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SOMMAIRE

A CYTOTAXONOMIC STUDY OF SOME MEMBERS OF THE TRIBE *OCIMOIDEAE (LABIATAE)*

BY

SIMA BHATTACHARYA
(Nee' Pal)

A detailed karyological analysis of fifteen taxa belonging to six genera of the tribe Ocimoideae under Labiatae has been carried out in order to get a comprehensive idea about the karyosystematic relationship and the mechanism of speciation among the members of the tribe. The present investigation showed that several chromosome numbers exist within the tribe. But in spite of the difference in chromosome numbers, it is quite obvious that there exists a relationship between the different genera and species as evidenced by their karyotypes and frequent occurrence of intraspecific or interspecific variations. Different degrees of polyploidy and structural alteration of chromosomes are found prevalently in this tribe and, therefore, their role in the mechanism of speciation is also obvious. The two subtribes are found to be with distinct prevalent chromosome numbers and their inclusion into two separate subtribes is justified on this basis, too.

The present investigation is a further extension of cytotaxonomical research made earlier by Garcia [13], Quézel [25], Morton [22], Bose and Choudhury [5], Bhattacharya [3], Pal [23], [24] on a few species and varieties of the genera belonging to the tribe *Ocimoideae* of the family *Labiatae*. Some other authors like Golubinski [15], [16], Takagi [27], Lapin [19], Vaarama [28], Mehra and Gill [21] and Vij and Kashyap [29] reported only the chromosome numbers of a few taxa of this tribe; the karyotype and cytotaxonomic analysis of the members of this tribe are still fragmentary approaches.

Members of this tribe are well known for their economic importance within the family; further thorough cytotaxonomic studies will, no doubt, provide an understanding of the phylogenetic relationships and means of speciation within the tribe. Moreover, the tribe is widely distributed throughout the world. Therefore, it is worthwhile collecting as much cytological information on various species and varieties as possible to use it in studies of phylogeny and hybridization.

MATERIALS AND METHODS

Twelve species and three cytotypes belonging to six genera of the tribe were collected mostly from the wild populations of Bengal, some parts of the Himalayas and the south of India. Seeds of *Plectranthus glaucocalyx* and *P. purpuratus* were obtained from Jardin Botanique de l'Université, France. Most of the collected plants were grown in earthen-

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ware pots in the experimental gardens of the Department of Botany, University of Calcutta. They were identified by courtesy of the herbarium authorities in the National Botanical Gardens, Sibpore.

In order to obtain scattered somatic chromosomes, chilled saturated aqueous solution of paradichlorobenzene and aesculine kept for one and a half hour at 10—12°C was found to be satisfactory. The root tips were then fixed in an acetic acid-ethyl alcohol (1 : 2) mixture for ten to fifteen minutes and were stained with aceto-orcein and subsequently squashed in 45% acetic acid.

For the study of meiotic chromosomes, flower buds were fixed in an acetic acid-ethyl alcohol mixture (1 : 2) for 24 hours and anthers were smeared in 1% aceto-carmine solution.

The figures were drawn at a table magnification of approximately ×2850 using Leitz microscope with an eye piece of 20 × and oil immersion objective with 1.3 N.A. and aplantic condenser. In the figures the chromosomes with secondary constrictions are outlined. Photographs of both somatic and meiotic plates were taken from temporary squash preparations at different magnifications and were suitably enlarged.

OBSERVATION

The different members under investigation show chromosome numbers ranging from 2n = 22 (*Plectranthus* sp.) to 2n = 44 (*O. gratissimum*). In general, the chromosomes are short ranging between 0.7—2.4 μ. There are size differences between the chromosomes of the different species and also among the chromosomes of the same karyotype. The types of chromosomes within the karyotype are denoted with reference to the previous classification considered by Bhattacharya [4] for the other members of the family. In addition to the primary constriction the types A, B, C are the bearers of secondary constriction or satellite. The type D is characterised by submedian-to-subterminal primary constriction and type E by median-to-nearly median constriction.

Details of karyotypes and chromosome numbers are summarised in Table 1. Members of the tribe are arranged according to Bentham and Hooker's system of classification [2].

The number written in parenthesis denotes the varied chromosome number observed in addition to the usual one in the same individual.

DISCUSSION

Among the fifteen taxa studied here, fourteen belong to the subtribe *Euocimeae* and one to the other subtribe *Lavanduleae* in Bentham and Hooker's system of classification.

The previous reports on the chromosome number in the genus *Ocimum* reveal not only a varying chromosome number but also the existance of both intra- and interspecific polyploidy. The chromosome number ranges from a haploid number n = 11 to n = 19 though n = 16 appears comparatively to prevail. In *O. sanctum*, *O. gratissimum* and *O. americanum* the present investigation reveals an intraspecific aneuploidy.

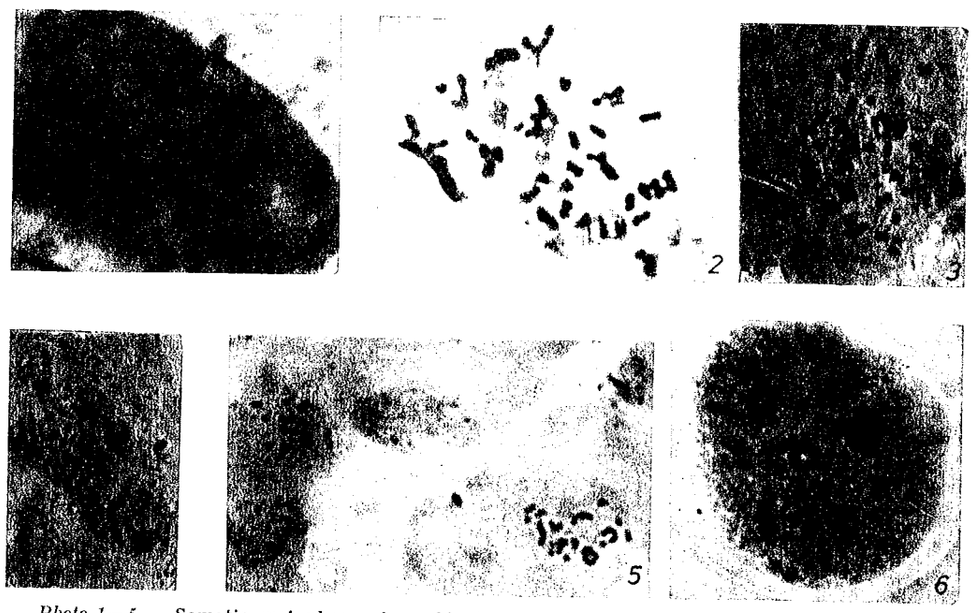

Photo 1—5. — Somatic metaphases 2n = 26, 2n = 44, 2n = 36, 2n = 36 and 2n = 22 chromosomes in *O. americanum* (Pop-I) ×1425, *O. gratissimum* ×1710, *O. sanctum* (Pigmented Type) X950, *O. sanctum* (Green Type) ×750, *Plectranthus* sp. ×750, respectively.

Photo 6. — n = 13 bivalents at diakinesis in *O. americanum* (Pop-I)X1531.

Fig. 1

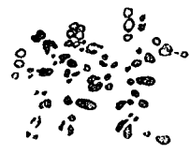

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

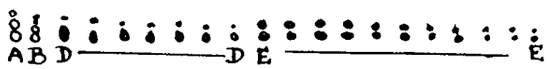

Fig. 1a

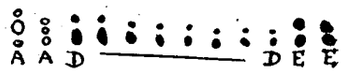

Fig. 2a

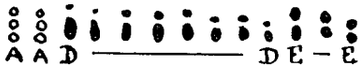

Fig. 3a

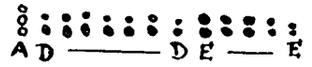

Fig. 4a

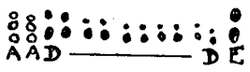

Fig. 5a

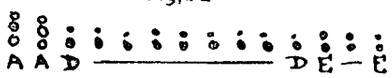

Fig. 6a

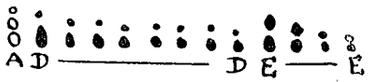

Fig. 8

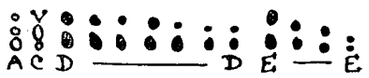

Fig. 9

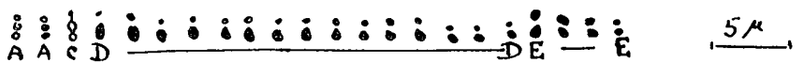

Fig. 10

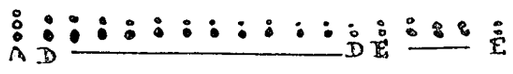

Fig. 11

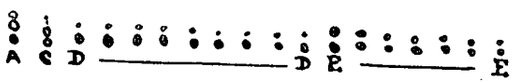

Fig. 12

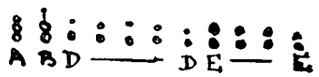

Fig. 13

Table 1

Details of karyotypes and chromosome numbers of different members of *Ocimoideae*

Name of the species	Chromosome number & type	Range in chromosome length (μ)	Total chromatin length in haploid complements (μ)
Tribe : *Ocimoideae*			
Subtribus : *Eucimeae*			
1. *Ocimum americanum* L. (Pop-I)	2n=26 ; A2+D16+E8 ; (Fig. 8 ; Photo 1,6) ; n=13, (16)	1.05—2.4	21
2. *O. americanum* L. (Pop-II)	2n=26 ; A2+C2+D14+E8 ; (Fig. 9) (2n=28) ; n=13, (II, 14, 17)	1.05—2.4	25.5
3. *O. gratissimum* L.	2n=44 ; A4+C2+D30+E8 ; (Fig. 10) ; (2n=34, 40, 46) ; n=22, (20, 21)	0.7—1.7	26
4. *O. sanctum* L. (Pigmented type)	2n=36 ; A2+D24+E10 ; (Fig. 11) ; (Photo 2) (2n=32) ; n=18, (16, 17) Photo 3)	0.7—1.7	19.5
5. *O. sanctum* L. (Green type)	2n=36 ; A2+C2+D18+E14 ; (Fig. 12) (2n=32) ; n=18 (Photo 4)	0.7—1.7	22
6. *Moschosma polystachyum* Benth.	2n=40 ; A2+B2+D14+E22 : (Fig. 1,1a)	0.7—1.7	22
7. *Orthosiphon rubicundus* Benth.	n=14, (13, 16), (Fig. 7)	—	—
8. *Plectranthus coetsa* Ham. (Type I)	2n=24 ; A4+D16+E4 ; (Fig. 2,2 a) (2n=26) ; n=12, (13)	1.4—2.2	18
9. *P. coetsa* Ham. (Type II)	n=13	—	—
10. *Plectranthus* sp.	2n=22 ; A2+B2+D10+E8 ; (2n=42, 44) (Fig. 13) (Photo 5)	0.8—2.1	15
11. *P. gerardianus* Benth.	2n=26 ; A4+D16+E6 ; n=13 (Figs 3, 3a)	1.05—2.4	23.5
12. *P. glaucocalyx* Max.	2n=26 ; A4+D20+E2 (Fig. 5, 5a)	0.8—2.1	17.5
13. *P. purpuratus* Harv.	2n=26 ; A2+D14+E10 ; (2n=36) (Fig. 4, 4a)	0.8—1.8	17
14. *Hyptis suaveolens* Poit.	2n=28 ; A4+D18+E6 (Fig. 6,6 a) n=14, (16)	0.8—1.9	17.5
Subtribus 2 : *Lavanduleae*			
15. *Lavandula burmanni* Benth.	n=18	—	—

Such wide aneuploid variation in the species suggests that the different chromosome numbers in the genus are all related and have been derived from each other. Under such circumstances it is difficult to work out the basic chromosome number in the genus even though in all probability it appears to be 8.

Figs 1—6a. — Karyotypes and idiograms of *Moschosma polystachyum* (2n=40) ; *Plectranthus coetsa* (Type I) (2n = 24) ; *P. gerardianus* (2n = 26) ; *P. purpuratus* (2n = 26) ; *P. glaucocalyx* (2n = 26) ; *Hyptis suaveolens* (2n = 28).

Fig. 7. — Metaphase 1 showing n = 14 bivalents in *Orthosiphon rubicundus*.

Figs 8—13. — Idiograms of *O. americanum* (Pop-I) (2n = 26) ; *O. americanum* (Pop-II) (2n = 26) ; *O. gratissimum* (2n = 44) ; *O. sanctum* (Pigmented type) (2n = 36) ; *O. sanctum* (Green type) (2n = 36) ; *Plectranthus* sp. (2n = 22).

It is remarkable that intraspecific variation as well as existence of cytotypes have been found to be mostly prevalent in India as reported in the present investigation. Such a situation may be explained in view of the diversity of adjacent climatic zones, specially in the Himalayas. It is likely that the wide ecological diversity has proved the successful survival of species adapted to such conditions. In any case, both aneuploidy and polyploidy may be considered to have played an important role in the evolution of species and genera.

Intra-individual variations in the chromosomes of *Ocimum sanctum* studied here have also been recorded. Such variations have been noted to occur both in the somatic and the meiotic cells. The difference in meiotic cells may either represent the continuation of a somatic variation in the germinal line or the absence of such somatic variation, may be due to premeiotic disturbances in chromosome behaviour. Such alteration in the pollen mother cells are expected to give rise to gametes with varying chromosome number, and hence the origin of aneuploids. The existence of aneuploids in nature may suggest that such irregular gametes remain viable and enter into successful fertilization.

A study of the karyotypes in different species of *Ocimum* in the present investigation shows that the chromosomes follow a common pattern in the morphology. The karyotype is graded and the chromosomes are mostly provided with median-to-submedian primary constriction. The size, too, varies from medium-to-small. Even though there is a similarity in the chromosome size, *O. americanum* collected from two different parts of India have comparatively long chromosomes in relation to the chromosome number as against those of other species of *Ocimum*. The total chromatin length in these taxa varies between 19.5 μ and 26 μ whereas the chromosome number from n=13 to n=22. This indicates that their total chromatin matter has not changed so much during speciation as the chromosome number. This shows, no doubt, a homogeneity within the genus. Darlington [10] and Stebbins [26] have shown how the basic chromosome number can be increased or decreased by chromosomal shifting and by translocation within a genome. Such phenomenon might have taken place during speciation in these taxa, too.

In spite of the basic uniformity in the general features of the karyotype, detailed chromosome morphology differs slightly from one species to another indicating that structural alterations of chromosomes have also played an important role in evolution.

In *Ocimum americanum* a secondary association of bivalents during meiosis has been recorded. Such associations are often claimed [9] as representing residual homology of chromosomes which in the course of evolution becomes somehow separated from one another. On the basis of the least grouping the basic number of chromosomes in *Ocimum* is indicated to be 4 and the present status is the result of amphidiploidy and subsequent changes. However, it is rather difficult to determine the basic number and it is not possible to ascertain the basic number on the ground of such association in one species only. The importance of amphidiploidy in the evolution of the genus is quite distinct.

Bhattacharya [3] suggested that species with a low number and large chromosomes, so far unexplored, might have been the progenitor

of other species of *Ocimum*. Moreover, its status in a different subfamily as assigned by Engler and Prantl was vindicated [12].

Moschosma is another genus of the same subtribe *Euocimeae* which has been investigated here for the first time. The chromosome number is quite high being 2n = 40. With only one report on chromosome number by Takagi [27] in *M. riparium* (2n = 30), it is difficult to ascertain its status though the chromosomes have the same pattern as that of other species of the subtribe. The chromosome number is, however, rather unusual for the subtribe, even though n = 20 chromosomes have been recorded in the aneuploid individuals of *O. gratissimum*. The total chromatin length (22 μ) of this taxon is, however, indicative of homogeneity with the species of *Ocimum*. One of the important characteristics of *Moschosma polystachyum* is the absence of any anther in the individuals cultivated in the Indian Botanical Garden, Sibpore. Absence of stamens may be due to remote hybridity as already claimed for similar conditions by *Mentha* [1]. The propagation is entirely vegetative. It would be worthwhile to investigate later the reason for the asexuality in this genus.

Only one species of *Orthosiphon* has been studied during the present investigation showing clearly 14 bivalents. All the other species of this genus studied so far, except *O. pallidus* contain the same chromosome number. This fact is no doubt indicative of the homogeneity of the genus. The chromosome number, too, is quite common for the subtribe. The occurrence of 2n = 24 chromosomes in *O. pallidus* [7] may suggest a derivation. The variant nuclei as found in few pollen mother cells in the present investigation may arise out of premeiotic disturbances or out of persistence of somatic variations up to germinal line. The role of such variant nuclei in the development of aneuploids is immense.

Five species of the genus *Plectranthus* have been studied here. Of these one species shows 2n = 22 chromosomes and *P. coetsa*, two populations (Type I and Type II) from different areas, show differences in external morphological characteristics and a different chromosome number. The somatic nuclear number 2n = 26 appears to be present in *P. gerardianus*, *P. glaucocalyx* and *P. purpuratus*. A survey of the chromosome number in different species of *Plectranthus* shows that multiples of 7 chromosomes are mostly prevaling, the other numbers noted being n = 11, 12, 13, 15 and occasionally 17. Similar to *Ocimum*, in this genus, too, intraspecific variations were recorded in the present investigation as well as in Morton's (1962). Occurrence of stray polyploid cells along with normal ones may be the case of the endopolyploidy recently reported by Georgi [14] in *Phaseolus vulgaris*. A similar case has been observed by the author in the study of DNA synthesis and karyotype in *Ph. vulgaris* L. (Unpublished). As such, the relationship between different numbers is quite clear.

The karyotype analysis, carried out in five species of *Plectranthus*, indicates their similarities, even though minute karyotype differences are present. Such differences bear clear evidence of the role of structural alterations of chromosomes in evolution. Total chromatin lengths found in these taxa are not highly variable (see Table 1), indicating thereby the homogeneity of the taxa within the genus. On the basis of the cytological data available on this genus, the present diversification of the

taxon seems to derive from a set of 7 chromosomes. In *P. gerardianus* and *P. coetsa* a secondary association of bivalents during meiosis was noted and the least grouping (i.e., maximum association) was found to be 7. If secondary association of bivalents is to be regarded as a criterion for working out the basic number of the species, then 7, no doubt, becomes the basic number of the genus. Occasionally, however, in a few pollen mother cells some indications of even lower number exist but these need confirmation. In any case, whatever the primary basic number, the amphidiploid origin of the species and the present straying of the genus from 7 chromosomes, whether primary or secondary, is quite evident. No doubt, considerable amount of structural alterations of chromosomes and sometimes numerical alterations, too, have taken place in the course of evolution following amphidiploidy.

The genus *Hyptis* of the same subtribe has been investigated by different authors and so far 7 species only are studied [11], [22], [8], [17]. Except for one report in *H. capitata* (n = 15) by Chuang *et al.*, the chromosome number of the other species is a multiple either of 14 or 16. The chromosome number of *H. capitata* might have originated either in the hybridization of the species with 2n = 28 and 2n = 32 chromosomes or might be an aneuploid derivation of these chromosomes.

In *H. suaveolens* intraspecific variations have been noted and individuals with either 2n = 28 or 2n = 32 chromosomes are on record. In the present investigation, 2n = 28 chromosomes were found only in meiotic cells both n = 14 and n = 16 bivalents were observed. Such irregularities may naturally originate in premeiotic disturbances. However, the occurrence of such pollen mother cells with varying chromosome numbers may lead ultimately to the production of plants with different chromosome numbers. It is very likely that species with different chromosome numbers in *H. suaveolens* might have had their origin in the successful fertilization of such abnormal gametes.

The karyotype of this species is also graded with medium-to-short chromosomes, the number of secondary constrictions in the haploid complement being only two. The total chromatin length of this species is 17.5 μ and similar to *P. glaucocalyx*. Moreover, the nature of the karyotype and the chromosome number do not differ widely from that of this species, too, justifying its systematic status in this subtribe.

In order to assess whether this species originates in *P. glaucocalyx* or in other allied species of *Plectranthus*, further cytogenetical study is needed. The very fact that 28 and 32 chromosomes have been observed in the same species, *H. suaveolens* certainly indicates a close relationship; the origin of one from the other is thus indicated. Such abnormal number may originate in non-disjunction. It is likely that the genus *Hyptis*, like most other genera of the subtribe Euocimeae, has the ancestral number x = 7 which ultimately has given rise to the other number in the course of evolution.

Another genus of the same tribe, Ocimoideae, is *Lavandula* which, no doubt, belongs to another subtribe, Lavanduleae. A large number of species of this genus have so far been studied, specially because of their commercial importance. In this genus, the chromosome number so far reported is a multiple of 9, 11, 12, 15 and 25, of which, however, multiples

of 9 appear to be comparatively prevalent. The chromosome number of *Lavandula burmanni* has been reported for the first time as showing n = 18 bivalents. It is rather difficult to ascertain clearly the basic set in *Lavandula* which may either be 6 [6] or 9 and the other numbers may be polyploid derivations of the same. Varietal difference in the chromosome number have been observed e.g., in *L. vera* [20], (Janaki Ammal, unpublished) and intraspecific variation has been recorded in *L. lanata* [13], [18]. These facts further indicate that the different numbers are related and might have diversified from a common point. It appears also that in this genus polyploidy and numerical alterations of chromosomes have quite an effective role in evolution. The prevalent chromosome number is also rather different from that of the subtribe Euocimeae, as such its inclusion under different subtribes based on other taxonomic considerations is also justified on cytological grounds.

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RELATIONS D'INTERDÉPENDANCE ENTRE LES ESPÈCES, LES PHYTOCÉNOSES ET LE SUBSTRAT DANS LES MARÉCAGES OLIGO- ET EUTROPHIQUES

PAR

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Dans cet ouvrage on essaie de mettre en évidence la dépendance entre les constituants chimiques N, P, K, Ca des plantes et le substrat (tourbe) dans les marais oligotrophe et les marais bas. On constate un rapport direct pour les éléments N (total) et Ca, et non pour P et K considérés comme facteurs limitatifs dans la tourbe oligotrophe. A certaines phytocénoses, l'on trouve des différences significatives, si l'on rapporte les cendres (brutes) de l'azote et du calcium des parties superterrestres à l'unité de superficie (m^2).

Nous considérons bien justifiée de restreindre notre attention à l'appréciation des différences pregnantes entre un grand nombre d'espèce qui poussent ou ne poussent pas dans un certain milieu [3]. Le groupement corrélatif des espèces qui poussent dans les marécages oligotrophiques (la dépression de Dorna) et des espèces qui poussent dans les marécages eutrophiques (la depression de Bilbor) nous offre des données significatives quant aux mêmes composants chimiques des feuilles vertes.

Le niveau de concentration des éléments nutritifs aux espèces provenant des marais ombrogènes décroît de 1,3% (azote total) à 0,1% (phosphore total, valeurs moyennes) (fig. 1). En ordre décroissant, se situent : N, Ca, K, P. Contrairement, aux espèces des marais bas on observe outre une concentration des éléments de la valeur moyenne de 2,2% (N total) à 0,2% (phosphore), un ordre d'accumulation différent : K (2,6%), N, Ca, P.

J'ai usé des méthodes courantes d'analyse chimique, autant pour les plantes que pour les sols [2], [4].

L'écart que l'on observe entre la valeur maximale et la valeur minime pour l'élément calcium aux espèces poussant dans les marécages oligotrophes s'explique par le fait qu'entre les espèces analysées — outre celles qui sont considérées strictement oligotrophes : *Andromeda polifolia*, *Vaccinium oxycoccos*, *Eriophorum vaginatum* — , sont entrées aussi des espèces à amplitude écologique plus large qui, dans la situation donnée, poussent dans le même domaine édaphique : *Vaccinium myrtillus*, *V. vitis idaea*, *Empetrum nigrum*, *Betula pubescens*, *Pinus silvestris*. De même, les extrêmes minimes-maximales (surtout maximales) pour le potassium déterminé du groupement des plantes à provenance du marécage bas, peuvent être attribués en partie à la particularité des espèces *Ligularia sibirica* et *Menyanthes trifoliata* de concentrer le potassium dans les organes superterrestres (selon nos données). Les considérations ci-dessus sur les

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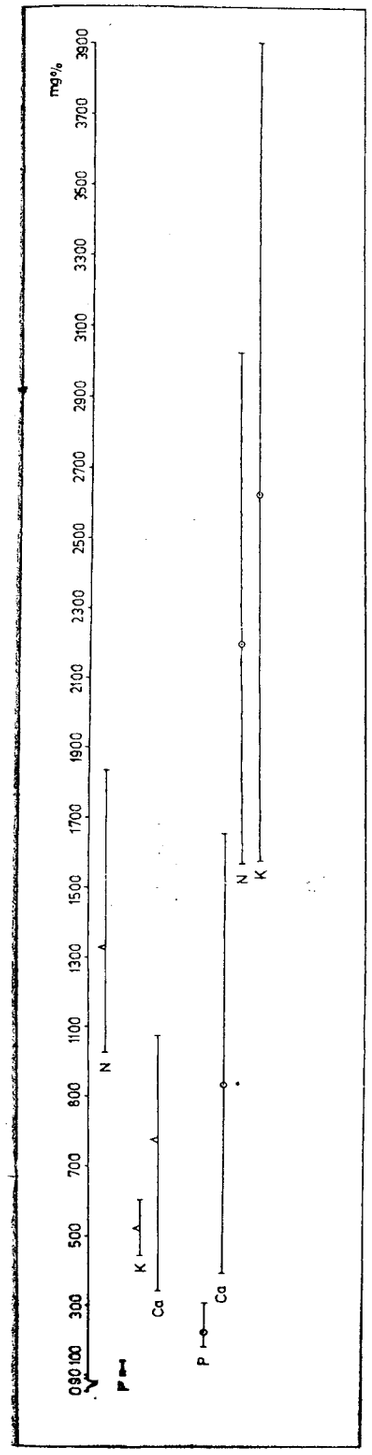
extrêmes minimes-maximales et les nombreuses différences interspécifiques bien connues ne changent pas pour autant la signification des différences entre les groupes de plantes localisées à deux extrémités édaphiques, fait démontré par le calcul de la signification statistique entre les moyennes de concentration des éléments N, P, K, Ca. La valeur t est supérieure à un coefficient de sécurité de 95% et même de 99% [1].

Une analyse identique a été faite pour P, K, Ca, N de la tourbe oligo- et eutrophique (fig. 2). La proportion d'azote total tient la première place, soit qu'il s'agisse de la tourbe ombrogène ou de la tourbe eutrophique. En ordre décroissant se situe le potassium à larges amplitudes minima-maxima, suivi de près par le calcium, le dernier étant le phosphore.

On observe l'appauvrissement graduel de la tourbe en éléments nutritifs le long du gradient marais bas — marais ombrogène, particulièrement dans le cas de l'azote total; on trouve des valeurs une ou trois fois plus grandes (supérieures) dans la tourbe eutrophique (on apprécie également les valeurs extrêmes); le calcium y est deux-trois fois plus abondant. La constatation est importante pour la discussion des corrélations plantes-substrat.

En quelle mesure la dépendance entre les constituants chimiques analysés, de la plante et de la tourbe, est-elle mise en évidence? On sait bien que l'absorption des sels par les plantes est étroitement liée aux ions concentrés tout près de leurs racines. La comparaison des données nous suggère une relation directe, causale entre la concentration accrue d'azote total de le tourbe eutrophique et la concentration relevée d'azote total dans le tissu des espèces poussant dans le marécage eutrophique. La concentration diminuée d'azote total de la tourbe ombrogène, correspond à la concentration plus basse d'azote total dans le tissu des espèces poussant dans le marais ombrogène. On constate une relation analogue pour le ion de calcium. Mais on ne trouve pas de relation bien définie entre les macroéléments phosphore et potassium du tissu des plantes oligo- et eutrophiques et les éléments identiques de la tourbe qui leur correspondent. Comme on l'a déjà vu, le phosphore et le potassium se trouvent dans une concentration deux-trois fois plus grande dans la tourbe oligotrophe par rapport à la tourbe eutrophique. Une explication plausible du manque de corrélation entre les plantes et le substrat pour ces éléments serait que, bien que dans la tourbe ombrogène le potassium et le phosphore existent (comme nos analyses le prouvent) en quantités suffisantes, cela n'implique pas qu'ils soit entièrement accessibles aux plantes; il y a la possibilité qu'ils soient fortement retenus au complexe absorbant de la tourbe. L'observation nous conduit à la conclusion que le phosphore et le potassium échangeables pourraient être des facteurs limitatifs dans la tourbe pauvre en sels et puissamment acide, par leur accessibilité réduite aux racines des espèces — quelques-unes calcifuges — de la marais haute. L'adaptation à la concentration diminuée de calcium et aux niveaux diminués de K et P accessibles, la capacité de prévenir la toxicité et la lésion due aux ions de Al représentent d'autres particularités. Le système radiculaire bien développé de beaucoup d'espèces de marais capable (en état) d'explorer plus de tourbe, éventuellement une efficience accrue d'absorption des sels dans



Poiana Cosnei (le marais de Singeorzana). ⊙ La valeur moyenne des 6 plantes dans le marais bas du ruisseau de Dobreanu: *Pedicularis sceptrum-carolinum*, *Phragmites australis*, *Blysmus compressus*, *Salix repens*, *Ligularia sibirica*, *Menyanthes trifoliata*. — La valeur minime et maxima (l'amplitude de variation minime-maxima).

Fig. 1. — Les composants chimiques des feuilles vertes: Δ la valeur moyenne des 8 plantes provenant des marais ombrogènes: *Vaccinium myrtillus*, *V. vitis idaea*, *V. oxycoccos*, *Andromeda prolifolia*, *Eriophorum vaginatum*, *Empetrum nigrum*, *Betula pubescens*, *Pinus silvestris*. Espèces des marécages oligotrophiques aux Poiana Stampei Pilugani, Cosna,

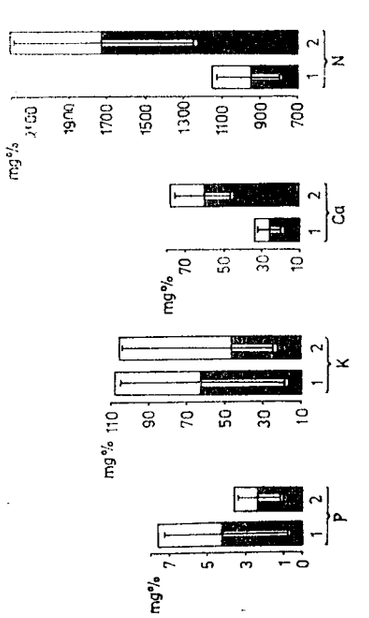


Fig. 2. — Composition chimique du tourbe ombrogène dans le marais de Singeorzana (*1*) et du tourbe eutrophique dans le marécage bas du ruisseau de Dobreanu (*2*). P, K, Ca sont exprimés comme formes mobiles et N total. Dans l'intérieur des histogrammes sont désignées les valeurs moyennes et l'amplitude de variation minime-maxima.

des conditions de nutrition minime, assure la croissance des espèces du marais mais ne favorise pas l'installation des taxons à exigences élevées pour le degré d'eutrophie (entre eux les espèces calciphiles).

En rapportant les cendres (brutes) provenant des parties superterrestres des phytocénoses installées dans le lagg de marécage oligotrophe (sur du sol gley) à l'unité de superficie, on met en évidence une croissance en poids deux fois et demie plus grande par rapport aux cendres appartenant aux cénoses existant sur la couche épaisse de tourbe oligotrophe (plantes herbacées et semiarbustes) (tableau 1). Mais même dans les tourbières

Tableau 1

Cendre et composition chimique du matériel végéta l (plantes supérieures) des faciès Echantillons des superficies de 625 cm² dans le marais ombrogène de Singeorzana

Faciès avec :	Cendre %	Azote total %	Calcium %	Cendre %/m² (g)	Azote total %/m² (g)	Calcium %/m² (g)
	mat. sec.					
Scirpus silvaticus (dans le lagg)	5,74	1,51	0,54	91,84	24,16	8,64
Betula pubescens codominante *Pinus silvestris*	2,31	1,18	0,37	36,96	18,88	5,92
Pinus silvestris *Vaccinium myrtillus*	2,71 3,65	0,97 1,18	0,64 0,88	43,36 58,40	15,52 18,88	10,24 14,08

basse on peut saisir des différences, comme par exemple entre deux complexes dominés par la même espèce *(Carex nigra)* installés dans des conditions édaphiques opposées, ou une différence statistiquement significative à DL 5% entre les valeurs moyennes de cendres des faciès *Carex nigra* (sur des croûtes calcaires) et *Menyanthes trifoliata* (tableau 2).

Si l'on compare la teneur en cendre par unité de surface des groupements de deux formations, l'on constate que dans le cas des phytocénoses à caractère eutrophique le poids des cendres est deux fois et demie plus grand que celui des phytccénoses typiquement oligotrophes (tableaux 1, 2).

L'amplitude de variation et la mise en rapport à l'unité de surface des constituants minéraux des parties superterrestres nous indiquent de petites valeurs pour l'azote et le calcium aux complexes de dessus la tourbe oligotrophe par rapport aux complexes de dessus la tourbe de bas-marais. L'azote total se trouve 1,3 fois et le calcium dans une quantité deux fois plus grande dans les groupements du bas-marais (Dobreanu). La situation est bien expressive pour le calcium de la cénose avec *Scirpus silvaticus* dans deux localisations différentes (tableaux 1, 2).

Outre l'estimation de la productivité du matériel végétal, la connaissance de sa teneur en sels minéraux représente une modalité de mettre en évidence directement la relation entre les groupements végétales ou les espèces et les facteurs du milieu qui en conditionnent l'existence et la persistance dans les systèmes tourbeux oligo- et eutrophiques.

Tableau 2

Cendre et composition chimique du matériel végétal (plantes supérieures) dans les faciès Echantillons des superficies de 625 cm² dans le marais bas du ruisseau de Dobreanu

Faciès avec :	Cendre % mat. sec		Azote total % mat. sec		Calcium % mat. sec		Cendre %/m² (g)	Azote total %/m² (g)	Calcium %/m² (g)
	val. moyenne (g)	L'amplitude de variation	val. moyenne (g)	L'amplitude de variation	val. moyenne (g)	L'amplitude de variation			
Scirpus silvaticus	10,12	9,15 – 11,17	1,72	1,53 – 2,07	1,31	1,25 – 1,42	161,92	27,52	20,96
Equisetum fluviatile	11,72	10,46 – 13,34	1,70	1,62 – 1,75	1,54	1,31 – 1,89	187,52	27,20	24,64
Carex nigra (sur des croûtes calcaires)	7,50	7,00 – 7,83	1,38	1,28 – 1,49	1,20	0,84 – 1,79	120,00	22,08	19,20
Carex nigra (sur la tourbe)	8,35	6,89 – 9,85	1,62	1,50 – 1,81	1,22	0,98 – 1,53	133,60	25,92	19,52
Carex appropinquata	7,62	7,06 – 8,09	1,32	1,26 – 1,39	0,94	0,73 – 1,28	121,92	21,12	15,04
Menyanthes trifoliata et Carex limosa	8,70	8,09 – 9,26	1,71	1,50 – 1,82	1,18	0,93 – 1,35	139,20	27,36	18,88
Salix repens	8,06	7,01 – 9,15	1,42	1,40 – 1,45	1,47	1,16 – 1,83	128,96	22,72	23,52

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THE CULTURE OF SOME FILAMENTOUS GREEN ALGAE IN DIFFERENT CONDITIONS OF LIGHT AND NUTRITIVE MEDIUM. III. ULTRASTRUCTURAL PECULIARITIES OF THE ALGAE *ULOTHRIX VARIABILIS* AND *STIGEOCLONIUM SUBSECUNDUM*

BY

C. CRĂCIUN, A. MARTON, Șt. PÉTERFI

In der Arbeit sind die ultrastrukturellen Eigentümlichkeiten der Algen *Ulothrix variabilis* und *Stigeoclonium subsecundum* wiedergegeben, die im Laboratorium in 4 experimentellen Varianten, unter verschiedenen Bedingungen der Beleuchtung und der Zusammensetzung des Nährmediums gezüchtet wurden. Im allgemeinen ist sich die Ultrastruktur der zwei Algenarten ähnlich : die Zellwand besteht aus 3 Schichten, mit Plasmodesmen in der Querwand, wandständigem Chloroplast, mit je 2 gruppierten Thylakoiden, manchmal längliche Grana bildend, 1—2 Pyrenoiden mit phragmentierter Amidon-Hülle, durch das Eindringen einiger Thylakoiden, usw.
Dauerndes Licht beeinflusst in geringerem Mass die Ultrastruktur der Alge *Stigeoclonium*, wobei die Veränderungen bei *Ulothrix* offensichtlich sind. In intensiven Bedingungen ist der Chloroplast gut entwickelt und die Thylakoiden erscheinen als gewellte Lamellen. Der Stickstoff-Mangel bedingt grössere Umänderungen der Chloroplast-Ultrastruktur, parallel mit einer massiven Anhäufung der Stärke und Fettstoffe.

The comparative research of some algae ultrastructure in different conditions of cultivation provide new details on the constancy or variability in certain cell structures, as well as their importance in modern taxonomy [11]. In a previous work [15] we thoroughly presented the cultivation conditions for the four experimental variants with the green algae *Stichococcus bacillaris*, *Gloeotila protogenita*, *Ulothrix variabilis*, *Stigeoclonium subsecundum*, and *Microthamnion kützingianum*. The research aimed at the effect of the modifications in the chemical composition of the nutritive medium and of the light on the biomass accumulation, the quantity of pigments and proteins, etc. The ultrastructure of the algae was also studied, underlining the resemblance of *Stichococcus* and *Gloeotila* [11] in what concerns the cell membrane structure, the chloroplast shape, the thylakoids range, the presence of the pyrenoid, the lack of a starch sheath, the crossing of the pyrenoid by single thylakoid parallel rows, etc. However the behaviour under continuous light is obviously different, pointing to physiologic differences with these algae.

Few references on the *Ulothrix* and *Stigeoclonium* ultrastructure are found in literature. Manton (1964) presents the zoospores ultrastructure in *Stigeoclonium*, Floyd and collab. (1971) point out the plasmodesmata in

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Ulothrix fimbriata and *Stigeoclonium helveticum*, describing comparatively the *Ulothrix* and *Stigeoclonium* cytology [4]. Cell ultrastructure peculiarities in various division phases are thoroughly studied by Pickett-Heaps (1975), and Mattox and collab. (1972); based on electron microscopy research, they point out the inclusions resembling virus in *Stigeoclonium farctum*.

MATERIAL AND METHOD

Ulothrix variabilis Kütz. was collected on the 6th of November 1970, out of a small lake near Dezmir (a village in the district of Cluj), where it vegetated epiphytically, at a depth of 30—50 cm and 7.5 pH. It has been cultivated for six years in laboratory on media of different compositions, pure cultures being obtained. *Stigeoclonium subsecundum* Kütz. is of the same source.

The four experimental variants were: I. static culture in liquid and agar Knop-Pringsheim nutrient medium, NV natural light, room temperature (20°C—24°C), for 21 days; II. static culture, in the same medium, at 6,000 lx intensity of fluorescent continuous light for 7 days and NV natural light for 14 days; III. intensive culture, bubbled with a mixture of air and 3—5% CO_2, 9,000 lx. intensity of fluorescent light, 12 hours light/12 hours dark work conditions, temperature of 22°C (±1°C), Knop-Pringsheim nutrient medium, for 14 days; IV. intensive culture identical to variant III, but using Bold medium with soil extract.

For the electron microscopy, the material was centrifugated, after a previous cooling at 0—4°C. The suspension was fixed with 6% glutaraldehyde and 1% phosphate buffer solution. After dehydration in successive acetone baths, W vestopal inclusion and LKB III ultramicrotome sections were performed. The sections contrast was obtained with uranyl acetate and lead citrate and were examined in BS-613 Tesla electron microscope.

RESULTS AND DISCUSSIONS

Ultrastructural characteristics of *Ulothrix variabilis*

Experiment variant I (I ab, Plate 1)

The filaments are formed of cells of variable length (11—12 mμ), more constant in point of thickness (6—8 mμ), surrounded by a cell wall, layered as follows: a less dense external layer, a more dense median one with amorphous structure, and an internal layer with numerous folds on the side of the plasmalemma. The presumed pectic-natured external layer contributes to the cells cohesion in the filament, the median and the internal one form the transverse walls, penetrated by many plasmodesmata. They have been pointed out with *Ulothrix fimbriata* too [3].

The chloroplast generally extends along the whole cell, often covering more than 2/3 of its circumference, and having lobated edges. The parietal shape is obvious, but the 1—2 pyrenoids in its mass contribute to a huge aspect. The thylakoids seem to be grouped two by two, in parallel rows, often with undulating aspect, from one end of the cell to the other.

In some cases, grouped thylakoids and long shaped grana are observed. Starch granules and plastoglobuli are present in the chloroplast. The pyrenoid is formed of a dense mass, more or less homogenous, sometimes with pyrenoglobuli and a kind of spheric formations which might be chloroplastic membranes diverticuli. The pyrenosome is covered by a starch sheath fragmented into 3—6 parts by the penetrating thylakoids, not more than one at a time. However, it seems that the thylakoids do not penetrate into the pyrenosome but they separate and the two leaflets surround the pyrenoid, and seem to have its own membrane.

The cytoplasm contains ribosomes, a variable number of osmiophilic grains, oval, oblong mitochondria profiles with numerous diverticuli, but lacking a light coloured lumen as in the *S. bacillaris* and *G. protogenita* [11], Golgi aparatus and small vacuoles with gas bubbles.

Experiment variant II (II ab, Plate 1).

The continuous light induced modifications refer especially to: the disturbance of the thylakoids arrangement, a higher accumulation of starch around the pyrenoid, and among the thylakoids, the presence of many dense osmiophilic grains in the cytoplasm and the removing of the nucleus membrane leaflets .The pyrenoid is irregular in shape, and uniform in structure, having a more pronounced density, it sometimes emerges the starch sheath and penetrates into the chloroplast stroma. These peculiarities underline some accelerated synthesis and accumulation processes, both of proteic substances and especially of lipides and carbohydrates, though the culture is negatively influenced by the continuous light: the algal filaments become yellowish and the quantity of pigments diminishes (15).

Experiment variant III (III, Plate 1)

The intensive culture in the Knop-Pringsheim medium, where the alga had a high accumulation of biomass and pigments [15], is ultrastructurally characterized by: changes of the cell membrane, interplastidic high starch accumulations, thickening of the pyrenoid starch sheath big osmiophilic grains accumulation in the cytoplasm and the presence of some crystalloids (C).

The external layer of the membrane is less dense, the median layer is thicker, the internal, folded one is thinner. The chloroplast is normal, but the accumulation of starch grains disturbs the thylakoids range. The pyrenosome seems smaller, while its starch sheath is thicker. The starch grains are set in zones.

The presence of some big osmiophilic globuli in the cytoplasm demonstrates that besides the stimulation of the synthesis and of the starch accumulation, the experimental conditions determine the speed of the lipides synthesis too. Crystalloids are accumulated in the vacuoles at different growth phases and in more advanced stages they are surrounded by a membrane which is the one of the vacuole they had been formed in.

Experiment variant IV (IV, Plate 1)

The ultrastructural changes consist in the total disturbance of the thylakoids order, their decreasing number (T), strong starch (S) and cytoplasm (cog) osmiophilic grains accumulation. The cell loses its normal structure, becoming a substance receiver.

Ultrastructural characteristics of *Stigeoclonium subsecundum*

Experiment variant I (I, Plate 2)

The cells are covered by a membrane resembling that of *Ulothrix*, but with an inner layer less folded in the side of plasmalemma. Numerous plasmodesmata are present. The parietal chloroplast extends from 1/3 to the whole length of the cell, 2/3 or more of its circumference, respectively. Large starch grains and plastoglobuli appear among the thylakoids. The pyrenoid structure is similar, of irregular shape, with a starch sheath divided by the penetration of a thylakoid. The nucleus with a nucleolus is central, on the internal side of the chloroplast, near the pyrenoid. The double nuclear membrane is externally trimmed with ribosomes. The cytoplasm has organellae resembling *Ulothrix*.

Experiment variant II (II, Plate 2)

Continuous light did not influence the *Stigeoclonium* cultures [15], a fact found out of the ultrastructural characteristics : a well developed chloroplast, with thylakoids grouped in long grana, a moderate quantity of starch grains and plastoglobuli, a big nucleus, numerous cytoplasm osmiophilic grains.

Experiment variant III (III.a.b., Plate 2)

The ultrastructure underlines optimum growth conditions for the alga. The well developed chloroplast has parallel rows of undulated thylakoids, grouped two by two. The interplastidic starch grains are bigger and set in a dark coloured inner zone and a less dense external one. The pyrenoid is big, with a thick starch sheath. Relatively big osmiophilic grains are accumulated in the cytoplasm.

Experiment variant IV (IV.a.b., Plate 2)

The decoloration of cultures, the diminishing quantity of pigments is correlated to a reduced chloroplast and number of thylakoids, parallel with a high starch accumulation as well as of cytoplasmic grains.

With *Stigeoclonium*, the electronmicrographs showed some spheric formations among the thylakoids, seeming cross sections of microtubes. To point them out a thorough research is necessary. Microtubes have also been detected in the plastids of *Chara fibrosa* and *Volvox* sp. (16), of *Dichotomosiphon tuberosus* (14), a.s.o.

The ultrastructural changes in the two studied algae were very alike. A correlation of the pigments quantity, the physiologic condition and structure of the chloroplast is especially observed. Our research [15] pointed out the *a* and *b* chlorophyll, characteristic for the *Chlorophyta* [13], in these algae, and out of the carotenoids, the *b*-caroten, the xanthophyll, the neoxanthin, and the violaxanthin, cited also by Goodwin (1974). We also demonstrated the resemblance between the two algae in what concerns the quality and quantity of pigments, and the appearance of degraded chlorophyll forms, in certain experimental conditions, which are not present in *Stichococcus* and *Gloeotila* [10].

Abbas and Godward (1963) pointed out the implications of light on the alga *Stigeoclonium;* our previous works [10] as well as most of the cited ones [15] underline the action of the nutritive medium on the studied algae too. The data concerning the algae ultrastructure, in

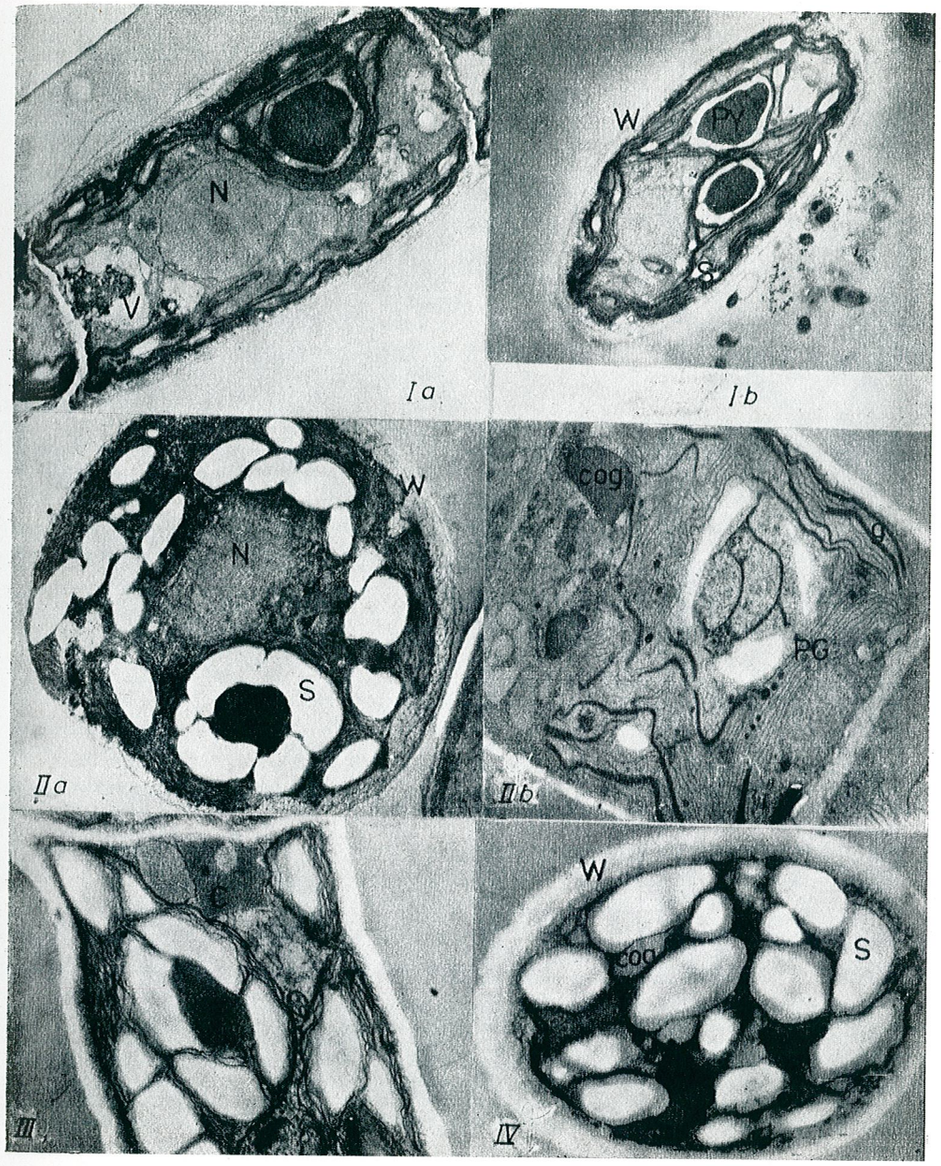

Plate 1

Ultrastructural aspects in *Ulothrix variabilis*

Ia Longitudinal section, × 5, 700, N = nucleus, CH = chloroplast, V = vacuole.
Ib Oblique section, × 4, 750, PY = pyrenoid, W = cell wall, S = starch.
IIa Cross section, × 10,300, N = nucleus, S = starch, W = cell wall.
IIb Longitudinal section, × 10,300, PG = plastoglobuli, g = granum, cog = cytoplasm osmiophilic grains.
III Longitudinal section, detail, × 14,200, T = thylakoids, C = crystalloids.
IV Cross section, × 10,300, S = starch, W = cell wall, cog = cytoplasm osmiophilic grains.

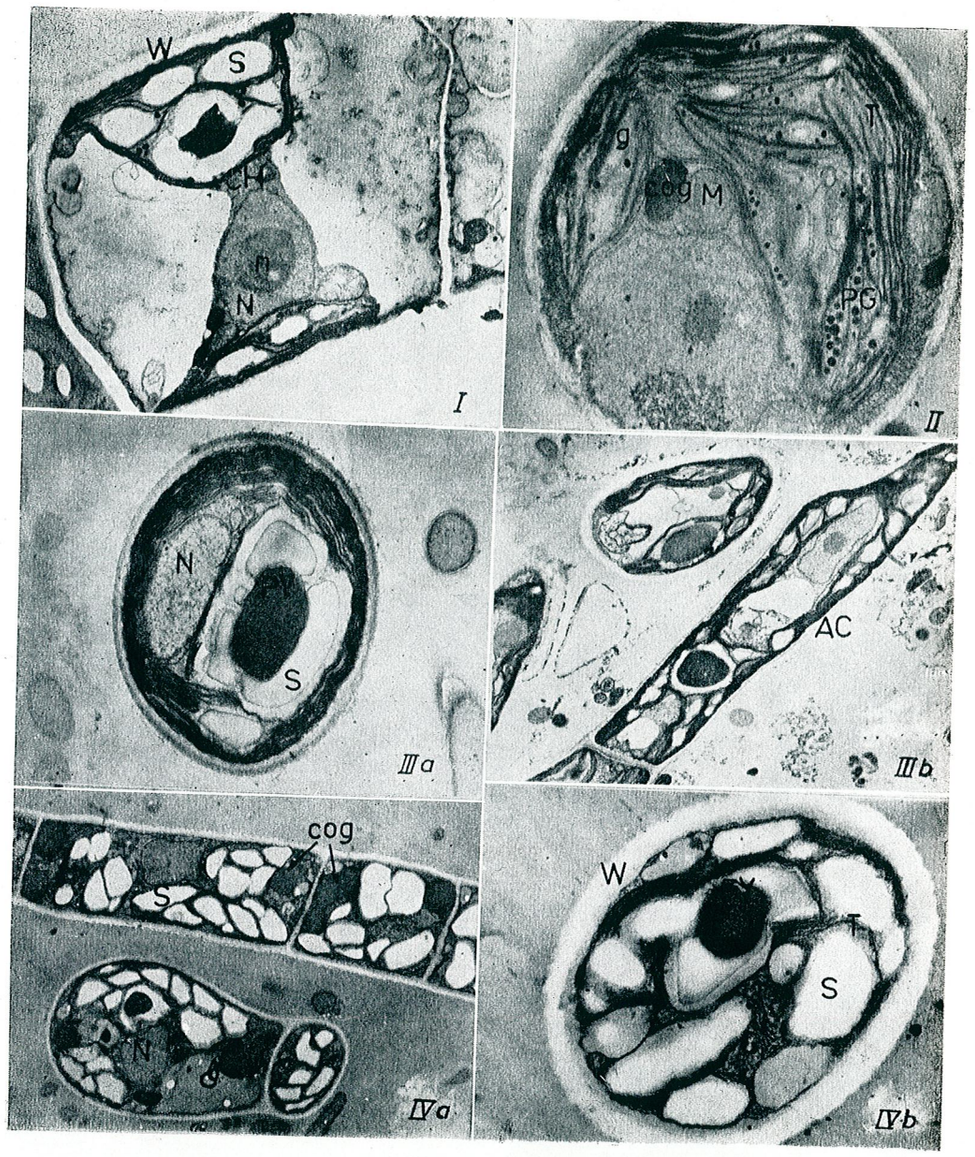

Plate 2

Ultrastructural aspects in *Stigeoclonium subsecundum*
I Longitudinal section, × 10,000, W = cell wall, CH = chloroplast, N = nucleus S = starch.
II Cross section, × 10,300, T = thylakoids, g = granum, PG = plastoglobuli, M = mitochondria, cog = cytoplasm osmiophilic grains.
IIIa Cross section, × 14,200, PY = pyrenoid, S = starch, N = nucleus.
IIIb Longitudinal section, × 4,800, AC = apical cell.
IVa Longitudinal section through a mature and a forming filament, × 4,800, S = starch, N = nucleus, cog = cytoplasm osmiophilic grains.
IVb Cross section, × 20,000, T = thylakoids, PY = pyrenoid, S = starch, G = Golgi apparatus, W = cell wall.

different experiment conditions, are very important for the thorough study of their structure and physiological behaviour, as well as for their taxonomy. Islam (1963) considered *Ulothrix* as a basis of the genus *Stigeoclonium* phylogenesis. The ultrastructural similarities between these algae confirm this idea. The presence of the plasmodesmata, as with other green algae [3], [5], [18], shows also the relationship with superior plants. Their lack in *Stichococcus* and *Gloeotila* [11] demonstrate an inferior character, though many other characters are close to those in *Ulothrix* and *Stigeoclonium*.

CONCLUSIONS

1. The algae *U. variabilis* and *S. subsecundum* have resembling ultrastructural characteristics: a layered cell wall, the internal layer, next to the plasmalemma, being folded; plasmodesmata in the transversal wall; parietal shaped chloroplast with thylakoids grouped two by two, sometimes combined in oblong grana; 1—2 pyrenoids with starch sheath, divided into 3—6 parts by the thylakoid penetration, but without the pyrenosome fragmentation, seeming to cover it in a pyrenoid membrane; starch accumulations in the chloroplast; osmiophilic globuli in the chloroplast and cytoplasm; large nucleus with a nucleolus, centrally set, near the pyrenoid.

2. The continuous light has a weak influence on *Stigeoclonium*, while it is obvious in *Ulothrix*, even in the ultrastructural characters.

3. Optimum growth conditions are seen in Knop-Pringsheim intensive cultures, especially by a well developed chloroplast with undulated thylakoids; in the Bold medium, with a decreased quantity of nitrogen, the synthesis and accumulation processes of starch and lipids in large quantities bring about the disturbance of the cell structure.

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PRODUCTS OF PHOTOSYNTHETIC $^{14}CO_2$ INCORPORATION IN THE LICHENS *PELTIGERA HORIZONTALIS* BAUMG. AND *USNEA FLORIDA* WIGG.

BY

GEORGETA FABIAN-GALAN and L. ATANASIU

The kinetics of photosynthetic $^{14}CO_2$ incorporation in sugar, amino acid and organic acid fractions was followed in *P. horizontalis* and *U. florida* after 1; 2; 3; 5 and 10 minutes incorporation periods. An increase was observed in the radioactivity of sugars, amino acids and organic acids with increasing time of exposure to $^{14}CO_2$ in both species. The radioactivity of the organic acids was considerably higher in the first minutes of exposure in contrast to the sugars and free amino acids in *P. horizontalis* (bluegreen algae symbiont). In *U. florida* (green algae symbiont) the sugars exhibited from the beginning the highest radioactivity.
Sucrose, glucose and certain unidentified compounds-probably polyols were radioactive after one minute for *U. florida* and, excepting sucrose, for *P. horizontalis*. Aspartic acid and alanine in *U. florida* and aspartic acid alone in *P. horizontalis* became labelled after one minute.
P. compounds, citric, tartaric, malic, glycolic, succinic and fumaric acids for P. horizontalis and only P-compounds and citric acid for *U. florida* became radioactive during the first minute following exposure.

The photosynthetic carbon assimilation pathways are not well characterized in lichens, as compared to the state of knowledge concerning free living algae and higher plants. Studying the physiology of lichens at the level of intact thalli may represent a fruitful way as against the situation when each of the partners are considered separately. Application of methods based on the use of ^{14}C, brought about a substantial contribution to the understanding of carbohydrate metabolism in lichens [20], [21], [22].

When thallus samples belonging to different lichen species were treated with $NaH^{14}CO_3$ the higher ^{14}C appeared in sugar alcohole [20], [6], [3], [16], [17], [18], [19]. One of the polyalcohols showing the widest distribution in lichens is mannitol, though its role is not known. Drew and Smith [7], [8] showed glucose is the main compound accumulating ^{14}C in *Peltigera polydactyla*. The labelled glucose was detected after one minute in the lichen algae symbiont while after 2–4 minutes it was detected in the fungus where it was converted to starch. However, Bednar [2] found that the $^{14}CO_2$ incorporation into the thallus of *Peltigera aphtosa* with *Coccomyxa* blue-green algae symbionts, resulted in the appearance of labelled sucrose and not of mannitol. Glucose, fructose, certain organic acids and amino acids exhibited radioactivity. A radioactive pentitol was found by Feige [10], [11] in *Scytonema*—the blue green symbiont of *Cora pavonia*—a short time after exposure to $^{14}CO_2$, while mannosidomannitol was present under the same conditions in *Calothrix* — the blue green symbiont of *Lichina pygmaea*.

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Highest radioactivity appeared in ribitol and sucrose, as mentioned by Richardson and Smith [16] in the case of *Trebouxia* green algae symbiont cells shortly after their exposure to labelled carbon dioxide. In *Hyalococcus*, the green-algae symbiont of *Dermatocarpon* sp., 70% of ^{14}C was fixed in sorbitol, 18% in sucrose while the remaining radioactivity was found in other compounds, as mentioned by Green (cited by Richardson, [19]). Analysing intact samples of *Collema* sp. *Parmelia furfuracea* and *Cladonia rangiferina*, exposed for 90 minutes under light in a $^{14}CO_2$ atmosphere, Fabian, Atanasiu and Sălăgeanu [9] identified the radioactive sucrose among the tested sugars. Aspartic and glutamic acid and alanine among amino acids, as well as malic and succinic acid among organic acids proved also to be labelled. This paper describes the ^{14}C-labelled products of photosynthesis in $^{14}CO_2$ obtained with two lichen species. The following aspects were studied, kinetics of ^{14}C incorporation into different compounds and the total soluble fractions accompanying these compounds.

MATERIALS AND METHODS

The lichens thalluses used in our work were taken in September from the mountain zone Sinaia — Valea Urlătoarei, at an altitude of about 1,000 m. The thalluses of *Usnea florida* containing a green-algae symbiont and *Peltigera horizontalis* containing a blue-green algae symbiont were used. The lichen thalluses have been maintained for 24 hours before the experiment under laboratory conditions under light intensity of 5,000 lux, obtained from fluorescent lamps, and at a temperature of 25°C.

Thallus samples kept in normal physiological conditions were introduced into assimilation chambers consisting of glass vessels disposed in series; an air stream containing $^{14}CO_2$, i.e.a. CO_2 concentration of 1% and a radioactivity of 262 μC/liter was circulated through the system in a closed circuit. Experiments were conducted in the laboratory at a 5,000 lux light intensity and 25°C. After 1, 2, 3, 5 and 10 minutes exposure-time, the assimilation chambers were separated from the circuit and the thallus samples were immersed in boiling methanol, 80%.

Sample preparation for chromatography was conducted after the method described by Champigny [5]. Total radioactivity of the soluble sugar fractions, amino acids and organic acids were evaluated with a Geiger Müller counter.

Radioactive compounds were separated by unidimensional chromatography on a 2043 Schleicher-Schule paper and subjected to radioautography. A water-n butanol-acetic acid mixture (5 : 4 : 1) was used for the irrigation of chromatograms separating the sugars and amino acids. An alcoholic solution of p-aminophenol and para-anisidine-phosphate was applied to reveal sugars, while a 0.1% ninhydrinbutanol solution was used for amino acids. The solvent system butanol-formic acid-water (75 : 18 : 9) was used to separate organic acids. Bromphenol-blue was sprayed to locate the organic acids. Labelled compounds were identified on radioautograms obtained after chromatogram exposure to an X-Röentgen plate. The position of separated compounds as against certain known substances used as controls and simultaneously separated from the chromatograms, was used as an identification criterion in the case of the analyzed samples.

RESULTS

The analysis of the time course depicted in Fig. 1 and 2 showed: (a) An increase in the radioactivity of sugars, amino acids and organic acids with the increase of $^{14}CO_2$-exposure time in both *P. horizontalis* (Fig. 1) and *U. florida* (Fig. 2); (b) The radioactivity of organic acids proved to be considerably higher in the early exposure as against the

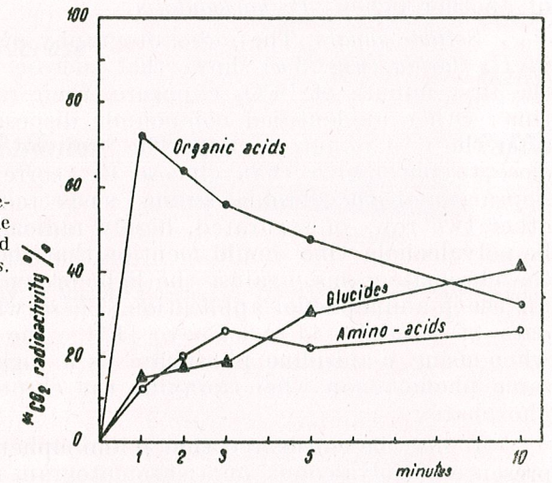

Fig. 1. — The kinetics of photosynthetic ^{14}C incorporation in the soluble fractions of sugars, amino acids and organic acids in *P. horizontalis*.

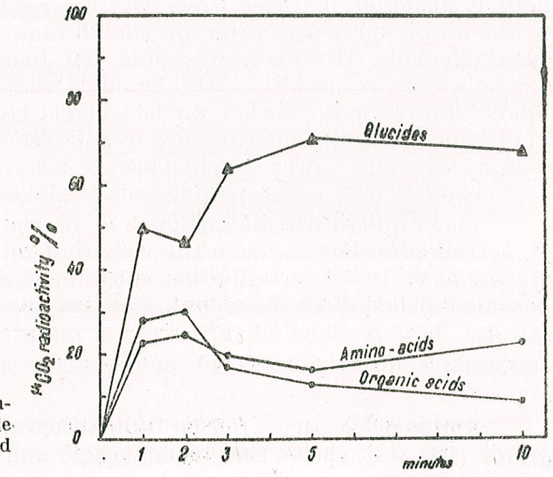

Fig. 2. — The kinetics of photosynthetic ^{14}C incorporation in the soluble fractions of sugars, amino acids and organic acids in *U. florida*.

sugars and free-amino acids in *P. horizontalis* (having a blue green algae symbiont); the radioactivity of sugars followed that of organic acids, the amino acid reaching higher values later (i.e. after 1 minute of exposure to $^{14}CO_2$). In the case of this lichen, we may conclude that the organic acids are the primary products of photosynthesis; (c) In *U. florida* (green algae symbiont), from the very beginning, the sugars exhibited the highest radioactivity level, followed by amino acids and organic acids (the latter showing the lowest radioactivity level). These data do not necessarily lead to the conclusion that in *U. florida* the sugars are primary products of photosynthesis, although such a conclusion is not absolutely impossible. The higher radioactivity shown by sugars could be explained in this case by the fact that organic acids — as primary photosynthesis products — are more rapidly subject to conversion as compared to the case of another lichen, *P. horizontalis*.

Soluble sugars. The radioautography of the glucide chromatograms in *U. florida* (Fig. 3 *a*) shows that sucrose and glucose appeared after the first minute of $^{14}CO_2$ exposure, their radioactivity increasing with time. Other unidentified compounds disposed in three rows appeared after the first minute as well. The first row characterized by Rf values close to but higher than glucose Rf, corresponds to the white spots appearing on the chromatograms, suspected to be sugar alcohols. The other two rows of separated, highly radioactive compounds could also be polyalcohols. One should mention that the white spots were noted on the chromatograms, against the light-brown background characteristics for the p-aminophenol application. These white spots show — after the data of Cerublis [4] and Pueyo [13] — the presence of sugar alcohols, when using p-anisidine phosphate as a sugar revelator. We noted the same phenomenon when spraying the chromatograms with p-anisidine phosphate.

It may be considered that p-aminophenol may show indirectly the presence of polyalcohols on a chromatogram made in view of sugar separation. For the above-mentioned reasons we believe that labelled compounds disposed in three rows the radioautography and corresponding to the white spots appearing on the chromatograms, are suspected to be sugar alcohols. However, we could not identify exactly to what polyalcohols they belong. It is also possible that these compounds represent lipids, sterols or any other unelectrolytic compounds showing solubility in organic solvents. The presence of rafinose and of other labelled compounds showing a lower Rf value may be a tetra or penta-sugar of stachyose or verbascose type, accompaning usually glucose was noted after 10 minutes.

The radioautography (Fig. 3 *b*) of the glucide chromatogram for *P. horizontalis* showed, from the very first minute, the presence of labelled glucose as well as of certain other substances, possibly polyalcohols; sucrose became labelled after the second, and not the first minute as in *U. florida*; rafinose became labelled after three minutes, while other unidentified compounds may be tetra or penta-sugars after five minutes of $^{14}CO_2$-exposure.

Amino acids. In *U. florida* radioautography of amino acid chromatograms (Fig. 4 *a*) shows that aspartic acid and alanine are radioactive after

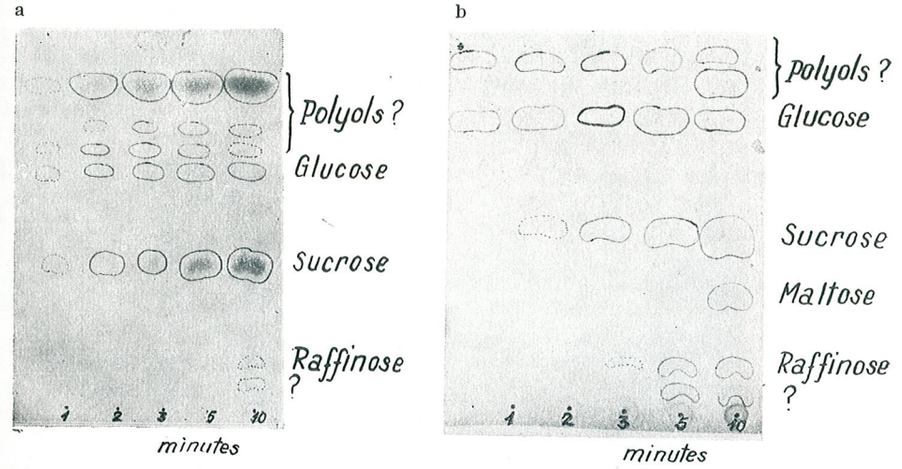

Fig. 3. — Radioautography of soluble glucides *a*: *U. florida*; *b*: *P. horizontalis*.

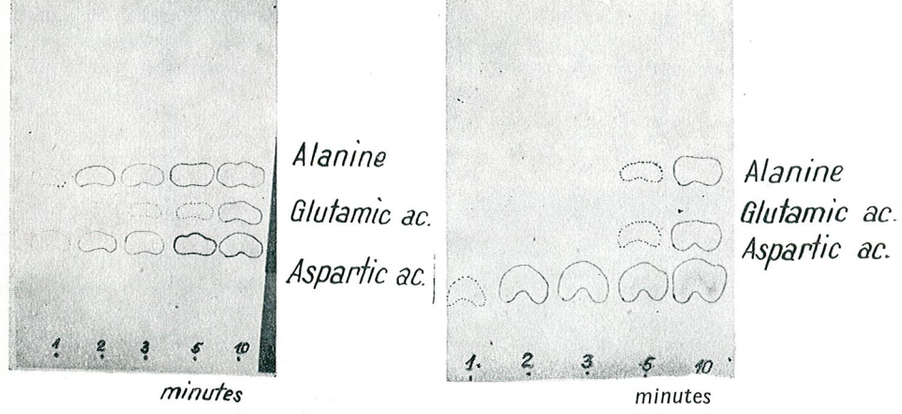

Fig. 4. — Radioautography of free amino acids. *a*: *U. florida*; *b*: *P. horizontalis*.

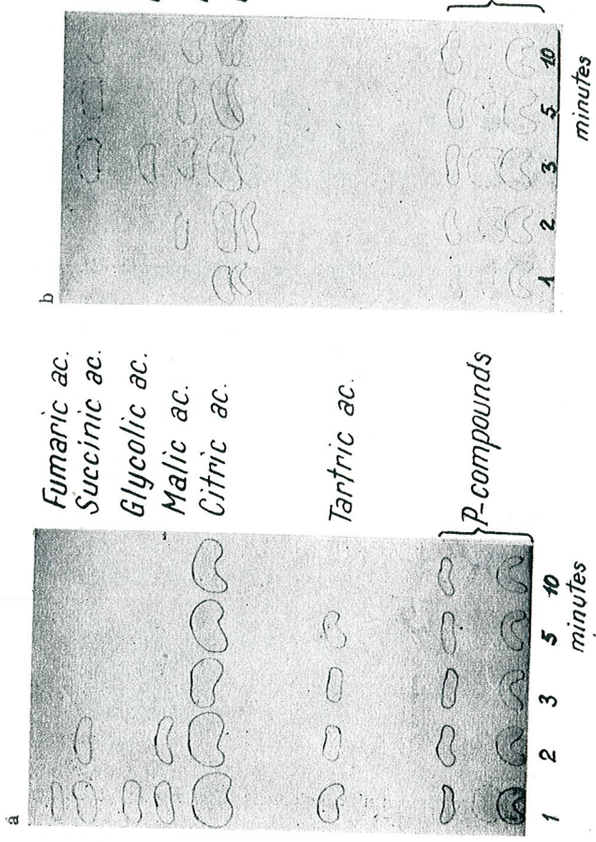

Fig. 5. — Radioautography of organic acids. *a* : *U. florida* ; *b* : *P. horizontalis*.

the first minute ; they accumulated an amount increasing with the exposure-time. Glutamic acid proved to be weakly labelled after three minutes. All the above-mentioned amino acids could be clearley distinguished.

In case of *P. horizontalis* only aspartic acid became labelled after the first minute as it could be noted in the radioautography of the chromatogram (Fig. 4 *b*) ; glutamic acid and alanine evidenced radioactivity only after five minutes. The aspartic acid and alanine became labelled after the first minute, while the glutamic acid after three minutes, the case of *U. florida*, evidencing a higher speed of synthesis as against *P. horizontalis*.

Organic acids. Fig. 5 *a* shows the radioautography of organic acids in *U. florida* : P-compounds and citric acid appeared as labelled after the first minute ; malic acid, after two minutes, glycolic and fumaric acid after three minutes ; however, the disappearance of glycolic acid was noted after five minutes. A very low radioactivity after all the experimented exposure periods, in case of an unidentified compound shows an Rf value close to, but lower than the citric acid.

The appearance of a great number of radioactive organic acids was noted after the first minute of exposure, in *P. horizontalis* (Fig. 5*b*), i.e. : tartric, citric, malic, glycolic, succinic and fumaric acids, as well as P-compounds. This finding is in agreement with the $^{14}CO_2$ incorporation (Fig. 1) in this species, which showed a considerably higher radioactivity of organic acids after the first minute of exposure, as against to sugars and free amino acids ; the radioactivity of sugars reached to the highest values only after a certain exposure period, followed by organic acids and amino acids. The disappearance of certain compounds from the radioautograms was noted with the increase of exposure time, i.e. : glycolic and fumaric acids after two minutes, malic and succinic acids after three minutes and tartric acid after ten minutes. P-compounds and citric acid were the only components which could be noted on the radioautograms exposure period.

DISCUSSIONS

A great number of radioactive compounds were noted among the soluble sugars separated from the extracts of the two lichen species. Among these, sucrose evidenced a quantitative preponderance, although glucose and probably polyalcohols were also present in appreciable amounts.

Norris, Norris and Calvin [12] conducted researches on the short-term photosynthesis products in plants of the nine phyla, except lichens. The great diversity of their data, resulting both from the metabolic differences existing between the plants and from the experimental conditions permitted to the authors to draw the general conclusion, that the amount of labelled sucrose is different in the two major groups of plants, i.e. : (a) plants made-up exclusively from photosynthesis tissues : algae bryophyte (protonema) and ferns (prothallus) ; (b) plants containing

both photosynthetic and non-photosynthetic tissues. A much higher radioactivity of sucrose was evident in the second, as against the first group.

In our previous research on the organic compounds appearing in lichens as a result of photosynthesis [1], [9], as well as in this works, we identified with certainty only sucrose — among sugars — a fact which although would suggest its direct synthesis, is very difficult to prove.

However, there are many studies whose authors seem to consider sucrose as an important product in the carbohydrate metabolism of lichens. It was found, for example, that $^{14}CO_2$ incorporation in Peltigera aphtosa resulted in the appearance of labelled sucrose and not mannitol [2].

Lichen thallus is made-up both from photosynthetic and nonphotosynthetic structures and the opinion of Norris, Norris and Calvin [12] seems to hold true in this case, too. The same conclusion may be drawn on the basis of the high concentrations of sugar alcohols found in lichens. This fact would bring closer the carbohydrate metabolism of lichens and the metabolism of similar compounds in the other plant groups. Anyway, both photosynthetic and sugar metabolism of lichens require supplementary investigation.

On the other hand $^{14}CO_2$ preponderant incorporation in organic acid fraction and not in sugars, after the first minutes of exposure, in case of P-orizontalis — in contrast to the reverse situation which was noted in *U. florida* suggests that the organic acids are first products of photosynthesis in lichens containing blue-green algae symbionts. This would be in disagreement with the more general view that the main labelled compound appearing in the blue-green algae symbionts is glucose. Nevertheless glucose appeared to be labelled on the sugar radioautograms during the first minute, preceding thus the sucrose which became evident only after the second minute of $^{14}CO_2$ exposure. In the lack of supplementary data it is difficult to establish if glucose is synthesized directly or via "Organic acids" as it occurs in the Calvin cycle.

The observation seems interesting if considering the fact that in free algae as well as higher plants organic acids are also found among the first products of photosynthesis.

The highest radioactivity was found in the sugar alcohols in case of green algae symbionts [3], [19]. In *U. florida* we found also that $^{14}CO_2$ incorporation was the highest in soluble sugar fraction.

A detailed study on the amino acids of lichens was conducted by Ramakrishnan and Subramanian [14], [15]. The amino acid fraction is well represented in lichens. Our data show that certain amino acids, i.e. aspartic acid and alanine are direct products of photosynthesis as they appear to be labelled from the first minute. Glutamic acid appears somewhat later. These amino acids are also regarded as primary products of photosynthesis in other plant groups.

As considering the occurrence of amino acids it may be noted that it is quicker in *U. florida* (green algae symbionts) as against *P. horizontalis* (blue-green algae symbionts), a fact which explains the more intense growth of *Usnea thalluses*. The low intensity of growth in lichens is generally due to the reduced protein synthesis in their thallus.

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GAMETIC PRODUCTION IN *ADONIS* (SUBSECT. VERNALES)

BY

A. T. SZABÓ

Further to previous studies [9], [10] this paper presents data referring to the gametic production in an *Adonis* topodeme (Finaţele Clujului, Transylvania, Romania). The gametic production of *A. Vernalis* exceeds by about 40 times the production of *A. volgensis* except the first flowering period, when *A. volgensis*, prevails. Hybrid plants with higher pollen sterility percentages have an intermediate position with respect to the gametic production measured on the sample area. Diagnema have been added and nomenclatural adjustments have been made as concerns the taxonomic differentiation of phenodemes, including hybrids.

The reasons for the expansion or restriction of the area of many spontaneous plant species are not sufficiently known. In panmictic populations one of the reasons may be the different gametic contribution of a considered genotype (species, variety, ecotype) to the descendent generations [2]. If different taxa belonging to two or more sympatric populations, which hybridize to a certain degree, differ in point of the number of available gametes, then these populations may have different gametic contributions to the descendent generations. Consequently, changes in the gene frequency — as well as in the genotype frequency occur on the level of gamete production and an expansion or dominance may result. This means that the effect of selection on the gene frequency may depend on the intensity and direction of the selection, and also on the amount of generated gametes carrying the information of the given genotypes.

On the Fînaţele Clujului (Transylvania, Romania) an interesting spontaneous *Adonis* topodeme (terminology according to Gilmour and Heslop-Harrison in Davis and Heywood) [1] was studied. This topodeme consists of relatively few individuals, belonging to different genodemes and phenodemes but to a single coenogamodeme. The topodeme is localized in a well delimited area [6], [10]; gene exchanges take place on the diploid level [9].

Excepting *A. vernalis* L., all the phenodemes have different taxonomic interpretations in literature [5], [7], [8], [12]. In our opinion the introgression followed by interbreeding and permanent back-crossing contributed to the almost complete disappearence of the original *A. volgensis* Stev.; the occurring phenodemes are in fact notomorpha of *A.* × *hybrida* Wolff em. *hoc loco*, representing different microevolutive stages of a complex evolutionary process. This point of view is supported both by high pollen sterility — an important differential character for the hybrids — and by chemotaxonomical evidences [Szabó T.A., 1977, Contr. bot., Cluj-Napoca, 231—241].

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In order to determine the degree of the gametic production of the main phenodemes, measurements have been performed in a sample area of 40 × 200 m in the western side of the previously plotted territory [10]. Phenodemes have been determined according to their character combinations given in Table 1.

Table 1

Characters used for the identification of the phenodemes

Characters used	Symbols used	1	2
Flower diameter (d)	A	d>35 mm	d<35 mm
Width of the leaf segments (w)	B	w>1,5 mm	w<1,5 mm
Hairiness	C	glabrescent	pubescent

Reproductive organs (flower buds, flowers, fruits) have been collected and counted for each phenodeme and phenological stage. The following data have been registered: average number of anthera per flower = a [Table 2]; average number of microspore per anthera, considering conventionally that a fertile microspore corresponds to a male gamete = b; pollen sterility = c. The gametic production has been estimated for each phenodeme and phenological stage, namely for plants with fruits (genetical information still intercepted, d), for flowers which represent the emission-reception period (e); and for flower buds in which genetical information is prepared (f). The sum of d, e and f is the total potential gametic production of each phenodeme to the descendent generations (g).

The different phenodemes — represented here by symbols and typological names — have significantly different gametic ratios (Table 2).

The $A_1B_1C_1$ phenodeme (*A. vernalis* L.) obviously overwhelms the $A_2B_2C_2$ phenodeme (*A. volgensis* Stev. var. *latisecta* var. nova) as concerns the number of anthera per flower (168 as against 61) and the average number of microspores per anthera (590 as against 2600). In spite of the similar pollen sterility percentage (4%) the *vernalis* phenodeme exceeds by about 40 times the *volgensis* phenodeme as to the total gametic production (291 million male gametes against 6.9 million male gametes). This rate tends toward infinity if we take into account the entire territory where *A. vernalis* is a common species but *A. volgensis* is absent altogether.

It should be noted that, in the first period, the relation is reversed while the rate tends to infinity in favour of *volgensis* phenodeme, which produces 3.55 million male gametest against 0 male gametes produced by *A. vernalis*. This first period represents the phenological isolation and ensured a long-term survival for the *volgensis* phenodeme.

The gametic production of $A_1B_2C_1$ phenodeme (*Adonis* × *hybrida* nm. *transsilvanica* (Simonovich) em. hoc loco is worthy of further notice. The relative abundance of individuals belonging to this phenodeme, the high number of microspores per anthera, lower pollen sterility etc. indicate a genetically better balanced notomorpha with higher vitality. Due to

Table 2

Gametic production of the different Adonis phenodemes (Finaţele Clujului, Transylvania, Romania, 16.IV.1976)

Phenodeme symbols (see Tab. I.)	Anthera per flower	Pollen per anthera	Pollen sterility %	Gametic production, Million male gamets (pollen) emited			
				prior to 16.Apr.	at 16 Apr.	after 16. Apr.	Total
	a	b	c	d	e	f	d e f
$A_1 B_1 C_1$ (A. vernalis)	168	5 900	4	—	161.30	130.00	291.30
$A_1 B_{1-2} C_1$ (nm. hybrida)	123	3 400	72	—	0.13	2.70	2.83
$A_1 B_{1-2} C_2$ (nm. walziana)	123	4 100	56	—	0.49	1.32	1.81
$A_1 B_2 C_2$ (nm. transsilv.)	83	3 100	34	3.62	7.38	2.40	13.30
$A_1 B_2 C_1$ (nm. transsilv.)	86	3 200	28	13.35	11.52	4.30	29.17
$A_2 B_2 C_{1-2}$ (nm. claudiopolitana)	78	3 100	18	2.60	0.34	0.40	3.34
$A_2 B_2 C_2$ (A. volgensis)	61	2 600	4	3.55	3.08	0.31	6.94
total male gamets per periods				23.12	184.24	141.43	348.69

hybrid vigour [3], this phenodeme is the most distinct as compared with *A. vernalis* L. with respect of leaf characters (Plate I.). Being relatively frequent on the territory (and also commonly collected for herbaria) this notomorpha has been identified by previous botanists as *A. volgensis* Stev. The nomotaxon *A. transsilvanica* Simonovich 1965 may be identical with this notomorpha.

Taking into account the biology (longevity, low germination rate) of the *Adonis* plants [4], the differences noticed in gametic production may represent cause-effect relations as far as dynamics and microevolution of the coenogamodeme are concerned.

Adjustments and diagnemas for the nomenclature of *Adonis* × *hybrida* Wolff em. hoc loco and *A. volgensis* Stev. in reference to the International Code of Botanical Nomenclature 13, paragraphs 32/3, 70 and H 10:

Adonis × *hybrida* Wolff 1957 nomen nudum, em hoc loco, syn. *A. volgensis* auct. non Stev., *A. transsilvanica* Simonovich 1965 *pro maiore parte:* planta perennis, caules glabri vel pubescentes. post anthesin ad 600 mm alti, folia caulina inferiora vaginiformia, lamina vix evoluta, folia media sessilia bipinnatisecta, circumscriptione late triangularia, vel fere rhomboidea, glabra vel pubescentia, laciniis lanceolatis 0,5—5 mm latis, multinucula ovato-elongata vel globosa, flores magni 50—70 (20—90) mm in diam. cum (80) 100—170 antheribus ovatis, pollen sterilis 10—80%.

nm. *hybrida*, syn. *A. hybrida* Wolff 1857 n.n. *pro parte*, Simonkai 1878 cum descriptione sub *A. volgensis* × *supervernalis* [7]: pedunculi recti vel sinuati, subglabri, folia caulina circumscriptione late triangularia, subglabra, laciniis ingustae 0,5—1 mm, flores magni 50—70 mm in diam., pollen sterilis 60—80% (Tab. II. $A_1B_{1-2}C_1$ Plate I, H). Typus: CLA (Herbarium Instituti Agronomici Cluj-Napoca) 06742.

nm. *walziana* (Simk. 1878), syn. *A. walziana* Simk. = *A. vernalis* × *supervolgensis* p.p., *A. hybrida* Wolff f. *walziana* (Simk.) A. Nyár. p.p.: caules floriferis post anthesin nutantes vel recurbatis, folia caulina circumscriptione rhomboidea, lacinii foliis caulinas 1—1,5 mm latis, pubescentis

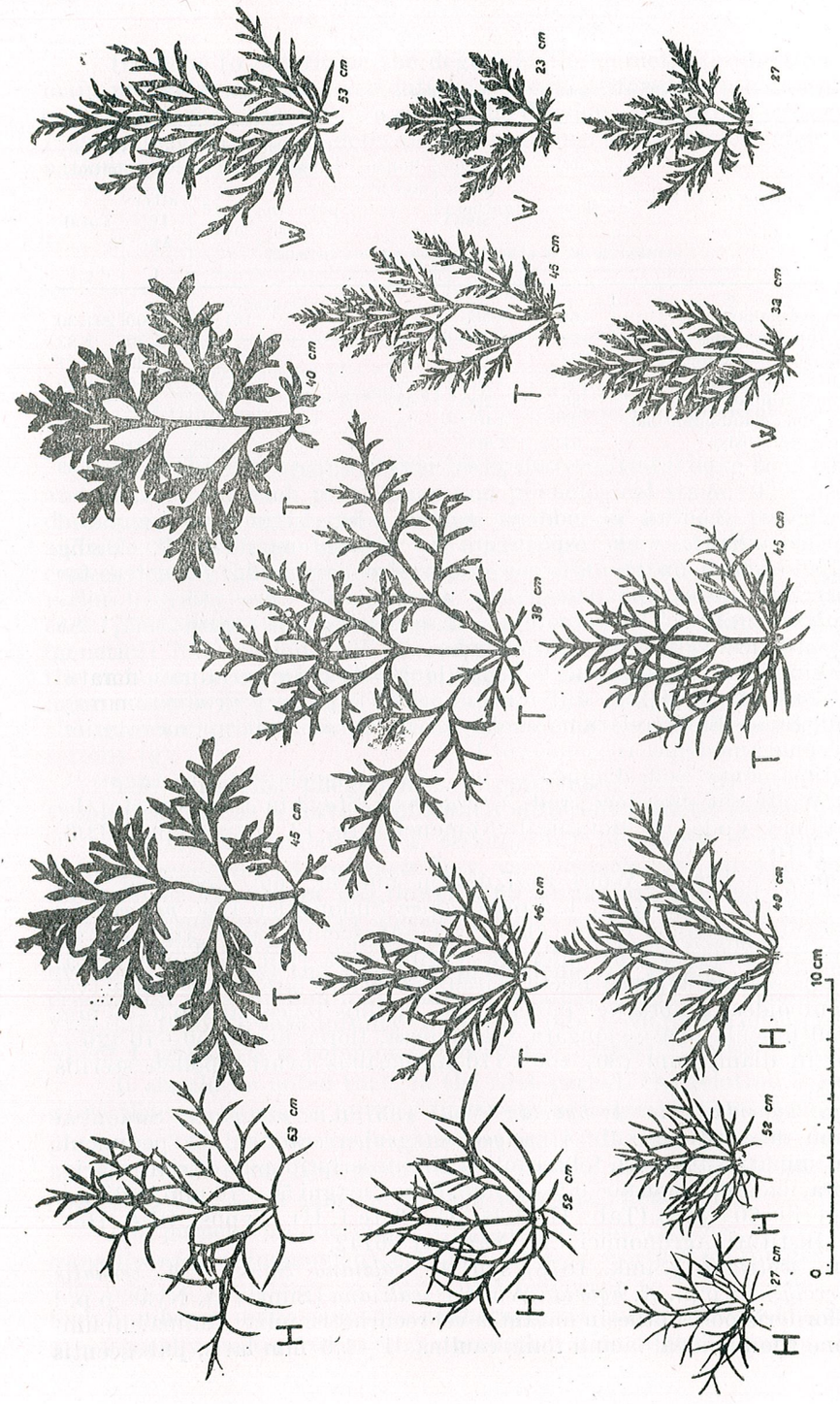

Plate 1

Leaf shape of the different notomorpha of *Adonis* × *hybrida* Wolff 1857 nomen nudum em. hoc loco cum notomorpha. H = *A. hybrida* nm. *hybrida*; T = *A. hybrida* nm. *transsilvanica* /Simonovich/; W = *A. hybrida* nm. *walziana* /Simk./; V = *A. volgensis* Stev. var. *latisecta* var. nova. The numbers indicate stem height in cm.

Plate 2

Flower size and stem position in *Adonis* subsect. *Vernales* 1. *A volgensis* Stev., flower and stem with mature fruit, from Dobrogea; 2. *A. volgensis* Stev. var. *latisecta* var. nova, flower, unmature fruit and mature achena; 3. *A.* × *hybrida* Wolff s.n. nm. *walziana* Simk. s.n., flower and unmature fruit; *A. hybrida* nm. *transsilvanica* /Simonovich/, flower, leaf and mature fruit; 5. *A. hybrida* nm. *claudiopolitana* nm. nova f. *comosa*, flower and unmature fruit; 6. *A. vernalis* L. flower, leaf, achena and mature fruit. Numbers 2—6 are from Finaţele Clujului.

vel subglabris, flores magni, pollen sterilis 50—60% (Tab. II., $A_1B_{1-2}C_2$, Plate I, W, Plate II, 3). Typus : CLA 18246.

nm. *transsilvanica* (Simonovich 1965) comb. nova, syn. *A. volgensis* auct. non Stev. f. *subglabra* Nyár. et f. *glaberrima* Nyár., *A. transsilvanica* Simonovich *pro maiore parte:* caules longioribus, post anthesin vel 600 mm altis, glabres vel subglabres (f. *glaberrima* Nyár., f. *subglabra* Nyár.) vel pubescentes (f. *pilosa* Schur), pedunculi in fructibus recti vel sinuati, multinucula ovata, folia caulina media circumscriptione late triangularia vel rhomboidea, 50—100 mm longa, laciniis 2—5 mm latis, flores 50—60 (80) mm in diam., cum 80—90 antheribus ovatis, pollen 20—40% sterilis. (Tab. II, $A_1B_2C_1$, $A_1B_2C_2$, Plate I, T, Plate II, 4). Typus : CLA 18251 (f. *subglabra*), CLA 18260 (f. *glaberrima*).

nm. *caludiopolitana* nm. nova : flores minoribus, 20—50 mm in diam. cum 60—80 antheribus globosus, pollen 10—50% sterilis, caulis glabris vel pubescentis, funiformibus cum laciniis foliis angustatis, vel caulis incrassatis et laciniis foliis latae (f. *crassa* f. nova), vel pedunculi floribus breves cum foliis involucratae (f. *comosa* f. nova, Plate II, 5 Tab. II. $A_2B_2C_{1-2}$). Typus : CLA 18245 (f. *crassa*) ; CLA 18263 (f. *comosa*).

A. volgensis Stev. non auct., syn. *A. hybrida* auct. incl. Wolff, *A. hybrida* f. *pilosa* Schur pro maior parte, *A. walziana* Simk. p.p. : caules breves ad 270—300 mm altis, gracillimus, ± pubescentes, flores minoribus 20—35 mm in diam. cum 60—70 antheribus globosus, pollen 4 (10) % sterilis, multinucula globosa, pedunculi in fructibus recurbatis, folia caulina late triangularia cum laciniis ad 2 mm latis (var. *latisecta* var. nova), vel rhomboidea cum rachis segmenti mediani ellongati (var. *moldavica* var. nova). Floret ante omnibus. (Tab. II, $A_2B_2C_2$, Plate I, V, Plate II, 1—2). Typus : CLA 18255 (var. *latisecta*) ; CLA 18241 (var. *moldavica*).

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CARACTÉRISTIQUES PHYSIOLOGIQUES DU SUC CELLULAIRE — INDICATEURS ÉCOLOGIQUES DU RÉGIME HYDRIQUE DES PLANTES DE RIVE BOCAGÈRE

PAR

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Le travail présente la pression osmotique et la teneur en sucre pour un nombre de 26 espèces herbacées et ligneuses de la prairie de Ciorogîrla (la Plaine Roumaine). La pression osmotique atteint des valeurs élevées pour la plus grande partie des espèces (11, 11—25, 39 bars) plus augmentées aux espèces ligneuses par rapport à celles herbacées, en corrélation avec la teneur en sucre compris entre 5,5 et 19,0% (le coefficient de corrélation r = 0,92).
L'équation de regression calculée pour la pression osmotique et la concentration en sucre des *Phragmites australis* est $y = 0{,}33\,x + 3{,}14$; celle de *Rubus caesius* est $y = 0{,}36\,x + 4{,}27$.
Les valeurs élevées de la pression osmotique indiquent une absorption plus difficile de l'eau du substrat, en reflétant la tendance de saturation et alcalinisation de celui-ci (le pH du sol varie autour de la valeur de 7,60).

L'analyse de la dynamique de certains facteurs internes des plantes, ainsi que les caractéristiques du suc cellulaire, permet la connaissance et l'approfondissement de certains processus physiologiques et, en même temps, fait ressortir l'influence du milieu et le caractère constant des adaptations des plantes en conditions répétables, ce qui donne la possibilité de leur utilisation en tant que test écologique.

Nos recherches menées sur les populations des plantes de bocage se proposent de présenter le régime hydrique d'un écosystème dans lequel l'eau — en tant que facteur du milieu — est une présence physique abondante, et d'indiquer le spécifique d'adaptation au niveau des producteurs primaires dominants.

MÉTHODES ET MATÉRIEL DE RECHERCHE

Le matériel végétal vivant a été récolté mensuellement, de 21 espèces de la plaine alluviale de Ciorogîrla (Com. Domneşti—Ilfov) dans la période mai — septembre 1976. Les espèces ont été choisies de la zone de rive bocagère et de la zone étroite du bord des cultures agricoles, de 3 catégories : *1*, espèces herbacées et mi-arbustives de rive bocagère ; *2*, espèces ligneuses de bocage ; *3*, espèces ligneuses du bord des cultures.

L'échantillon de matériel végétal a été récolté de minimum 10—20 individus. Le suc cellulaire est extrait par l'extorsion du matériel végétal, détruit par cuisson, dans un pressoir prototype exécuté par l'Académie Roumaine.

La concentration en sucre a été déterminée par la méthode réfractométrique avec le réfractomètre portable type Abbe. La pression osmotique a été déterminée par la méthode cryoscopique, le gelé se réalisant avec de la neige carbonique.

RÉSULTATS ET DISCUSSIONS

Dans la plaine alluviale de Ciorogîrla, l'approvisionnement du substrat avec de l'eau, pendant l'été, est toujours bon, compris entre 15 et 25%, grâce au rapprochement de la couche phréatique (fig. 1); c'est la raison pour laquelle l'humidité relative maintient des valeurs augmentées même dans les conditions de manque de précipitations abondantes. Il faut préciser pourtant que la texture du sol est sableuse, ayant donc une faible capacité de rétention de l'eau. La réaction du sol est neutre-alcaline, avec une tendance d'alcalinisation augmentée vers l'automne. On remarque que l'alcalinisation augmente aussi du bord de l'eau vers le terrain agricol, les valeurs étant comprises entre 7,3 et 7,9 pH; l'alcalinisation est d'habitude en corrélation avec la salinité; il existe donc certaines prémisses qui puissent indiquer un niveau réduit de l'eau accessible dans cette région bien que l'humidité totale présente un niveau élevé.

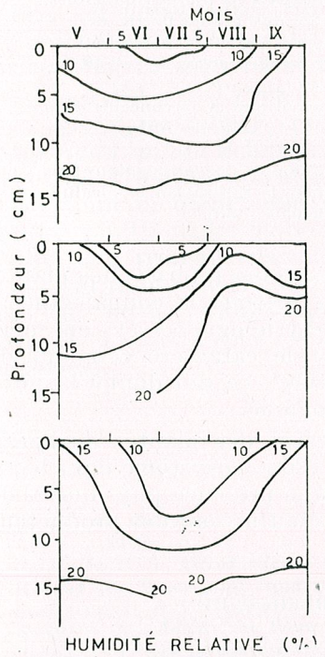

Fig. 1. — Chronoïsoplète du substrats de stations.

1. LA PRESSION OSMOTIQUE DU SUC CELLULAIRE

A la suite de l'examen des valeurs moyennes de la pression osmotique du suc cellulaire des populations de bocage (Tableau 1) on peut constater qu'elles diffèrent assez, en fonction de l'espèce, entre 11,11 bars à *Lysimachia nummularia* et 27,50 bars à *Populus nigra*. Les espèces herbacées ont, en général, des valeurs plus réduites que celles ligneuses; entre les herbacées, les espèces némorales, avec un tissu du soutènement réduit et des racines moins profondes, présentent les valeurs les plus réduites (par. ex. *Aegopodium podagraria*, *Galium palustre*).

Les plantes situées dans la zone du bord des cultures agricoles, plus ensoleillées, présentent des valeurs plus augmentées que celles des bocages, plus ombragées, mais en général plus réduites que celles des ligneuses (*Setaria glauca*, *Cichorium intybus*).

Tableau 1

Caractéristiques du suc cellulaire des espèces herbacées et ligneuses

Espèce	Station	Pression osmotique $\bar{x} \pm s_{\bar{x}}$	Contenu en sucre $\bar{x} \pm s_{\bar{x}}$
Espèces herbacées et mi-herbacées de bocage			
— *Phragmites australis*	A	21,9 ±24,80	10,90±12,30
,, ,,	M	21,68±22,90	11,10± 8,69
— *Lysimachia nummularia*	M	11,11± 5,50	5,80± 0,21
— *Aegopodium podagraria*	M	13,41± 6,26	7,70± 0,33
— *Galium palustre*	M	18,89	6,80
— *Aristolochia clematites*	M	20,31±18,72	8,80± 8,20
— *Rubus caesius*	A	20,17±14,64	9,30± 0,91
,, ,,	M	20,82± 9,70	10,80± 1,25
Espèces ligneuses et mi-ligneuses de bocage			
— *Populus nigra*	M	27,50± 8,29	13,90± 0,88
— *Alnus glutinosa*	A	24,04±11,52	13,80± 7,20
— *Salix alba*	A	22,29± 9,20	12,20± 5,82
— *Amorpha fruticosa*	M	24,41±22,70	11,90± 6,20
— *Cornus sanguinea*	M	25,39±12,82	15,90± 7,80
— *Crataegus monogyna*	M	31,17± 0,30	19,08± 3,17
— *Clematis vitalba*	M	31,21	17,00
— *Humulus lupulus*	M	22,55± 5,80	10,30± 4,40
Espèces ligneuses du bord des cultures			
— *Asparagus tenuifolius*	m	24,76± 1,75	10,90± 1,20
— *Chaerophyllum temulum*	m	26,57	10,40
— *Poa pratensis*	m	15,88	9,00
— *Lolium perenne*	m	18,02± 8,80	9,36± 7,75
— *Setaria glauca*	m	13,00	5,20
— *Cichorium intybus*	m	12,52	5,50
— *Veronica chamaedrys*	m	18,36	10,00

A = près de l'eau; M = milieu du bocage; m = haut rivage, bord des cultures.

Dans une première synthèse, les recherches concernant la pression osmotique [6] présentent pour les espèces mésophylles des valeurs moyennes de 10,4 bars pour les herbacées et de 14,4 bars pour les feuilles des arbres. Les valeurs que nous venons d'obtenir indiquent un niveau bien plus élevé, en reflétant le spécifique des conditions concrètes, locales.

Un travail plus récent [3] publie le spectre osmotique de Walter, qui indique les limites connues pour différentes espèces européennes. Nous avons constaté que, par rapport au spectre de Walter (fig. 2), les valeurs déterminées par nous ont une amplitude saisonnière large pour la plupart des espèces et s'intègrent aux limites supérieures de la catégorie des plantes de forêt (10 jusqu'à 30 bars).

Les espèces des plantes d'eau présentent des valeurs plus réduites dans ce spectre (2—27 bars) rapportées à celles déterminées par nous pour les populations du voisinage de l'eau (10—30 bars) mais on remarque une zone de superposition, comprise entre 10 et 15 bars; il est possible que les plantes avec une pression osmotique plus réduite soient celles submersibles.

A Ciorogîrla, les individus du voisinage immédiat de l'eau, ainsi que ceux plus éloignés de l'eau, au milieu du bocage, ne présentent de différences quant à la pression osmotique, celles existantes (Tableau 1) s'encadrant dans la variation statistique des données, en s'agissant d'une seule population statistique qui ne se sépare pas à la suite de la modification des microconditions. Donc, la consommation d'eau des plantes du bocage de Ciorogîrla est plus grande et l'absorption est plus difficile ; il faut une force osmotique plus élevée que d'habitude pour l'extraction

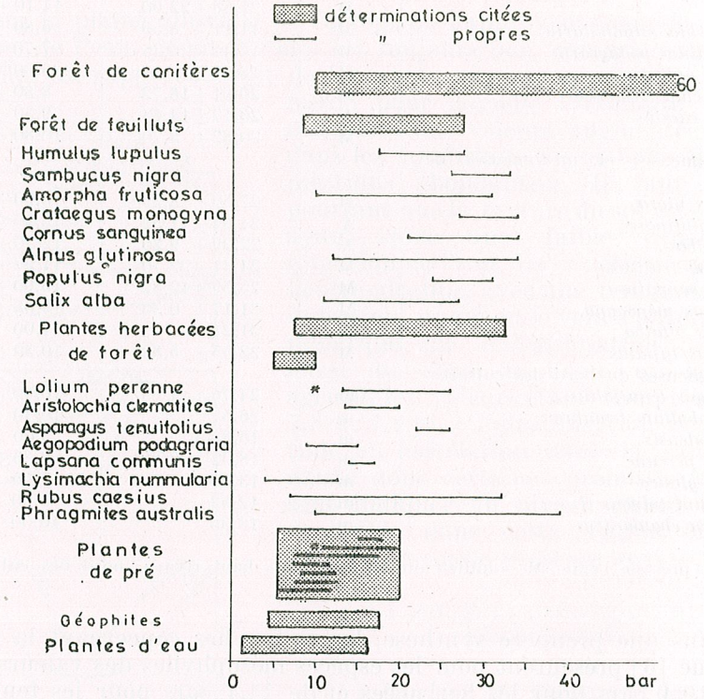

Fig. 2. — Séries des valeurs de la pression osmotique des feuilles des espèces examinées par nous, comprises dans le spectre osmotique constitué par Walter (1960).

de l'eau dans cette station. L'eau est retenue dans le substrat à l'aide des forces augmentées à cause des sels solubles de la solution du sol, de l'existence desquels nous nous rendons compte dans le substrat à la suite des déterminations du pH alcalin, les deux étant en corrélation. Du point de vue écophysiologique, on a pu mettre en évidence, directement ou par voie synthétique, ce complexe des facteurs du milieu.

La pression osmotique du suc présente des valeurs différentes au cours de la saison de végétation, ayant une dynamique forte et étroitement liée à la dynamique des facteurs du milieu (fig. 3). Aux espèces

de bocage on observe que la période de l'été s'intègre dans le type classique du courbe, mais pour toutes les espèces surgit pendant l'été un moment maximum, à la fin du mois de juillet. Ce moment marque la période la plus difficile à laquelle la plante s'adapte à la suite de l'augmentation de la pression osmotique. Les espèces herbacées sont plus constamment affectées que celles ligneuses ; on peut supposer donc que le niveau de l'eau soit baissé et que les plantes avec des racines moins profondes aient un approvisionnement d'eau plus faible.

On remarque une augmentation intense de la pression osmotique au cours du mois de septembre chez les individus des *Phragmites australis* et *Rubus caesius*, étant atteint le plus faible potentiel de l'eau = pression osmotique grande + contenu réduit d'eau.

En vue d'une caractérisation des conditions abictiques du milieu, les espèces sténohydriques nous semblent très importantes (fig. 2) ; à savoir *Asparagus tenuifolius*, *Lapsana communis*, lesquels, strictement limités à un intervalle étroit de valeur de la pression osmotique, signaleront toujours, par leur présence, *un certain niveau de l'humidité accessible au substrat*. D'autre part, la présence, à Ciorogîrla, d'un grand nombre d'espèces eurihydriques, reflète la grande capacité d'adaptation de la majorité des espèces de bocage aux conditions variables de vie.

2. LA CONCENTRATION EN SUCRE DU SUC CELLULAIRE

Pour la plupart des espèces de la plaine alluviale de Ciorogîrla, nous avons constaté que le sucre a le rôle principal dans le réglage de la pression osmotique ; la figure 4 montre le degrès de corrélation entre la pression osmotique et le sucre des *Phragmites australis* et *Rubus caesius*. La relation entre les 2 paramètres exprime la dépendance directement proportionnelle ; la valeur du coefficient de corrélation de 0,931 pour *Phragmites* et de 0,927 pour *Rubus*, exprime une corrélation forte (distinctement significative selon le test de Fisher). Pour ces deux espèces on a pu déterminé aussi l'équation de la droite de régression selon laquelle varient les deux paramètres. Les équations sont rapprochées, ce qui nous permet de croire qu'on pourrait les généraliser pour les populations de la même station : *Phragmites australis* $y = 0{,}33\,x + 3{,}14$; *Rubus caesius* $y = 0{,}36\,x + 4{,}27$.

La variation du contenu en sucre pour les espèces de la prairie de Ciorogîrla est évidente dans le tableau 1 ; on remarque un pourcentage élevé pour *Crataegus monogyna* (19%, en moyenne) et une quantité plus réduite pour *Setaria viridis* (5,2%) — les valeurs extrêmes des populations que nous avons analysées.

Les espèces ligneuses et mi-ligneuses présentent une quantité plus grande de sucre que celle herbacées, en augmentant d'une manière claire la capacité osmotique de ces plantes. L'amplitude de la variation du sucre est modérée pour la plupart des populations analysées (fig. 5).

Pendant la saison de végétation, la dynamique du sucre est plus active et moins régulière que la pression osmotique ; la plupart des espèces présentent un maximum estival assez ample (fig. 5). L'accumulation grande de sucre au mois de septembre s'explique au cas des plantes péren-

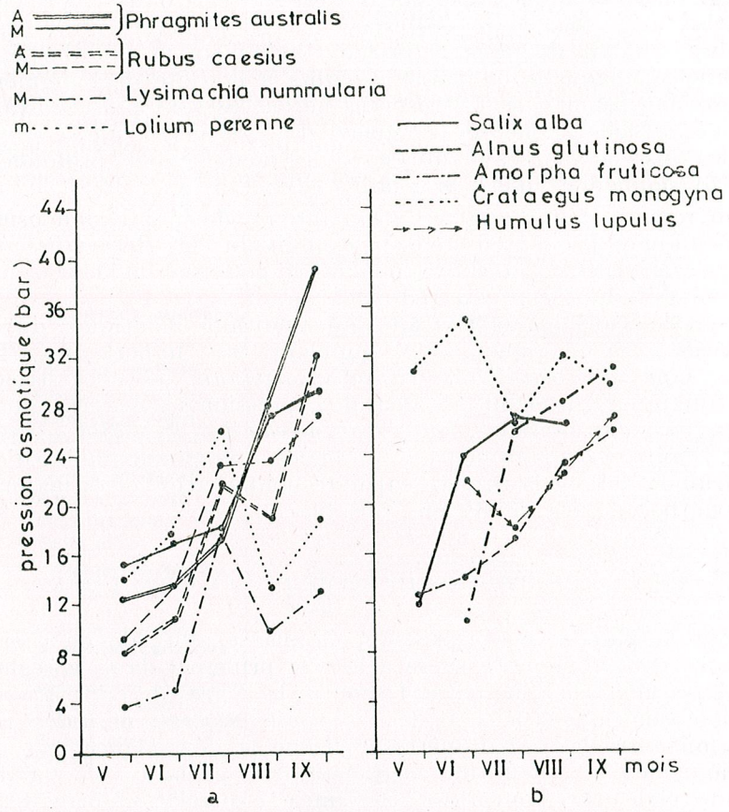

Fig. 3. — Dynamique saisonnière de la pression osmotique ; *a*, espèces herbacées, *b*, espèces ligneuses.

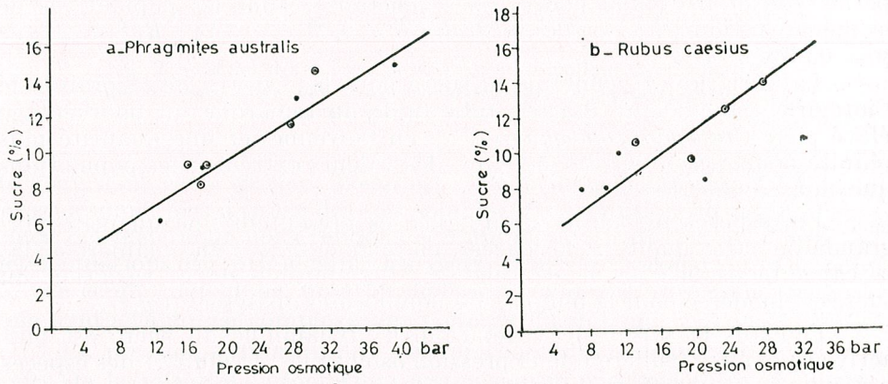

Fig. 4. — *a*, *b*, Droites de regression qui expriment la corrélation du contenu en sucre avec la pression osmotique.

nantes à l'aide de leur arrangement pour la saison froide. Cette accumulation explique aussi l'augmentation de la pression osmotique de cette période.

Un aspect plus particulier de la dynamique saisonnière est remarqué à *Alnus glutinosa*, auquel paraît un maximum estival en sucre mais on n'observe pas une nouvelle augmentation au mois de septembre comme pour les autres espèces.

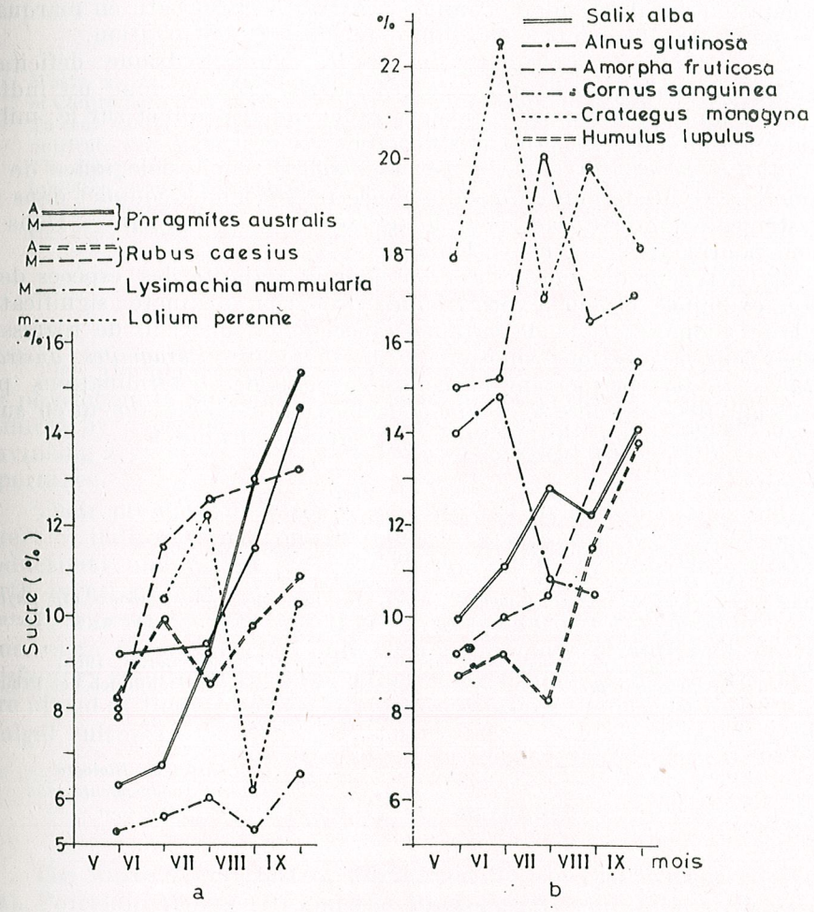

Fig. 5. — Dynamique saisonnière du contenu en sucre ; *a*, espèces herbacées, *b*, espèces ligneuses.

On remarque même dans le cas du sucre de grandes valeurs pour les espèces de bocage, comparables à celles des régions froides, où elles jouent un rôle énergétique de protection [1], [2], [4]. Dans cette station, le contenu augmenté reflète la même adaptation des plantes aux condi-

tions moins favorables du milieu, déterminées par la retention de l'eau avec des forces augmentées dans le substrat salé, bien que sa quantité absolue soit plus grande.

CONCLUSIONS

1. La pression osmotique dans les conditions de la plaine alluviale de Ciorogîrla a eu des valeurs plus grandes que celles des populations de la végétation zonale de forêt, comprises entre 10 et 30 bari, en marquant de cette manière le caractère de salinité du substrat de la station.

2. La pression osmotique a surpris le régime hydrique déficitaire physiologiquement dans la station de Ciorogîrla ; elle est donc un indicateur écologique sensible et plus exact, grâce au test direct sur le milieu à l'aide des producteurs primaires de l'écosystème.

Il est nécessaire d'élargir les recherches écophysiologiques de ce paramètre en vue de l'obtention d'un spectre écologique complet dans les écosystèmes caractéristiques pour le territoire de la Roumanie. Dans ce cas, on pourra arriver à un étalonnage.

3. La teneur en sucre est grand pour la majorité des espèces de la station examinée et elle exprime une corrélation distincte significative avec la pression osmotique de certaines espèces. Les équations de régression établies pour la pression osmotique et le sucre aux *Phragmites australis* et *Rubus caesius*, permettent l'accomplissement des déterminations précises de la teneur en sucre selon une méthode plus facile. La teneur en sucre devient indirectement un indicateur du régime hydrique.

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SOME METABOLIC CHARACTERISTICS OF *SCENEDESMUS ACUTUS* CULTIVATED IN MEDIA PREPARED FROM WASTE WATERS

BY

ŞT. PÉTERFI, FR. NAGY-TÓTH, ADRIANA BARNA, M. ŞTIRBAN, V. BERCEA

Waste waters from the Brewery, Porcelain Works, and Pharmaceutical Works of Cluj-Napoca city, mixed and diluted with the waters of the Someş, were tested. To each mixture $(NH_4)_2HPO_4$ an amount of 3.30 g per liter, as only exogeneous nutrient, were added.
The data recorded show that the waste waters from the Pharmaceutical Works inhibited strongly the multiplication of *Scenedesmus acutus*, but the other types of waste waters mixed in the culture media ensured a satisfactory growth of this alga, though they did not stimulate it.

INTRODUCTION

The abundance of the *Scenedesmus* species in several biotopes, the physiological flexibility (nutrition, photosynthesis, respiration, etc.) manifest by a prodigious morphological variability (polymorphism), are convincing arguments in favour of their ecological and possible economic importance.

Their presence in water reservoirs, and oxidation basins and their (direct or indirect) involvement in the purification of waste waters, high productivity and use of their biomass poses many interesting problems worth investigations.

It was found [2], [15] that certain solutions obtained by mixing some waste waters enriched only with a minimum of nutrients, provided satisfactory results for the production of algal biomass. These experiments were aimed at finding some more possibilities for the use of waste waters in algal cultures.

MATERIALS AND METHODS

The waste waters tested, discharged by the Pharmaceutical Works (Ph), Porcelain Works (P) and the Brewery (B) were diluted in various proposition in water samples from upstream (S-up) and downstream (S-down) the Şomeş river (the Cluj-Napoca area). The mixtures thus obtained were enriched with 3.30 g $(NH_4)_2HPO_4$ per liter, the only exogeneous substance added. As control, the modified Tamiya urea EH

solution was used. In this way the following experimental variants were prepared :

1. S-up + B in proportion of . . . . 3 : 1 ;
2. S-up + B + P „ „ „ . . . . . 2 : 1 : 1 ;
3. S-up + B + P + Ph „ „ „ . . . . . 1 : :1 :1 :1 ;
4. S-down + B „ „ „ . . . . . 3 : 1 ;
5. S-down + B + P „ „ „ . . . . . 2 : 1 : 1 ;

6. Tamiya urea EH modified nutrient solution (control).

The liquid mixtures were not sterilized. The pH was adjusted to 7.0—7.5. The material was grown in vertical columns [18]. As biotest *Scenedesmus acutus*, "Fortuna" strain, was used [17]. The initial density was 250 cells per µl. The cultures were bubbled (10—15 ml per 100 ml suspension per min air + 3—5 CO_2) daily for 13 hrs under light (Electrofar Daylight 40 W tubes, 5000+—2001x). It is considered that bubbling develops in the cultures rheoplanktonic properties, which together with the original (polluted, and unpolluted) composition of waters might enable the modelling of the spontaneous conditions of algal growth in rivers, as well as the self-purification of waters.

Determinations involved :

1. cell density (with Bürker haemocytometer) ;
2. optical density (photocolorimeter FEK-56 M) ;
3. dry weight ;
4. protein (aminic groups) content of cells and of cultural liquid (supernatant), according to Lowry applied by Fogg (1966) [6];
5. pigment content (chlorophyll *a* and *b*, carotene, luteine, violaxanthine, neoxanthine) according to Hager and Meyer-Bertenrath (1966) [9];

From the data recorded the growth-rate constant (k) and the *t*-test of significance were computed. Some morphological modifications of the cells and the coenobia (*polymorphism*) were also noted.

RESULTS AND DISCUSSIONS

The growth of microbial and implicitly algal populations [8] is generally viewed in a broader sense than in physiology. In fact it is conceived as an increase of biomass instead of volume. Accumulation of synthesized substances (cell-mass) in a batch-culture is indicative first of cell multiplication, without considering the enlargement of cells, i.e., physiological growth. The synchronized cultures of *Chlorella*, *Scenedesmus* [13], [21], [22], [29] have demonstrated that the development (ontogenetic growth) of unicellular microalgae (alike to other living organisms) takes place in several stages each with its own morphological, physiological and biochemical characteristics. According to *Hrib et al.* [4], [11], [12] the growth (expansion) of cells, as well as of coenobia in *Scenedesmus quadricauda* occurs in several discontinuous stages, similar to that in *Avena coleoptiles* reported by Cleland (1971) [5].

The kinetics of microbial growth is explained more conveniently by the growth-rate constant (k). It is the biomass production ratio per time unit, expressed by a first-order equation. However, the direct

(arithmetic) plots of the values of biomass accumulation in a batch-culture (of microbes or algae alike) gives a sigmoid line [3], resembling the "great period of growth" (die grosse Periode des Waschstums) in vascular plants, a notion introduced by Sachs [25], [27]. Actually, the growth of microalgae always follows this course when life conditions differ from previous ones. In these cases one can distinguish a lag phase, an exponential phase, a stationary phase and a death phase, each corresponding to a well-known portion of the growth curve [30]. The variants formed of the waste waters from the Brewery and the Porcelain Works approach a sigmoid growth curve, but those consisting of waste waters from the Pharmaceutical Works, as well as in the control variants because of inhibition, and the lack of the lag phase, respectively such curves do not occur (Fig. 1).

A semilogarithmic plot of data on algal multiplication (biomass accumulation), irrespective of variables, supplies the growth rate of the process, the growth rate of the population either fixed or variable, indicates the sensitivity of the reaction, demonstrates the behaviour of cells under the impact of the factors acting upon them, distinguishes more readily the phases of growth and, affords a simpler calculation of the growth-rate constant of the slope [26]. In this respect the growth curves of the cultures indicate the extreme values : strongly positive in controls compared to the other variants, and strongly negative in the waste waters from the Pharmaceutical Works. Although, at the beginning, the growth-rate showed different values, in the end very close indices were recorded (Fig. 2). This kind of plotting shows more pregnantly than the arithmetic one the absence of the lag phase, i.e., the pretty fast cell multiplication in the control, and its presence i.e., cessation of growth, in the variant containing waste water from the Pharmaceutical Works. These differences also are demonstrated by the daily changes in the growth-rate constant (Fig. 3, Tabel 1).

Table 1

Daily values of the growth-rate constant (*k*) in *Scenedesmus acutus* cultivated in waste water media

Media \ Days	2	3	4	5	6	7	8	9	10	11
S-up+B 3 :1	0.046	0.113	0.192	0.220	0.110	0.130	0.051	0.035	0.036	0.030
S-up+B+P 2 :1 :1	0.012	0.089	0.103	0.262	0.064	0.105	0.060	0.075	0.007	0.063
S-up+B+P 1 :1 :1 :1 +Ph	−0.120	0.000	0.119	0.073	0.033	−0.020	0.039	0.059	−0.040	0.023
S-down+B 3 :1	0.050	0.087	0.132	0.213	0.110	0.145	0.043	0.065	0.014	0.066
S-down+B+P 2 :1 :1	−0.070	0.084	0.135	0.254	0.060	0.129	0.068	0.055	0.034	0.063
Tamiya urea EH modified (control)	0.800	0.300	0.076	0.098	0.103	0.050	0.076	0.025	0.026	0.008

Due to its adaptability (genetically defined) *Scenedesmus acutus* can use as nutrients the substances existent in waste waters, except for the Pharmaceutical Works wastes which contain nitrous derivatives (Table 2), that have an inhibiting effect on metabolism despite some probable alteration of its composition caused by dilution, illumination,

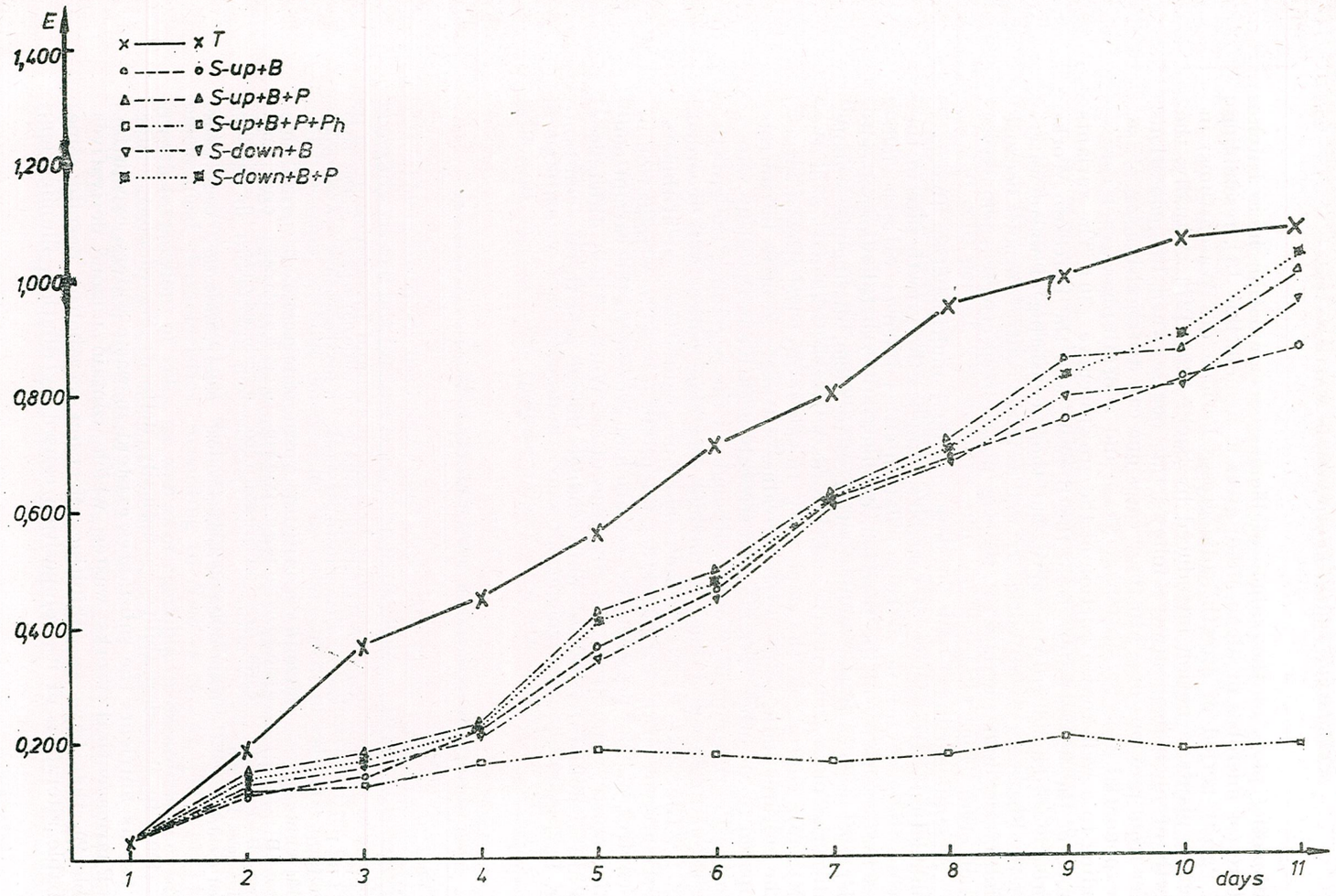

Fig. 1. — The accumulation of biomass (growth preiod) of the cultures of *Scenedesmus acutus* in media based on waste waters.

T — Tamiya urea EH modified nutrient solution; S-up — the Someş river water (upstream); S-down — Someş river water downstream Cluj-Napoca city; B — Brewery; P — Porcelain

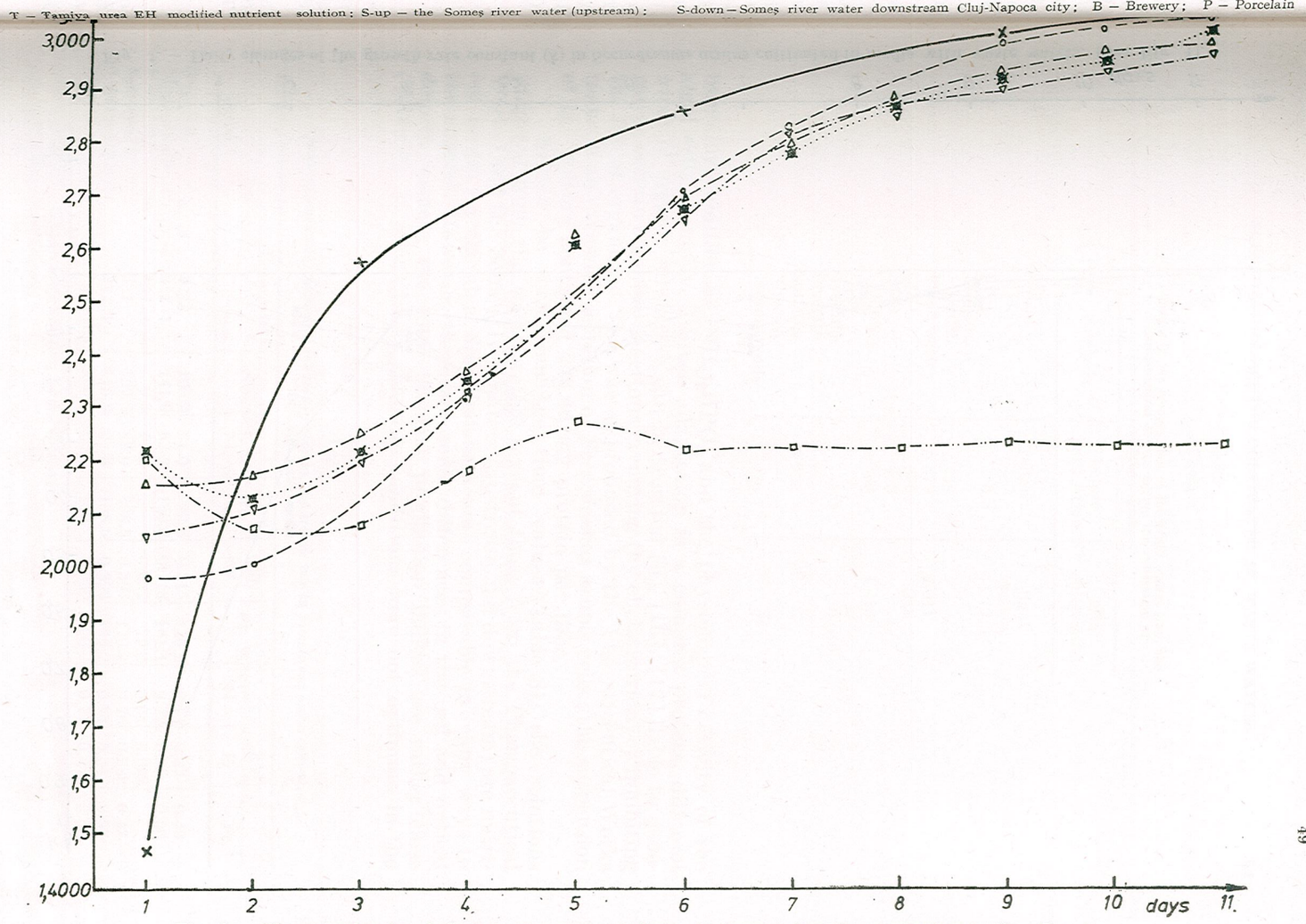

Fig. 2. — The growth-rate of *Scenedesmus acutus* cultures in terms of media composition. (See Fig. 1).

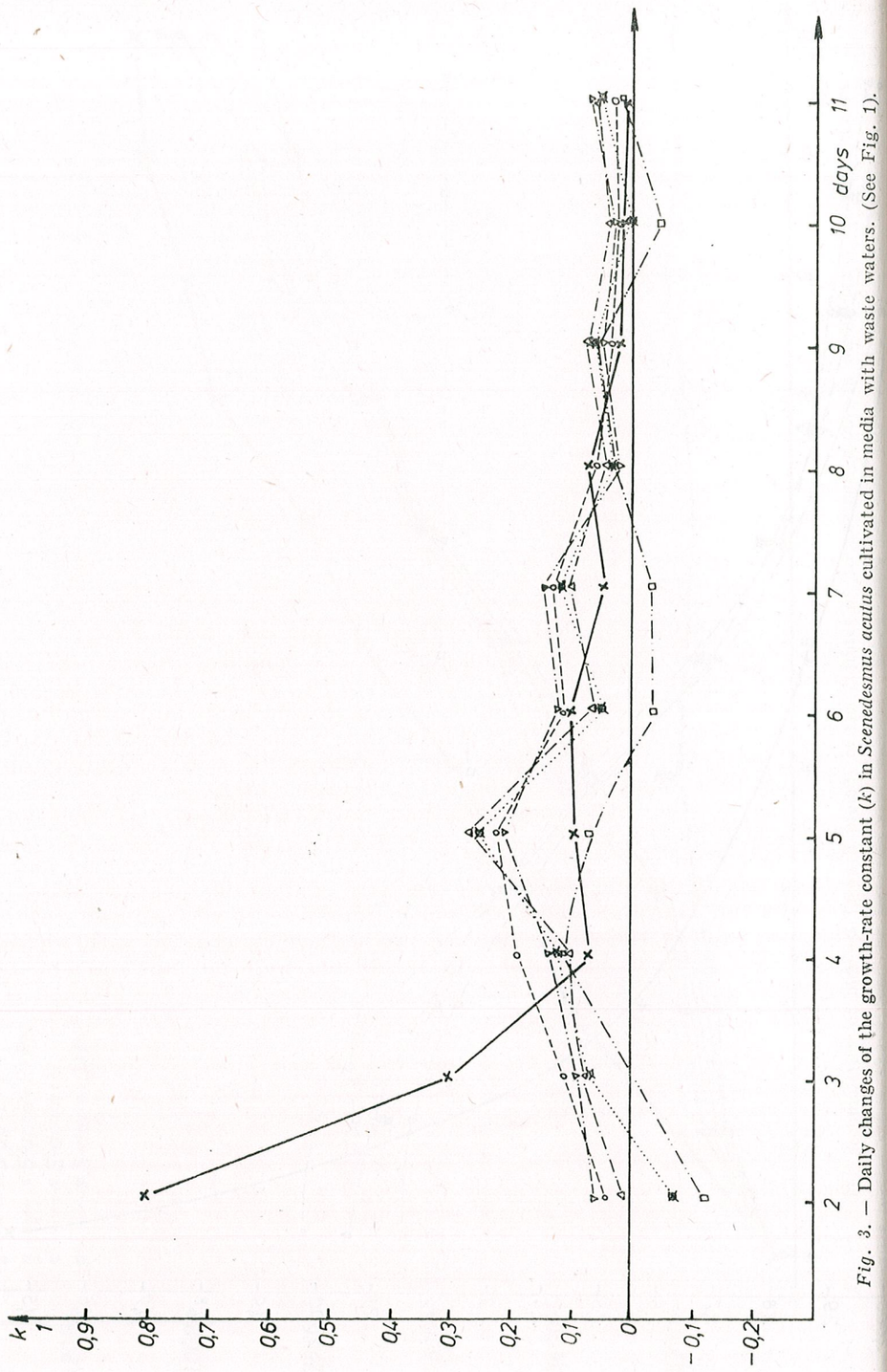

Fig. 3. — Daily changes of the growth-rate constant (*k*) in *Scenedesmus acutus* cultivated in media with waste waters. (See Fig. 1).

Table 2

Changes in the composition of the waste waters from the Pharmaceutical Works during the cultivation of *Scenedesmus acutus*

Analysis	before cultivation	after cultivation
Colour	yellow	light yellow
pH	6.5	7.5
Suspended solids	768	—
Fixed wastes	8 873	1 011
COD	896	248
BOD	815	—
Chlorates	4 822	1 418
H_2S	—	—
Cyanides	4.0	0.019
Phenols	—	—
Sulfates	8 935	—
Nitrous compounds	420.7	?

bubbling, addition of $(NH_4)_2HPO_4$ and the physiological activity of the algal cells themselves. Since adaptation is a complex process, different kinds of organic substances can be secreted [6], [16], [17] liable to generating certain chemical reactions favouring (by chelatization) or inhibiting the life of organisms. The wastes produced by the Pharmaceutical Works induced an excessive secretion of *Scenedesmus acutus* cells; the suspension of the culture contained 236.66 mg protein per liter.

As can be seen from the final values (Table 3), the solutions prepared from the waste waters (except those from the Pharmaceutical Works) and the Someș river waters (upstream and downstream) are nutrient media for the alga *Scenedesmus acutus* comparable in many ways to the control. However, there are some differences in the cell and optical density values; the total biomass production indices are rather close (Table 3). It seems likely that life conditions were not optimum in the

Table 3

Biomass production, chlorophyll and protein content, and growth rate in *Scenedesmus acutus* **cultivated in waste water media**

Media * \ Parameters		N %	E %	Dry wt. %	Chl. *b/a*	Pr. %	k**		
							N	E	Pr.
S-up+B	3 :1	100.3	81.0	92.3	0.315	136.9	0.73	0.29	0.49
S-up+B+P	2 :1 :1	141.9	92.8	100.8	0.318	161.1	0.77	0.25	0.50
S-up+B+P+Ph	1 :1 :1 :1	0.3	17.8	—	0.632	29.2	−0.04	0.025	—
S-down+B	3 :1	109.3	88.0	108.5	0.307	170.0	0.76	0.28	0.43
S-down+B+P	2 :1 :1	116.8	95.8	108.5	0.396	196.2	0.73	0.24	0.52
Tamiya urea EH nutrient solution		100	100	100	0.350	100	0.71	0.47	0.55

* To each mixture 3.30 g $(NH_4)HPO_4$ per liter were added

** Calculated on base $\log_2$

N — cell density; E — optical density; Chl — chlorophyll; Pr — protein

control either. The growth-rate constant of some *Scenedesmus* species varies between 2.0 and 2.4 [10], [31], but in the present experiments it did not exceed 1; it is calculated either from the cell density or from the optical one, and the protein content (Table 3). However, the k-values of these cultures are comparable with those in aerobic oxidation basins (0.127—0.286) published by Arceivala [1].

The growth-rate constant, if based on $\log_2$, expresses the doubling of the time of an algal population growth. Doubling of the culture growth time in the control solution reached 2.13, days in the S-up + + B + P mixture 4 days whereas in the variant containing waste waters from the Pharmaceutical Works 40 days. It is clear that this last mixture strongly inhibited the cell multiplication of *Scenedesmus acutus*, though it was not toxic. Brezina et al. [4] found that the generation time in *Scenedesmus quadricauda* was 40 hrs. Taking into account that every *Scendesmus* cell generates a four-celled *scenedesmoid* (*tetradesmoid*) coenobium, this record means that the population increased four times in 40 hrs. The longer generation time in these experiments could be due to the light system used for cultivation. It was found [20] that *Scenedesmus acutiformis* multiplication was lower in the vertical column than in the parallelipipedic one. In the vertical column, illuminated from the centre until the stationary phase of growth, the necessary time is 17—19 days, whereas in the parallelipipedic one, illuminated on either side, the period required for the cultivation of a batch is of only 7—9 days. Lower biomass multiplication and production might be due to non-sterile conditions of cultivation, which in their turn could be detrimental to the alga.

The convergence of the final values of algal production in the experimental variants (except those with waste waters from the Pharmaceutical Works) could be the consequence of the better adaptation of photosynthetic pigment components to the changed conditions of life [14], [23], [28]. The proportion of chlorophyll *b/a* in the algae, generally, is 0.3, but it can vary widely [24]. In *Chlorella pyrenoidosa*, grown at 500 1× this proportion was 0.68, but at the light intensity of 1500 1× it dropped to 0.58; it follows that the chlorophyll *b* content is higher under weaker light. In addition, unfavourable conditions (especially nutritional) increase the carotenoid content in algae. In the present experiments the proportion of chlorophyll *b/a* was 0.30 to 0.39, thus being within the limits of the published data, but in the cells grown in the mixture with waste waters from the Pharmaceutical Works the chlorophyll *b* amount was somewhat higher (the ratio of chlorophyll *b/a* was of 0.63). This could be a consequence of the indirectly weaker light intensity caused by the shadow of the coloured mixture of the liquid (of the shade-bearing).

Differences in the value of the pigment content can be followed also by the extraction procedures [7]. Various life conditions bring about different biochemical structures in the protein-pigment complex, and, therefore, the same technical procedure would not yield the same results. This opinion can be supported by the changing values of the protein and pigment content of this *Scenedesmus acutus* species grown in different nutrient media.

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ВЛИЯНИЕ ХРОМИСТЫХ СОЛЕЙ ИЗ ОСАДОЧНЫХ ВОД НА ФИЗИОЛОГИЧЕСКИЕ ПРОЦЕССЫ У ВОДОРОСЛЕЙ *CHLORELLA VULGARIS*

В. ПЕТРИЯ

Les expériences effectuées avec des sels de chrome recueillis des eaux résiduelles provenant des fabriques de peaux ont montré que ces sels, en concentration faible 10^{-4} et 10^{-5} stimulent facilement la croissance et la photosynthèse de l'algue *Chlorella*, alors que des concentrations fortes (10^{-3} 10^{-2}) inhibent ces processus. Dans les milieux de culture qui contiennent de faibles quantités de sels de chrome, la valeur du pH croît plus vite que dans le cas des concentrations fortes. L'intensité de la respiration a été très peu influencée.

В данной работе изучалось влияние хромистых солей в осадочных водах, происходивших из кожевенных заводов, где по Русановски М. (7) колебается 50 и 228 мг/л.

Для установления токсичности этих солей изучалось их влияние на рост, фотосинтез, дыхание и колебание pH из культурной среды. Использовались водоросли, так как они более чувствительны, чем бактерии или рыбы как установили Мэлэчя И. и Ионеску М. (5).

Водоросли были культивированы при флюоресцентном свете (8000 люксов) в питательном растворе Кноп-Прингхейм, к которому добавлялись хромистые соли в разных количествах, для того чтобы иметь концентрации между 1.10^{-5} гр. и 1.10^{-2} гр. на 100 мл. раствора.

Через 15 дней фильтрировались культурная среда и установился сухой вес водорослей.

Достигнутые результаты изображены на рис. 1; В малых концентрациях 5.10^{-5} и 1.10^{-4} замечается легкое стимулирование; по мере увеличения концентраций хромистых солей рост водорослей подавлен, так как в концентрациях 6.10^{-3} и 1.10^{-2} вес водорослей уменшается на половину.

Интенсивность фотосинтеза определяли методом Варбурге в течение трех дней при освещений 8000 люксов. Полученные данные изображены на

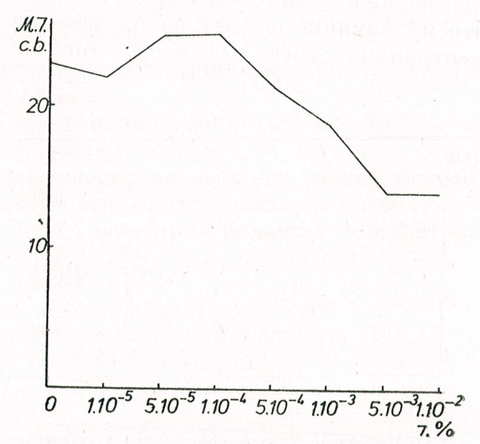

Рис. 1 — Сухой вес водоросли через 15 дней

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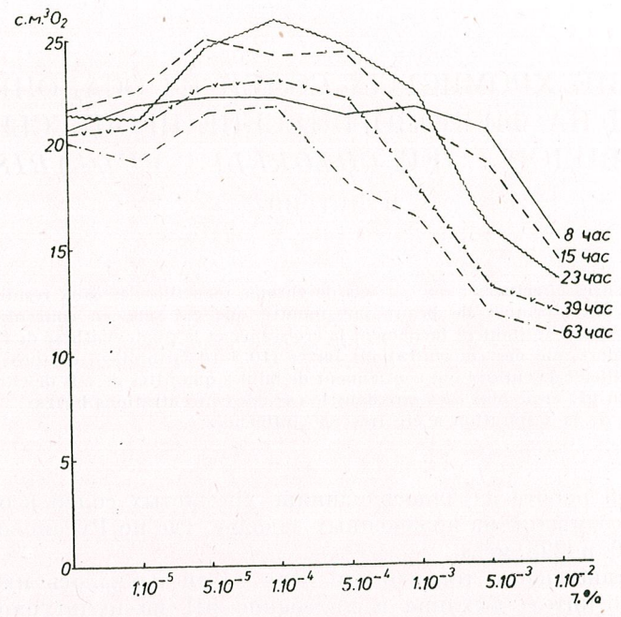

Рис. 2 — Влияние хромистых солей на фотосинтез

рис. 2; замечаем, что хромистые соли в больших концентрациях ингибируют фотосинтез, а это действие усиливается со временем; малые концентрации стимулируют этот процесс. Влияние хромистых солей на дыхание изучалось методом Варбурга и установилось, как заметно на рис. 3, что их влияние более слабое, чем при фотосинтезе. Но в больших концентрациях замечалось легкое торможение этого процесса.

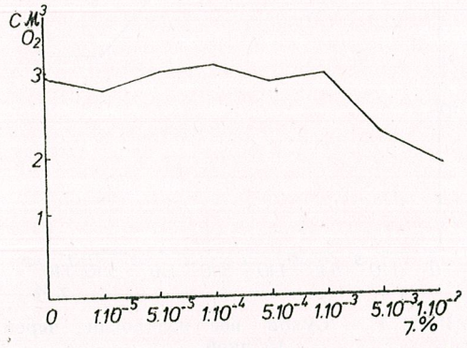

Рис. 3 — Влияние хромистых солей на дыхание

Изучалась также динамика pH культурных сред, содержавших разные дозы хромистых солей.

Данные представлены в таблице 1; замечается, что в первый день, когда добавились хромистые соли, pH среды находился между 7,27—7,46. В больших концентрациях pH был низкий, а именно 6,80 в концентрации 5.16^{-3} и 5.97, и 5,97 в концентрации 1.10^{-3} в растворе 100 мл.

В следующие дни pH увеличился, особенно в контрольном питательном растворе и

Таблица 1

Изменение pH в культурной среде водоросли *Chlorella*

Время в сутках / Конц. г%	1	2	3	4	5	6	8	10	11	20
Контроль	7,40	8,18	8,38	8,35	8,16	8,05	7,51	7,51	7,50	7,86
1.10^{-5}	7,45	8,07	8,16	8,24	8,20	8,07	7,58	7,45	7,50	7,68
5.10^{-5}	7,43	8,09	8,19	8,20	8,07	7,98	7,51	7,50	7,50	7,69
1.10^{-4}	7,44	7,95	8,10	8,07	8,05	7,90	7,50	7,51	7,51	7,72
5.10^{-4}	7,42	7,89	8,00	8,04	8,00	7,89	7,49	7,50	7,50	7,65
1.10^{-3}	7,27	7,73	7,88	7,94	7,94	7,86	7,45	7,47	7,50	7,60
5.10^{-3}	6,80	7,10	7,24	7,40	7,50	7,52	7,42	7,44	7,48	7,58
1.10^{-2}	5,97	5,95	6,07	6,24	6,42	6,62	7,40	7,42	7,46	6,90

при низких концентрациях хромистых солей. В больших концентрациях рост pH реализовался более медленно; через 6—7 дней pH достиг инициального значения.

Эти результаты утверждают, что в начале происходит абсорбция анионов в питательной среде как в контрольном растворе, так и в растворах малой концентрации хромистых солей.

Растворы с большой концентрацией хрома, имеющие кислый pH, имеют ингибирующее действие на обмен веществ; это приводит к медленному росту pH в среде.

Наши результаты подтверждают выводы других исследователей: например, Мэлэчя И. (4) исследуя влияния хрома и других металлов, заметил ингибирующий эффект на рост фотосинтеза, а Ранел [6] констатировал замедление деления клеток.

Ингибирующее действие хромвистых солей на фотосинтез замечается в большей степени на фотосинтез, чем на дыхание. Это соответствует с результатами Бирхницена Ж. Ф. [1] и Гаффрона [3]; Бувере Келлей и Авери [2]. Мэлэчя И. [4] объясняет ингибирующее действие тем, что эти соли реагируют с протопластом и особенно с белками. Хромистые соли влияют и на уменьшение pH.

ВЫВОДЫ

1. Хромистые соли в концентрациях выше чем 1.10^{-3} г. на 100 мл ингибирует рост водоросли *Chlorella.*

2. Фотосинтез торможен концентрациями 5.10^{-3} и 1.10^{-2}, а малые дозы легко стимулируют этот процесс.

3. Хромистые соли влияют меньше на дыхание, чем на фотосинтез.

4. В питательных средах, которые содержат малые дозы хромистых солей, pH увеличивается быстрее, чем при больших концентрациях.

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RYTHMICITÉ CIRCADIENNE ET HEBDOMADAIRE DE QUELQUES PROCESSUS PHYSIOLOGIQUES CHEZ LES ALGUES *CHLORELLA COELASTROIDES*

PAR

LUCIA POLESCU-IONĂȘESCU

Les observations que nous avons faites ont permis de mettre en évidence la rythmicité circadienne et hebdomadaire des certains processus physiologiques : synthèse de pigments, de protéines et des acides nucléiques. Dans tous les cas, il y avait une concordance parfaite entre la synthèse de ces substances ; les algues effectuaient un rythme toutes les 8 heures et tous les 3 jours pendant une semaine. A remarquer que les oscillations de la synthèse des pigments caroténoïdes sont plus faibles comparativement aux chlorophylles *a* et *b*.

Le présent article touche à quelques problèmes concernant la rythmicité de la synthèse chlorophyllienne, protéique et des acides nucléiques. Les recherches sur la rythmicité cyrcadienne et hebdomadaire ont commencé en soumettant les algues à l'influence contrôlée de certains facteurs tels la lumière et la température. Après quelques cycles irréguliers, transitoires, le système atteint une nouvelle fréquence qui se maintient invariable.

MATÉRIEL ET MÉTHODES

Les algues *Chlorella coelastroïdes* provenant de l'algothèque de l'Université de Genève, ont été synchronisées d'après la méthode de Lorenzen [2]. La culture synchrone était maintenue après, dans des conditions de culture constantes (25°C température, 3 % CO_2, et un régime d'obscurité, après une exposition de 4 heures à la lumière).

La quantité de pigments a été déterminée par spectrophotométrie différentielle, après leur extraction en acétone.

L'incorporation des acides aminés et des acides nucléiques a été suivie en administrant du triptophan, de la thimidine et de l'uridine radioactive dans le milieu de culture des algues. De temps en temps, la culture d'algues traversait la membrane du filtre Millipore, dont le diamètre des pores ne dépassait pas 0,45 μ. Le milieu de culture filtré était ensuite déposé sur des rondelles en papier filtrant dont on déterminait la radioactivité.

RÉSULTATS ET DISCUSSIONS

Nos recherches ont permis la mise en évidence de la rythmicité circadienne et hebdomadaire de quelques processus physiologiques. La quantité de chlorophylle *a* per cellule commence à augmenter 4 heures

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après avoir passé les algues à l'obscurité, et elle atteint le niveau maximum à la 8e heure. Après une chute de la courbe, qui se produit pendant les 6 heures suivantes, la synthèse de chlorophylle recommence et atteint le maximum à la 20e heure. Les algues ont fait donc un rythme de la synthèse de chlorophylle toutes les 8 heures (fig. 1).

Les mêmes observations ont été faites concernant la rythmicité circadienne de la synthèse de chlorophylle *b* et des pigments caroténoïdes.

Nos recherches sur la rythmicité hebdomadaire de la synthèse chlorophyllienne ont démontré qu'après une chute de la courbe qui se produit pendant les 4 premiers jours, celle-ci commence à monter et le maximum est enregistré au 6e jour ; une nouvelle chute se produit après (fig. 2). Les algues ont effectué donc un rythme de la synthèse chlorophyllienne tous les 3 jours. A signaler que les oscillations de la synthèse des pigments caroténoïdes sont plus faibles comparativement aux chlorophylles *a* et *b*.

Afin de mettre en évidence la synthèse des protéines, le triptophan radioactif a été administré au milieu de culture des algues et de temps en temps on effectuait la détermination de la radioactivité du surnageant. On peut constater la même rythmicité circadienne de 8 heures (avec le maximum à la 8e heure d'obscurité). A remarquer qu'il y a une concordance parfaite entre la synthèse des pigments chlorophylliens et la synthèse des protéines (fig. 3).

Les oscillations de la synthèse hebdomadaire des protéines sont plus amples et elles se produisent tous les 3 jours (fig. 4).

Concernant la synthèse d'ADN, nous avons établi une rythmicité circadienne de 8 heures — dont la synthèse maxima se produit à la 8e heure et celle minima à la 16e heure (fig. 5) — , ainsi qu'une rythmicité hebdomadaire dont les oscillations sont plus amples, et le rythme s'effectue tous les 3 jours (fig. 6). La synthèse d'ADN se produit donc parallèlement avec la synthèse de pigments chlorophylliens et de protéines.

En suivant la synthèse d'ARN, nous avons constaté la même rythmicité circadienne et hebdomadaire (figs. 7 et 8).

Les données que nous avons présenté viennent de confirmer les observations faites par J. F. Feldman [1], qui a établi une oscillation circadienne des processus métaboliques chez les algues *Euglena*. Il s'agit de l'incorporation des acides aminés dans les cultures d'*Euglena gracilis*, maintenues à la température de 25°C et au régime de 12 heures de lumière et 12 heures d'obscurité. Il enregistrait le maximum entre 6 et 12 heures et le minimum entre 8 et 24 heures. D'après Feldman, cette oscillation circadienne de l'incorporation des acides aminés pourrait être déterminée par les modifications de la synthèse des protéines, ou bien elle pourrait être le résultat des oscillations de l'assimilation des acides aminés.

B. M. Sweeney et J. W. Hastings [3] ont remarqué chez *Gonyaulax* une rythmicité circadienne des processus métaboliques, qui continue pendant le maintien des algues à une lumière d'intensité faible mais constante.

G. Walther et E. Edmunds [4] ont déduit que les ocillations circadiennes de la photosynthèse se produisent par suite de la modification de la concentration de la chlorophylle *a*. Mais, pourtant, dans la plupart des cas, il n'y avait pas une corrélation entre l'intensité de la photo-

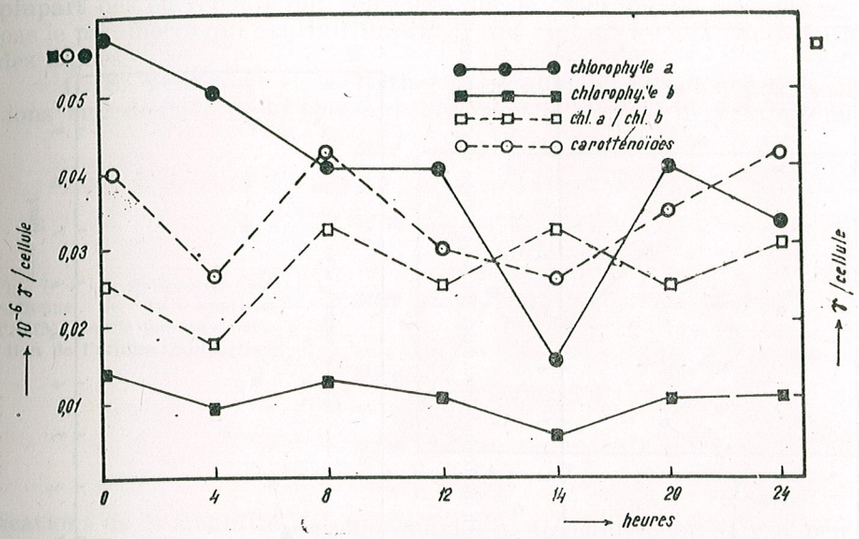

Fig. 1. — La rythmicité circadienne de la synthèse des pigments chlorophylliens et caroténoïdes, chez les algues *Chlorella coelastroides*

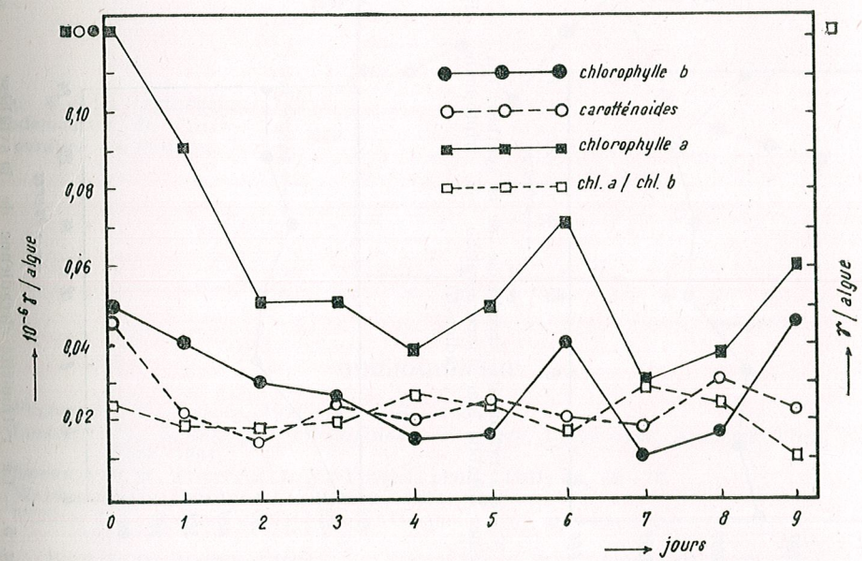

Fig. 2. — La rythmicité hebdomadaire de la synthèse des pigments chlorophylliens et caroténoïdes

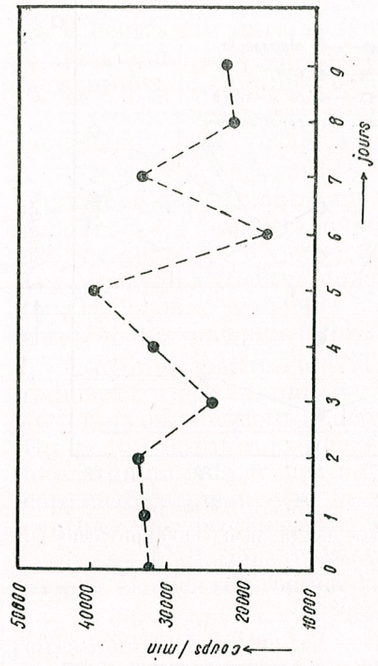

Fig. 4. — La rythmicité hebdomadaire de la synthèse des protéines

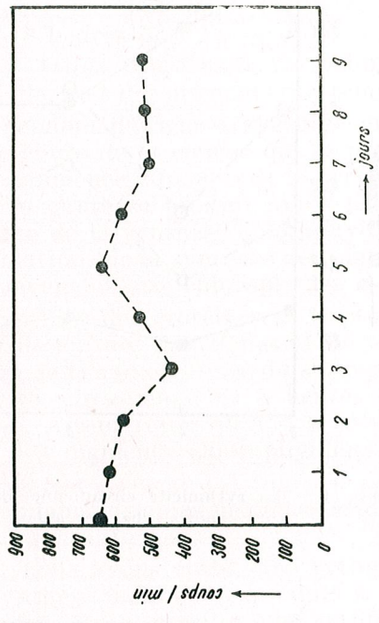

Fig. 6. — La rythmicité hebdomadaire de la synthèse d'ADN

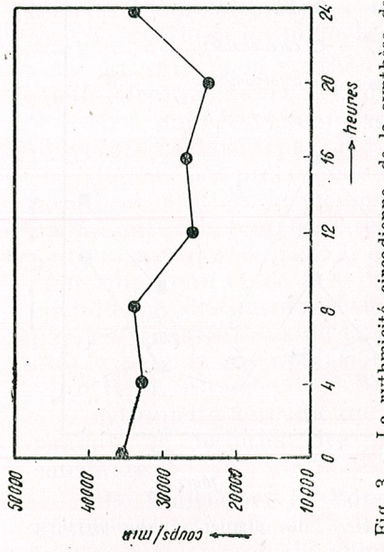

Fig. 3. — La rythmicité circadienne de la synthèse des protéines, établie par l'incorporation du triptophan radioactif

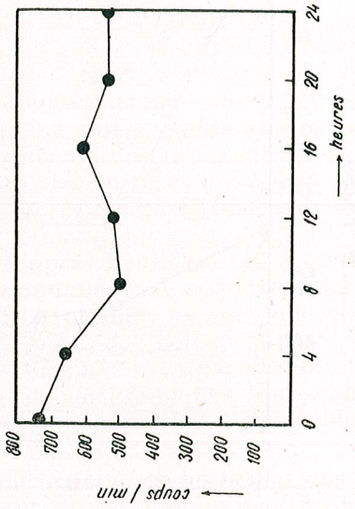

Fig. 5. — La rythmicité circadienne de la synthèse d'ADN, établie par l'incorporation de la thimidine radioactive

synthèse des algues et la quantité de chlorophylle. Contrairement, la plupart des chercheurs ont considéré que la chlorophylle ne représentait pas le paramètre qui pourrait imprimer une rythmicité à la photosynthèse des algues.

C. S. Yentch et H. R. Ryther [5] soulignent le fait que les oscillations circadiennes de la photosynthèse sont le résultat de certaines modifications de la quantité de chlorophylle *a*. Généralement, il y a peu de données sur les aspects biochimiques des processus rythmiques chez les êtres vivants.

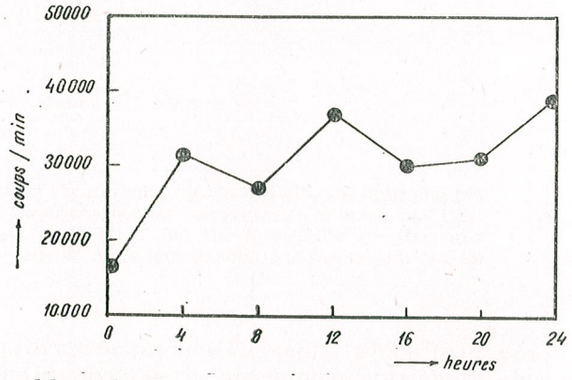

Fig. 7. — La rythmicité circadienne de la synthèse d'ARN, établie par incorporation de l'uridine radioactive

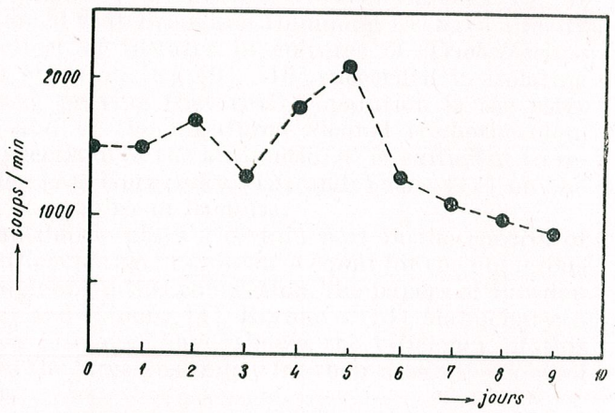

Fig. 8. — La rythmicité hebdomadaire de l'incorporation de l'uridine

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IMPROVEMENT OF TRICHOTHECINE BIOSYNTHESIS BY USING ULTRAVIOLET IRRADIATIONS AND DIFFERENT CULTURE MEDIA

BY

M. RUSAN and AL. MANOLIU

The work includes some aspects on the suitable culture medium, the optimum pH as well as the improvement of the trichothecine biosynthesis process by U.V. irradiations. The modified Freeman medium and the R medium are the most suitable for trichothecine biosynthesis. After four irradiations the variant No. 68 has the highest titer.

Attempts are made to provide some new elements, which may possibly elucidate certain problems involved in the mechanism of trichothecine biosynthesis. The size of the present paper does not permit us to expound on the problems concerned with the biogenesis and chemistry of this antibiotic produced by fungus *Trichothecium roseum* Link ex. Fr. It should, however, be mentioned that Brian and Hemming [5] have already reported the fungicidal effect of filtrates in cultures of *Trichothecium roseum* and Freeman and Morrison [8], [9], [10] succeeded in isolating the trichothecine antibiotic, proving that this preparation is the ester of the isochrotonic acid and of the unsatured alcohol trichothecolon. As regards the fungicidal spectrum of the antibiotic, it is sufficient to recall the papers of Caltrider [7], Korzybsky [12] and Yasne [17] on the trichothecine behaviour to a number of bacteria.

The fact that this antibiotic plays a certain part in the control of a large number of phytopathogenic agents, would account for the numerous studies devoted to this problem. In this connection, the papers of Bawden and Freeman [3], Bradley and Ganong [4], Koroleva [13], Manucharyan [14], Săvescu and Hulea [16] etc. have shown the efficiency of this fungicidal preparations and the large possibility for their use in the control of phytopathogenic agents.

MATERIAL AND METHOD

Trichothecium roseum was cultivated on the already described M.D.A. culture medium [15], considered as control for all further experiments.

The culture media used were : the *modified Freeman medium*, pH=6, with the following composition : NH_4 tartrate=2g, glucose=50 g, SO_4Mg-0.5 g, PO_4H_2K=lg, ClK=0.5 g, SO_4Fe=0.01 g, casein hydrolysate =4g, yeast extract=4g, distilled water at 1000 cc. The *Wollenweber medium*, pH-8,

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Raulin medium pH=4, *Bates medium*, pH=4, *M medium*, pH=7.5, *The original medium*, developed by the authors, pH=6, has the following composition: arginine=1g, fructose=25 g, malt extract=10 g, yeast extract=5 g, niacin=400/1, distilled water at 1000 cc.

Trichothecine extractions from the culture liquid or from the mycelium at different pH values as well as from different organic solvents were made. The presence of the active substance was determined by means of microbiological dosing in Petri dishes, on double agar layer in inoxidable steel cylinders, according to the method of Grove and Randall [11]. The test-germ used was *Candida albicans* and the culture media for the microbiological dosing of fungicidal antibiotics was the agarised potato medium.

Termostation of *T. roseum* cultures was performed at 25°C during 24 hrs, while that of *C. albicans* cultures at 37°C during 18 hrs. The microbiological dosings were performed at 24 hrs interval starting from the 72-th hr of thermostation of *T. roseum* cultures.

To ascertain the endocellular or extracellular origin of this active principle, microbiological dosings were made in culture fluids as well as in well-dried mycelium by means of a vacuum pump. For those experiments aimed at inducing some improved characteristics in trichothecine biosynthesis by means of certain mutagenic factors, an ultraviolet light device was fitted into a sterile box. Four cultures obtained by 4 former irradiations were used as biological material, details have already been published [15].

RESULTS AND DISCUSSIONS

In order to ascertain the location of trichothecine biosynthesis — exocellularly or endocellularly, microbiological dosings were carried out for 7 days from the culture liquid and the mycelium at different pH values. The dynamics of trichothecine biosynthesis is represented in Fig. 1 and the values of the active principle extracted from the mycelium are shown in Fig. 2.

In both cases, maximum trichothecine values were recorded when *Trichothecium roseum* was cultivated in media with an unchanged pH. It was also established that the values of the inhibition zones recorded following dosing of the culture liquid were higher than those obtained from the mycelium extracts previously dried by means of a vacuum pump.

Methanol, and especially butanol-acetate, proved almost inefficient in the extraction of the investigated active principle.

Using a wide range of culture media of variable content, we found that the modified Freeman medium together with the R medium could provide the most suitable conditions for the development of the fungus *Trichothecium roseum*. This fact was testified by the high amount of biomass together with the enhanced development dynamics (Table 1). Owing to the very insignificant variations of the pH during biosynthesis, these media could offer evident advantages when compared with the other media used up to now. Thus, in the modified Freeman medium, the pH varied between 5—5.5 and in the R medium between 6—6.5 (Fig. 3). The modified Freeman medium together with the R medium

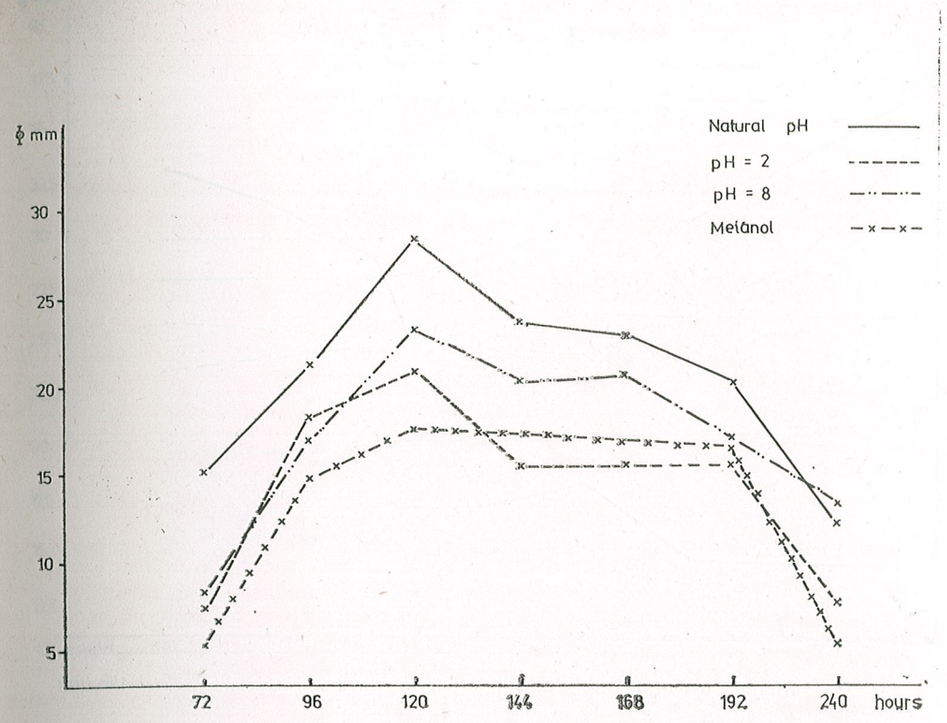


Fig. 1. — Maxima and minima of trichothecine biosynthesis by *Trichothecium roseum* Link from the culture liquid, expressed by the size of the inhibition zones in mm as against *Candida albicans*.

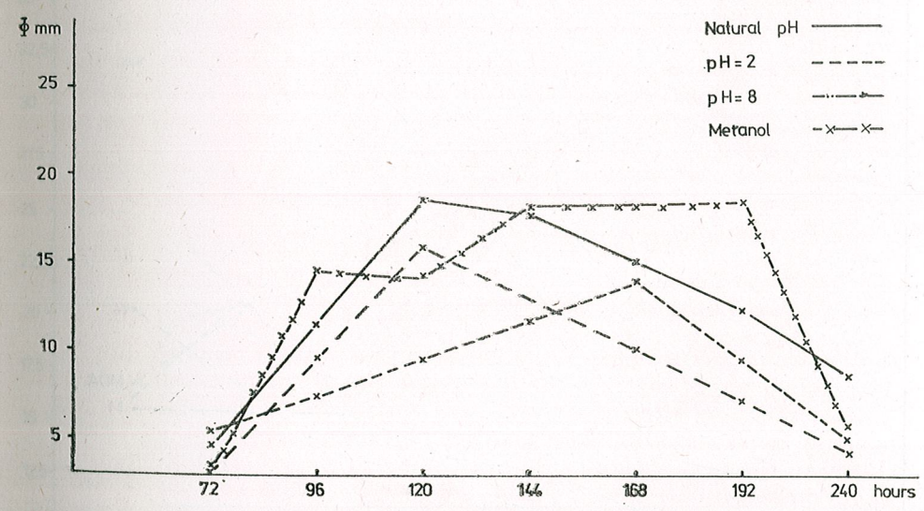


Fig. 2. — Maxima and minima of trichothecine biosynthesis by fungus *Trichothecium roseum* from the mycelium, expressed by the size of the inhibition zones in mm as against *C. albicans*.

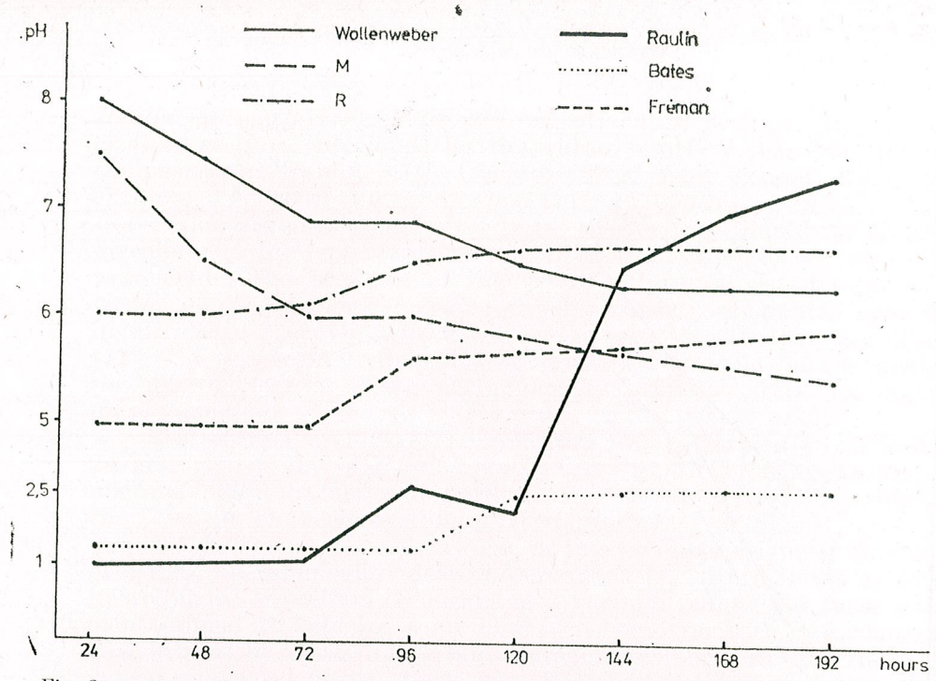


Fig. 3. — pH variation in culture media during trichothecine biosynthesis by *Trichothecium roseum*.

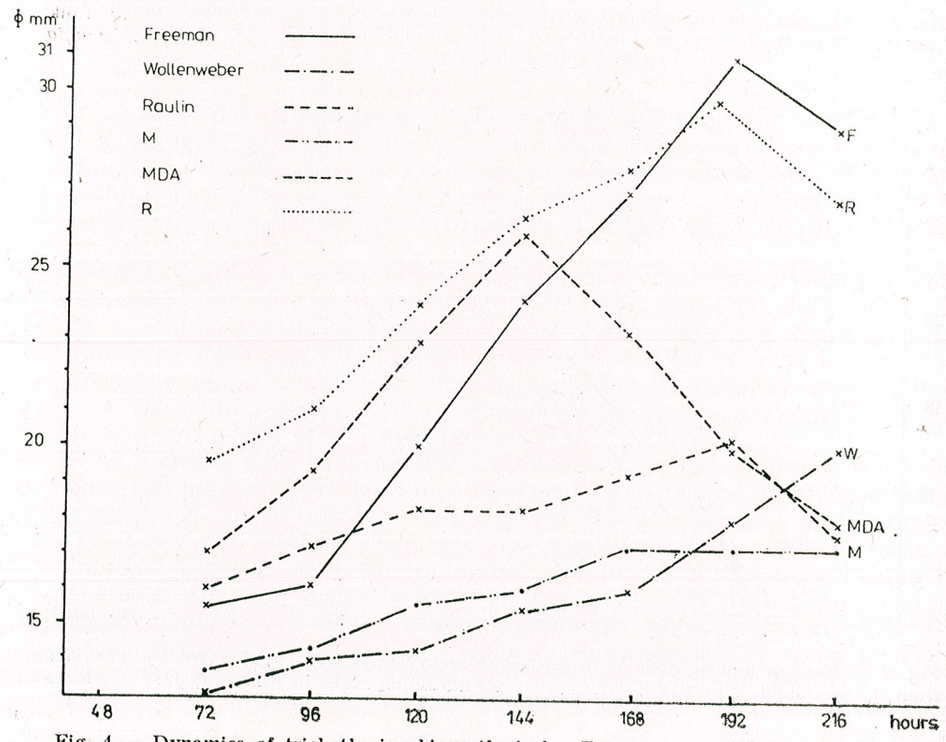
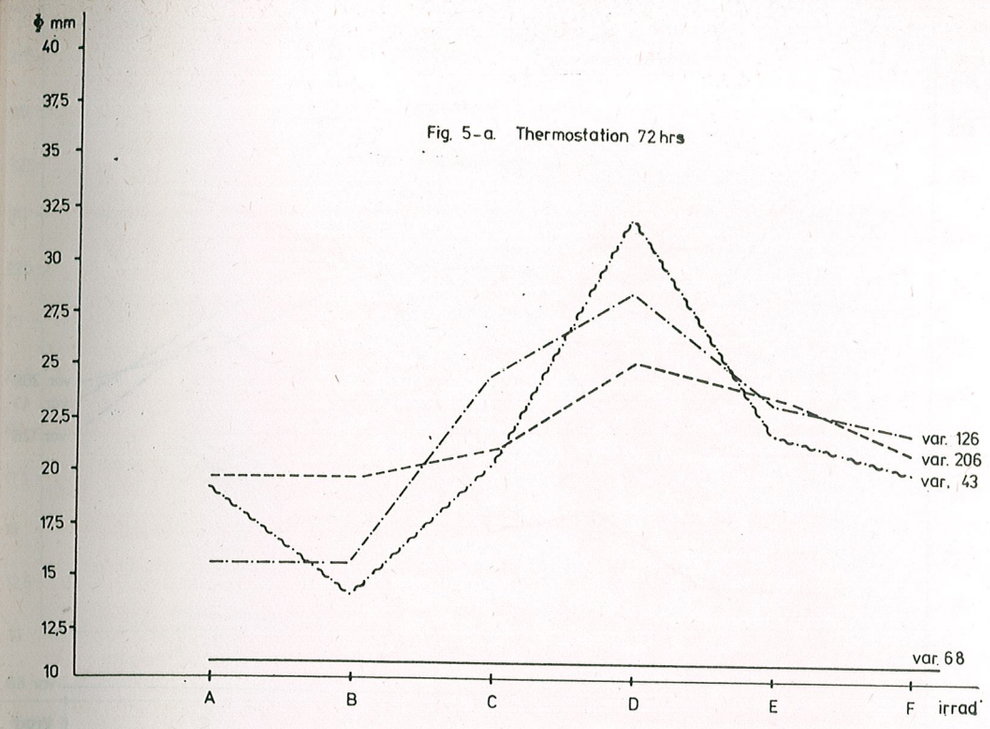


Fig. 4. — Dynamics of trichothecine biosynthesis by *T. roseum* on different culture media, expressed by the size of the inhibition zones in mm as against *Candida albicans*.



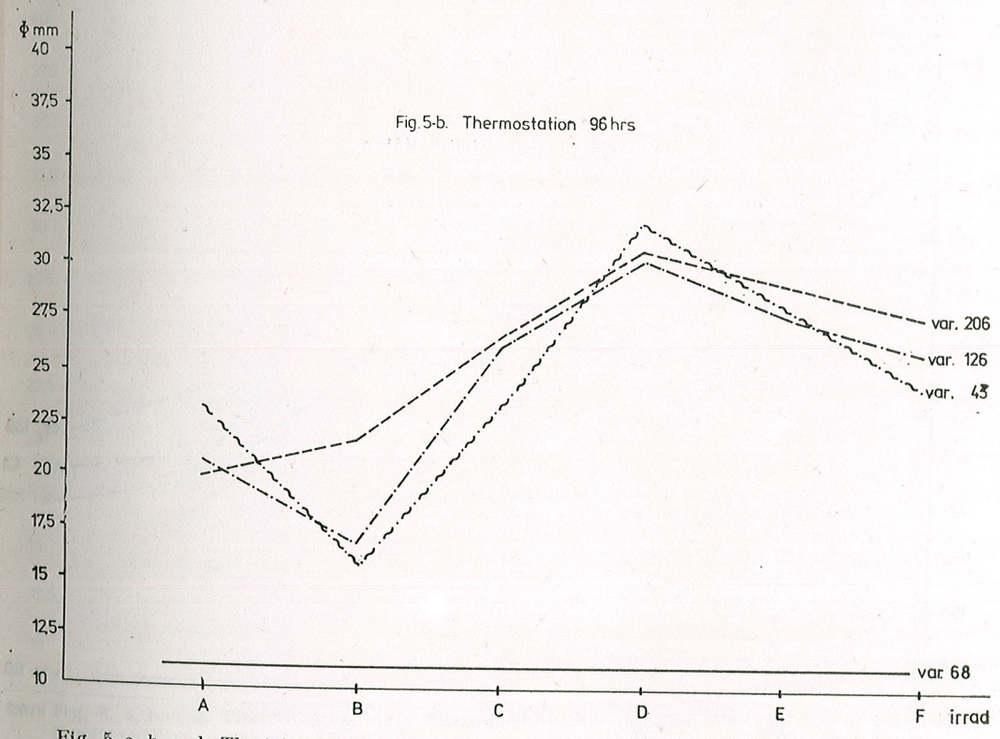


Fig. 5. a, b, c, d. The inhibition zones determined by thermostation (72—144 hrs) of the test *Candida albicans* in the presence of culture extracts from variants Nos. 43, 68, 126, 206, resulted from *Trichothecium roseum* conidia u.v. irradiated for 50 min.

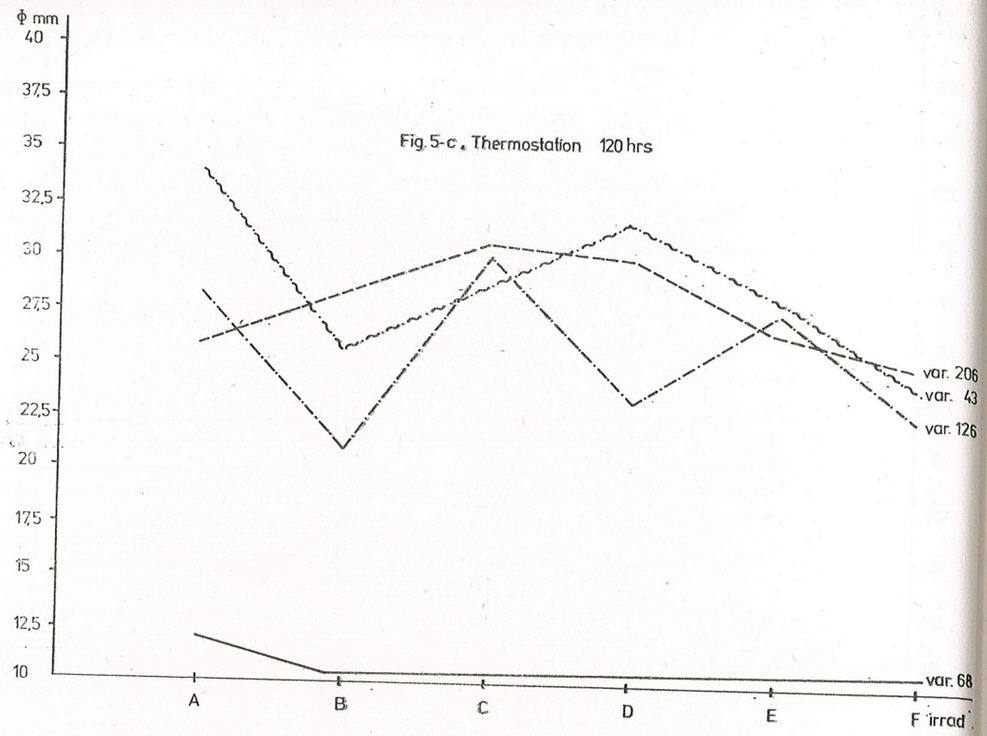


Fig. 5-c. Thermostation 120 hrs

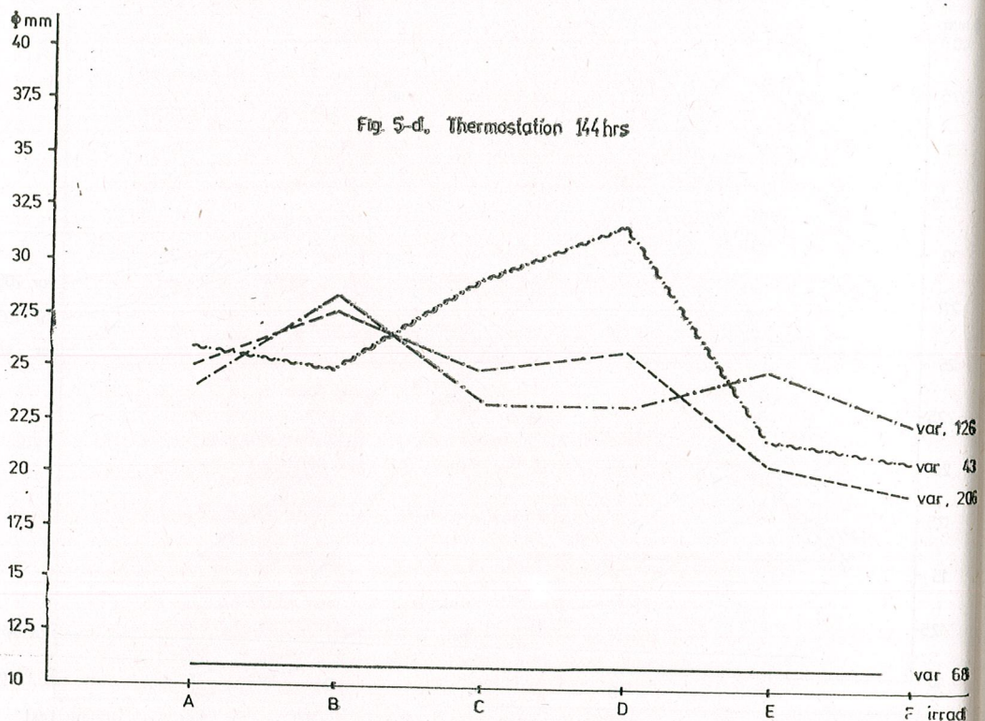


Fig. 5-d. Thermostation 144 hrs

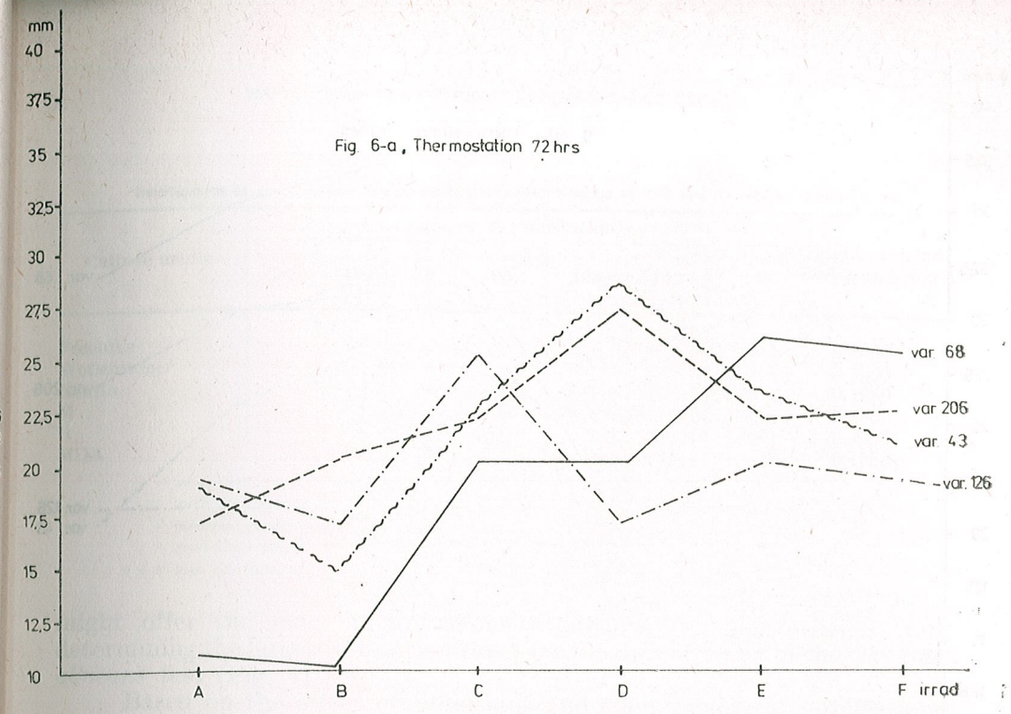


Fig. 6-a, Thermostation 72 hrs

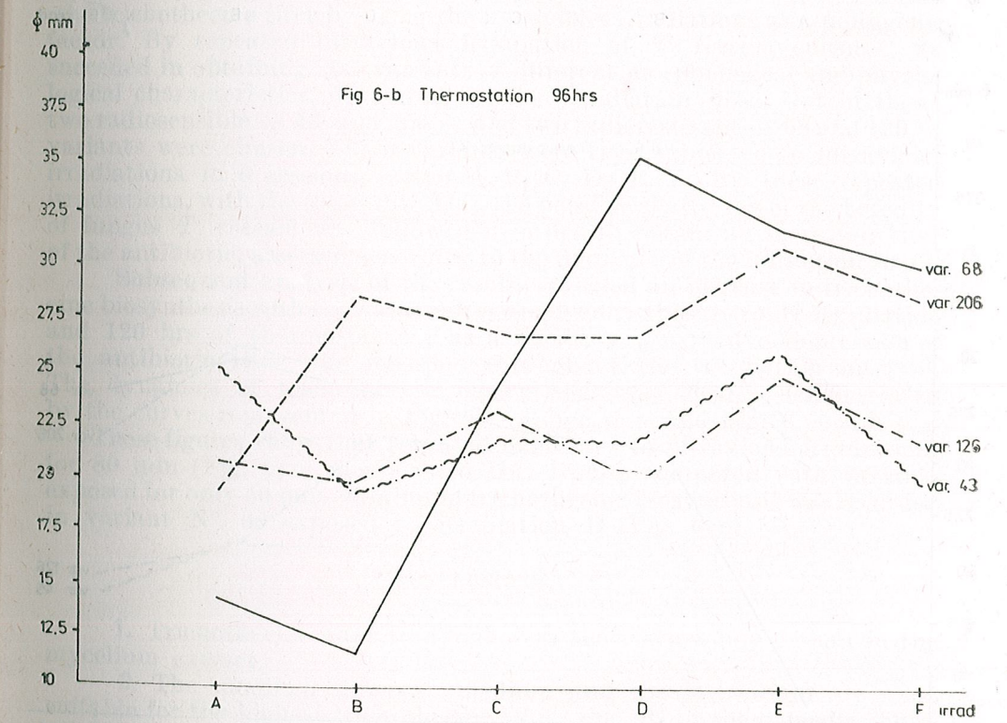


Fig 6-b Thermostation 96hrs

Fig. 6. a, b, c, d. The inhibition zones determined by thermostation (72—144 hrs) of the test *Candida albicans* in the presence of culture extracts from variants Nos. 43, 68, 126, 206, resulted from *T. roseum* conidia u.v. irradiated for 80 min.

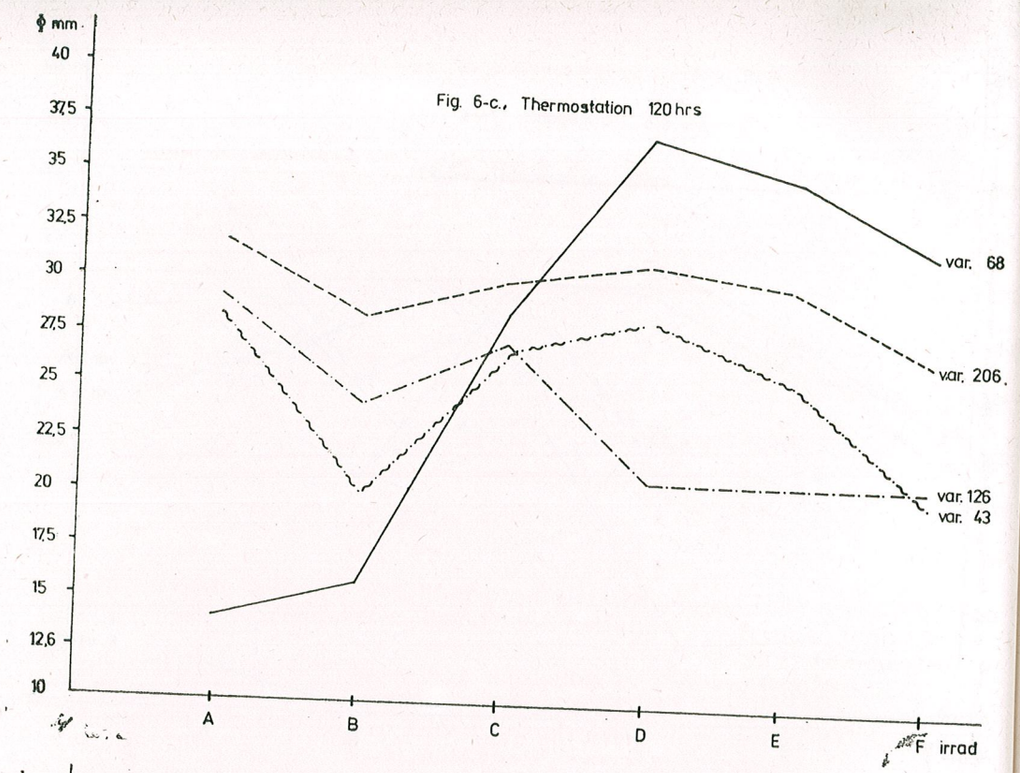


Fig. 6-c., Thermostation 120 hrs

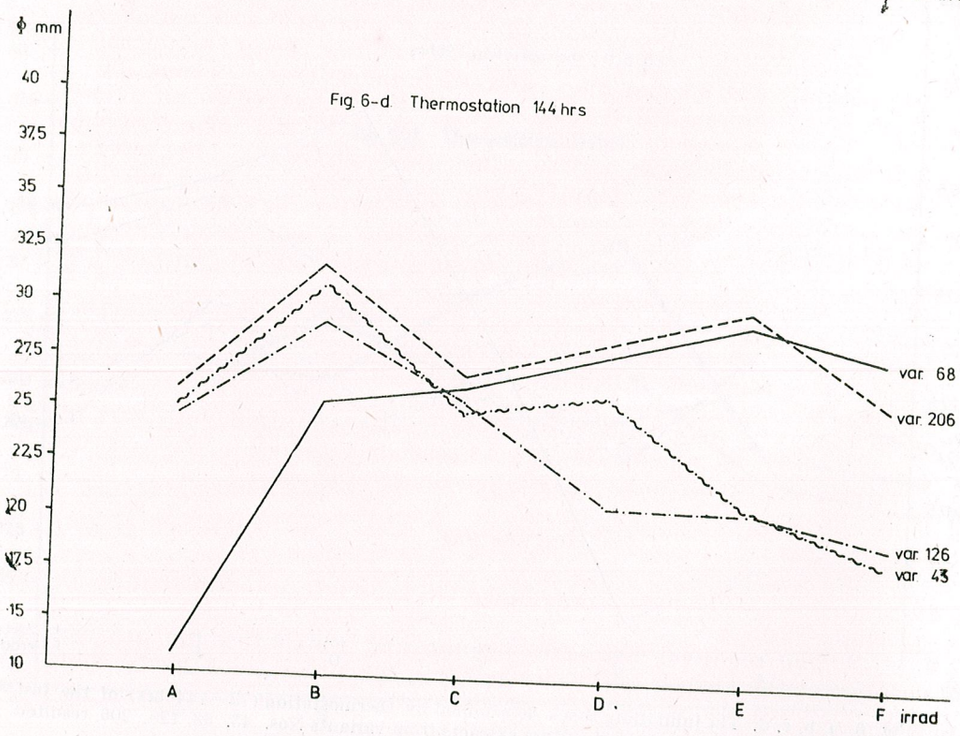


Fig. 6-d. Thermostation 144 hrs

Table 1

Development of *Trichothecium roseum* on different culture media at 25°C and the weight of its biomass

Culture media	Time of thermostation/h							Weight of dried mycelium/mg
	24	48	72	96	120	144	168	
Freeman	−+	+	++	++	+++	+++	+++	0.7060
Wollenweber	−	−+	+	++	+++	+++	+++	0.3200
Raulin	−	−+	+	++	+++	+++	+++	0.1950
M	−	−+	+	++	+++	+++	+++	0.1800
R	−+	+	++	+++	+++	+++	+++	0.4800
MDA	−	−+	+	++	++	+++	+++	0.1580

− no growth
−+ weak development
+ moderate development
++ good development
+++ high development

might offer the most advantageous conditions for biosynthesis, thus determining the largest inhibition zones for the development of the *Candida albicans* test (Fig. 4).

Based on the effect of radiations and their biological implications Baraboi [2], Buxton [6], Bacq and Alexander [1] we tried to increase the trichothecine titer by using the ultraviolet irradiations as a mutagenic factor. By repeated ultraviolet irradiation of *T. roseum* cultures, we succeded in obtaining 207 variants of different morphological and physiological characteristics, depending on the irradiation dose. Out of these, two radiosensible — 43 and 206 — and two radioresistant — 68 and 126 — variants were chosen. These variants were then subjected to ultraviolet irradiations in 6 sessions, noted A, B, C, D, E, F. By these repeated irradiations, with the same variables, the possible shattering of the heredity of fungus *T. roseum* was followed in order to obtain an increased titer of the antibiotic, assessed according to the diameter of the inhibition zones.

Subsequent analysis of the results revealed an increase in trichothecine biosynthesis with top values after an 80 min exposure to D irradiation and 120 hrs of thermostation; as a result, a progressive depression of the antibiotic titer was recorded throughout the irradiation interval. The dynamics of trichothecine biosynthesis was clearly demonstrated by the curves represented in Fig. 5 — a, b, c, d — and Fig. 6 — a, b, c, d —. These figures show that repeated exposures to ultraviolet irradiations for 80 min (Fig. 6, c) might yield best results compared with variants exposed for only 50 min. Maximum trichothecine biosynthesis was recorded in variant N°. 68 exposed to irradiation D (Fig. 6, c).

CONCLUSIONS

1. Trichothecine titer was higher in the culture liquid than in the mycelium extract.

2. The modified Freeman medium and the R medium are most suitable for the highest trichothecine titer; the pH of these media shows very small variations.

3. The unchanged pH of the culture medium may provide the best trichothecine yields.

4. The rate of development of *Trichothecium roseum* Link cultures during 6 irradiations with ultraviolet light slowed down following the 4-th irradiation — D —.

5. Variant No. 68 exposed for 80 min to ultraviolet light exhibited the highest trichothecine biosynthesis ability.

6. The optimum irradiation dose for variant No. 68 was 360 min — 4 irradiation session of 80 min each.

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ВОЗДЕЙСТВИЕ ГЕРБИЦИДОВ КАРАГАРДА И ЭТАЗИНА НА РОСТ, ФОТОСИНТЕЗ И ДЫХАНИЕ ВОДОРОСЛИ *CHLORELLA*

ИОАНА СПИРЕСКУ

L'auteur présente les resultats de certaines experiences effectuées avec les herbicides Caragard et Etazine, en vue de d'établir leur toxicité et en employant les algues Chlorella comme indicateur. Les concentrations qu'on a employé ont été comprises entre 0,0001 et 5 mg/l.
Nous avons établi pour les concentrations au dessus de 0,0005 mg/l, une inhibition de la croissance, de la photosynthèse et de la synthèse de chlorophylle.
Les petites concentrations, de 0,0001 à 0,0005 mg/l ont une action simulatrice sur ces procéssus.
Les substances ont peu influencé la respiration par rapport aux autres procéssus physiologiques.

В данной работе мы даём результаты, полученные с гербицидами: Карагард (тербуметон, 2-третичный-бутиламино-4-этиламино-6-метокси-1, 3, 5 -триазин) и Этазин (секбуметон, 2-вторичный бутиламино-4-этиламино-6-метокси-1, 3, 5-триазин).

Карагард используется в борьбе с однодольными и двудольными, однолетними и многолетними сорняками, произрастающими в насаждениях фруктовых деревьев и в виноградниках, а Этазин в посевах люцерны.

В проведенных опытах наблюдали за влиянием, оказываемым этими веществами на рост, фотосинтез, дыхание и на количество пигментов хлорофилла у водоросли хлореллы.

Водоросли выращивали в питательном растворе Кноп-Принсхейм, к которому добавлялось различное количество гербицидов, и таким образом получалась концентрация между 0,0001 и 5 мг/л.

Для культуры были использованы колбы Эрленмеера в 300 мл, причем каждая из них содержал по 200 мл питательного раствора. Водоросли содержались при флюоресцентном свете в 4000 люксов в течении 15 дней, после чего определялась интенсивность роста — критерием служило количество биомассы.

Полученные данные указаны на рис. 1, откуда и можно установить, что при очень малых дозах происходит слабое стимулирование при концентрации в 0,0001 мг/л в случае Карагарда, а в случае Этазина при концентрации в 0,0001 и 0,0005 мг/л.

При более повышенных концентрациях замечается торможение роста, усиливающегося согласно концентрации. При концентрациях, превышающее 1 мг/л рост прекращается.

Интенсивность фотосинтеза была определена манометрическим методом Варбурга, при освещении в 8000 люксов, при различных сроках

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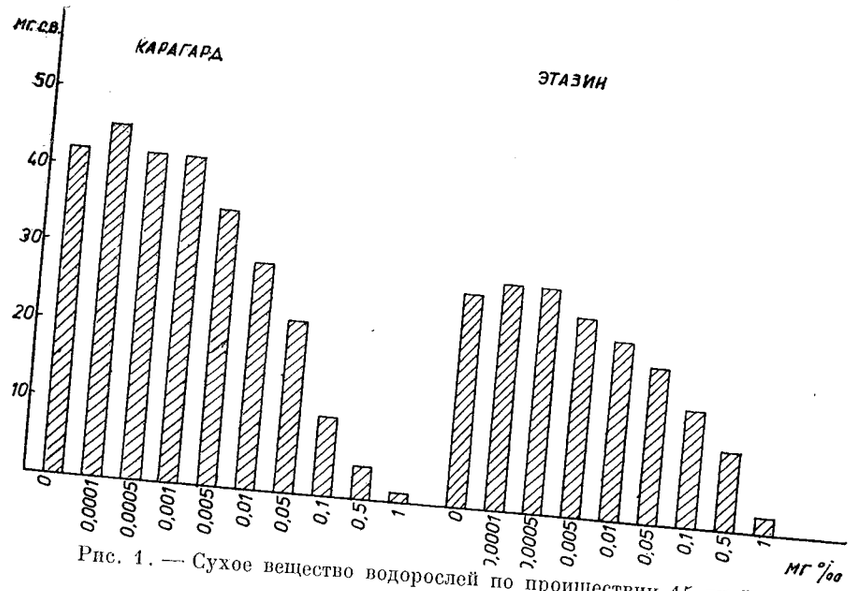

Рис. 1. — Сухое вещество водорослей по проишествии 15 дней

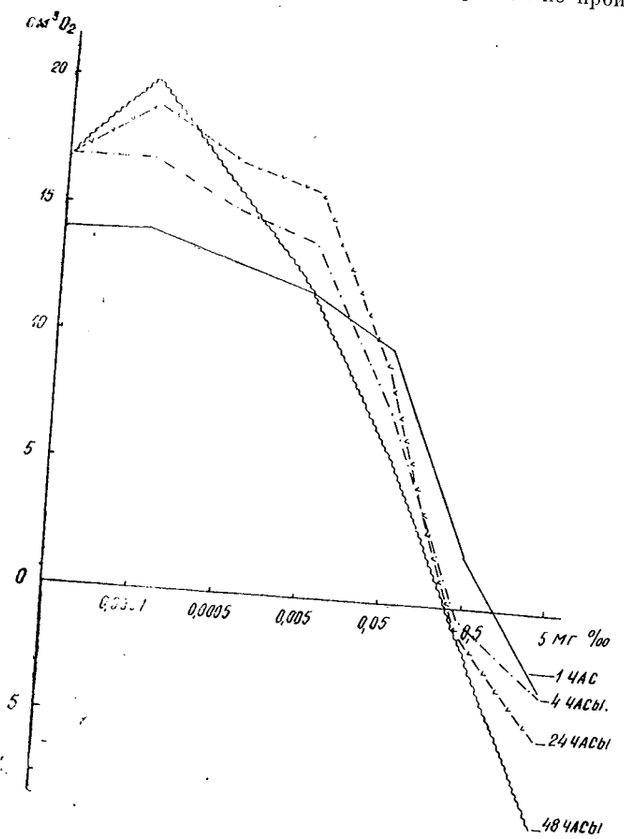

Рис. 2. — Воздействие Карагарда на фотосинтез

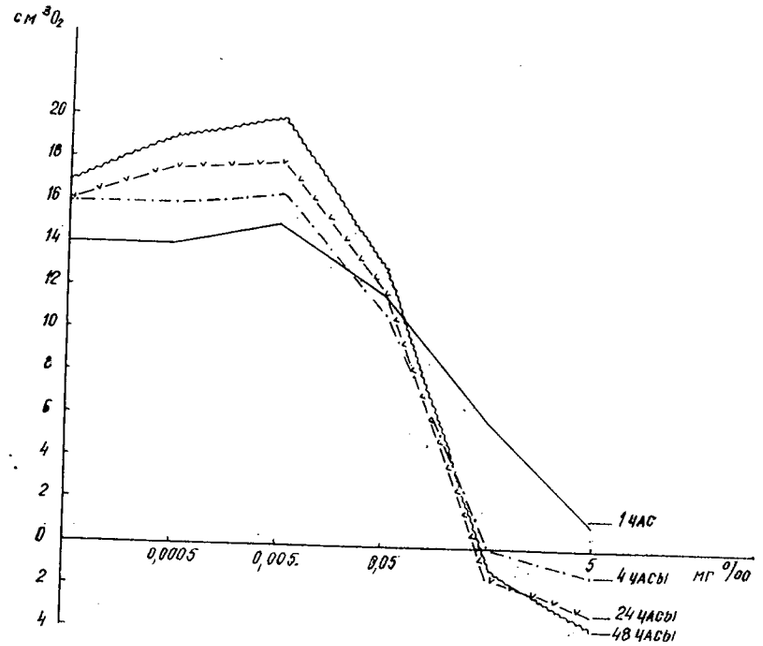

Рис. 3. — Воздействие Этазина на фотосинтез

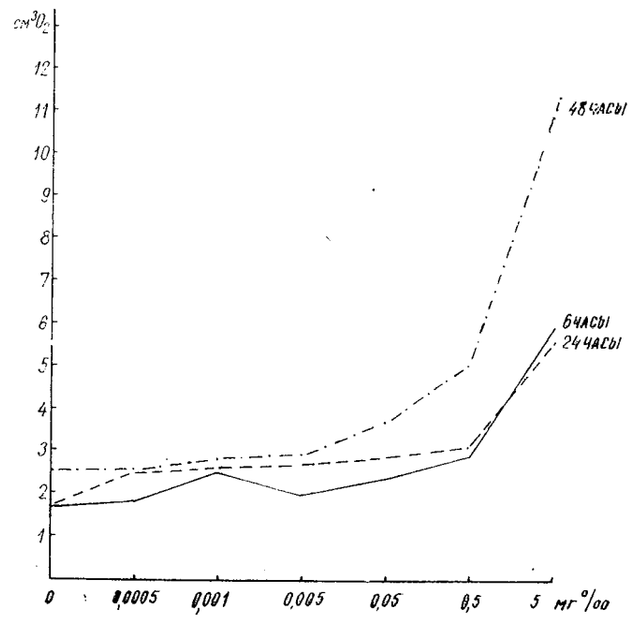

Рис. 4. — Воздействие Карагарда на дыхание

времени: 1,4,24,48 часов. Полученные результаты указаны на рисунках 2 и 3. На рис. 2 представлены результаты опытов, произведенных над гербицидом Карагард, по которым можно убедиться, что при концентрации в 0,0001 мг/л фотосинтез слегка стимулирован, в то время как более повышенные концентрации производят торможение этого процесса. При концентрациях в 0,5 и 5 мг/л получаются отрицательные величины и происходит лишь процесс дыхания. Констатируется также, что торможение усугубляется и согласно длительности воздействия гербицидов.

Влияние Этазина на интенсивность фотосинтеза указано в графике 3, из которого виден ход подобный Карагарду, с тем различием, что Этазин менее токсичен.

Дыхание было определено тем же методом Варбурга при расстоянии времени в 6, 24, 48 часов с момента добавления гербисида.

Результаты представлены на рисунках 4 и 5, из которых можно видеть, что слабые концентрации мало влияют на дыхание, а при более повышенных концентрациях произошло стимулирование — в случае с Карагардом.

Содержимое пигментов хлорофилла определялось фотоэлектро-колориметрическим методом посредством аппарата ФЕК. 56.

Определения были произведены по истечении 15 дней с момента добавления гербицидов.

Данные указаны на рисунках 6 и 7, по которым можно констатировать, что в разбавленных растворах в 0,0001 мг/л в случае Карагарда и в 0,0005—0,005 мг/л в случае Этазина, количество хлорофилла немного больше, чем у контрольных водорослей. В средах с большими дозами гербицида синтез этих пигментов заторможен.

Полученные нами результаты соответствуют с результатами других исследователей. Лужнова М. И. с сотруд. [3], Лалова М. [1], Ладонин В.Ф. Спесивцев Л. Г. [2], Паул В. [4], Сперлинг Д. [5], которые произвели опыты над гербицидами: Метурин, Триазин, Атразин Амитрол, наблюдали торможение роста и фотосинтеза у подопытных растений.

ЗАКЛЮЧЕНИЕ

Опыты проведенные над гербицидами: Карагард и Этазин привели к следующим выводам:

1) Дозы, превышающие 0,0001 мг/л у Карагарды и 0,0005 мг/л для Этазина, производят тормозящее действие на рост, являющийся более сильным в случае Карагарды.

2) Процессы фотосинтеза также задерживаются концентрациями, превышающими 0,0005 мг/л у Карагарды и 0,005 мг/л у Этазина. При очень слабых концентрациях замечается лёгкая стимуляция.

3) Интенсивность дыхания менее подвержена влиянию этих веществ, чем фотосинтез; только лишь при сильной концентрации констатируется некоторое стимулирование.

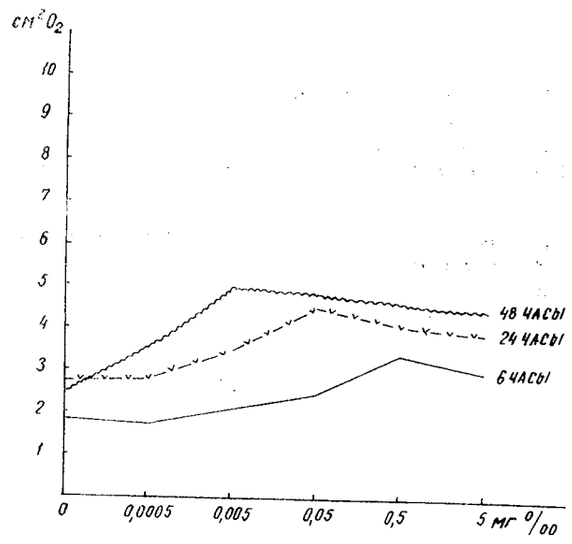

Рис. 5.—Воздействие Этазина на дыхание

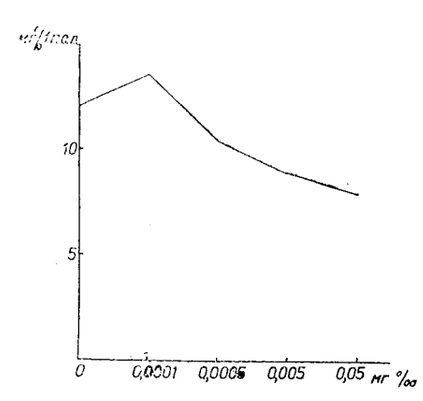

Рис. 6. — Воздействие Карагарда на количество пигментов хлорофилла

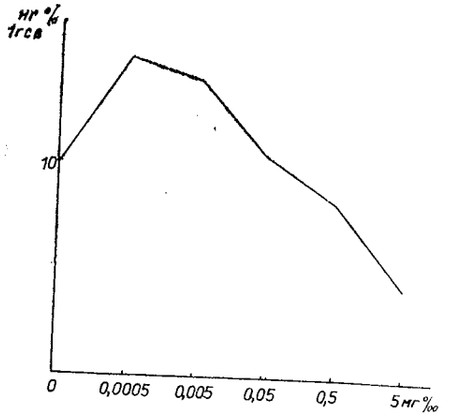

Рис. 7. — Воздействие Этазина на количество пигментов хлорофилла

4) Синтез пигментов хлорофилла тормозится гербицидами, над которыми проводились опыты. Только лишь при концентрациях 0,0001 мг/л Карагарда и 0,0005—0,005 мг/л Этазина отмечается легкое повышение количества пигментов хлорофилла.

5) Произведенные опыты показали, что гербицид Карагард содержит токсины в большей степени, чем Этазин.

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THE ACTION OF VARIOUS NITROGEN SOURCES ON THE PHOTOSYNTHESIS OF *DUNALIELLA*

BY

N. PRISTAVU and K. WEGMANN

The influence of various nitrogen sources on growth rate, oxygen evolution, and on the ^{14}C distribution patterns after $^{14}CO_2$ assimilation was investigated in two *Dunaliella* species. The results are discussed in the view of compartmentation of the cells and the effects of the nitrogen sources on enzymes involved in the nitrogen metabolism.

Algae can grow on various nitrogen compounds as a sole nitrogen source [26], [42], the most important being nitrate, nitrite, ammonia, urea and certain amino acids. However, there are differences in the utilization by alga species for the spectrum of nitrogen compounds. While most algal cultures can be maintained in the presence of nitrate or ammonia, the latter being preferred in the presence of both compounds [14] this is not generally true for the other nitrogen compounds. Urea is consumed by a number of *Chlorophyceae*, but not by *Euglena gracilis*, *Anacystis nidulans*, *Synechococcus cedrorum* or *Porphyridium cruentum* [5]. Certain amino acids or glutamine are favorable nitrogen sources for *Chlorella vulgaris* and *Bumilleriopsis brevis* [4]. The nature of the nitrogen source interferes with the carbon metabolism. The assimilation of nitrate or ammonia requires a proper carbon source in *Chlamydomonas reinhardi* [44], preferably CO_2 in the light, or acetate in the dark. In the presence of CO_2 as the carbon source the uptake of nitrogen is inhibited by DCMU. Similar results have been obtained with *Dunaliella tertiolecta* [15]; CO_2 could not be substituted, however, by glucose, glycerol, acetate, pyruvate, or α-ketoglutarate [12]. In *Dunaliella tertiolecta* 10^{-4} M DCMU did not totally inhibit NO_3^- assimilation [11].

In the present paper the question is posed in which way growth, photosynthetic oxygen evolution, and $^{14}CO_2$ incorporation patterns are influenced by the availability of a variety of nitrogen compounds. *Dunaliella* was used as an experimental organism because its metabolism has been extensively studied in the author's laboratory [7], [8], [28], [46], [47].

MATERIALS AND METHODS

Dunaliella spec., strain 19-6 (cf. *D. tertiolecta*), and *D. salina*, both obtained from the algal collection of the University of Göttingen, were grown in culture tubes placed in a light thermostate [39]. The cultures

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were aerated with CO_2-enriched (2.0%) compressed air. For further details see [47]. The cells were always harvested during the logarithmic growth phase, preferably on the 3rd day after inoculation.

Growth rates were determined by daily counting aliquots in a microscopic counting chamber. From the logarithmic part of the growth curves the number of divisions per day were calculated

$$n = \frac{\log N - \log N_0}{\log 2 \cdot t},$$

where N and N_0 are the cell numbers in the end and at the beginning of the logarithmic phase, t is the number of days. Chlorophyll determinations were performed according to Metzner et al. [25].

Oxygen evolution was determined by a Clark type electrode (YSI 4004) inserted into a 4 ml acrylic glass cuvette. The cell suspension was agitated by a magnetic stirrer. The cuvette with the algae was illuminated by an Attralux incandescent lamp (300 W), the light intensity was 55.000 lux. During the investigation time the suspension contained sufficient CO_2 for maintaining photosynthesis; addition of $NaHCO_3$ did not affect the oxygen evolution rate. $^{14}CO_2$ incorporation was carried out

Table

$^{14}CO_2$ incorporation into *Dunaliella tertiolecta*

Experiment No.	1		2	
Dunaliella spec.	*D. tertiolecta*		*D. tertiolecta*	
$^{14}CO_2$ Fixation time (min)	10		10	
N source	2 mM KNO_3	2 mM NH_4Cl	20 mM KNO_3	20 mM NH_4Cl
Total fixation rate (DPM·10^6)	33.81	41.64	25.80	37.20
^{14}C Distribution : (DPM·10^6)				
Glycerol	4.75	3.31	3.79	5.51
Alanine	1.91	7.50	1.24	2.31
Glycine	3.82	3.60	0.10	1.04
Serine	0.51	3.69	0.10	2.38
Threonine	0.25	0.79		0.22
Aspartate	2.67	1.96	2.01	4.05
Leucine + Valine	0.68	0.46	0.28	0.33
Glutamate	5.71	6.43	5.01	4.05
Asparagine	0.08	0.69	0.36	
Glutamine	0.07	0.40	0.28	0.33
Malate	2.72	0.73	8.88	8.93
Glycolate	4.51	4.93	0.41	0.67
Fumarate			0.10	0.15
Succinate	0.10	0.48	0.23	
Citrate	0.57	0.50	0.98	1.04
3-PGA	0.30	0.62	0.54	0.07
Phosphoenolpyruvate	0.03	0.04	0.08	0.08
Triose phosphates	0.08	0.08		
Sugar monophosphates	1.50	1.69	0.67	1.64
Sugar diphosphates	0.81	0.85		
Uridine diphosphoglucose	0.63	1.33		0.48
Sucrose	0.08	0.19	0.10	0.04
Total amino acids	15.70	25.52	9.38	14.71

in 50 ml Erlenmeyer flasks. Prior to the experiments the algae were centrifuged and resuspended in (a tenth of the original volume) fresh medium containing the nitrogen compound. The media did not contain soil extract in order to avoid contamination by uncontrolled nitrogen compounds. The algae were preilluminated for 5 min. by an Attralux incandescent lamp (300 W); the light intensity was 55.000 lux. Then 80 μCi $NaH^{14}CO_3$ were added and the illumination continued. $NaH^{14}CO_3$ has been purchased from Amersham-Buchler, Braunschweig. After 3 or 10 min the incorporation was stopped by pourring the algal suspension into 50 ml boiling ethanol. The extract was centrifuged, the sediment was successively extracted by 20 ml 50% ethanol and 20 ml dist. water. The extracts were unified, brought to dryness in a flash evaporator and the solubles were taken up into 2 ml dist. water. Aliquots were counted for total incorporation rates (Beckman Liquid Scintillation Counter DPM 100). Further aliquots were two-dimensionally separated by thin-layer electrophoresis/chromatography according to Schürmann [38]. The radioactive spots were detected and identified by autoradiography on X-ray films, scraped into counting vials and the radioactivity was measured by liquid scintillation counting.

3

and *D. salina* in the presence of nitrate or ammonium

3	3	4	5	5	
D. tertiolecta		*D. salina*		*D. salina*	
3		3		3	
20 mM KNO_3	20 mM NH_4Cl	2 mM KNO_3	2 mM NH_4Cl	20 mM KNO_3	20 mM NH_4Cl
14.42	17.89	21.36	41.49	23.34	46.12
4.07	0.48	7.61	10.37	7.61	9.50
1.07	2.09	0.45	3.28	0.56	4.20
1.14	0.27	0.88	4.26	0.84	2.44
0.71	0.41	1.82	0.25	1.52	3.23
			1.08		
0.92	8.03	1.22	1.49	1.49	6.04
		0.26	0.95		0.97
0.16	0.82	1.03	1.41	0.40	1.71
0.10					
0.14					
0.27	2.24	1.28	3.82	0.91	4.20
	0.14	0.66		0.41	
	0.05				
	0.66				
0.52				0.19	
0.89	0.93	1.26	3.32	2.64	4.61
0.06	0.09	0.19	0.17	1.82	0.18
		0.15		0.26	0.18
2.91	1.36	1.09	8.96	2.31	6.69
		1.39	1.24		
0.46		0.56		0.84	0.83
	0.11				
4.50	11.62	5.66	12.82	4.81	18.59

EXPERIMENTS AND RESULTS

Growth rates of *Dunaliella tertiolecta* in the presence of a number of nitrogen compounds are given in Table 1. The cells can grow on several inorganic and organic compounds. The growth rate is somewhat increased in the presence of ammonia. The amino acids glycine, glutamate and aspartate are poor nitrogen sources, while the amides glutamine and asparagine appear to be relatively good sources. Attention must be payed, however, to the fact that these growth rates are only obtained if the pH of the culture is kept constant. Otherwise the cells cease growing after a short time of low pH e.g. in the presence of NH_4Cl. Oxygen evolution (Table 2) resulted in a similar picture. The lowest values were obtained in the presence of glutamate or aspartate, while there is little

Table 1

Growth rate of *Dunaliella tertiolecta* in dependence on the nitrogen source during logarithmic growth. Continuous illumination 8 000 Lux, 25°C, aerated with 2% CO_2 in compressed air

N source	growth rate n
Nitrate	1.25
Nitrite	1.11
Urea	0.45
Ammonium	1.42
Glycine	0.29
Glutamate	0.38
Glutamine	0.63
Aspartate	0.23
Asparagine	0.87

Table 2

Oxygen evolution from *Dunaliella tertiolecta* and *D. salina* in dependence on the nitrogen source. $2{,}4 \cdot 10^5$ ergs. cm^{-2} sec^{-1} incandescent light. O_2 evolution in µMoles. hr^{-1}. mg $Chl,^{-1}$

N source	D. tertiolecta	D. salina
Nitrate	220	220
Nitrite	150	30
Urea	210	220
Ammonium	200	120
Glycine	200	180
Glutamate	30	110
Glutamine	210	230
Aspartate	20	130
Asparagine	240	140

reduction of oxygen evolution in the presence of nitrite observed with *D. tertiolecta*. On the contrary the experiment with *D. salina* shows that this species seems to be much more sensitive to nitrite.

$^{14}CO_2$ fixation rates and ^{14}C distribution patterns for a number of experiments with *D. salina* and *D. tertiolecta* in the presence of nitrate and ammonia are shown in Table 3. Under the experimental conditions the total fixation rates appear increased in the presence of NH^+_4, the increase being much higher in *D. salina* than in *D. tertiolecta*. A dramatic increase in all the experiments was observed in the amino acid fraction; their sums are also given in Table 3. A more detailed consideration shows alanine and glutamate as the most favoured amino acids for additional synthesis in the presence of NH^+_4, however, other amino acids can also be increased.

In Tables 4 and 5 the ^{14}C distribution patterns in the presence of a number of nitrogen compounds are compared for *D. salina* and *D. tertiolecta*. In order to get a better possibility of comparison the labeled compounds are presented in groups. The total fixation rates vary with the nitrogen source supplied, but they are not conform in all cases with the growth rates and oxygen evolutions. The increased labelling rates of

Table 4

$^{14}CO_2$ incorporation into *Dunaliella tertiolecta* in the presence of various nitrogen sources

N source ($2{,}5 \cdot 10^{-3}$M)	KNO_3	$NaNO_2$	NH_4Cl	Urea	Gly	Glu	Gln	Asp	Asn
Total fixation rate (DPM · 10⁶)	3.55	0.12	5.16	3.06	5.07	2.75	3.16	3.56	3.39
Percentage of ^{14}C in :									
Glycerol	23.9	50.8	13.2	23.0	38.7	51.8	25.1	54.2	42.4
Amino acids	20.4	24.9	52.2	29.7	24.5	12.4	46.0	25.7	22.5
Sugar phosphates + 3-PGA	32.7	10.7	8.6	17.4	15.4	28.9	10.4	16.5	16.3
Organic acids	16.5	3.4	22.0	27.5	18.4	5.3	14.7	3.1	14.1

Table 5

$^{14}CO_2$ incorporation into *Dunaliella salina* in the presence of various nitrogen sources

N source ($2{,}5 \cdot 10^{-3}$M)	KNO_3	$NaNO_2$	NH_4Cl	Urea	Gly	Glu	Gln	Asp	Asn
Total fixation rate (DPM · 10⁶)	21.36	0.12	41.49	28.86	28.82	20.84	23.33	32.36	17.55
Percentage of ^{14}C in :									
Glycerol	35.6	25.8	25.0	2.6	41.8	3.9	42.9	43.9	46.8
Amino acids	26.4	26.1	30.9	34.4	23.0	56.4	22.3	19.6	21.5
Sugar phosphates + 3-PGA	20.8	28.8	32.6	36.7	28.5	25.9	27.8	26.6	22.9
Organic acids	9.1	7.8	9.2	24.4	3.6	2.8	3.9	3.4	3.4

the various amino acids are different for the two *Dunaliella* species. The percentage of organic acids is particularly increased in both species in the presence of urea.

DISCUSSION

While the incorporation pathways of organic nitrogen compounds into algae are poorly investigated, there are a number of publications concerning nitrate and ammonia. Obviously the path of carbon is changed by the nature of the nitrogen source. Reisner et al. [31] found that in *Chlorella vulgaris* nitrate is incorporated into glutamate, in contrast ammonia is used for the synthesis of alanine and glutamine after nitrogen starvation. Baker and Thompson [2] observed an increase of pyruvate in nitrogen starved *Chlorella vulgaris* by about 50% within 5 min after the addition of NH_4^+. According to Kanazawa, Kirk and Bassham [20] NH_4^+ does not only contribute to reductive amination in *Chlorella*, but also by enzyme regulation. In the presence of NH_4^+ the 3-PGA and PEP levels are reduced, pyruvate and alanine contents are increased, sucrose synthesis

is inhibited, and the ^{14}C incorporation into malate and citrate via TCA cycle is stimulated. This regulation was also observed in the dark [19]. The activity of pyruvate kinase [6] cannot explain the higher malate concentration. The increased malate content might be caused either by the stimulation of reductive pyruvate carboxylation or by increased condensation of acetyl-CoA with glyoxylate [18].

As far as *Dunaliella* is concerned, there are older investigations by Gibor [9] on the utilization of nitrate and ammonia. He observed a 2.8 times greater growth rate of *D. viridis* in the presence of ammonia instead of nitrate, while the growth rate of *D. salina* was little influenced. Paasche [30] obtained similar results with *D. tertiolecta*, whose growth rate, photosynthetic oxygen evolution, protein content, and particularly the activity of ribulose diphosphate carboxylase (52%) were increased by NH^+_4 nutrition. Paasche explained the higher growth rate by the increased synthesis of Calvin cycle enzymes in the presence of ammonia, thus causing a more effective CO_2 incorporation. In our experiments with *D. tertiolecta* and *D. salina* the increased CO_2 fixation rates could be confirmed. However, Paasche's explanation cannot be applied to our results, because the stimulated incorporation rates can be already observed a few minutes after changing the nitrogen source, too early for a *de novo* synthesis of the enzymes. The evaluation of the ^{14}C distribution patterns shows — beside some stimulation of the Calvin cycle — an important increase of carbon flow into the compounds synthesized through the 2-PGA—PEP—pathway, by the presence of NH^+_4. This is in agreement with the results of Kanazawa et al. [20] obtained with *Chlorella pyrenoidosa*, and those of Winkenbach et al. [50] with *Dunaliella* and *Acetabularia*.

As a rule ammonia is introduced into the algae metabolism either by reductive amination of α-ketoglutarate yielding glutamate, or by glutamine synthesis. Then the amino groups are transferred by amino transferases upon the keto acids, other than α-ketoglutarate. In this connection two aspects should be considered : the behaviour of the nitrogen metabolism, and the possible effects of the nitrogen source on enzyme synthesis or activities.

Isolated *Vicia* chloroplasts photoreduce α-ketoglutarate to glutamate in the presence of NH^+_4 [10]. Glutamic and alanine dehydrogenases are present in spinach chloroplasts [45]. There is no full agreement in respect to the presence of amino transferases in chloroplasts [36]. In *Vicia* and *Nicotiana* [49], and in *Zea mays* [1] glutamate oxalacetate aminotransferase and glutamate pyruvate aminotransferase was found to be distributed between chloroplasts and cytoplasm, however, Heber [17] and Kirk and Leech [21] reported that only glutamate oxalacetate aminotransferase was distributed among the two fractions, while glutamate pyruvate aminotransferase was only present outside the chloroplasts. Aspartate synthesis was observed in isolated chloroplasts [35]. Heber [17] demonstrated that aspartate and alanine in vivo were partly synthesized in chloroplasts. By the way, nitrate and nitrite reductases are also located in chloroplasts [40], where they probably supply ammonium ions for reductive amination. There is no reason to believe that the subcellular distribution of enzymes is different in algae. Smith et al. [41] found

amino acid synthesis in *Chlorella* chloroplasts. However, from their kinetics they concluded that at least two amino acid pools must exist in *Chlorella*. They could not establish reductive amination of pyruvate or PEP in *Chlorella* cells by ^{15}N, ^{14}C incorporation experiments [3]. However, there are indications that this is different in *Dunaliella* [48].

Another point of interest is the influence of the nitrogen source on enzymes. Nitrate and nitrite reductases are induced by their substrates in *Anabaena cylindrica* [29], but not in *Cyanidium caldarium*, where nitrate reductase is also synthesized in nitrogen starved cells [34].

It seems most remarkable that *Cyanidium caldarium* contains more than double the nitrate reductase activity when grown on glutamate instead of nitrate [32], [33]. Nitrite reductase was studied in *Anabena cylindrica* [16] and *Dunaliella tertiolecta* [13]; its activity seems not to be affected by the nitrogen source [27]. Nitrate reductase is inactivated in *Chlorella fusca* within one hour by the addition of NH^+_4, and its synthesis is repressed [24], [27]. This is not valid for higher plants [37]. It was observed [43] that the inhibition occurs only under conditions under that NH^+_4 is assimilated. Losada et al. [23] explain the nitrate reductase inhibition by the increase of reducing power of the cell caused by the uncoupling of the photophosphorylation in the presence of ammonia. NADH dependent glutamic and alanine dehydrogenases in *Chlorella* were only little influenced by NH^+_4, while NADPH dependent glutamic dehydrogenase was stimulated by NH^+_4 and inhibited by NO^-_3 [22].

Taken all together, more detailed information on the role of the nitrogen source in the carbon metabolism should be obtained by the determination of the involved enzymes in addition to ^{14}C incorporation experiments.

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BIODETERIORATION OF WOOD IN MINES I. MICROMYCETES ISOLATED FROM WOOD

BY

MARILENA IOACHIMESCU-DINULESCU

The results of the studies initiated between 1971 and 1973 at the Institute of Biology on the mycoflora of wood in mines and the possible role of different agents in the selfignition phenomena occuring in the pyrite mines, determined the extension of the studies also on the mycoflora of the coal mines.
The fungi isolated belong to 41 species, among which *Circinella umbellata* van Tieghem and Monnier, *Bispora betulina* (Corda) Hughes, *Dyctiosporium elegans* Corda, *Gliomastix murorum* (Corda) Hughes, *Humicola grisea* Traaen, *Ophiostoma stenoceras* (Robak) Melin and Nannf, *Petriella setifera* (Schm) Curzi, are new taxons in the mycoflora of Romania.
The presence and frequency of the isolated is discussed in relation to the wood essence, site of sampling, pH of sample.

Due to their structure and chemical composition, the wood structures used in mines to reinforce the galleries and working faces are a favourable substrate for the development of microorganisms and, implicitly, of fungi.

In the course of the wood biodeterioration phenomenon, in particular the one occuring in mines, the action of different biological agents and their development on such a substrate is influenced by several factors of the respective ecological habitat such as temperature, humidity, air currents, different operations required by deposit exploitation etc. Thus, the climate conditions to mines and the interrelationships between different biodeterioration agents, plead in favour of a new branch of microbiology — *the microbiology of mines* [19].

Most of the studies on the fungi deteriorating the wood in mines are devoted to the *Basidiomycetes* [5], [6], [14], [18], less attention being given to the micromycetes of the class *Ascomycetes* and Fungi Imperfecti, known (1950—1954) as agents of wood biodeterioration.

The first studies on the mycoflora of mine wood were conducted in Romania between 1970 and 1973 at the Institute of Biology [10], [12], and brought evidence on some physiological characteristics of the species isolated from the pyrite mines [11].

The present paper deals about the fungi isolated between 1973 and 1975 from the wood structures of the coal mine, of which seven are new taxons in the mycoflora of Romania.

MATERIAL AND METHODS

Wood pieces were sampled from different galleries and working faces and plated in Petri dishes acting as moist chambers. The isolations

were made either from the outer or inner part of the samples on culture media, or by passage of the fructifications that were visible on the wood samples plated on the respective media.

The following media were used: PDA, malt-extract 2%, Czapek-Dox, Abrams-cellulose, Eggins and Pugh, malt-cellulose, yeast-extract-cellulose.

The isolated were incubated at 24—26°C.

RESULTS AND DISCUSSIONS

A large number of strains belonging to 31 genera (Table 1) were isolated from the wood of the coal mine. As seen in Table 1, the prevalence of certain genera is evident, e.g. *Trichoderma viride* (*T. lignorum*), *Ophiostoma stenoceras*, *Aspergillus versicolor*, *Dyctiosporum elegans*, *Aspergillus ustus*, *Gliomastix murorum*, *Chaetomium globosum*, *Ulocladium botrytis* etc.

As to the essence, most mine galleries and working races are reinforced with fir wood (S_1, S_3, S_4, S_5), and a single one with oak wood (S_2); from this latter only a few isolates were obtained, oak wood being more resistant than the coniferous species [5].

Table

Fungi isolated from wood

Sample	Site of sampling	Wood essence	pH of sample	*Absidia sp.*	*Alternaria alternata*	*Aspergillus ochraceus*	*A. nidulans*	*A. niger*	*A. versicolor*	*A. ustus*	*Bispora betulina* *	*Botrytis cinerea*	*Cephalotrichum stemonitis*	*Chaetomium funicolum*	*Ch. globosum*
Fungi															
S_1 (a-c)	Mine 3 Access plane to the working face	fir wood	6,0—6,6	1	4	2	1	1	5	4	3	3	2	3	7
S_2 (a-c)	Gallery I 4703	oak wood	6,2—6,4	—	—	—	—	1	2	3	—	—	—	—	—
S_3 (a-j)	Side gallery between entrance and gallery I 101	fir wood	5,8—6,7	4	2	—	2	1	6	6	3	2	6	2	2
S_4 (a-c)	Gallery I 102 (Abundant Macromycetes)	fir wood	5,8	—	—	1	1	—	2	1	—	1	—	1	4
S_5 (a-b)	Ceiling Gallery I 101	fir wood	5,8—6,0	—	1	1	—	1	3	2	—	1	2	—	1
TOTAL				5	7	4	4	4	18	16	6	7	10	6	14

*New taxons for Romania's mycoflora

The wood samples showed pH values ranging between 5.4 (S_4) and 6.7 (S_3), larger numbers of isolates being obtained from the samples with pH values of 6.6 and 6.7 (S_1 and S_3), generally considered as the most favourable for the growth of fungi from mines [2].

With regard to the new taxons in the mycoflora of Romania, their description is as follows:

— *Bispora betulina* (Corda) Hughes forms colonies effused on wood. Mycelium partly superficial. Conidiophores usually short, straight or flexous, unbranched, pale-brown or brown, smooth, 4—16 × 3—5 μ. Conidia catenate acrogenous, simple or cylindrical rounded at the ends, brown, usually 1-septate, with a very dark band at the septum, smooth, 8—12 (13) × 4—5 μ in size (Plate II, 6).

— *Circinella umbellata* van Tieghem and Monnier [syn. *Mucor umbellatus* (van Tieghem and Le Monnier) Schrotes; *Circinella aspera* Lendner] is characterized by fine sporangiophorus filaments [17], 60 mm to 1 cm high, with short branchings covering the surface of the wood (Plate I, 2). The sporangiophores are delicate and bent, with an encrusted membrane, 20 μ thick. Sporangia dark grey, with fragile wall. The columel is narrow, cylindrical, conical, 84—90 μ in size. The spores are small, 4.5—10 μ in size, round to oval, brownish-blue (Plate I,1).

1

structures in the mine

Ch. indicum	*Circinella umbellata* *	*Cladosporium herbarum*	*Dictyosporium elegans* *	*Epicoccum nigrum*	*Fusarium solani*	*Geotrichum candidum*	*Gliocladium deliquescens*	*G. roseum*	*Gliomastix murorum* *	*Humicola grisea* *	*Melanospora zamiae*	*Ophiostoma stenoceras* *	*Papulaspora rubida*	*Penicillium expansum*	*P. frequentans*	*P. ochraceum*	*P. variabile*	*Petriella setifera* *	*Rhizopus nigricans*	*Sordaria fimicola*	*Stachybotris atra*	*Tamnidium elegans*	*Trichoderma viride*	*Tricholecium roseum*	*Trilirachium dependens*	*Verticillium albo-atrum*	*V. tenerum*	*Ulocladium botrytis*	TOTAL
2	4	1	4	1	—	1	—	—	3	—	1	7	2	3	1	1	2	—	4	5	1	2	3	—	—	—	3	1	89
—	—	1	—	1	—	—	—	—	—	—	—	—	—	2	1	1	1	—	—	—	3	—	2	—	—	—	1	1	20
1	6	3	10	3	3	4	—	8	6	4	4	8	4	—	2	4	6	—	4	7	5	6	9	4	10	—	2	6	164
1	—	—	1	—	—	2	7	5	—	—	—	4	—	—	1	1	2	6	2	—	2	—	3	2	—	4	2	3	59
—	2	3	2	1	1	2	2	2	1	1	—	—	—	1	3	2	—	—	—	—	—	—	4	1	—	1	—	2	43
4	12	8	17	6	4	9	9	15	10	5	5	19	6	6	8	9	11	6	10	12	11	8	21	7	10	5	8	13	375

— *Dictyosporium elegans* Corda, covers the wood with small dark brown spots (Plate I, 4), from which dark-brown conidia were isolated, 50—80 × 24—31 μ in size and flattened at one end, usually consisting of 5—6 rows of cells of about the same length (Plate I, 3). The conidia contain 51 to 96 cells. Ellis, 1971, reported the presence of the fungus on degraded wood.

— *Gliomastix murorum* (Corda) Hughes, forms on PDA dusty dark green colonies, with abundant hialine phialides, smooth on the basis and more rough towards the apex, where a small coliar can be noticed. The phialides are 20—30 μ long and 2—3 μ thick. Frequently, conidia are subspherical, catenulated, olive-brown, 2.0—5.5 × 2.0—4.5 μ in size, usually rough (Plate II, 1).

— *Humicola grisea* Traaen, forms on PDA cottony, white to pale grey colonies which later turn to dark grey with a darkish lower side. Conidia are dark brown, 12—17 μ in diameter. Ellis reported the fungus on wood (Plate II, 3).

— *Ophiostoma stenoceras* (Robak) Melin and Nannf [syn. *Ceratostomella stenoceras* Robak; *Ceratocystis stenoceras* (Robak) C. Moreau] The colonies have a varied aspect, smooth, floccose, white, turning to dark. The opposite side colourless or light yellow. It shows the conidial state of *Sporothrix schenckii* Hektoen and Perkins (Plate II, 2). Conidia of 2.5—4.0 × 1.5—3.0 μ in size, smooth, hialine, guttulate or spindle-shaped, frequently thrust in clusters in the slightly conidiophore tip. Peritecia black, globose, spherical with long necks and the ends surrounded by ostiolate hairs (Plate II, 2). The ascospores 2.5 × 1.2 μ in size are hialine, grouped in clusters in a yellow-white mucus at the end of the neck [9].

— *Petriella setifera* (Schm) Curzi*, formed on wood sphaerical, pale-brown to dark-brown perithecia, 70—125 μ in diameter, with a short neck, covered with simple, rigid, ligthly pigmented hairs (Plate II, 4); ascospores asymmetrical, convexely-shaped with pointed ends and thinner walls coloured in red-brown, containing numerous oil droplets, measuring 7—11 × 5—6 μ (Plate II, 5). Conidial stage: *Sporothricum* and *Graphium* [1].

The abundance of the isolates obtained from the sample S_3 could be accounted for not only by the large number of samplings from the same place, but also by the site of sampling since the circulation of the fungal spores from the air is possible (S_3 being close to the entrance in the mine).

The activities in the mine favour a specific climate, characterized by a certain temperature, relative humidity and speed of the air. There are data indicating that in different countries the temperatures are different within one year [19]. In the case of the coal mine of Romania the temperature varied between 15 and 18°C in 1975, and the humidity between 90 and 95%.

Our results on the mycoflora of the coal mines are in agreement with the data in the literature, according to which the genera *Aspergillus*, *Alternaria*, *Chaetomium*, *Penicillium*, *Trichoderma* are involved in the deterioration of wood in general [3], [4], [8], [9], [15], and of the wood

*The recent taxonomy works kindly supplied by Dr. O. Constantinescu is acknowledged.

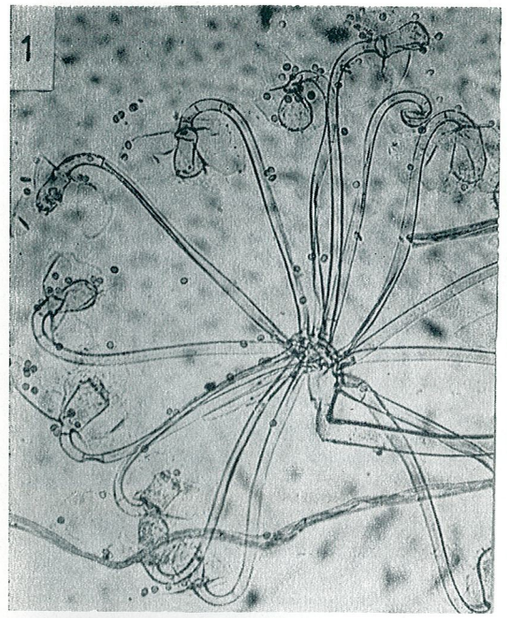

Plate I

Fig. 1. — *Circinella umbellata* — sporangiophores with sporangia and spores
Fig. 2. — *Circinella umbellata* — mine wood covered by the fungus
Fig. 3. — *Dictyosporium elegans* — conidia
Fig. 4. — *Dictyosporium elegans* — mine wood covered by the fungus

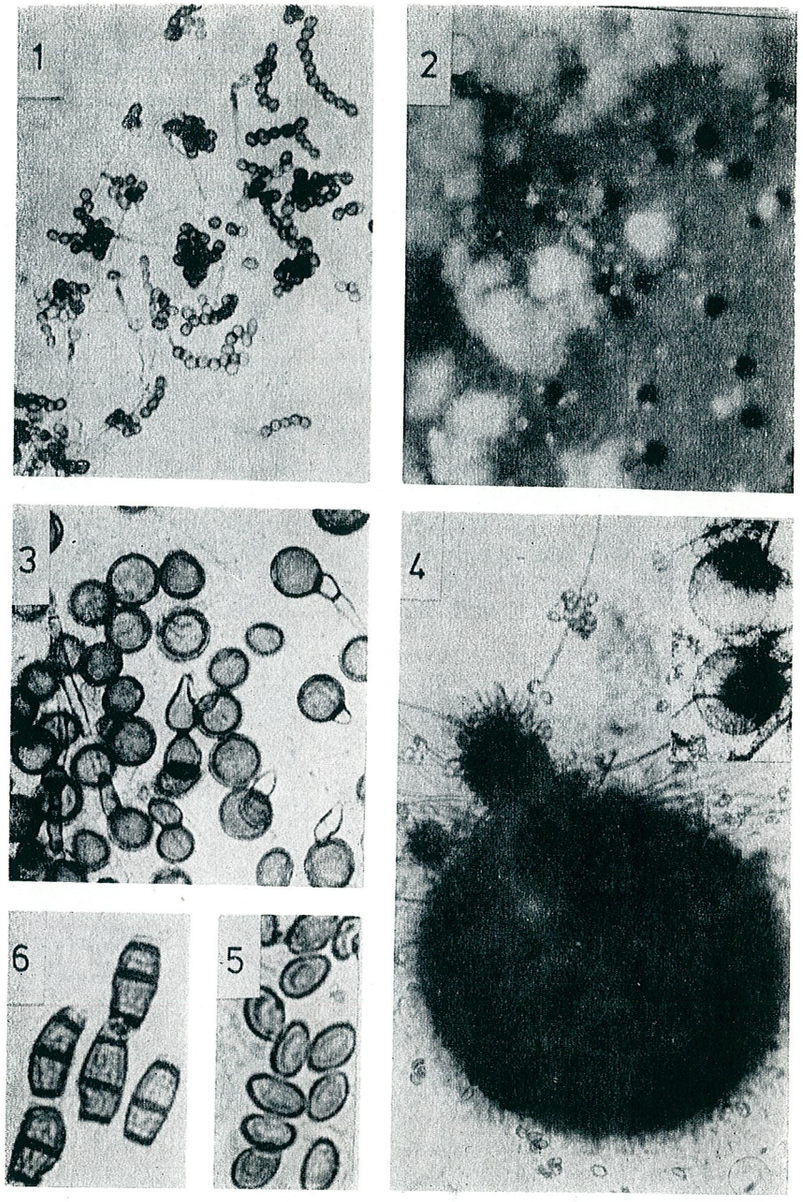

Plate II

Fig. 1. — *Gliomastix murorum* — conidiophores and conidia
Fig. 2. — *Ophiostoma stenoceras* — perithecia and conidial stage (Sporothrix schenckii), on Abrams — cellulose medium
Fig. 3. — *Humicola grisea* — conidiophores and conidia
Fig. 4. — *Petriella setifera* — perithecia
Fig. 5. — *Petriella setifera* — ascospores
Fig. 6. — *Bispora betulina* — conidia

from the mines in particular [10], [12], [13], [16], [18], [19], the structural changes noticed during the microscope examination of the degraded mine wood being even described [7].

Initially, the spores of *Rhizopus*, *Penicillium*, and *Trichoderma* can penetrate in the mine together with the woody materials used in reinforcing the galleries and working faces [13]. Further, they find in the mine conditions that are favourable to their development : high temperatures and humidity, as well as a poor ventilation [16].

Based on the classification of Ryctera and Bartakova, 1963 [19], we may conclude that in most of the mines in Europe, the environmental conditions are resembling those of the tropical climate.

Thus, the presence of the micromycetes at the level of this habitat, represented by the wood structures of the galleries and working faces, can be accounted for by the conditions of this ecological habitat represented by the mine, the more so as the respective biodeteriogens have a particularly complex and labile enzyme system, allowing them to settle on different substrates, on which they can act in different ways (a mechanical, chemical, biochemical action).

CONCLUSIONS

1. Fourty-one species belonging to 31 genera have been isolated from the coal mine. The most frequent were the following : *Trichoderma viride*, *Ophiostoma stenoceras*, *Aspergillus versicolor*, *Dictyosporium elegans*, *Chaetomium globosum*, *Gliomastrix murorum*.

2. Among the species isolated, the following seven represent taxons that are new for the mycoflora of Romania : *Bispora betulina* (Corda) Hughes, *Circinella umbellata* van Tieghem and Le Monnier, *Dyctiosporium elegans* Corda, *Gliomastix murorum* (Corda) Hughes, *Humicola grisea* Traaen, *Ophiostoma stenoceras* (Robak) Melin and Nannf and *Petriella setifera* (Schm) Curzi.

3. The frequency of the fungi varied in terms of the site of sampling, the essence and the pH values of the samples.

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CHEMICAL COMPOSITION OF THREE RAISED PEAT BOG WATERS FROM ROMANIA

BY

GH. COLDEA and F. MARKIEVICI

INTRODUCTION

The special microclimatic and pedo-ecologic conditions of the ombrophilous allowed the surviving in their floras, sometimes as compact populations, of certain relict species of northern origin. The lack of data concerning the chemical composition of waters for such bogs in Romania, as well as the unexplained absence of some oligotrophic species in geomorphologically similar bogs made us to investigate the relationship between the floristic composition of plant communities and the chemical composition of water.

Our results are comparable with those obtained and published by Gorham (1956), (1957), Gorham and Cragg (1960), and Tolpa and Gorham (1961) for other areas in Europe.

DESCRIPTION OF THE AREAS

For the purpose of our research three characteristic ombrophilous bogs have been selected, which are morphologically and geobotanically very similar, but are situated in different mountain regions of Romania.

The Vlăşchinescu high moor is situated in the Oaş-Maramureş plateau, in the north-west of Romania, 20 km from Baia Mare; the Mohoş raised peat bog, from the eastern Carpathians is located at the south-east extremity of the Harghita Mountains, about 8 km from Tuşnad; the Izbuc high moor, from the Romanian Western Mountains, situated in the Izbuc Valley (on the tributary brooks of the Someşul Cald Valley) about 28 km south of Huedin.

It should be mentioned, from a geobotanical point of view, that all the three bogs are found in the spruce fir (*Picea abies*) forests zone of the mountains, having mostly the same vegetation cover. Some of the typically oligotrophic species, which sometimes form compact communities, are : *Eriophorum vaginatum*, *Empetrum nigrum*, *Scheuchzeria palustris*, *Carex limosa*, *Rhynchospora alba*, *Vaccinium oxycoccos*, *Carex rostrata*, *Andromeda polifolia*, *Sphagnum cuspidatum*, *Sphagnum magellanicum*, *Sphagnum fuscum*, *Drepanocladus fluitans*. The three bogs here have in common some oligotrophic algal species too (Table 2). The bogs are different in point of their tree layers : in the border zone of the Mohoş peat bog there are compact populations of *Pinus sylvestris* and *Betula pubescens*, while in the central part of the Izbuc high moor small areas are covered by *Pinus montana ssp. mughus*.

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Table 1

Characteristics of the three Romanian bogs

Location :	Vlăşchinescu, 20 km east of Baia-Mare	Mohoş, 8 km east of Tuşnad	Izbuc 28 km south of Huedin
Altitude :	930 m	1 050 m	1 100 m
Area (ha) :	2.5	80	8
Substratum :	andesite	volcanic tuff	crystalline schist
Surface configuration :	Raised approx. 3.5 m above surrounding mineral soil	Raised approx. 10 m above surrounding mineral soil	Raised approx. 4 m above surrounding mineral soil
Dominant vegetation :	Communities : *Eriophorum vaginatum, Carex limosa-Scheuchzeria palustris, Carex rostrata — Scheuchzeria palustris*	Communities : *Eriophorum vaginatum, Scheuchzeria palustris, Pinus sylvestris, Betula pubescens*	Communities : *Eriophorum vaginatum, Carex limosa-Scheuchzeria palustris, Rhynchospora alba*
Mean annual temperature :	+4°C	+2°C	+2°C
January isotherm :	—4.5°C	—5.5°C	—5°C
July isotherm :	+14°C	+12°C	+12°C
Mean annual precipitation :	1 200 mm	1 000 mm	1 100 mm
Mean annual air humidity :	>80 %	<80 %	>80 %

The surface configuration of these bogs show a strong convexity, the peat layer being 3.5—10 m deep. The water supply of bogs comes only from rainfalls. The main data concerning habitat conditions of the bogs are given in Table 1.

METHODS

In August 1975, 15 water samples — 5 samples for each of the studied bogs — have been collected in polyethylene canisters for quantitative chemical analyses. The analyses were made by employing the standard methods used in our laboratory. The pH was electrometrically determined in aqueous solution; the Na, K, and Ca — by flame photometry; Mg — through atomic absorption; Fe — colorimetrically, with O-phenentrolyne; Mn — colorimetrically, as permanganate; the chlorides — volumetrically after Mohr; and the sulphates — by iodometry.

At the same time, the plant species growing in the sampling sites have been noted, according to Braun-Blanquet's method. Based on floristic composition, the stand samples have been grouped in communities dominated by *Rhynchospora alba*, *Carex limosa — Scheuchzeria palustris*, and *Carex rostrata — Scheuchzeria palustris* (see Table 2). In such a way we tried to detect the possible correlation which might exist between the floristic composition of the oligotrophic communities and the chemical composition of the water.

Table 2

The vegetation of the hollows of bogs examined
Column 1, communities of ***Rhynchospora alba***
2—3, communities of ***Carex limosa-Scheuchzeria palustris***
4—5, communities of ***Carex rostrata-Scheuchzeria palustris***
6, communities of *Scheuchzeria palustris*

Bog	Izbuc		Vlăschinescu		Mohos	
Community	1	2	3	4	5	6
Rhynchospora alba (L.) Vahl	4.5	.	.	.	.	.
Carex limosa L.	1.5	3.5	2.5	.	.	.
Carex rostrata Stokes	.	.	.	3.5	1.2	.
Scheuchzeria palustris L.	+	2.5	2.5	1.5	1.3	2.5
Drosera rotundifolia L.	+.3	+.4	+3.	+.3	.	+.3
Drosera obovata Mert. et Koch	.	.	.	.	.	1.4
Andromeda polifolia L.	+	+.3	.	.	.	+.1
Eriophorum vaginatum L.	+.2	+	+.2	1.5	1.2	+.1
Carex pauciflora Host	+.3	.	+.4	1.4	.	.
Vaccinium microcarpum Ait.	.	+.2	+.3	.	+.1	.
Vaccinium oxycoccos L.	.	.	+.2	+.4	+.5	+.4
Empetrum nigrum L.	.	.	+	.	.	.
Lycopodium inundatum (L.) Hol.	.	.	+.4	.	.	.
Molinia caerulea (L.) Moench	.	.	+	.	.	.
Sphagnum cuspidatum Ehrh.	4.5	4.5	.	.	5.5	5.5
Sphagnum magellanicum Brid.	1.2	+.2	.	.	+.3	.
Sphagnum nemoreum Scop.	.	.	.	.	.	1.2
Drepanocladus fluitans Warnst.	.	.	3.5	3.5	.	.
Cladopodiella fluitans (Nees) Buch	.	.	.	+.3	.	+.5
Staurastrum spinosum (Bréb.) Ralfs	1.5	2.5	+	.	.	+
Staurastrum quadrispinatum Turn.	1.5	+	.	.	.	.
Penium polymorphum (Perty) Perty	+	+	+	.	.	+
Actinotaenium cucurbita (Bréb.) Teil.	+	+	+	.	.	+
Chroococcus turgidus (Kütz.) Näg.	+	+.5	+	.	.	.
Netrium oblongum (De Bary) Lütk.	2.5	+	.	.	.	+
Teilingia granulata (Roy et Biss.) Bour.	+	.	+	.	.	+
Frustulia rhomboides var. saxonica (Rabenh.) De Toni	+	+	+	.	.	.

RESULTS

Since the bogs have been selected of the same type — ombrophilous bogs — showing only slight floristical differences, the anions and cations content of the waters exhibited general similarities. In all samples (Table 3) the pH was of 3.5—4.5, the highest values being recorded in the samples collected from the Mohoş peat bog (pH : 3.5—4.2). The same samples exhibited the highest content of chlorides (30—51 p.p.m.), sulphates (0.5—0.7 p.p.m.) as well as the maximum concentration of Na (6.0—11.0 p.p.m.), Ca (4.0—6.0 p.p.m.) and Mg (2.4—5.0 p.p.m.) cations.

The high chlorides content in these samples is correlated with the Na, Ca, and Mg ions and is due to the chemical composition of the substratum at Mohoş — the volcanic tuff.

Table 3

Chemical composition of waters from the three Romanian bogs

Bog		Community	pH	Na	K	Ca	Mg	Fe	Mn	Cl	SO_4
				Parts per million							
Izbuc	1	*Rhynchospora alba*	4.18	trace	trace	2.0	0.12	0.86	0.062	—	—
	2	*Rhynchospora alba*	4.25	,,	,,	2.0	0.20	1.28	0.070	—	—
	3	*Carex limosa-Scheuch.*	4.12	,,	,,	trace	trace	0.75	0.120	—	—
	4	*Carex limosa-Scheuch.*	4.10	,,	,,	,,	,,	0.54	0.024	—	—
	5	*Carex limosa Scheuch.*	4.05	,,	,,	,,	,,	0.46	0.062	—	—
Vlăş-chinescu	6	*Carex limosa-Scheuch.*	4.08	0.90	trace	3.0	0.7	0.40	0.050	0.1	0.2
	7	*Carex limosa-Scheuch.*	4.15	1.70	0.20	3.0	1.2	0.46	0.036	0.2	0.2
	8	*Carex limosa-Scheuch.*	4.52	0.80	0.60	2.5	1.2	0.50	0.030	0.2	0.4
	9	*Carex limosa-Scheuch.*	4.45	1.50	0.80	2.0	0.6	0.40	0.060	0.2	0.3
	10	*Carex rostrata-Scheuch.*	4.32	3.25	0.30	2.0	0.6	0.60	0.050	0.3	0.3
Mohoş	11	*Carex rostrata-Scheuch.*	4.20	11.0	0.12	6.0	4.8	0.71	trace	35	0.6
	12	*Scheuch. palustris*	4.00	8.0	0.20	4.0	3.6	0.40	,,	35	0.6
	13	*Scheuch. palustris*	3.85	10.0	0.70	4.0	3.6	0.50	0.060	30	0.5
	14	*Scheuch. palustris*	3.50	8.0	0.08	4.5	5.0	0.40	0.040	51	0.7
	15	*Scheuch. palustris*	3.80	6.0	0.16	4.0	2.4	0.44	0.040	40	0.65

According to the correlations observed between the occurrence of oligotrophic species and some ecological factors, the absence of *Rhynchospora alba* and *Carex limosa* in the Mohoş peat bog is probably due to a higher Na and Mg ions concentration. In the stations in which these species are present, the concentration of Na and Mg ions is lower (Table 3). The assertion is more plausible when we compare the climatic conditions of Izbuc — with luxuriant vegetation of the two species — to those of Mohoş (see Table 1) which in both cases are very similar; on the other hand it is known that the two species form compact populations in bogs situated both north and south of Mohoş (POP, 1960).

Compared to the two above — mentioned oligotrophic species, the characteristic species of hollous — *Scheuchzeria palustris* — has a wider ecological tolerance range. It is present in all the studied stations, whatever the water anions and cations concentration would be. On the basis of these findings, we think that when describing the oligotrophic communities consisting of *Carex limosa* and *Scheuchzeria palustris*, the name *Scheuchzerio-Caricetum limosae* (Br.-Bl. 21) Libb. 32 should be accepted, since the association is geobotanically more justified, having a larger content than *Caricetum limosae* Br.-Bl. 21 [1].

DISCUSSIONS

Our results are both similar with and different from the ones published by Gorham (1956) and Gorham and Cragg (1960) in England, and by Tolpa and Gorham (1961) in Poland (Table 4). In all the cases the pH values are low, representing a strong acid medium. Close values have been found in K ions concentration, and less close in Na and Ca. In what concerns the cation concentration our data are nearer to those published in Poland. Only the raised peat bog Mohoş has a higher cation

Table 4

A comparison of waters from bogs in Romania, Poland and the British Isles

Location		pH	Na	K	Ca	Mg	Cl	SO_4
			Parts per million					
Izbuc	Romania	4.14	trace	trace	0.80	0.06	—	—
Vlăşchinescu	Romania	4.30	1.63	0.38	2.50	0.86	0.2	0.3
Mohoş	Romania	3.87	8.6	0.25	4.5	3.88	38.2	3.1
Kolnica	Poland	3.8	0.6	0.2	0.7	0.4	0.7	10.1
Wieliszewo	Poland	3.8	2.2	1.0	0.8	0.7	2.8	11.6
Zieleniec	Poland	3.9	0.2	0.2	0.5	0.3	0.3	7.7
British Isles : Max.		4.5	13.9	0.6	1.0	1.8	23.6	11.4
: Min.		3.9	2.2	0.1	0.3	0.3	4.3	2.7

concentration, the data being more similar to those published in England.

Data differing from those in literature are found in the anions content. In our samples the sulphates are extremely low or they are absent. The chlorides are also low, excepting the bog in Mohoş, with values resembling the data published in England.

As a conclusion it may be stated that the bogs having similar floristic composition have generally similar chemical composition of the waters too, whatever their geographical position would be.

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Le 90e ANNIVERSAIRE DU PROFESSEUR ALEXANDRU BORZA

Le 20 mai 1977, dans la salle de conférences de la Bibliothèque centrale universitaire de Cluj-Napoca, se sont déroulés les travaux du symposium rendant hommage au 90e anniversaire de la naissance du professeur Alexandru Borza, illustre savant botaniste, homme de science émérite et organisateur remarquable.

Cette manifestation qui a réuni nombre de botanistes et de naturalistes de Cluj-Napoca et d'autres centres du pays a remporté beaucoup de succès. Il y a lieu à mentionner l'excellente organisation due à la Chaire de Biologie végétale, au Jardin botanique de la Faculté de Biologie-Géographie de l'Université « Babeş-Bolyai » et au Comité départemental de la culture et de l'éducation socialiste de Cluj-Napoca.

Les travaux du symposium se sont déployés sous la présidence de l'acad. Şt. Péterfi, l'acad. Şt. Pascu, l'acad. V. Preda, des professeurs I. Pop, I. Hodişan, O. Raţiu.

Fig. 1. — Inauguration du monument élevé à la mémoire du professeur Alexandru Borza, dans le Jardin botanique de Cluj-Napoca (le 9 juillet 1977).

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Les 11 rapports (élaborés par 21 auteurs) ont présenté une grande partie de la vaste œuvre botanique du professeur Al. Borza en tant que fondateur d'institutions, auteur de nombreuses monographies, maître accompli, vulgarisateur, protecteur de la nature et créateur d'école botanique.

Dans son discours introductif, ainsi que dans la séance de clôture l'acad. Şt. Péterfi a relevé la personnalité de ce botaniste distingué. Ont été soulignés les principaux moments de la vie, de la formation et de l'œuvre du savant Al. Borza (I. Hodişan, O. Raţiu), son activité de mémorialiste (M. Zaciu), de créateur et d'organisateur de l'Institut, du Jardin et du Musée botanique (O. Raţiu, F. Micle, I. Gergely), d'éminent professeur et pédagogue (A. Crişan, M. Ciurchea, M. Bechet). On a relevé sa contribution au développement de la cryptogamie (Tr. I. Ştefureac), au progrès de la phytotaxonomie (E. Ghişa), son activité comme fondateur de l'école roumaine de géobotanique (Şt. Csűrös, I. Pop, V. Cristea), d'ethnobotaniste (V. Codoreanu, Şt. Şuteu, L. Turcu) de promoteur de la nature (N. Boşcaiu, Al. Filipaşcu, F. Täuber), de même que ses rapports avec les scientifiques roumains et étrangers (I. Resmeriţă).

A cette occasion Tr. I. Ştefureac a exprimé les sentiments d'admiration et de respect de tous les botanistes du pays pour l'activité prodigieuse du professeur Al. Borza dans le domaine de la botanique en Roumanie.

La figure du professeur Al. Borza, taillée dans le calcaire éocène de la Valea Baciului (près de Cluj) par le sculpteur Fulicea (Bucarest), domine avec prestance l'entrée du Jardin botanique, témoignant d'une étape de gloire de la botanique roumaine. L'inauguration solennelle du monument (1977) (fig. 1) symbolise, avec des aspirations élevées, la continuation de son œuvre, dans l'époque contemporaine, vers de nouveaux sommets.

Le professeur Al. Borza a connu de près le trésor de la flore de la Roumanie, de sorte que, de la multitude des observations sur les plantes, il a élaboré avec maîtrise de nouveaux principes, capables de fructifier, avec une large générosité, dans l'œuvre de ses élèves et ses collaborateurs.

L'exemple prestigieux du professeur Al. Borza animé par un sentiment élevé du devoir, par une passion et une honnêteté scientifique remarquables, serve admirablement à l'unité, à l'évolution, au prestige et au développement continu des sciences botaniques roumaines.

Le professeur Al. Borza restera pour nous tous, comme pour les nouvelles générations de botanistes qui vont venir une figure féconde et prestigieuse, toujours vivante par son œuvre vaste, d'une réelle valeur scientifique, mise avec dévouement au service de la science et du bien.

Traian I. Ştefureac

AVIS AUX AUTEURS

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

Les tableaux et l'explication des figures seront dactylographiés sur pages séparées et les diagrammes exécutés à l'encre de Chine noire, sur papier calque.

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

Les références bibliographiques, citées par ordre alphabétique des auteurs, comporteront le nom de l'auteur, l'initiale du prénom, le titre de la revue, abrégé conformément aux usances internationales, l'année, le tome, le numéro, la page. Les travaux seront accompagnés d'un court résumé, de maximum 10 lignes. Les textes des travaux ne doivent pas dépasser 7 pages dactylographiées (y compris les tableaux, la bibliographie et l'explication des figures).

La responsabilité concernant le contenu des articles revient exclusivement aux auteurs.

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