.

At the same time, we must consider the fact that the chemicals used for treatment can change the normal rhythmicity of the functions in all the organisms involved.

CROP PROTECTION PERSPECTIVES

For the development of crop protection studies according to the new discoveries in other scientific domains, many interesting actions were proposed, such as the use of polyclonal antisera in plant bacteriology, genetic engineering in the tissue culture methodology, incorporation of genetic resistance in host species, regeneration of infected plants (20, 22, 23, 24). Regarding all these new domains, I think that Biorhythmogenetics and mainly the new concept of "starting time" (6) can have great applications, the validity of this concept being experimentally proved in chronotoxicological genetical studies at *Pisum sativum* L. (7).

Also, in my view the development of Biorhythmotechnology can be very useful for agriculture and plant protection – the programmes should include rhythmical investigations, evidencing the deploy of defence mechanisms in plants, for identifying, monitoring and controlling a variety of plant diseases and pests as well as harmful agricultural chemicals. The hours for determinations must be observed for a correct analysis and comparison.

The importance of Chronophytopathology is supposed to increase, as agriculture is developing intensively. Thus, biorhythmological studies in all agriculture domains, with a view to efficient rational and scientific combat of the harmful organisms are proposed. Useful for short or long time echophysiological prognosis, the knowledge and the biorhythmological laws must be considered.

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ONE HUNDRED YEARS SINCE THE DISCOVERY OF THE DOUBLE FERTILIZATION OF ANGIOSPERMS

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Double fertilization to angiosperms was independently discovered by Navashin (1898) and Guignard (1899), with a priority for the first, although his paper published in Russia was known by the botanists after the one published by Guignard, a reason for which in most of the specialised treatises the image of the double fertilization to *Lilium martagon* is presented after the paper of Guignard.

Key words: double fertilization, angiosperms.

Today it is widely recognised by all botanists that the angiosperms have a double fertilization, a phenomena discovered at the end of the 19th century (1898-1899) by Navashin and Guignard independently. During this process one of the spermatic nuclei (male gametes) gets into the egg and unites with its nucleus resulting the main diploid zygote (2n), the first cell of a new sporophyte; the second spermatic nucleus gets united with the polar nuclei, sometimes already fused of the embryo sac, resulting the accessory triploid zygote (3n), in the case of the monosporic embryo sac (*Polygonum* type), or pentaploid (5n), in the case of the embryo tetrasporic sac (*Fritillaria*, *Lilium* type). Out of the diploid zygote, through repeated divisions, the proembryo will result and then the embryo, out of which a new plant will result. Out of the accessory zygote (3n, 5n), through repeated divisions, the albumen (the secondary endosperm) will result with a role in the embryo feeding.

The biological importance of the double fertilization lies in the fact that during process there are formed not only the double heredity zygote (maternal and paternal) but also the albumen with double origin and mixed characters (from two gametes). It is considered that this fact has increased both the viability and the adaptation capacity of the angiosperms to the most varied existence conditions.

One century after this important discovery the botanists' opinions are still divided concerning the priority of its author.

In Volume III of the collection "Biologie végétale" (Champagnat, Ozenda, Baillaud, 1969) it is shown that the double fertilization was first observed by Guignard (1889) to *Lepidium* and by Navashin (1890) to *Lilium* and *Helianthus*; it

was observed to some other angiosperms among *Liliaceae*, *Orchidaceae* and *Ranunculaceae* families; approximately at the middle of the 20th century, the double fertilization was generalized to plants belonging to the most diverse groups (monocotyledons and dicotyledons).

Today it is said that even though it cannot be universal, it represents at least the normal case of angiosperms, as stated by some Romanian authors (Natalia Rădulescu-Mitroiu, 1976), with the distinction that this botanist gives priority to Navashin in the discovery of the double fertilization in 1898 at *Fritillaria tenella*, sustaining that almost simultaneously Guignard has observed this phenomenon to *Lilium martagon* in 1899.

Immediately after the publication, Goebel (1899) reviewed L. Guignard's paper "Sur les anthérozoïdes et la double copulation sexuelle chez les végétaux angiospermes" in the prestigious German magazine "Flora", showing that: "Guignard is studying the double fertilization to *Lilium martagon*, explaining that the first gamete is fecundating the egg (this being a sexual fertilization), and the second one is fecundating the secondary nucleus of the embryo sac, which nucleus is considered to be vegetative and this is why this second fertilization is considered to be a "pseudofertilization". It is surprising that Goebel didn't know yet the paper published by S. Navashin in 1898 in "Izvestia Akademii Nauk Rosii". More careful, although without specifying the discoverers, are Demalsy and Feller-Demalsy (1990) who had mentioned that the process of double fertilization was discovered in 1898.

Most botanists underline Navashin's priority in the discovery of the double fertilization in 1898. We are referring, first of all, to Bonnier and Leclerc du Sablon (1905) who had written in their "Botany" book that Navashin had discovered the antherozoids at angiosperms; this author and Guignard have demonstrated the role of the male gametes after their penetration in the embryo sac.

Maheshwari (1950), talking about the double fertilization refers to the studies of Navashin (1898) to *Lilium martagon* and *Fritillaria tenella*; a few months later (1899), Guignard was confirming the discovery of this process, working independently upon *Lilium martagon*.

Grințescu (1928-1934, 1985), referring to the double fertilization said that it was observed for the first time by Navashin (1898) and Guignard (1899), adding that Strasburger considers the fusion of the second male gamet with secondary nucleus of the embryo sac as a "vegetative fertilization". Also in the Romanian literature, Andrei (1978), Șerbănescu-Jitariu and Toma (1980) mention that the double fertilization (a general phenomenon with angiosperms) was discovered by S.G. Navashin (1898) and confirmed one year later (1899) by L. Guignard. Thus the process of the double fertilization was discovered by Navashin in 1898 and confirmed by Guignard in 1899 both botanists making independent researches upon the same species: *Lilium martagon*; the first one had also worked with the species

Fritillaria tenella; this is why, in agreement with several other authors, we think that we did not make any mistake saying that the double fertilization to angiosperms was discovered in 1898-1899, independently, by the Russian researcher S.G. Navashin and by the French one L. Guignard with priority of the first in the discovery of this important process which characterizes almost all angiosperms.

In what follows we shall make a more detailed presentation of the discovery of the double fertilization by the Russian botanist S.G. Navashin who was professor at Kiev University and a well-known researcher in the fields of anatomy, embryology and vegetal cytology. This great botanist had held at the 10th Congress of the Naturalists and Physicians of Russia on the 24th of the August 1898 the paper "New Observation Upon the Fertilization of *Fritillaria tenella* and *Lilium martagon*". In November of the same year the paper was published in German ("Resultate einer Revision der Befruchtungsorgane bei *Lilium martagon* und *Fritillaria tenella*) in Bull. Acad. Imp. des Sci. de Petersburg, t. IX, 189 : 377-382 (Izvestia Akademii Nauk Rosii).

The report of this scientific event was published in "Dvenik" Szieda, and in nr. 6, page 164 it said: "Professor S.G. Navashin had mentioned that the cell tyres of the ovular apparatus, eventually developed over the whole surface don't keep their initial status because before entering the pollen tube in the embryo sac, they suffer a total resorption in their apical part, a fact which was not observed previously. In the protoplasm of the embryo sac are entering two identical fusiform mobile male cells. One of the sperms gets into the ovule (egg), and the second one unites with one of the two polar nuclei of the central cell of the embryo sac".

At the same Congress, commenting on S. G. Navashin's paper, the embryologist E. Beleaeu shows that the discovery of the double fertilization will totally change the general opinion about the process and confirms one of the common features of the angiosperms.

The discovery of the double fertilization in angiosperms has drawn the attention of the scientific world and first of all of the academic circle of the Russian Imperial Academy. Among the first advocates of the new discovery we can mention the academicians M.S. Voronin and A.S. Fomintin who in a report concerning S.G. Navashin's paper "ob oplodotvorenii u slojnotvetnyh i orhidnyh" presented on the 23rd of September 1898 at the Naturalists Society of Sankt Petersburg (see "Trudy", t. 29, vyp. I, the opening session of the botanical section, nr. 5 : 166-167) had mentioned that S.G. Navashin's observations were contrary to the ones made by L. Guignard, E. Overton and D. Mottier.

S.G. Navashin proved the following: 1. Out of the pollen tube get into the embryo sac not one but two sperms. 2. These cells are identical and worm like. 3. One of the two sperms gets into the egg and the second one gets united with the superior polar nucleus of the central cell of the embryo sac. 4. After that the two nuclei emerge and after the fertilization with the male gamete it results the

accessory zygote which is at the origin of the albumen formation (the secondary endosperm). 5. This, the albumen proceeds, as the embryo, following a sexual process; the first one results from the divisions of the main zygote and the second one from the divisions of the accessory zygote. 6. In other words, following the process of double fertilization is results a normal embryo and an "embryo" (albumen) with an abnormal development. 7. The double fertilization remains forever one of the most specific features of the angiosperms.

We have mentioned the "double fertilization" but this notion was finalized later in the article "Ob oplodotvorenii u slojtovetnyh i orhidnyh" published by S.G. Navashin in the magazine "Isvestia Imp. Akad. Nauk Rosii", t. XIII, nr. 3 : 335-340, 1900.

But as I wrote at the beginning of this article, Navashin's discovery of the double fertilization (1898) didn't receive immediate and irrevocable "green light" from the botanists, as it usually happens with most of the discoveries.

One year later (1899), more precisely five months later, it appeared the article "Sur les anthérozoïdes et la double copulation sexuelle chez les végétaux angiospermes" published by L. Guignard in the magazine "Rev. gén. de Bot.", t. 11 : 129-135. This article was immediately reviewed by the well-known German botanist Goebel in the magazine "Flora", t. 86, p. 235, 1899, which determines some authors to consider that L. Guinard has the priority of the double fertilizations discovery; this author considers the fecundation of the secondary nucleus of the central cell as a "pseudofecundation" because it is a vegetative one; based on this idea, the well-known German botanist Strasburger calls it vegetative fecundation (vegetative Befruchtung).

Based on the above our conclusion is that the double fertilization to angiosperms was independently discovered by Navashin (1898) and Guignard (1899) with a priority for the first, although his paper published in Russia was known by the botanists after the one published by Guignard, a reason for which in most of the specialised treatises the image of the double fertilization to *Lilium martagon* is presented after the paper of Guignard (8, 17).

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