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# REVUE ROUMAINE DE BIOLOGIE

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# DIE TORFMOORE „LA LACURI" VON BISOCA (KREIS BUZĂU)

VON

G. DIHORU, R. WALLFISCH, C. PÎRVU

The flora *(Bryophyta* and *Tracheophyta)* of three peat-bogs in the Bend-Subcarpathians (Bisoca, Buzău county) placed at an altitude of 900 m is presented. *Brachydontium trichodes* (Web.) Milde was also found among the bryophytes collected there. It is a species mentioned only a few times in our speciality literature.

Hinweise auf die Torfmoore von Bisoca, Kreis Buzău, finden wir bei S. Paşcovschi (1935), T. Mărăşescu (1939), C. C. Georgescu (1940), E. Pop (1950), E. Pop, I. Ciobanu (1957) und E. Pop (1960). Während in den ersten drei Arbeiten nur ganz allgemeine Daten, fast belanglos vom botanischen Standpunkt, über diese Moore enthalten sind, werden in den letzten drei jedoch genaue botanische Angaben gemacht.

Die Anwesenheit seltener Moorpflanzen (E. Pop, 1960) veranlasste uns die Moore in der Sicht des Naturschutzes zu untersuchen. Aus unserer Durchmusterung der Flora *(Bryophyta* und *Tracheophyta)* ergab sich die Ausweisung einiger bisher nicht bekannter Arten, insbesondere *Bryophyta* (s. Tabellen I und II), was schon von E. Pop angeregt worden war.

*Physisch-Geographische Gegebenheiten.* Im West-Nordwestteil des Bisocawaldes befindet sich ein von den Ortsansässigen „La Lacuri" genanntes Plateau („La Brigada-Hügel"). Auf einer Wiese steht hier ein Forsthaus mit einem Acker und einigen Obstbäumen. Nicht weit von hier liegen innerhalb eines herrlichen Kiefernwaldes *(Pinus sylvestris)* von ca. 20 ha Fläche, zwischen 850 und 987 m Höhe, vier kleine Becken, jedes den Namen eines Sees tragend.

Eines der Becken, der „*Lacul Limpede*", hat eine Ausdehnung von 2800 m², eine grösste Tiefe von 2,4 m und enthält schwach mineralisiertes Wasser (82 mg/l); der Wasserspiegel ist frei von Vegetation. Die anderen drei Becken sind ganz oder grossenteils verlandet. Der erste dieser Seen, der „*Lacul cu Mușchi*", hat eine ovale Form von ungefähr 1,5 ha Oberfläche und ist vollständig von Moosen, Kraut- und Gehölzpflanzen bedeckt *(Pinus sylvestris*, *Betula pendula*, *Alnus glutinosa* usw.). Die Torfschicht soll eine Mächtigkeit von 4,5 m haben und einer Gesamtmenge von 45.000 m³ entsprechen. Der zweite See, „Lacul Negru" genannt, ist nierenförmig im Umriss, bedeckt eine Fläche von annähernd 1 ha und weist im nordwestlichen Teil eine offene Wasserfläche auf. Das Gehölz ist ansehnlich und besteht vorrangig aus *Salix cinerea*. In der Mitte beträgt die Tiefe 7,20 m. Die Torfmenge wird auf 22.000 m³ geschätzt. Der dritte Moor-See, der „*Lacul Sec*", ist höher gelegen als die ersten beiden und ist fast frei von Holzpflanzen. Seine Form ist kreisrund, die Fläche beträgt 4000 m², die Trofmenge entspricht ungefähr 5000 m³.

Die Gesamttorfmenge in den drei Mooren wurde auf 72.000 m³ geschätzt. Die Ablagerung begann in der Kiefern-Zeit, aber der grösste Teil wurde in der Buchen-Zeit (Subatlantik) abgelagert [10].

Die Seen entstanden infolge karstischer Vorgänge im Kliwa-Sandstein, welcher den „La Brigada"-Hügel aufbaut [2].

Abb. 1. — *Pinus sylvestris*—Gehölz.

*Die Flora der Torfmoore.* Die Moosflora mit 39 Arten und einer Varietät (Tabelle I) ist nicht besonders interessant. Es soll aber die verhältnismässig geringe Meereshöhe dieser Hochmoore hervorgehoben werden, worin die Torfmoose eine hervorragende Rolle spielen *(Sphagnum fallax* und *S. flexuosum* am Rande der offenen Wasserflächen, *S. magellanicum* und *S. palustre* an den übrigen Stellen). An der Oberfläche der Torfschicht treten noch verschiedene *Polytrichum*-Arten auf *(P. commune, P. alpestre* und sogar *P. longisetum)*. Die übrigen Moose wurden von Steinen und Baumstümpfen, vor allem gegen die Ränder der Moore zu, gesammelt (Tabelle I).

*Tabelle I*

BRYOPHYTA

| Art | Moor: M | N | S |
|---|---|---|---|
| + *Amblystegium serpens* (Hedw.) B.S.G. | C | C | |
| – *Blepharostoma trichophyllum* (L.) Dum. | | C | |
| *Brachythecium rutabulum* (Hedw.) B.S.G. | | | |
| var. *rutabululum* | C | +C | |
| var. *densum* B.S.G. | | C | |



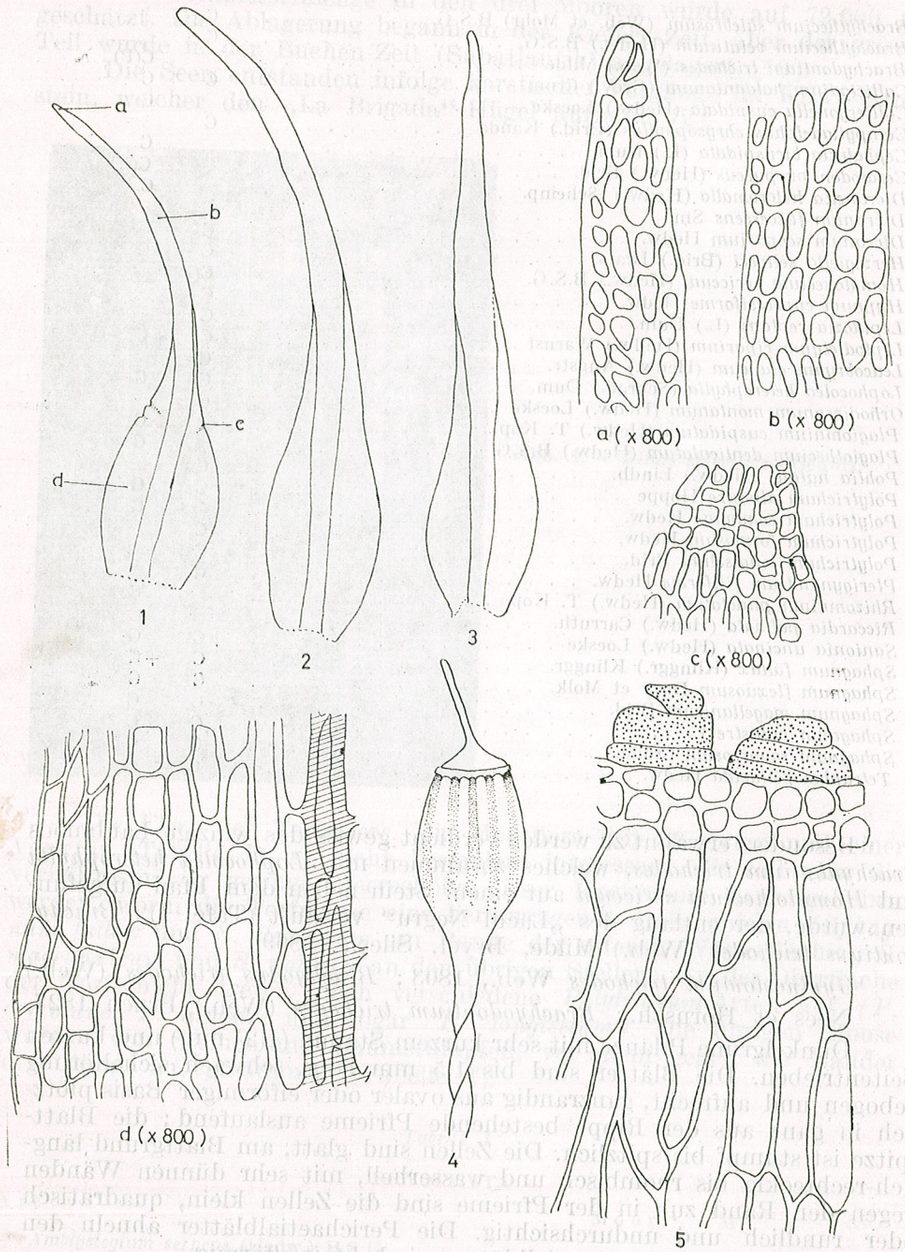
| | | | |
|---|---|---|---|
| + *Brachythecium salebrosum* (Web. et Mohr) B.S.G. | C | | |
| + *Brachythecium velutinum* (Hedw.) B.S.G. | C | | |
| + *Brachydontium trichodes* (Web.) Milde | | C(P) | |
| + *Calliciadium haldanianum* (Grev.) Crum. | C | C | |
| *Calliergonella cuspidata* (Hedw.) Loeske | | C | |
| + *Campyliadelphus chrysophyllus* (Brid.) Kanda | C | | |
| – *Cephalozia bicuspidata* (L.) Dum. | | C | |
| *Ceratodon purpureus* (Hedw.) Brid. | | C(P) | |
| + *Dicranella heteromalla* (Hedw.) Scheinp. | | C | |
| + *Dicranum fuscescens* Sm. | C | | |
| + *Dicranum scoparium* Hedw. | C | | |
| + *Herzogiella seligeri* (Brid.) Iwats. | C | C | |
| *Homalohecium sericeum* (Hedw.) B.S.G. | C(P) | | |
| *Hypnum cupressiforme* Hedw. | C | | |
| – *Lepidozia reptans* (L.) Dum. | C | | |
| *Leptodictyum riparium* (Hedw.) Warnst | +C | +C | C |
| *Leucobryum glaucum* (Hedw.) Ångstr. | C | | |
| +– *Lophocolea heterophylla* (Schrad.) Dum. | C | C | |
| *Orhodicranum montanum* (Hedw.) Loeske | C | | |
| + *Plagiomnium cuspidatum* (Hedw.) T. Kop. | | C | |
| + *Plagiothecium denticulatum* (Hedw.) B.S.G. | | C | |
| + *Pohlia nutans* (Hedw.) Lindb. | C | | |
| + *Polytrichum alpestre* Hoppe | C | C | |
| *Polytrichum commune* Hedw. | | C | |
| + *Polytrichum formosum* Hedw. | C | | |
| + *Polytrichum longisetum* Brid. | C | | |
| *Pterigynandrum filiforme* Hedw. | C | | |
| *Rhizomnium punctatum* (Hedw.) T. Kop. | | C | |
| – *Riccardia palmata* (Hedw.) Carruth. | | C | |
| *Sanionia uncinata* (Hedw.) Loeske | +C | C | |
| *Sphagnum fallax* (Klinggr.) Klinggr. | C | +C | |
| *Sphagnum flexuosum* Dozy et Molk. | C | C | |
| *Sphagnum magellanicum* Brid. | C | C | |
| *Sphagnum palustre* L. | C | C | |
| *Sphagnum squarrosum* Crome | +C | C | |
| *Tetraphis pellucida* Hedw. | C | | |

Besonders erwähnt zu werden verdient gewiss das winzige Laubmoos *Brachydontium trichodes*, welches zusammen mit *Lophocolea heterophylla* und *Homalothecium sericeum* auf einem Stein neben dem Pfad aufgefunden wurde, der entlang des „Lacul Negru" verläuft (Tafel I): *Brachydontium trichodes* (Web.) Milde, Bryol. Siles., 1869.

*Gymnostomum trichodes* Web., 1803; *Brachyodus trichodes* (Web.) Nees et Hornsch.; *Brachyodontium trichodes* (Web.) Bruch 1827.

Dunkelgrüne Pflanze mit sehr kurzem Stamm (1(2) mm) und kurzen Seitentrieben. Die Blätter sind bis 1,5 mm lang, schwach sichelförmig gebogen und aufrecht, ganzrandig aus ovaler oder eiförmiger Basis plötzlich in ganz aus der Rippe bestehende Pfrieme auslaufend; die Blattspitze ist stumpf bis spitzlich. Die Zellen sind glatt, am Blattgrund länglich-rechteckig bis rhombisch und wasserhell, mit sehr dünnen Wänden gegen den Rand zu; in der Pfrieme sind die Zellen klein, quadratisch oder rundlich und undurchsichtig. Die Perichaetialblätter ähneln den Stammblättern; die Perigonialblätter sind breit-eiförmig.

Der Kapselstiel ist aufrecht, feucht verbogen, trocken am Grund nach rechts verdreht, gegen das Ende zu nach links; er hat eine Länge von 2—3 mm und ist verhältnismässig dick und gelblich. Die Kapsel




Tafel I Morphologie des Mooses *Brachydontium trichodes*
1 — Unteres Blatt (× 210); 2 — Mittleres Blatt (× 210); 3 — Perichaetialblatt (× 210); 4 — Kapsel (× 75); 5 — Teil der Kapselwand mit Peristomzähnen (× 800).



ist aufrecht, eiförmig bis länglich, mit dünnen Wänden von hellbrauner Färbung; die Kapsel ist gestreift und bei der Reife gefurcht. Die Zellen des Exotheziums sind länglich und besitzen in den Rippen verdickte Längswände. Die Peristomzähne, 16 an der Zahl, sind kurz — den Anulus nicht überschreitend, breit, stumpflich oder gestutzt, bleich und warzig, und sind manchmal durchbrochen oder unregelmässig gespalten. Der Anulus weist 2(3) Zellreihen auf und ist abfallend. Der Deckel besitzt einen langen und geraden pfriemenförmigen Schnabel. Die Haube ist glatt, hochkegelförmig, mützenförmig und am Rand 5-lappig. Die Sporen sind gelblich und glatt bei beiläufig 10 (12) μ Durchmesser, und reifen im Sommer und Anfang Herbst; n = 11. Autözisch.

*Bemerkung.* Die wichtigsten Merkmale zur Unterscheidung von *Seligeria* (aus derselben Familie der *Seligeriaceae)* sind die gestreifte Kapsel, die mützenförmige und gelappte Haube, die äusserst kurzen, gestutzten und warzigen Peristomzähne.

Die Chorologie des Mooses in Rumänien ist nur spärlich bekannt, wozu wahrscheinlich auch seine Winzigkeit beiträgt. Gewöhnlich wird es ganz zufällig, zusammen mit grösseren Moosen eingesammelt und wird erst im Laboratorium bei der Analyse erkannt. Soweit uns bekannt ist, wurde das Moos in der älteren Literatur aus dem Făgăraș-Gebirge [3], [13] und aus dem Retezat-Geb. (Judele-Tal [3], [13], [8] * gemeldet und neuerdings aus dem Rodna-Geb. [15].

Auf dem Gebiete der Moore wurden 96 Tracheophyten-Arten (Tabelle II) festgestellt; davon sind bemerkenswert und kennzeichnend die folgenden: *Molinia caerulea, Eriophorum vaginatum, E. gracile, Drosera rotundifolia, Peucedanum palustre, Menyanthes trifoliata, Calamagrostis canescens, Sparganium minimum, Carex elongata, Epilobium palustre, Ponentilla erecta.*

Viele der früher nur mit dem Gattungsnamen angeführten Pflanzen sind genau bestimmt worden, andere hingegen von grosser Bedeutung für das Gebiet, konnten nicht wieder aufgefunden werden, wie z.B.: *Betula pubescens, B. carpatica, Alnus viridis.* Wir haben uns bemüht die Moor-Birke aufzufinden; das ganze gesammelte Material weist aber Harzdrüsen auf und gehört also zu *Betula pendula,* mit veränderlichen Blättern, mit keil- bis herzförmigem Grund:

f. *pendula* — Blattgrund kurz keilförmig bis abgerundet, doppelt gesägt;

f. *cuneata* Schneid. — Blätter rhombisch, auffällig keilförmig am Grund, lang zugespitzt; Hauptzähne sichelförmig gebogen;

f. *subcordata* Lindq. — kurze und breit-eiförmige Blätter, am Grund schwach herzförmig;

*f. ovata* Nyárády — Blätter eiförmig, fein und fast gleichmässig gesägt.

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* Die chorologischen Daten aus der älteren Literatur wurden uns liebenswürdigerweise von Prof. Dr. Doz. Tr. I. Ștefureac mitgeteilt, wofür wir Ihm auch an dieser Stelle unseren Dank bekunden.

*Tabelle II*

TRACHEOPHYTA

| *Art* / *Moor:* | M | N | S |
|---|---|---|---|
| *Abies alba* Miller | A | | |
| *Acer pseudoplatanus* L. | C | | |
| *Agrostis capillaria* L. | | C | |
| *Agrostis gigantea* Roth | | C | |
| *Agrostis stolonifera* L. | B | B | A |
| *Alisma plantago-aquatica* L. | | C | |
| *Alnus glutinosa* (L.) Gaertn. | A | A | |
| *Alnus viridis* DC | D | | |
| *Alopecurus aequalis* Sobol. | | C | |
| *Athyrium filix-femina* (L.) Roth | A | B | |
| *Betula hybrida* Bechst. (pendula × pubescens) | D | D | |
| *Betula pendula* Roth | A | B | B |
| *Betula pubescens* Ehrh. | D | | |
| *Bidens tripartita* L. | C | C | |
| *Calamagrostis arundinacea* (L.) Roth | D | | |
| *Calamagrostis canescens* (Weber) Roth | C | C | C |
| *Calamagrostis epigeios* (L.) Roth | C | | |
| *Callitriche cophocarpa* Sendtner | | C | |
| *Campanula patula* L. subsp. *abietina* (Griseb.) Simonkai | | | C |
| *Carex curta* Good. | B | A | A |
| *Carex elongata* L. | C | C | |
| *Carex ovalis* Good. | | C | |
| *Carex pseudocyperus* L. | | A | |
| *Carex rostrata* Stokes | A | | |
| *Carex vesicaria* L. | B | B | A |
| *Cerastium fontanum* Baumg. subsp. *triviale* (Link) Jalas | C | | |
| *Deschampsia caespitosa* (L.) Beauv. | C | C | |
| *Drosera rotundifolia* L. | A | A | |
| *Dryopteris lanceolato-cristata* (Hoffm.) Alston | A | | |
| *Epilobium angustifolium* L. | A | | |
| *Epilobium montanum* L. | C | C | |
| *Epilobium obscurum* (Schreb.) Roth | | C | |
| *Epilobium palustre* L. | C | C | C |
| *Eriophorum angustifolium* Roth. | C | C | |
| *Eriophorum gracile* Koch ex Roth | | | A |
| *Eriophorum vaginatum* L. | A | A | A |
| *Fagus sylvatica* L. | A | | |
| *Galeopsis bifida* Boenn. | C | C | C |
| *Galeopsis tetrahit* L. | D | | |
| *Galium aparine* L. | C | | |
| *Galium mollugo* L. | | C | |
| *Galium palustre* L. | | C | |
| *Geranium robertianum* L. | C | | |
| *Glyceria fluitans* (L.) R.Br. | | | C |
| *Hieracium rotundatum* Kit. ex Schultes | C | | |
| *Hypericum maculatum* Crantz | | C | |
| *Impatiens noli-tangere* L. | | C | |
| *Juncus effusus* L. | B | A | A |
| *Lemna minor* L. | | C | |
| *Leontodon autumnalis* L. | | C | |
| *Luzula luzuloides* (Lam.) Dandy et Wilm. | C | | |
| *Lycopus europaeus* L. | | C | |
| *Lysimachia nummularia* L. | | C | C |
| *Lysimachia punctata* L. | | C | |
| *Lysimachia vulgaris* L. | A | A | A |
| *Lythrum salicaria* L. | | C | |
| *Majanthemum bifolium* (L.) F. W. Schmidt | C | | |
| *Menyanthes trifoliata* L. | | A | |



| | | | |
|---|---|---|---|
| *Molinia caerulea* (L.) Moench. | A | A | |
| *Mycelis muralis* (L.) Reichenb. | | | C |
| *Myosotis scorpioides* L. | C | C | C |
| *Oenanthe aquatica* (L.) Poir. | C | | |
| *Oxalis acetosella* L. | C | | |
| *Persicaria hydropiper* (L.) Opiz | C | C | |
| *Peucedanum palustre* L. | A | A | |
| *Picea abies* (L.) Karsten | A | A | |
| *Pinus sylvestris* L. (Abb. 1) | A | A | A |
| *Poa palustris* L. | C | C | |
| *Populus tremula* L. | A | A | |
| *Potamogeton natans* L. | | A | |
| *Potentilla erecta* (L.) Räuschel | C | | |
| *Ranunculus repens* L. | C | C | |
| *Rosa canina* L. | C | | |
| *Rubus canescens* DC | | C | |
| *Rubus hirtus* Waldst. et Kit. | C | | |
| *Rubus idaeus* L. | C | | |
| *Rumex obtusifolius* L. | C | C | |
| *Salix caprea* L. | C | C | |
| *Salix cinerea* L. | B | A | |
| *Scirpus sylvaticus* L. | C | C | |
| *Sorbus aucuparia* L. | C | C | |
| *Sparganium erectum* L. | C | | C |
| *Sparganium minimum* Wallr. (Abb. 2) | | C | |
| *Spirodela polyrhiza* (L.) Schleiden | | C | |
| *Stellaria graminea* L. | | C | |
| *Stellaria media* (L.) Cyr. | | C | |
| ? *Typha latifolia* L. | | C | |
| *Urtica dioica* L. | A | B | B |
| *Utricularia vulgaris* L. | | A | |
| *Vaccinium myrtillus* L. | A | B | |
| *Vaccinium vitis-idaea* L. | A | | |
| *Veronica scutellata* L. | | C | |
| *Vicia sepium* L. var. *montana* Koch | | | D |
| *Viola tricolor* L. | | C | |

*Die Vegetation.* Am Aufbau der Vegetation der drei Moore sind folgende Assoziationen (oder Assoziations-Fragmente) beteiligt: *Carici (canescenti) — Sphagnetum flexuosae* Dihoru (63) 75, *Carici-Menyanthetum* Soó (38) 55, *Caricetum vesicariae* Br.-Bl. et Denis 26, *Eriophoro vaginato-Sphagnetum recurvi-magellanici* (Weber 1902) Soó(27) 54, *Salici cinereae-Sphagnetum recurvi* (Zólyomi 31) Soó 54, *Carici elongatae-Alnetum* Koch 26, *Calamagrostetum canescentis* Simon, *Sparganietum minimae* (Abb. 2)

*Bedeutung für den Naturschutz.* Die drei Moore sind zwar auf der Buchenstufe gelegen, befinden sich aber inmitten eines stattlichen *Pinus sylvestris*-Gehölzes, und stellen ein sozologisches Objekt erster Grösse dar.

— Die Pufferzone, bestehend aus dem hochragenden *Pinus sylvestris*-Wald kann vollständig oder zumindest teilweise erhalten werden.

Die drei Moore enthalten eine für Hochmoore charakteristische Flora und Vegetation, einschliesslich einiger Seltenheiten der Flora Rumäniens und zwar auf geringer Höhe ü.d.M.

— Die Moore können für das Publikum des Gebiets (einschl. der Schuljugend) eine für Bildung und Unterricht wichtige Rolle spielen, sowohl dank der floristischen Seltenheiten und der Hochmoorvegetation,

als auch im Zusammenhang mit dem Ursprung der Moore und der Ablagerung des Torfes.

Die Bestimmung zum Naturschutzgebiet erfordert keinerlei Anstrengung zur Erhaltung (Tiere und Menschen betreten das Gebiet selten oder gar nicht); ausserdem wird der Wirtschaft kein nutzbares Gelände entzogen.

Abb. 2. — *Sparganium minimum* im „Lacul Negru"-Moor.

ABKÜRZUNGEN

A — in der Literatur angeführt und wieder aufgefunden
B — wie A, aber auch in anderen Mooren vorkommend
C — von uns festgestellt
D — im Schrifttum angeführt, aber nicht wieder aufgefunden
M — See „Lacul cu Muşchi"
N — See „Lacul Negru"
P — Waldrand
S — See „Lacul Sec"
\+ — Exemplar mit Sporogonen

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# RECHERCHES SUR LA MORPHOLOGIE DU POLLEN ET LA TAXONOMIE DE CERTAINS REPRÉSENTANTS DE LA FAMILLE *CARYOPHYLLACEAE*

PAR

GABRIELA ȘERBĂNESCU-JITARIU

In this paper there are studied 52 units belonging to 10 species from Romanian flora.
There are examined the exterior aspect of the microspores, the structure, the thickness of the sporoderm in optical section, the dimensions, the shape.
The pollen morphology of *Sperguleae* from the subfamily *Paronychioideae* determines us to plead for including *Sperguleae* in another subfamily.

## INTRODUCTION

La famille *Caryophyllaceae* occupe dans les divers systèmes phylogénétiques des *Angiospermae* une position centrale dans les limites de l'ordre auquel elle appartient *(Centrospermae, Caryophyllales, Chenopodiales)* et constitue l'une des directions d'évolution dans le cadre des *Dicotyledoneae*.

Par suite de ce fait, les recherches effectuées sur les représentants étudiés ont compris presque la majorité des caractères de premier ordre dans la description et la délimitation des taxons respectifs. Dernièrement, pour la clarification de la position taxonomique et des liens phylétiques dans les limites de cette famille, les caractères morphologiques du pollen ont été également pris en considération.

En ce sens cet ouvrage constitue une contribution à la connaissance du pollen des *Caryophyllaceae* par l'étude morphopollinique effectuée sur 52 unités systématiques appartenant à 10 genres de la flore roumaine.

## MATÉRIEL ET TERMINOLOGIE

On a utilisé surtout du matériel d'herbier et, en partie, du matériel frais. Le matériel d'herbier étudié dans cet ouvrage (d'après les méthodes mentionnées dans les ouvrages antérieurs) provient de l'herbier de l'Institut botanique de Bucarest, l'herbier du Jardin d'essai de Bucarest, l'herbier de l'Institut de biologie de Bucarest ainsi que de l'herbier de l'Université de Cluj-Napoca.

Le matériel a été analysé dans l'eau et dans de l'hydrate de chloral.

La terminologie utilisée est celle proposée par Erdtman [4], [7] et par Straka [11], qui est très répandue.

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L'ordre de la présentation des genres, ainsi que la systématisation du matériel par unités supérieures aux genres sont ceux proposés par Engler [3], tandis qu'à l'intérieur des genres la systématisation est celle de la « Flore de la R.P.R. » [8].

## LA MORPHOLOGIE DES GRAINS DE POLLEN

Le pollen est, généralement, sphéroïdal—sous-sphéroïdal, de taille moyenne jusqu'à grande, polyporé (panthoporé) et operculé, rarement 3 — 4 colpé ou 6—8 rugate. Le pore est d'habitude pourvu d'annulus, et l'opercule ne dépasse pas généralement la surface du tectum. Dans l'eau le pollen est brun-orange-jaune, tandis que dans de l'hydrate de chloral il est jaune, jaune pâle jusqu'à incolore.

L'exine crassisexinée, d'épaisseur moyenne, seulement dans certains cas ténuiexinée ; en coupe optique, elle est tectée-baculée, avec des éléments supratectaux plus évidents chez le pollen sec ou demi-sec, et, dans certains cas, pilée-sympilée.

### SOUS-FAMILLE PARONYCHIOIDEAE

Pollen polyporé et operculé (tr. *Paronychieae*) ; 3—4 colpé, 6—8 rugate (Tr. *Sperguleae*). Pores avec annulus, l'opercule orné de verrues évidentes chez *Paronychia* ; en coupe optique, l'exine est pilée-sympilée.

Fig. 1. — *Paronychia cephalotes* M.B. ; aspect extérieur et coupe optique (1450 × orig.).

Fig. 2. — *Spergula morisonii* Boreau ; *a*, aspect extérieur et le sporoderme en coupe optique ; *b*, coupe optique agrandie (*a*, 1250 × ; *b*, 2525 ×, orig.).

Fig. 3. — *Minuartia sedoides* (L. Hiern ; *a*, aspect extérieur et le sporoderme en coupe optique ; *b*, pore et aspect extérieur du sporoderme ; *c*, coupe optique (*a*, 1250 × ; *b*, *c*, 2525 ×, orig.).

Fig. 4. — *Minuartia laricifolia* (L.) Schinz et Thell. ; *a*, pore et aspect extérieur du sporoderme ; *b*, coupe optique (2525 ×, orig.).

Fig. 5. — *Minuartia verna* (L.) Hiern ; pore et aspect extérieur du sporoderme (2525 ×, orig.).

Fig. 6. — *Minuartia viscosa* (Schreb.) Schinz et Thell. ; aspect extérieur et le sporoderme en coupe optique (1250 ×, orig.).

Fig. 7. — *Stellaria aquatica* Scop. ; *a*, aspect extérieur et le sporoderme en coupe optique ; *b*, le sporoderme en coupe optique autour du pore (*a*, 875 ×, *b*, 2525 ×, orig.).

Fig. 8. — *Stellaria nemorum* L. ; *a*, aspect extérieur et le sporoderme en coupe optique ; *b*, coupe optique (*a*, 1250 ×, *b*, 2525 ×, orig.).

Fig. 9. — *Stellaria holostea* L. ; pore (1280 ×, orig.).

Fig. 10. — *Stellaria longifolia* Mühlenb. ; pore et l'ornementation de l'exine (2525 ×, orig.).

Fig. 11. — *Stellaria palustris* Ehrh. ; aspect extérieur et le sporoderme en coupe optique (1280 ×, orig.).

Fig. 12. — *Cerastium cerastioides* L. ; *a*, aspect extérieur et le sporoderme en coupe optique ; *b*, pore et l'ornementation de l'exine autour de lui ; *c*, coupe optique (*a*, 1450 × ; *b*, *c*, 2125 ×, orig.).



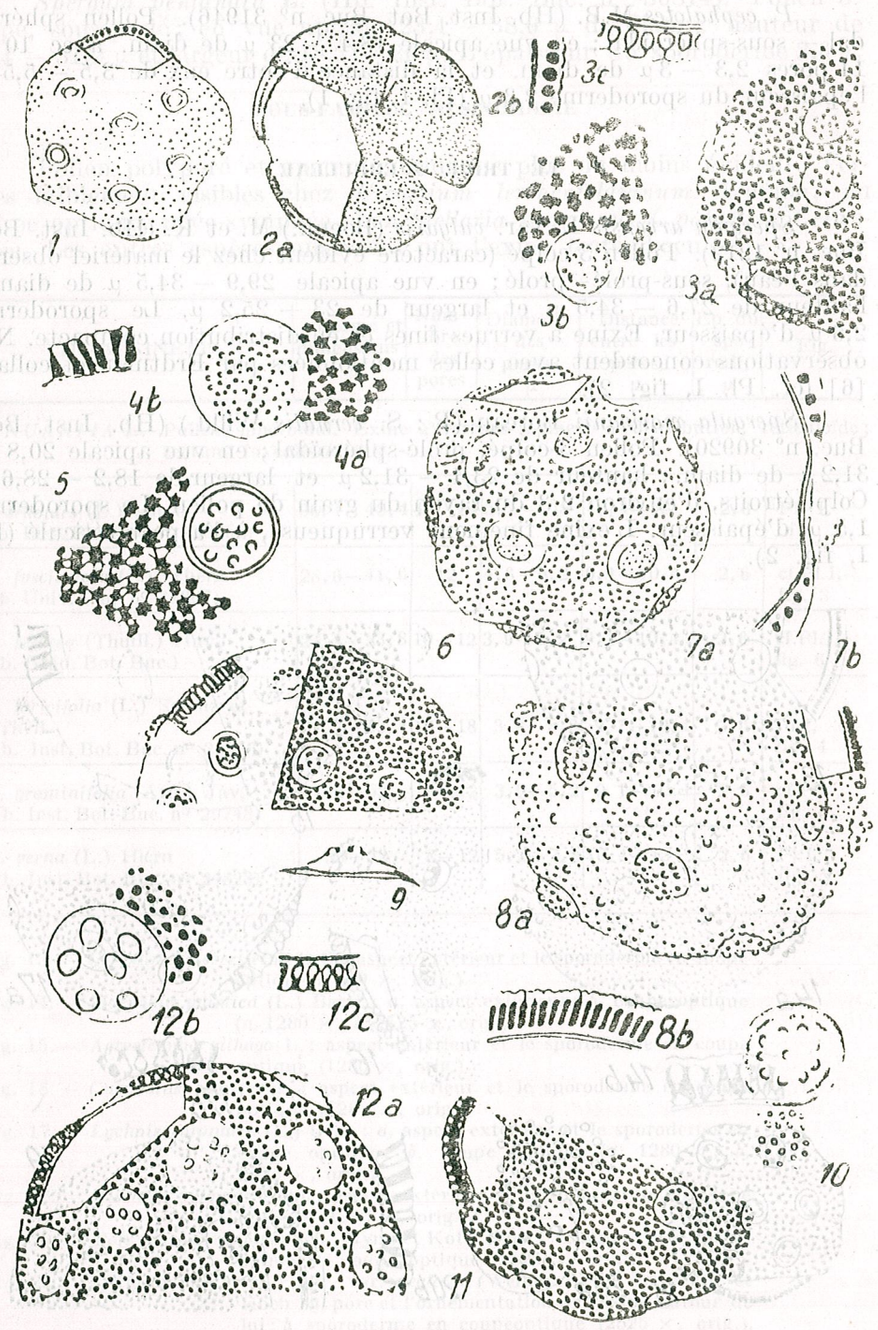


Planche I

*P. cephalotes* M.B. (Hb. Inst. Bot. Buc. nº 31946). Pollen sphéroïdal — sous-sphéroïdal; en vue apicale 18,4 — 23 μ de diam. avec 10 — 14 pores 2,3 — 3 μ de diam. et la distance d'entre eux de 3,5 — 5,5 μ, l'épaisseur du sporoderme 2,3 μ (Pl. I, fig. 1).

### LE TRIBE SPERGULEAE

*Spergula arvensis* L. var. *vulgaris* (Boenn.) M. et K. (Hb. Inst. Bot. Buc. nº 1212). Pollen 3-colpé (caractère évident chez le matériel observé dans l'eau), sous-prolé—prolé; en vue apicale 29,9 — 34,5 μ de diam.; hauteur de 27,6 — 34,5 μ et largeur de 23 — 25,2 μ. Le sporoderme 2,6 μ d'épaisseur. Exine à verrues fines et en distribution compacte. Nos observations concordent avec celles mentionnées par Erdtman et collab. [6] (cf. Pl. I, fig. 2).

*Spergula morisonii* Boreau (R : S. *vernalis* Willd.) (Hb. Inst. Bot. Buc. nº 30920). Pollen 3-colpé, prolé-sphéroïdal; en vue apicale 20,8 — 31,2 μ de diam.; hauteur de 23,4 — 31,2 μ et largeur de 18,2 — 28,6 μ. Colpi étroits, d'environ 2/3 du rayon du grain de pollen. Le sporoderme 1,5 μ d'épaisseur. L'exine finement verruqueuse, à l'aspect réticulé (Pl. I, fig. 2).

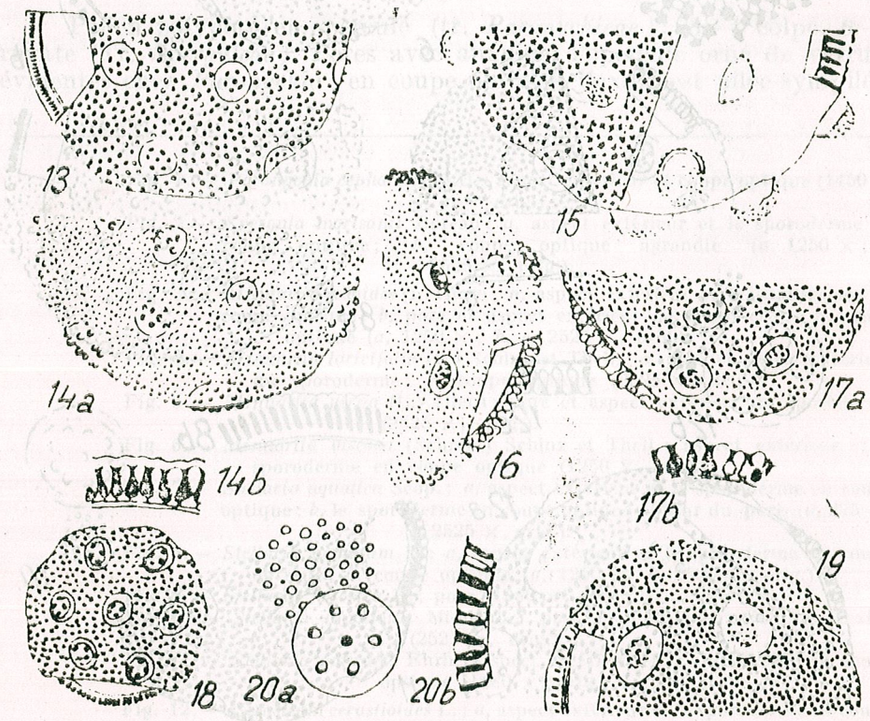


Planche II



*Spergula pentandra* L. (Hb. Inst. Bot. Buc. nº 30874). Pollen 3-colpé, sous-prolé; en vue apicale 23,4 — 38,6 μ de diam.; hauteur de 23,4 — 31,2 μ et largeur de 18,2 — 26 μ. L'épaisseur du sporoderme 2,6 μ.

### SOUS-FAMILLE ALSINOIDEAE

Pollen polyporé et operculé. Annulus plus ou moins évident. Verrues faiblement visibles chez *Cerastium lerchenfeldianum*. L'exine, en coupe optique, pilée-sympilée chez *Stellaria nemorum*, *S. palustris*, *Cerastium*. Les autres espèces analysées ont l'exine tectée-baculée.

| TR. ALSINEAE | Forme et dimensions μ | Nombre des pores | Diamètre des pores, μ | Distance entre les pores, μ | Ep. du sporod., μ | Fig. |
|---|---|---|---|---|---|---|
| *MINUARTIA* L. Pollen sphéroïdal. Exine à verrues (épines) à distribution réticuloïde; tectum fovéolé-réticulé | | | | | | |
| M. *sedoides* (L.) Hiern (Hb. Inst. Bot.Buc nº 28451) | 36,4—49,4 | 10—24 | 5,2—7,8 | 10,4—13 | 2,6 | Pl. I, fig. 3 |
| *M. fasciculata* (L.) Hiern (Hb. Univ. Cluj nº 11807) | 28,6—41,6 | 12 | 4,6—5,2 | 9,1—10,4 | 2,6 | cf.Pl.I, fig. 3 |
| *M. setacea* (Thuill.) Hay, (Hb. Grăd. Bot. Buc.) | 23,4—33,8 | 10—12 | 3,9—5,2 | 9,1—10,4 | 2,6 | cf.Pl.I, fig. 6 |
| *M. laricifolia* (L.) Schinz et Thell (Hb. Inst. Bot. Buc. nº 29266) | 31,2—49,4 | 16—18 | 3,9—5,2 | 9,1—10,4 | 2,6 | Pl.I, fig. 4 |
| *M. graminifolia* (Ard.) Jáv. (Hb. Inst, Bot. Buc. nº 29748) | 31,2—52 | 8—12 | 3,9—5,2 | 9,1—10,4 | 2,6 | cf.Pl.I, fig. 6 |
| *M. verna* (L.) Hiern (Hb. Inst. Bot. Buc. nº 24829) | 39—52 | 8—12 | 5,2—7,8 | 10,4—13 | 2,6 | Pl.I, fig. 5 |

Fig. 13. — *Cerastium biebersteinii* D.C.; aspect extérieur et le sporoderme en coupe optique (1280 ×, orig.).
Fig. 14. — *Moenchia mantica* (L.) Bartl.; *a*, aspect extérieur; *b*, coupe optique (*a*, 1280 ×; *b*, 2525 ×, orig.).
Fig. 15. — *Agrostemma githago* L.; aspect extérieur et le sporoderme en coupe optique (1280 ×, orig.).
Fig. 16. — *Cucubalus baccifer* L.; aspect extérieur et le sporoderme en coupe optique (1280 ×, orig.).
Fig. 17. — *Lychnis coronaria* (L.) Desr.; *a*, aspect extérieur et le sporoderme en coupe optique; *b*, coupe optique (*a*, 1280 ×; *b*, 2525 ×, orig.).
Fig. 18. — *Viscaria vulgaris* Röhl.; aspect extérieur et le sporoderme en coupe optique (1280 ×, orig.).
Fig. 19. — *Polyschemone nivalis* Sch., Nym. et Kot.; aspect extérieur et le sporoderme en coupe optique (2525 ×, orig.).
Fig. 20. — *Stellaria media* (L.) Cyr. var. *neglecta* (Weihe) Weihe ap. Mert. et Koch: *a*, pore et l'ornementation de l'exine autour de lui; *b*, sporoderme en coupeoptique (2525 ×, orig.).

| | Forme et dimensions μ | Nombre des pores | Diamètre des pores, μ | Dist. entre les pores, μ | Epais. du sporod., μ | Fig. |
|---|---|---|---|---|---|---|
| *M. gerardii* (Willd.) Hay. (Hb. Inst. Bot. Buc. nº 30517) | 23,4—36,4 | 12—18 | 7,8 | 7,8—15,6 | 2,6 | cf.Pl.I, fig. 6 |
| *M. frutescens* (Kit.) Tuzson (Hb. Inst. Bot. Buc. nº 28235) | 28,6—36,4 | 10—12 | 5,2 | 10,4 | 2,6 | cf.Pl.I, fig. 3 |
| *M. cataractarum* Jka. (Hb. Inst. Bot. Buc. nº 28218) | 31,2—36,4 | 12—14 | 5,2 | 10,4 | 2,6 | cf.Pl.I, fig. 3 |
| *M. recurva* (All.) Schinz et Thell. (Hb. Inst. Bot. Buc. nº 29590) | 31,2—36,4 | 12—18 | 5,2—7,8 | 10,4—13 | 2,6 | cf.Pl.I, fig. 3 |
| *M. tenuifolia* (L.) Hiern (Hb. Inst. Bot. Buc. nº 30291) | 23,4—36,4 | 14—16 | 5,2 | 10,4 | 2,6 | cf.Pl.I, fig. 6 |
| *M. viscosa* (Schreb.) Schinz et Thell. (Hb. Univ. Cluj nº 503375) | 31,2—46,8 | 20—30 | 5,2 | 10,4 | 2,6 | Pl.I, fig. 6 |
| *M. glomerata* (M.B.) Deg. (Hb. Inst. Bot. Buc. nº 28770) | 26—44,2 | 10—12 | 5,2 | 10,4 | 2,6 | cf.Pl.I, fig. 6 |
| *M. capillacea* (All.) Graebn. (Portes de fer, leg. et det. N. Roman) | 28,6—41,6 | 12 | 5,2—7,8 | 10,4—13 | 2,6 | cf.Pl.I, fig. 6 |
| *STELLARIA* L. Pollen sphéroïdal. Exine à verrues à distribution réticuloïde. *S. nemorum* a parmi des verrues (épines) irrégulièrement distribuées de grandes verrues claviformes | | | | | | |
| *S. aquatica* Scop. (Hb. Univ. Cluj nº 420189) | 33,8—52 | 12—18 | 4,6 | 2,6—6,6 | 3,9—5,2 | Pl.I, fig. 7 |
| *S. nemorum* L. (Hb. Inst, Bot. Buc. nº 25273) | 39—52 | 12 | 6,5—7,8 | 10—13 | 2,6—3,9 | Pl.I, fig. 8 |
| *S. media* (L.) Cyr. (Hb. Grăd. Bot. Buc.) | 31,2—39 | 18 | 3,9—5,2 | 7,8—10,4 | 2,6 | cf.Pl.I, fig. 8 |
| *S. media* (L.) Cyr. var. *neglecta* (Weihe) Weihe ap. Mert. et Koch (Hb. Inst. Bot. Buc. nº 25171) | 33,8—41,6 | 8—14 | 7,8 | 7,8 | 2,6 | Pl.II, fig. 20 |
| *S. pallida* (Dum.) Piré f. *flaccida* Haek. (Hb. Inst. Bot. Buc. nº 25314) | 31,2—36,4 | 12 | 5,2—6,9 | 0,4—11,7 | 2,6 | cf.Pl.I, fig. 11 |
| *S. holostea* L. (Cult. Grăd. Bot. Buc.) | 28,6—39 | 12 | 3,7—5 | 8,7—10 | 2,6 | Pl.I, fig. 9 |
| *S. longifolia* Mühlenb. (Hb. Inst. Bot. Buc. nº 25039) | 33,8—46,8 | 18 | 5,2—7,8 | 10,4 | 2,6 | Pl.I, fig. 10 |
| *S. graminea* L. (Hb. Inst. Bot. Buc. nº 24819) | 15,6—41,6 | 12 | 5,2 | 10,4 | 2,6 | cf.Pl.I, fig. 11 |



| | Forme et dim. en μ | Nombre des pores | Diamètre des pores μ | Distance entre les pores, μ | Épais. du sporod. μ | Fig. |
|---|---|---|---|---|---|---|
| *S. alsine* Rchb. (Hb. Inst. Bot. Buc. nº 24539) | 28,6—36,4 | 11 | 5,2 | 7,8 | 2,6 | cf.Pl.I, fig. 11 |
| *S. palustris* Ehrh. (Hb. Inst. Bot. Buc. nº 25323) | 33,8—44,2 | 12 | 5,2—7,8 | 10,8 | 2,6—3,9 | Pl.I, fig. 11 |
| *CERASTIUM* L. Pollen sphéroïdal. Exine euréticulée et, chez certaines espèces, aux murs irrégulièrement verruqueux | | | | | | |
| *C. cerastioides* L. (Hb. Univ. Cluj nº 19774) | 36,4—52 | 8—16 | 3,9—7,8 | 3,9—7,8 | 2,6—3,9 | Pl.I, fig. 12 |
| *C. anomalum* W. et K. (F.R.E. nº 400) | 26—41,6 | 12—14 | 5,2—6,5 | 10,4—11,7 | 2,6 | cf.Pl.I, fig. 12 |
| *C. glomeratum* Thuill. (Hb. Univ. Cluj nº 274220) | 33,8—39 | 14—20 | 5,2 | 10,4 | 2,6 | cf.Pl.II, fig. 13 |
| *C. brachypetalum* Desp. (Hb. Univ. Cluj nº 274252) | 33,8—46,8 | 16—18 | 5,2—6,5 | 7,8—13 | 2,6 | cf.Pl.I, fig. 12 |
| *C. semidecandrum* L. (Hb. Inst. Bot. Buc. nº 26845) | 23,4—31,2 | 12 | 3,9—5,2 | 6,5—7,8 | 2,6 | cf.Pl.II, fig. 13 |
| *C. pumilum* Curt. (Hb. Inst. Bot. Buc. nº 26395) | 36,4—44,2 | 18—20 | 5,2 | 10,4 | 2,6 | cf.Pl.II, fig. 13 |
| *C. caespitosum* Gilib. (Cult. Grăd. Bot. Buc.) | 31,2—44,2 | 16—24 | 5,2 | 10,4 | 2,6 | cf.Pl.I, fig. 12 |
| *C. fontanum* Baumg. (Hb. Univ. Cluj nº 561499) | 46,8—54,6 | 26—28 | 5,2 | 10,4 | 2,6 | cf.Pl.II, fig. 13 |
| *C. bulgaricum* Uechtr. (Hb. Univ. Cluj nº 125991) | 31,2—41,6 | 8—12 | 5,2 | 10,4 | 2,6 | cf.Pl.II, fig. 13 |
| *C. sylvaticum* W. et K. (Hb. Inst. Bot. Buc. nº 26967) | 36,4—44,2 | 20—26 | 5,2 | 10,4 | 2,6 | cf.Pl.II, fig. 13 |
| *C. banaticum* (Rochel) Heuff. (Hb. Grăd. Bot. Buc.) | 33,8—46,8 | 16—18 | 5,2 | 10,4 | 3,9 | cf.Pl.II, fig. 13 |
| *C. alpinum* L. (Cult. Grăd. Bot. Buc.) | 28,6—46,8 | 18—22 | 3,9—7,8 | 3,9—7,8 | 2,6 | cf.Pl.I, fig. 12 |
| *C. lanatum* Lam. (Hb. Inst. Bot. Buc. nº 26585) | 28,6—39 | 18 | 3,9—5,2 | 7,8—10,4 | 2,6 | cf.Pl.I, fig. 12 |
| *C. latifolium* L. (Hb. Univ. Cluj nº 274211) | 39—54,6 | 10—12 | 5,2—6,5 | 10,4—11,6 | 2,6 | cf.Pl.I, fig. 12 |
| *C. transsilvanicum* Schur (Hb. Univ. Cluj nº 420428) | 39—57,2 | 12—22 | 5,2 | 10,4 | 2,6 | cf.Pl.I, fig. 12 |
| *C. arvense* L. (Cult. Grăd. Bot. Buc.) | 36,4—41,6 | 18 | 3,9—5,2 | 6,5—7,8 | 2,6 | cf.Pl.I, fig. 12 |

| | Forme et dimensions μ | Nombre des pores | Diamètre des pores, μ | Distances entre les pores, μ | Épais. du sporod. μ | Fig. |
|---|---|---|---|---|---|---|
| *C. lerchenfeldianum* Schur (Hb. Grăd. Bot. Buc.) | 31,2—44,2 | 16 | 5,2—7,8 | 10,4—11,7 | 2,6 | cf.Pl.I, fig. 12 |
| *C. biebersteinii* D.C. (Cult. Grăd. Bot. Buc.) | 39—59,8 | 24 | 3,9—5,2 | 6,5—7,8 | 2,6 | Pl.II, fig. 13 |
| *MOENCHIA* Ehrh. Pollen sphéroïdal—sous-sphéroïdal. Exine finement fovéolée | | | | | | |
| *M. mantica* (L.) Bartl. (Hb. Univ. Cluj nº 153826) | sphéroid. 26—46,8 sous-sphér. 26—44,2/ 28,6—52 | 14—18 | 5,2 | 10,4 | 2,6 | Pl.II, fig. 14 |

**SOUS-FAMILLE SILENOIDEAE**

Pollen polyporé et operculé. L'opercule, qui occupe env. 1/2 ou toute la surface de l'ouverture du pore, est ornementé d'éléments supratectaux (épines-verrues *(Agrostemma)* ou verrues) distribués circulairement, parfois 1—2 centraux; rarement plus nombreux et irrégulièrement distribués.

Exine en coupe optique tectée-baculée ou pilée-sympilée *(Cucubalus, Lychnis)*.

| | Forme et dimens. en μ | Nombre des pores | Diamètre des pores en μ | Epaisseur du sporoderme | Fig. |
|---|---|---|---|---|---|
| *TR. LYCHNIDEAE CUCUBALUS* L. Pollen sphéroïdal. Exine à verrues (épines) à distribution irrégulière ou ± réticuloïde | | | | | |
| *C. baccifer* L. (Hb. Grăd. Bot. Buc.) | 20,8—46,8 | 18—22 | 5,2 | 2,6 | Pl.II, fig. 16 |
| *LYCHNIS* L. | Exine euréticulée | | | | |
| *L. coronaria* (L.) Desr. (Hb. Grăd. Bot. Buc.) | sphéroïdal 26—41,6 | 16—22 | 3,6 | 2,6 | Pl.II, fig. 17 |
| *L. flos-cuculi* L. (Cult. Grăd. Bot. Buc.) | sphéroïdal 23,4—46,8 sous-sphér. 23,4—31,2/ 20,8—28,6 | 30—32 | 3,9—7,8 | 2,6 | cf.Pl.II, fig. 17 |



| | Forme et dimens. en μ | Nombre des pores | Diamètre des poresen μ | Epaisseur du sporoderme | Fig. |
|---|---|---|---|---|---|
| *POLYSCHEMONE* Sch., Nym. et Kot. | Exine euréticulée | | | | |
| *P. nivalis* Sch., Nym. et Kotschy (Hb. Inst. Niol. Buc. nº 45497) | sphéroïdal 29—42,9 | 18—22 | 5,2—7,8 | 2,6 | Pl.II, fig. 19 |
| *VISCARIA* Rohl. | Exine euréticulée | | | | |
| *V. vulgaris* Röhl. (Cult. Grăd. Bot. Buc.) | 11,7—36,6 | 18 | 2,6—3,9 | 2,6 | Pl.II, fig. 18 |
| *V. atropurpurea* Griseb. (Hb. I.N.C.E.F. nº 17282) | sphéroïdal 26—52 sous-sphér. 31,2—52 26—45,8 | 16—18 | 2,6—3,9 | 2,6 | cf.Pl.II, fig. 18 |
| *AGROSTEMMA* L. | Exine euréticulée | | | | |
| *A. githago* L. (Cult. Grăd. Bot. Buc.) | sphéroïdal 23,4—62,4 sous-sphér. 33,8—59,8 28,6—54,6 | 26—30 | 7,8 | 2,6 | Pl.II, fig. 15 |

**DISCUSSIONS**

Takhtajan [12], Engler [3] et Hutchinson [10] considèrent la famille *Caryophyllaceae* comme appartenant à l'une des lignes d'évolution des *Dicotyledonatae*. Ainsi Hutchinson considère l'ordre *Caryophyllales* comme fondamental dans la division *Herbaceae*, dérivant de *Ranales*, mais plus évolué que l'ordre de base *Saxifragales*.

Au point de vue de la morphologie du pollen, la grande majorité des espèces analysées ont le pollen polyporé-panthoporé (omniaperturé) et operculé, à l'exception de celles des tribus *Sperguleae* et *Polycarpeae* (sous-famille *Paronychioideae)* dont le pollen est 3—4 colpé ou 6—8 rugate.

La taille du pollen, en général moyenne (sous 50 μ), parfois petite *(Paronychia)* ou grande, paraît n'avoir aucune liaison avec la taxonomie, mais avec certains caractères morphologiques de la plante (hauteur de la plante, grandeur de la fleur, grandeur du périanthe).

Le nombre des pores, lequel dans le cadre de la famille est compris entre 8 — 32, varie même dans le cadre des genres, par exemple chez *Minuartia* 8—18. De plus, le nombre des pores est variable aussi dans le cadre de la même espèce.

Le diamètre des pores est compris, en général, entre 4—8 μ; on observe des dimensions beaucoup plus petites chez *Paronychia*, dues

à la petite taille du pollen et au grand nombre de pores, ainsi que chez *Viscaria*.

L'ornementation de l'exine est déterminée par la distribution des éléments supratectaux et par la perforation du tectum. Chez la sous-famille *Paronychioideae* prédomine le type ayant le tectum compact et non perforé, chez la sous-famille *Alsinoideae* apparaissent des perforations du tectum, soit sous la forme de fovéoles *(Minuartia)*, rarement avec des verrues *(Stellaria nemorum)*, soit sous la forme de microréticules.

L'ornementation de l'exine paraît justifier l'ordre des sous-familles citée par Engler [3] : chez *Paronychioideae* (la première sous-famille) apparaît le type d'exine ayant le tectum compact, plus primitif, chez *Alsinoideae* (la deuxième) apparaissent de petites perforations, tandis que chez *Silenoideae* (la troisième), apparaissent simultanément plusieurs types qui marquent des tendances d'évolution en plusieurs directions. Dans la dernière sous-famille on remarque le genre *Lychnis*, qui présente seulement le type fovéolé.

L'ensemble des caractères morphopolliniques analysés pour un grand nombre d'unités systématiques appartenant à la famille *Caryophyllaceae* permet certaines considérations taxonomiques concernant cette famille.

L'existence de la sous-famille *Paronychioideae* (cf. Engler [3] est confirmée en partie également par la morphologie du pollen. Toutefois, la tribu *Sperguleae*, ayant le pollen colpé ou rugate, se détache de cette sous-famille et pourrait éventuellement constituer une autre sous-famille.

## CONCLUSIONS

De l'analyse morphopollinique des unités mentionnées, on dégage les conclusions suivantes : le pollen est en général sphéroïdal—sous-sphéroïdal, polyporé (panthoporé) et operculé ; le nombre des pores variant entre 3 et 32 caractérise les sous-familles *Alsinoideae* et *Silenoidaee*, ainsi que la tribu *Paronychieae* de la sous-famille *Paronychioïdeae*. La tribu *Sperguleae* appartenant à la même sous-famille *Paronychioideae*, dont les grains de pollen sont colpés ou rugates, fait exception à ce type dominant.

Un trait également specifique de la majorité des taxons étudiés est l'exine euréticulée, à structure pilée-sympilée, plus rarement avec des verrues, épines supratectales, à disposition réticuloïde ; en ce dernier cas, l'exine, en coupe optique, est tectée-baculée. En ce qui concerne la taxonomie de la famille *Caryophyllaceae*, la morphologie du pollen plaide pour la séparation de la tribu *Sperguleae* de la sous-famille *Paronychioideae* et pour son inclusion dans une autre sous-famille.

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# POLLENMORPHOLOGIE IN DER TAXONOMIE UND PHYLOGENIE DER PFLANZEN

VON

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Nach einer kurzen Darlegung der Entwicklung der Kenntnisse über die Charakteristiken der Pollenkörner werden die zahlreichen Anwendungen der Pollenmorphologie in der Taxonomie und Phylogenie der Pflanzen vorgestellt.

Bildung und Fortschritt der mikroskopischen Biologie, einschliesslich der Morphologie des Pollens, sind eine direkte Folge der Ausbildung der technischen Optik, aber die Geschichte der Pollenmorphologie ist tatsächlich, im allgemeinen, die Geschichte der Palynologie.

Von den zahlreichen mikroskopischen Untersuchungen die neulich nach den Entdeckungen von R. Hoocke (1875) gemacht wurden, sind einige eine Beschreibung des Blütenstaubes verschiedener Pflanzen.

Die ersten Betrachtungen über die Bedeutung des Pollens in der Pflanzensystematik finden wir schon in den Arbeiten von I. T. Koelreuters (1761—1766), indem man verhältnismässig vor langem feststellte, daß die Form des Pollens für jede Familie, Gattung und sogar Art charakteristisch ist. Dies bedeutet, daß die taxonomische Orientierung oder Lage in der Palynologie eine der ältesten darstellt.

Der Verdienst, die Morphologie der Pollenkörner als einen wichtigen Charakter in der Beschreibung der Pflanzen vorzuschlagen, gehört dem Forscher J. B. Guillemin (1825), dennoch der erste erfolgreiche Versuch, die Charaktere des Pollens für die Klassifikation anzuwenden, scheint der von J. Lindley (1830) bei den *Orchideen* zu sein.

Im gleichen Sinne sind auch die Arbeiten Hugo v. Mohl (1834), C. J. Fritsche (1837) und A. Hassel (1842) zu betrachten, welche detaillierte, reich illustrierte Analysen betreffend die Morphologie der Pollenkörner zahlreicher Pflanzen darbringen. Ausgehend von dem Prinzip, die Struktur des Pollens als ein Klassifikationsmittel zu gebrauchen, hat A. Hassel versucht die *Nymphaeazeen* nach ihrem Pollen zu den *Monocotyledonaten* zu stellen.

Gleichfalls erwähnen wir den Beitrag den L. Radlkofer (1884) gebracht hat, der zum erstenmal eine umfangreiche Monograpahie "Über den systematischen Wert des Zustandes des Pollens bei den *Acanthaceae*" veröffentlichte, indem er feststellte, daß jede Hauptgruppe durch eine bestimmte Pollenform charakterisiert ist. Bedeutende Beiträge über die Wichtigkeit des Pollens für die Systematik enthalten auch die Arbeiten von H. Fischer (1890), J. D. Hooker (1872—1897), A. A. Kuznezov (1916) u.a., die es versuchten die Eigentümlichkeiten der Pollenkörner in ihrem

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taxonomischen und phylogenetischen Studium der Spermatophyten anzuwenden.

In diesem Sinne bemerken wir das Eindringen der Pollenmorphologie als bedeutende Hilfswissenschaft für die Systematik der höheren Pflanzen in dem wertvollen Werk von A. Engler — A. Prantl "Die natürlichen Pflanzenfamilien", in welchen einige Taxonomisten in ihrer Klassifikation der Pflanzen auch morphopollinische Merkmale gebrauchen, sowie in anderen neueren Synthese-Arbeiten, z.B.; A. Engler "Syllabus der Pflanzenfamilien", G. Hegi "Illustrierte Flora von Mitteleuropa" etc.

Diesen Vorgängern verdanken wir, daß in der Palynologie sich der Gedanke des taxonomischen Pollencharakters und der unveränderlichen Eredität desselben im 20. Jahrhundert als durchdringend erzeigt hat.

Die gegenwärtige Geschichte der Pollenmorphologie und die der Sporen für die Systematik der Pflanzen hat mit der monographischen Arbeit von R. P. Wodehouse "Pollengrains" (1935) begonnen, die sich als Referenzwerk und als fundamentale Informierung über den Pollen weltberühmt erwies. Die aktuelle Pollenmorphologie einer sehr großen Anzal von Pflanzen analysierend, bemerkt der Verfasser, daß der Ursprung und die Entwicklung der morphologischen Form des Pollens in Verbindung sowohl mit den phylogenetischen Faktoren, als auch mit den äußeren und inneren Bedingungen des Mediums stehen. Auf diese Weise drückt die Verschiedenheit der Pollenkörner von eng verwandten Arten einer Gattung eine phyletische Erscheinung aus.

Wodehouse hat ebenfalls die entwicklungsgeschichtlichen Prinzipien des Pollens (1936) sowie deren Bewertung in der Klassifikation der Pflanzen klar ausgedrückt, indem er bemerkte, daß die Pollenkörner mit einem einzigen Kolpus, charakteristisch für die *Bennettitales*, auch bei den primitiven *Gymnospermen* und den primitiven *Dicotyledonaten* vorhanden sind; der Kolpus des Pollens bei den *Magnoliaceae* erscheint als eine phylogenetische Brücke, welche die große genetische Lakune zwischen den Pollenkörnern mit einem einzigen Kolpus mit den Pollenkörnern mit 3 Kolpussen der *Angiospermen* verbindet.

Der Beginn einer neuen Etappe in der Geschichte der Pollenmorphologie bildet die Erscheinung der Arbeit G. Erdtman's, des Gründers der modernen Palynologie "Pollen morphology and plant taxonomy, Angiosperms" (1952). Diese grundlegende Arbeit stellt eine der ersten allgemeinen Übersicht der Pollenmorphologie fast aller Vertreter der höheren Pflanzen dar und enthält die Erwähnung von genetischen Verbindungen unter Familien auf Grund von morphopollinischen Angaben. Er hat die Grundlage und die Prinzipien der Untersuchungsmethoden in der Pollenmorphologie entwickelt, indem er eine besondere Terminologie ausarbeitete, die sich mit der Zeit erweitert hat und von den meisten Pollenforschern angenommen wurde.

Die Entwicklung der Kenntnisse betreffend die Pollenmorphologie hat der Morphologie und der fundamentalen Anatomie, im allgemeinen, einen großen Beitrag gebracht und hat es ermöglicht, neue Ansichten auszuarbeiten, sowie eine neue Orientierung in der Pflanzenphylogenie zu geben. Auf diese Weise wurden die Pollendiagnosen bei der Vervollständigung der Diagnosen neuer Pflanzen allmählich ergänzt und die erzielten Ergebnisse auf Grund der Pollen- und Sporenmorphologie sind



gewöhnlich in Einklang mit denen in der Anatomie (C. R. Metcalfe, 1961) oder mit den erzielten Ergebnissen auf anderen Gebieten der Botanik.

Auf Grund von morphologischen Merkmalen der Pollenkörner und einer entsprechenden Terminologie für die Beschreibung des Pollens wurden dichotomische Bestimmungsschlüssel aufgestellt mit deren Hilfe die Pollen-Angehörigkeit verschiedener Klassen festgestellt wurde (H. J. Beug, 1961) oder verschiedene Pollen-Typen erkannt (K. Faegri, J. Iversen, 1964; H. T. Waterbolk, 1964; M. Th. Cerceeau, 1956, etc.), welche eine Identifizierung des Pollens der Familien, Gattungen und selbst der Arten, möglich machten.

In den vergangenen Jahren G. Erdtman und H. Straka (1961) arbeiteten ein System von nummerischen Formeln für eine genauere Charakterisierung versschiedener Pollen-Typen, N.P.C. genannt, aus, welches sich in der Anwendung der Morphologie auf die Pollenkörner in der Makro-Taxonomie der Pflanzen stützte und in die Benützung der morphopollinischen Angaben in der Klassifikation von Taxonen mit kleinerem Rang in der Familie ermöglichte.

Dieses System betrifft die Anzahl, Stellung und den Charakter der Keim-Apperturen die H. Straka (1964) mit den Kennzeichen im Dezimal-System stellt und erweitert. Auf diese Weise hat sich ein System der morphologischen Analyse des Pollens gebildet, das (obwohl so ziemlich schwierig) die Möglichkeit gibt, "palynologische Lochkarten" aufzustellen, die eien Vereinigung von ähnlichen Pollen-Typen erleichtern in ihrer Morphologie, Aufstellung von genetischen Verhältnissen bei verschiedenen Taxonen, als auch ihre spätere Vervollständigug mit neu entdeckten Angaben, besonders auf Grund einer elektronmikroskopischen Forschung.

Gegenwärtig sind zahlreiche bedeutende wissenschaftliche Ergebnisse vorhanden die auf Grund einer erfolgreichen Zusammenarbeit zwischen der Makro- und Mikromorphologie für die Erledigung von Problemen der Pflanzensystematik angewandt werden. Mit den neuen, immer umfangreicheren Kenntnissen über die Pollenmorphologie ergaben sich zahlreiche morphopollinische Angaben betreffend einige Gattungen, Familien, Ordnungen und selbst grössere systematische Einheiten. Diese Angaben haben zur besseren taxonomischen Kenntnis verschiedener systematischen Kategorien beigetragen, und selbst zu einer richtigen Würdigung der Stellung von Pflanzengruppen, sowie deren Angehörigkeit zu Taxonen einer bestimmten systematischen Gruppierung geführt.

Die palynologischen Untersuchungen einer großen Anzahl von Vertretern vaskulärer Pflanzen haben zur Feststellung von bestimmten Regeln in der Variabilität des Pollens und der Sporen in ihrer natürlichen Grenze geführt. Auf diese Weise haben sich die charakteristischen Eigenschaften für eine Art, Gattung und Familie abgezeichnet und es ging hervor, wie sich der Grad dieser Variabilität im Rahmen verschiedener Klassen ändert; aber die Kenntnis dieser Regeln der morpho-sporo-pollinischen Variabilität hat zu einer genaueren Bestimmung verschiedener Taxone beigetragen, beziehungsweise geführt.

Auf Grund der Pollen- und Sporenmorphologie können in einer systematischen Einheit bestimmte "pollinische Formen", auch "Pollen-Typen" benannt, unterschieden werden, charakterisiert durch eine ganze

Vielfältigkeit von eigentümlichen Komplexen, die manchmal einer einzigen Gattung entsprechen können. In einigen Fällen ist die taxonomische und evolutive Wichtigkeit des Pollens bzw. der Sporen grösser bei der Familie, der Ordnung und selbst bei dem Phylum. So wurde es festgestellt, daß die Lage der Appertur im Tetradenzustand der Mikrosporengenese proximal ist, bei den *Bryophyten* und *Pteridophyten*, distal bei den *Gymnospermen* und den monapperturaten *Angiospermen*. Trotzdem fehlen oft nur scheinbar die Apperturen bei den *Equisetinen* und bei den *Bryophyten*.

Ausnahmsweise eine oder mehrere proximale Apperturen oder ähnliche Appertur-Zonen kann man an der proximalen Ansicht selbst bei dem Pollen höherer Pflanzen feststellen, z.B. : einige *Annonaceae* besitzen eine dünne proximale Zone, sowie auch einige *Mimosaceae* mit Pollen in Polliaden, welche eine oder mehrere Apperturen in Form von Poren auf der proximalen Stelle aufweist. Bei einigen fossilen *Gymnospermen* besitzen die Mikrosporen eine bipoläre Appertur, die ungenügend erforscht ist und so eine Übergangsform von den Sporen mit proximalen zu jenen mit distaler Appertur sein können.

Eine wesentliche Charakteristik bei den Pollenkörnern des grössten Teiles der *Angiospermen* bildet die Anwesenheit mehrerer Apperturen ; von diesen haben die *Monocotyledonatae* Pollenkörner mit einer einzigen distalen Appertur ; dieser Pollen-Typus kann bei den *Magnoliaceae* der *Dicotyledonaten*-Reihe begegnet werden ; bei den typischen *Dicotyledonaten* jedoch weist der Pollen mit mehreren Apperturen eine zonale Aufstellung auf.

Oft wurde es festgestellt, daß der Pollen-Typus eines Taxons charakteristisch und konstant (stenopalyn) selbst nur für jene Gruppe ist, welche gewöhnlich eine natürliche Gruppierung aufweist.

In anderen Fällen, selbst in den Grenzen einer natürlichen systematischen Einheit, können die Pollen-Typen wesentlich variieren ; dieser Taxon ist *euripalyn* und evident *heterogen*. In diesem Falle beweist die große Diversität von morphopollinischen Typen in einer Familie, daß diese aus einer verschiedenen heterogenen Gruppe ausgebildet ist. Auf diese Art sind einige Familien *homogen* und andere jedoch *heterogen*, welch letztere aufgeteilt werden müssen, denn die Morphologie des Pollens weist oft Anzeige auf, in welche Gruppe die Gattung mit einer unklaren Stellung gestellt werden kann, so z.B. : die Separation der Familie *Altingiaceae* von der Familie *Hamamelidaceae*, die Familie *Irvingiaceae* von der Familie *Simarubaceae* (G. Erdtman, 1969) ; die Gattung *Paeonia* von der Familie *Ranunculaceae* (N. Mitroiu, 1970), die Unterfamilie *Allioideae* von der Familie *Alliaceae* (D. Rădulescu, 1973), die Umbildung der Familie *Plantaginaceae* in ein selbständiges Taxon der Ordnung *Plantaginales* (G. Şerbănescu-Jitariu, 1971) etc.

Die Pollenmorphologie kann ebenfalls für eine Verschmelzung von Familien sprechen u.zw. : die Fam. *Diclinteraceae* mit den *Polygalaceae*, *Heteropyxidaceae* mit den *Myrtaceae* (G. Erdtman, 1969) ; dann die Zusammenfassung der Familien *Moraceae* und *Cannabinaceae* in die Familie *Urticaceae* ; die Familie *Zingiberaceae*, *Cannaceae* und *Musaceae* in die Familie *Scitamineae*, sowie auch andere Beispiele, welche aus den Arbeiten der rumänischen Palynologen ersichtlich sind.



Es ist offenbar, daß die Feststellung der systematischen Position verschiedener Taxone nicht exclusiv nach den morphopollinischen Kriterien gemacht werden muß sondern, nur unter Berücksichtigung von mehreren verschiedenen Bemerkungen aus dem vegetativen Zustand und aus dem Rahmen ihrer Fortpflanzung. In diesem Sinne haben wir zahlreiche Beispiele gebracht, von welchen wir die Begrezung der *Abelia* der *Caprifoliaceae* erwähnen und die Erhöhung beider Sektionen : *Euabelia* und *Zabelia* zum Grade einer Gattung. Die neue taxonomische Stellung beider haben zu Grunde sowohl die Morphologie der Pollenkörner, als auch morpho-anatomische Merkmale (G. Erdtman, 1967).

Die Existenz einer morpho-pollinischen Variation bei verschiedenen Arten derselben Gattung kann viel schwerer anhand von Untersuchungen erkannt werden, die noch grösstenteils mit Hilfe der optischen Mikroskopen durchgeführt werden, obwohl diese noch immer ihre Bedeutung in der palynologischen Forschung haben. Details der Exinestruktur, die manchmal bei dem gewöhnlichen Mikroskop noch sichtbar sind, können auf Grund von elektronmikroskopischen Untersuchungen ergänzt werden und sie ermöglichen die feine Struktur der Sporodermis zu erkennen, sowie den Prozeß der Differenzierung der Sporen- und Pollenhülle zu beobachten.

In einigen Fällen hat nur die Elektronmikroskopie die sehr feinen Unterschiede in den Wänden der Sporen- und Pollenkörner an den Tag gelegt. Auf diese Weise, im Rahmen einiger Familien,welche bei uns *stenopalyn* betrachtet werden, wie z.B. die Familie *Mesembryanthemaceae*, konnten in ihrer Exine submikroskopisch mehrere Pollen-Typen unterschieden werden, die primitive oder entwickelte Gattungen charakterisieren (Straka, 1971). Ähnliche Ergebnisse auf Grund von elektronmikroskopischen Untersuchungen haben die Existenz verschiedener Pollen-Typen und in dem Umriss anderer Familien, wie *Didiereaceae* und *Pedaliaceae* entdeckt, welche mit den makromorphologischen Merkmalen vergliechen, haben die Feststellung von natürlichen phyletischen Verhältnissen, im Rahmen der jeweiligen Taxone, möglich gemacht, die von H. Straka (1971) in einem genealogischen und phylogenetischen Stammbaum der morphopollinischen Merkmale wiedergegeben wurden.

Die Pollenmorphologie hat eine große Anwendung auch in der Taxonomie von Kulturpflanzen gefunden : bei *Canna* und anderen Dekorationspflanzen (Nair, 1960, 1961), bei *Zea mays* und anderen Getreidearten (Nair, 1962 ; Withedheat und Langam, 1965,etc.), sowie bei Zitronenarten und verschiedenen Medizinalpflanzen (Nair und Kaul, 1965, etc.). Die morphologischen Eigentümlichkeiten der Pollenkörner wiederspiegeln sich in dem hybridogenen Charakter einiger Pflanzen, in der Ableitung von Kulturpflanzen aus spontanen Pflanzen und in ihrem gegenseitigen genetischen Verhältnis. So einige Arten die als *rein* betrachtet werden, besitzenheterogenen, polymorphen Blütenstaub auf Grund einer wahrscheinlichen meiotischen Trübung oder deren Degeneration, die eine sogennante reine Art als Hybrid darstellt, z.B. : die Bastarde von *Betula nana* × *B. tortuosa* mit sehr unterschiedlichen Pollen-Typen, eine Erscheinung die auch bei *Triticale* und bei verschiedenen Dekorationspflanzen begegnet werden kann.

Eine bemerkenswerte Feststellung im Studium des Pollens von Kulturpflanzen ist die Tatsache, daß die Morphologie des Pollens auch ein Ausdruck des physiologischen Verhältnisses darstellt, die morpho-physiologische Korrelation offenbart sich aber verschiedenartig (Pollen-dimorphismus, Sterilität deselben, etc.).

Zytopalynologische Korrelationskomplexe haben gezeigt, daß die Pollenmorphologie oft den Polyploidie-Grad einer Pflanze aufweist, der in der Pollenmorphologie erschienenen Variationen, z.B. : bei *Sysimbrium irio* und *Oldenlandia corymbosa* mit verschiedenen Pollen-Typen bei der diploiden und tetraploiden Art oder Sorte (Nair, 1970) etc. Es wurde bemerkt, daß das Anwachsen der Chromosomenzahl zu einer Erscheinung von Pollenkörnern mit grösseren Dimensionen führt, die mehrere Apperturen haben oder eine Veränderung ihrer Stellung aufweisen (Maurizio, 1957).

Die konstanten oder veränderlichen ökologischen Bedingungen in denen sich die Pflanzen entwickeln, Bedingungen die ein Erscheinen von neuen Ökotypen die sich in der Pollenmorphologie wiederspiegeln und in diesem Sinne ein Beispiel der Pollenvariation, zufolge einer Pflanzenmigration, bildet der Blütenstaub bei *Caltha palustris*. Dies ist eine wesentliche europäische Art mit 3-zonokolpaten Pollenkörnern in allen Zonen der Erde, welche jedoch nach einer Migration nach Kaschmir eine Varietät *alba* hervorbringt mit pantoporaten Pollenkörnern (Nair, 1961). Der Pollen von *Gnetum* scheint gleichfalls von drei Typen zu sein u.zw. : südamerikanisch, afrikanisch und asiatisch (G. Erdtman, 1969).

Die Pollenmorphologie ist auch als ein Ausdruck der Evolution und der Phylogenie der Pflanzen zu berücksichtigen, so daß die Kenntnis der Entwicklung der Sporen-und Pollenmorphologie eine grundlegende Wichtigkeit für die Erklärung der Pflanzenphylogenie, miteingerechnet der Ursprung der *Angiospermen* darstellt.

Im Laufe der Evolution des Pflanzenreiches, gleich mit der Diversifikation und des stufenweisen Anwuchses der strukturellen Komplexität der vegetativen und Fortpflanzungsorgane ist die Anzahl, Position und Varietät der morpho-strukturellen Apperturen angewachsen, sowie die der Sporen- und Pollenkörnerhülle. Abhängig von der Evolution der Appertur-Form sind drei Grund-Typen vorhanden, welche die großen Gruppen der Pflanzen charakterisieren u.zw. : *trilete*, *monolete* und *non-aperturate* Formen, die vereinzelt bei gutbegrenzten (fossilen und aktuellen) Taxonen unter den *Bryophyten*, *Pteridophyten*, *Gymnospermen* und bei einigen *Angiospermen*, während letztere eine grosse Verschiedenheit von apperturellen Formen kennzeichnet, vorgefunden werden. Die trilete Form der Sporen wird als eine der primitivsten angesehen, nur ist sie bei den aktuellen und fossilen *Pteridophyten*, sowie bei einigen fossilen *Gymnospermen*, bei den *Cycadofilicales*, vorhanden, die *monolete* Form ist jedoch nur bei den evolutiven Gruppen beschränkt.

Auf Grund dieser wesentlichen Eigenschaften der Apperturen, welche mit den Eigentümlichkeiten der Struktur und Epistruktur der Sporodermis korreliert ist, wurden verschiedene Evolutions-Schemen des Pollens gebildet, welche die phyletischen Zusammenhänge in den Grenzen der betreffenden Taxone wiederspiegeln (R. P. Wodehouse, 1935, 1936 ;



L. D. Kuprianova, 1954 ; A. Takhtajan, 1959 ; G. Erdtman, 1965 ; N. Mitroiu, 1968 ; P. K. K. Nair, 1968 ; u.a.).

Die elektronmikroskopischen Untersuchungen betreffend die Struktur der Sporen- und Pollenhülle haben letzthin eine neue Orientierung der phylogenetischen Ansichten bewirkt, welche sich auf morphopollinischen Angaben stützen, indem sich eine bestimmte phylogenetische und in der submikroskopischen Struktur der Sporodermis äußert. Auf diese Weise sind den Sporen der Moose und Farnpflanzen, im allgemeinen, homogene Schichten eigen ; die Lamellierung der Exine-Schichten wurde zum erstenmal bei einer sehr alten heterosporen Farnpflanze, *Archaeopteris*, bemerkt und nachher bei den Sporen der heterosporen aktuellen Farnpflanzen. Der lamelläre Charakter der inneren Exine-Schichten wurde häufig bei dem Gymnospermen-Pollen gefunden ; bei den Angiospermen jedoch ist die lamelläre Exine (Endexine) nur bei bestimmten Stadien der Ontogenese vorhanden, die nachher verschwindet und nur bei dem reifen Pollen bestimmter Gattungen unter den *Degeneriaceae* und *Liliaceae* (Yucca, Lilium) weiter zu finden ist.

Das Pollen-Studium fossiler und aktueller Pflanzen, ergibt, daß im Laufe der Zeit sich zwei evolutive Linien entwickelt haben u.zw. : die Linie der *Magnoliaceae* und *Ranunculaceae* durch die Vorherrschung eines monocolpaten und 3-zonocolpaten Pollens charakterisiert, jedoch die verschiedenen Familien der Angiospermen haben eine Affinität zu dieser phylogenetischen Gruppe. Die Anwesenheit von monocolpaten Pollen, sowohl in den Familien der *Magnoliinae*-Gruppe, als auch bei den meisten *Monocotyledonaten* möchte suggerieren, daß letztere enger mit den *Magnolien-Dicotyledonaten* und weniger mit denen der *Ranalen-Dicotyledonaten* verwandt sind. Eher kann man sagen, daß die Gruppe der *Monocotyledonaten*, die *Magnolien*-und *Ranalien*-Gruppe, würde drei independente phylogenetische Linien vorstellen, die einen phylogenetischen Ursprung vielleicht bei den *Magnolien*- und *Ranalien-Dicotyledonaten* haben (Nair, 1970).

Da aber palynologische Angaben betreffend den Ursprung der Angiospermen noch abhanden sind, da man ihren uralten Typus nicht kennt, die vorhandenen (fossilen und aktuellen) Sporen und Pollenköner bieten genügende Beweise zu Gunsten eines polyphyletischen Ursprunges vieler vaskulärer Pflanzengruppen, die von L. Kuprinova (1973), in der letzten Zeit, argumentiert wird. Die Verfasserin unterscheidet mehrere Haupt-Etappen in der Entwicklung der Sporen und des Pollens als auch in anderen Systemen der Organe entsprechend den geologischen Zeitabschnitten der Erde. Die in den entscheidenden Momenten erschienenen Formen haben sich parallel und konvergent entwickelt und sie ergaben viele verschiedene evolutive Stufen, beginnend mit den einfachsten proximalen bis zu jenen mit äquatorielen komplexen Apperturen. Auf diese Weise entwickelt sich der Pollen der *Angiospermen* mit einer großen Anzahl von Appertur-Typen der Pflanzen vom Ende des Mesozoikums und des Anfanges des Känozoikums, eine Verminderung der Variabilität der Appertur-Typen aufweisend.

Die jüngsten Errungenschaften in der Morphologie, auf Grund von neuen Methoden und Untersuchungstechniken, besonders der Elektronmikroskopie, welche die Verbindungen der Palynologie mit anderen

Wissenschaften (Zytologie, Embryologie, Biochemie) vertieften, werden neue objektive Kriterien bilden, sowohl für die Festlegung der taxonomischen Stellung verschiedener systematischer Einheiten, als auch neuer phylogenetischer Verbindungen.

Es wird gleichfalls bemerkt, daß die Pollenkörner einen Ausdruck der strukturellen und funktionellen Evolution der Pflanzen bilden und so die Möglichkeit besteht, den Pollen als Untersuchungsmaterial für biologische Forschungen zu verwenden, besonders in der Methodologie der Kultur der Pflanzen, in der Pollen-Selektion von den gewünschten Formen bis zu deren dirigierten Hybridsations-Prozessen der ökonomischen Pflanzen.

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# ISOLATION AND CULTURE OF CELL PROTOPLASTS FROM THE MESOPHYLL CALLUS OF *VITIS VINIFERA* L.

BY

AURELIA BREZEANU, ANA ROŞU

The aim of the paper is to discuss some methodological aspects regarding the isolation and culture of protoplast from the mesophyll callus of grape vine (cv. Feteasca Regală). Protoplasts were obtained after overnight incubation at 26°C in 2% Cellulase Onozuka R—10 plus 1% Macerase Onozuka or in 2.5% Cellulysin, 1% Macerosyme and 0.05% Dryselase dissolved in a mixture of 0.14 M KCl and 0.10 M $CaCl_2$ at pH 5.5. The liquid nutrient medium which is a Linsmeyer—Skoog medium with 1 mg/l kinetin, 2 mg/l NAA and 90 g/l mannitol as osmotic stabilizer, stimulated the regeneration of the new cell wall, followed by cell division which took place within 2—3 days since inoculation. The subsequent transfer of the culture on the surface of the same medium solidified with 0.7% agar, or on a variant of the basal Murashige—Skoog (1962) medium with 2 mg/l BAP and 0.18 mg/l NAA in which mannitol was omitted, resulted in the development of microcolonies and small masses of callus.

The regenerative capacity is a main feature of protoplasts and it determined a wider range of their utilisation in experimental biology. The investigation regarding the behaviour in culture of the higher plant protoplasts, on specific nutrient media, pointed out their capacity to remake the cell wall, to divide and form cell colonies from which autonomous plants can be obtained by organogenesis and somatic embryogenesis [5]. These features supported the enhancement of the investigations to obtain protoplasts for hybrid cell cloning, resulted either from interspecific or intergeneric fusions [4], or for the multiplication of the mutant cell lines and the regeneration of plants with new characteristics.

Although good progress was made in plants regeneration from protoplasts, the number of species, mainly wooden plants where this process took place, is limited [3]. The species *Vitis vinifera* is not much studied from this point of view. However, the investigations of Skene [8], [9] should be mentioned, who described for the Sultana cultivar the isolation and culture conditions of protoplasts originating from the pericarp callus cells. The previous investigations preformed by us on the cvs. Feteasca Regală, Riesling and Dattier and by Hasler et al [11] on the cv Riesling×Silvaner, pointed out the conditions that assure a viable population of mesophyll protoplasts and the ranges of ultrastructural modifications which accompany the isolation and reversion processes. Because of the reduced regenerative capacity of the mesophyll protoplasts our attention was directed to another source of biological material, namely the callus culture, which was started from explants of the leaf mesophyll.

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## MATERIAL AND METHODS

Mesophyll callus belonging to the Fetească Regală cultivar was used as a protoplast source. The culture initiation was performed on the basic Murashige-Skoog medium (1962) with 0.1 mg/l BAP and 1mg/l 2,4-D, in 16 hours daily illumination and 26—29°C temperature. For the obtainin of protoplasts, 2g of callus (fresh weight) were incubated in 15 ml enzyme solution, sterilised by filtration. Two enzymatic formulae were tested: —formula I: Cellulase Onozuka R—10—2% plus Macerase Onozuka—1%, dissolved in a solution of salt prepared from 0.1 M $CaCl_2$ and 0.14 M KCl, in the presence of 0.5% dextran sulfate, with a pH adjusted to 5.5 [8]; — formula II: Cellulysin™ — 2.5%, Macerosyme™—1%, Dryselase—0.05% in the same salt solution and with the same pH (5.5) — original formula.

The enzyme solution was incubated for 16 hours, under static conditions in the dark and at a temperature of 26°C. The protoplast suspension was filtered through two nylon cloth layers to remove the coarse impurities, then it was rinsed by slight centrifugation (200 r.p.m.) and successively resuspended in the same salt solution in which the enzyme mixture was prepared. In order to obtain a good osmolarity, mannitol was added in a concentration of 9%. The protoplasts suspension obtained after washing is impurified with numerous cellular debris, resulted after the enzymatic digestion, being either dead cells or cells with a partly or totally removed wall. Therefore, the purification of protoplasts by their resuspending in a purification solution containing 0.1 M $CaCl_2$ plus 0.14 M KCl and 23% sucrose, followed by recentrifugation for 10 minutes at 200 rotation p.m., is an essential stage in the isolation process. After this operation, in the higher part of the centrifugation test tube, an intensely green pellet of about 1 cm in height, which represents a concentrated suspension of viable protoplasts, was noticed. The concentrate layer was collected from the surface of the purification solution and it was resuspended in a previously known volume of culture medium so that a dilution of $2 - 5 \times \times 10^2$ protoplasts per milliliters was obtained.

The nutritive medium used for regeneration is represented by the Linsmeyer-Skoog basal medium, with 1 mg/l kinetin, 2mg/NAA, 20 g/l sucrose, 90 g/l mannitol. In order to establish the best culture conditions the efficiency of the following procedures was tested:

— *Culture in liquid nutritive medium*, under static conditions 2 ml of protoplast suspension was transferred into 25 ml Erlenmeyer flasks or glass tubes of 3/1.5 cm size;

— *Culture on solid medium support*, 5 drops of protoplast suspension were placed on the surface of a solidified nutritive medium (with 0.7% agar). The nutritive medium where the protoplast suspension was prepared and a variant of the Murashige-Skoog medium with 2 mg/l BAP and 0.18 mg/l NAA were used at the same time.

— *The microdrop array method*. By means of a Pasteur pipette with a very thin point, small suspension drops were applied on the lid of a Petri plate. In order to preserve an adequate atmospheric humidity, sterile distilled water or sterile nutritive medium was introduced inside the Petri plate [7];

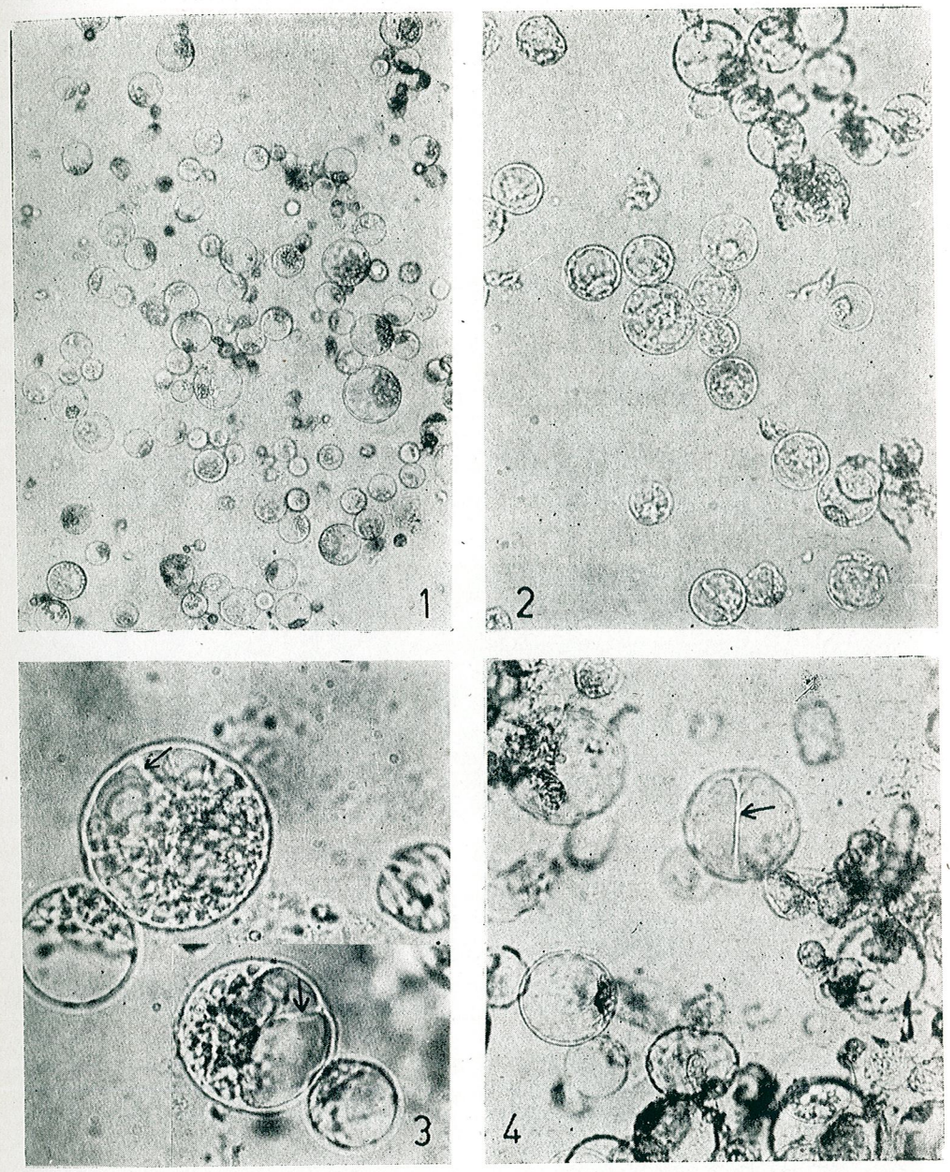


PLATE I — Fig. 1. — Protoplasts recently isolated from cells of the mesophyll callus in *Vitis vinifera L.*, cv. Fetească regală; (× 250)
Fig. 2. — Protoplasts after two days of culture in a liquid medium; (× 250)
Fig. 3. — Incipient stage of division septum formation (see arrow) after four days of culture; (× 315)
Fig. 4. — First cell division after seven — ten days of culture; (× 315)

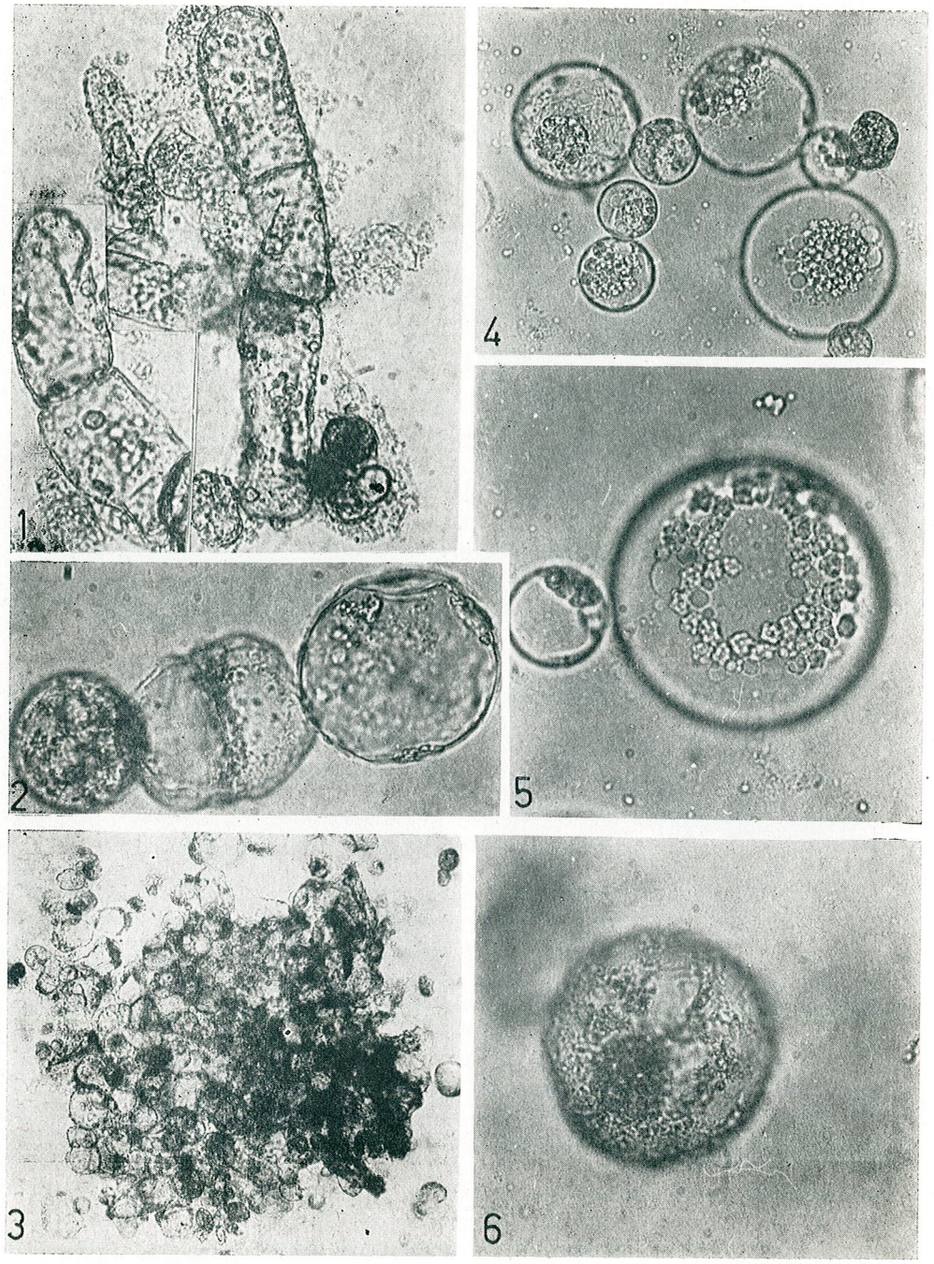

PLATE II — Fig. 1. — Multiple divisions after the first four weeks of culture ; (× 315)
Fig. 2. — First cell divisions of protoplasts cultivated on filter paper support ; (× 800)
Fig. 3. — Microcolonies after 5 weeks of culture (250 ×)
Fig. 4. — Groups of cells from the peripheral area of the colony, with numerous amyloplasts ; (× 315)
Fig. 5. — Detail ; (× 800)
Fig. 6. — Morphology of a cell with meristematic features, from the central area of the colony ; (× 800)



— the culture on filter paper support [6]; 3 ml of medium solidified with 0.6% agar were placed in Petri plates with a diameter of 6 cm. Small round discs of chromatographic Wattman 1 paper were placed on the medium so carefully that they should not sink into it. A 0.5 ml suspension of protoplasts was dripped on this support.

In all cases the incubation was performed at a diffuse light of 500 lx and a temperature of 26—28°C. 7—10 days after incubation when the microscopic examination showed the first cell divisions, the suspensions were transferred on the same solid nutritive medium, with a half-strength mannitol concentration.

## RESULTS AND DISCUSSIONS

Protoplast isolation was normally performed in case of using both enzyme combinations, (Plate I, Fig. 1). The differences consisted in the fact that Cellulysin (2.5%) with Macerosym (1%) and Dryselase(0.05%) hastened the lysis process of the cell wall with about 4—5 hours. Therefore, the release of naked cells started in this variant in the first six hours of incubation and ended completely after 10—12 hours. The slight stirring (50 r.p.m.) had a stimulating effect. This remark is important, because by using more rapid methods of isolation the longer contact of cells with cellulosolytical enzymes is avoided, as it generally affects the viability and regeneration capacity of cells.

The protoplast suspensions obtained were slightly impurified with cell debris and were characterized by a high density of cells. From a morphological point of view the cells, with a perfectly spherical shape, present a well preserved membrane system. The heterogeneity of cell population called our attention. At the same time small sized protoplasts with a rich cytoplasmic content, similar to embryogenic cells as well as large-size, highly vacuolised protoplasts, were present.

The Linsmeyer-Skoog nutritive medium with 1 mg/l kinetin, 2 mg/l NAA and 90 g/l mannitol, supported the regeneration process of the cell-wall and the initiation of cell divisions, in case of all experimented culture conditions. Among variants, clear differences appeared after 6—8 days. From the four analysed modalities of culture, the method of culture in liquid medium, under static conditions and the placing of suspension drops on a support of agar medium showed the best results. From these variants after 4—5 days, a great number of cells divided 2 or 3 times, with the formation of groups or "filamentous" cells contained 3—4 cells (Pl. II, Fig. 1). The first cells to enter the division stage were the small ones with embryogenic character (Pl. I, Fig. 3, 4). The large cells with a well-expressed vacuolar system divided to a less extent. The majority started a rapid degeneration process and died.

The phenomenon is somehow similar to that one noticed by Vasil and Vasil [10] in *Penisetum americane*. The embryogenic cells with a high capacity of division are expected to manifest also a morphogenetic response, after being transferred on specific nutritive media.

Five weeks later, visible, well individualised microcolonies were noticed, Figure 1, consisting of cell clusters. (Pl. II, Fig. 3). The central area of these colonies is represented by a "nucleus" of small embryogenic cells under permanent division. (Pl. II : Fig. 6). At their periphery, large

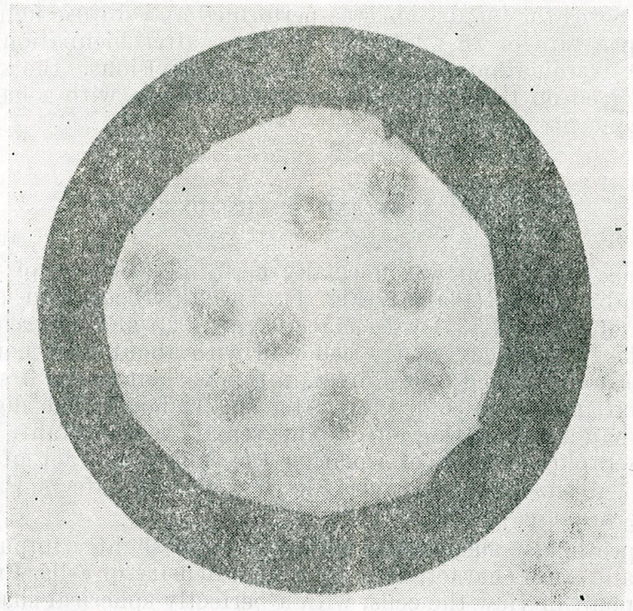

Fig. 1. — Macroscopic aspect of microcalluses.

cells poor in cytoplasm, with a reduced mitotic activity, are noticed. The cell colonies that were obtained were achlorophyllous. The abundance of amyloplasts placed in the nucleus neighbourhood was evident, having contiguity relations with it. The amyloplasts, containing large starch grains and a poor stroma, are characterized by a weak differentiated membrane system (Pl. II, Fig. 4, 5).

The initiation of cell divisions and formation of microcolonies were noticed on a Murashige-Skoog medium with 2 mg/l BAP and 0.18 mg/l NAA, in which mannitol was omitted.

The microdrop array method was not satisfactory for this species. It is possible that by increasing the number of cells per drop to obtain a too high density of cells. Consequently, the cells felt the shortage of nutrients and $O_2$ and the accumulation of metabolic products released in the medium. The impossibility to renew the medium with a fresh nutritive medium and the concentration of the initial one due to evaporation, is another element that influences negatively cell viability. The method can be used more successfully in case of other species such as *Nicotiana* and *Datura* where the regeneration and division processes take place more rapidly (between a few hours to a few days).



The culture on filter paper support used with good results by Partanen [6] for *Pteridium aquilinum* was not successful although the first cell divisions were initiated in this case as well (Pl. II, Fig. 2).

We may conclude that :

— Mesophyll callus cells belonging to *Vitis vinifera*, cv. Fetească Regală, are a good biological material for isolating significant populations of viable protoplasts with a high regenerative potential;

— Association of Cellulysin (2.5%) with Macerosym (1%) and Dryselase (0.05%) as well as a slight stirring (50 r.p.m.) hasten with 5—6 hours the process of enzymatic degradation of the cell wall;

— The Linsmeyer-Skoog culture medium with 1 mg/l kinetin, 2 mg/l NAA, 20 g/l sucrose and 90 g/l mannitol as an osmotic stabiliser stimulates the regeneration of cell divisions;

— The method of culture in liquid medium under static conditions or on a support of agar medium provide the best conditions for the development of microcolonies and of the callus.

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# THE CORRELATION BETWEEN RADIOSENSITIVITY AND THE DNA AMOUNT PER CHROMOSOME WITHIN THE *NIGELLA* L. GENUS (*RANUNCULACEAE*)

BY

GABRIEL C. CORNEANU [1], VIOREL SORAN [2], HAROLD H. SMITH [3], ANDREI LAZÁNYI [4]

The paper presents the values of the DNA amount per nucleus and per chromosome (expressed in DNA arbitrary units) with ten species and one variety of *Nigella* genus, as well as the doses of X-rays or fission neutrons which bring about the slight stem growth inhibition or $D_{50/30}$ values. The existence of a negative correlation between the DNA amount per chromosome and the $D_{50/30}$ value was found, both in case of irradiation of dry seeds with X-rays ($r = -0.78$) and in case of fission neutrons irradiation ($r = -0.54$).

The results of the experiments performed by A. H. Sparrow and his school showed that between the DNA content per chromosome and the radiosensitivity of plants, examined through different radiobiological indices, there is a clear negative correlation, the regression line slope being of about —1 [1], [6], [7], [8], [9], [10].

Other experiments revealed the existence of a low correlation or the lack of the correlation between the radiosensitivity and the DNA amount. Thus K.- H. von Wangenheim and F. Walther [11] analysing three kinds of wheat (Bayro, Walthari and Peragis) found that to the small differences recorded between the nuclear volume values (577, 529.3, respectively 554.5 $\mu m^3$) and the DNA content (192, 189.5, respectively 197.5) there correspond high differences in radiosensitivity. Also J. P. Miksche and T. D. Rudolph [5] studying the radiosensitivity of nine gymnosperm species whose chromosome somatic number is similar (22 or 24) did not remark the existence of a significant relationship between the nuclear volume or the DNA content, on one hand, and the radiosensitivity of the studied species (examined through the inhibition of the dry weight by 50%, respectively of the germination; survival; the length of the plantlets and their dry weight).

Since important changes can be produced in radiosensitivity without changes in the DNA content as well, the possibility of a simple general relationship between the radiosensitivity and the DNA content can be excluded. The fact that all species of a plant group (gymnosperms or angiosperms) produce the same radiobiological response to absorption

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of an equivalent energy quantity per chromosome, suggests that in the average chromosome of a species the same lesion number is produced, although a variation in the DNA content may exist between species.

In this paper, the existent relationship between the DNA amount (calculated from nuclei in the $G_1$-stage, represented by the chromosome groups in the ana-telophase) and the radiosensitivity of some *Nigella* species, examined through different degrees of the growth inhibition, was studied.

## MATERIAL AND METHOD

### THE BIOLOGICAL MATERIAL AND ITS IRRADIATION

The following species were utilized: *Nigella arvensis* L. ssp. *aristata* (Sibth. & Sm.) Nym., *N. arvensis* L. ssp. *arvensis*, *N. ciliaris* D.C., *N. damascena* L. — single flower, white, *N. damascena* L. — double flower, *N. degenii* Vierh. ssp. *jenny* Strid, *N. doerfleri* Vierh., *N. hispanica* L., *N. nigellastrum* (L.) Willk (= *Garidella nigellastrum* L.), *N. orientalis* L. and *N. sativa* L. (all the species presenting 2n = 12), whose dry seeds were irradiated with X-rays or fission neutrons at Brookhaven National Laboratory, Upton (USA). The X-rays irradiation was performed with the help of an G.M. Maxitron apparatus having the parameters: 250 kVp, 30 mA, filter of 1 mm Al, the dose rate ranging between 800—1200 R/min(depending on the size of the seeds and their amount). The doses used were: 0, 3500, 4500, 5500, 6500 and 11 000 R. The fission neutrons irradiation was performed between golden folioles by the reactor from Brookhaven National Laboratory utilizing the doses: 0, 100, 200, 400, 600 and 1000 R. Both the irradiated variants and the control variants came by air from Upton (USA) to Craiova (Romania).

Previous researches on the radiosensitivity of the *Nigella* genus [2], [3] showed that the "storage effect" and the conditions during the transport do not modify the relative radiosensitivity of the species studied, as compared to the recorded values in case of the seeds germination soon after irradiation.

### GROWTH TESTS

The seed germination and the plantlet growth took place on a sand layer, in the greenhouse, at a temperature of 20°—24°C, utilizing 100 seeds per variant. The biometric observations on plants were performed 30 days after the sprouting of the plants, the values recorded being statistically interpreted. From the growth curves of the plant length, there were established, for each species, the radiation doses (in R), which determine the slight inhibition of the growth (SGI, corresponding to a 15% reduction of the stem length in comparison with the control) and the radiation



doses which determine the $D_{50/30}$ value (respectively a 50% reduction of the stem size of 30 day-old plants, in comparison with the control; Fig. 1).

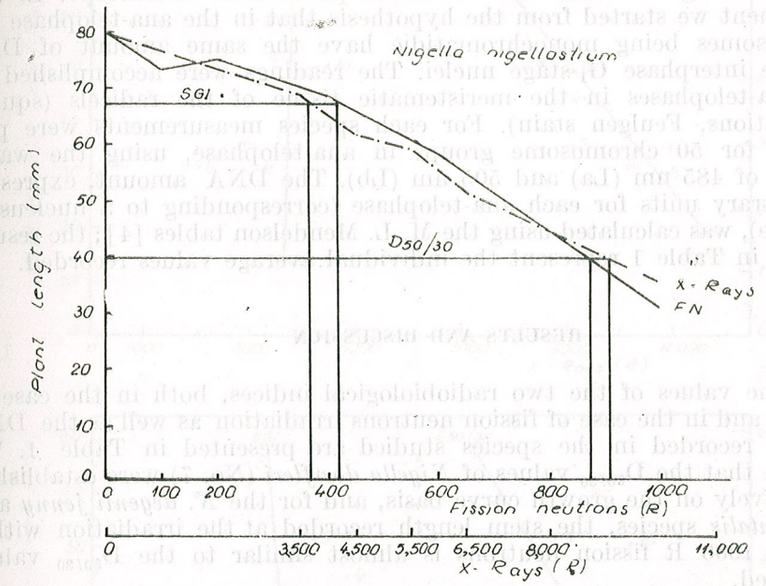


Fig. 1. — Establishment of slight growth inhibition values and $D_{50/30}$ values on the basis of stem growth test in *Nigella nigellastrum* (X-rays) and fission neutrons).

*Table 1*

The correlation between the DNA estimated amount and different values of growth inhibition in 11 *Nigella* species and varieties

| No. | Species | estimated DNA (arbitrary units) | | Slight growth inhibition of stem (R) | | $D_{50/30}$ values (growth inhibition of stem, R) | |
|---|---|---|---|---|---|---|---|
| | | DNA/nucleus | DNA/chromosome | Fission neutrons | X-rays | Fission neutrons | X-rays |
| 1 | *N. arvensis aristata* | 35.22±1.07 | 2.94±0.09 | 405 | 3,150 | 800 | 10,850 |
| 2 | *N. arvensis arvensis* | 35.34±0.88 | 2.94±0.07 | 425 | 3,750 | 930 | 9,150 |
| 3 | *N. ciliaris* | 59.15±1.64 | 4.93±0.14 | 200 | 3,200 | 750 | 7,600 |
| 4 | *N. damascena* — single flower | 44.83±1.39 | 3.74±0.12 | 485 | 5,300 | 910 | 9,300 |
| 5 | *N. damascena* — double flower | 65.63±1.86 | 5.47±0.16 | 300 | 3,000 | 595 | 8,700 |
| 6 | *N. degenii jenny* | 30.86±1.29 | 2.57±0.11 | 305 | — | 1,010 | — |
| 7 | *N. doerfleri* | 39.46±1.10 | 3.29±0.10 | 510 | 5,500 | 1,090 | 11,500 |
| 8 | *N. hispanica* | 31.05±0.99 | 2.59±0.08 | 200 | 2,100 | 820 | 10,800 |
| 9 | *N. nigellastrum* | 51.63±3.51 | 4.30±0.29 | 420 | 3,700 | 880 | 8,700 |
| 10 | *N. orientalis* | 50.48±1.35 | 4.21±0.12 | 370 | 4,500 | 1,020 | 9,100 |
| 11 | *N. sativa* | 33.84±1.40 | 2.82±0.12 | 280 | 3,500 | 880 | 10,500 |
| | Correlation coefficient (± *r*) | | | −0.09 | +0.02 | −0.54 | −0.78 |

## DNA AMOUNT

The DNA amount was determined by the double wavelength method, utilizing the MPE Lutz Ortholux photometer microscope. In this experiment we started from the hypothesis that in the ana-telophase the chromosomes being monochromatidic have the same amount of DNA like the interphase $G_1$-stage nuclei. The readings were accomplished on the ana-telophases in the meristematic tissue of the radicels (squash preparations, Feulgen stain). For each species measurements were performed for 50 chromosome groups in ana-telophase, using the wavelengths of 485 nm (La) and 505 nm (Lb). The DNA amount, expressed in arbitrary units for each ana-telophase (corresponding to a nucleus in $G_1$-stage), was calculated using the M. L. Mendelson tables [4]; the results showed in Table 1 represent the individual average values recorded.

## RESULTS AND DISCUSSION

The values of the two radiobiological indices, both in the case of X-rays, and in the case of fission neutrons irradiation as well as the DNA amount recorded in the species studied are presented in Table 1. We mention that the $D_{50/30}$ values of *Nigella doerfleri* (No. 7) were established estimatively on the growth curve basis, and for the *N. degenii jenny* and *N. orientalis* species, the stem length recorded at the irradiation with a dose of 1000 R fission neutrons is almost similar to the $D_{50/30}$ values calculated.

The existent correlation between the DNA amount per nucleus or per chromosome and the radiobiological indices studied are shown in Fig. 2. Its analysis points out the fact that both in the case of X-rays and in the case of fission neutrons, no correlation between the slight growth inhibition and the DNA amount per nucleus or per chromosome is found (the correlation coefficients : $r = + 0.02$, respectively $r = - 0.09$). Between the $D_{50/30}$ value and the DNA amount, however, the existence of a good negative correlation is found both in the case of X-rays ($r = - 0.78$), and the fission neutrons ($r = - 0.54$, or in the case of the omission of the *Nigella doerfleri* species, $r = - 0.50$).

The fact that the value of the correlation coefficient is different from — 1, shows that between the DNA amount and the plant radiosensitivity there is not a simple linear relation, as also other parameters, besides the DNA amount, intervene in determining the radiosensitivity. The established correlation coefficient can be influenced by the limits of the method used for the DNA amount determination, too.

## CONCLUSIONS

The study of the correlation between the radiosensitivity (examined by the two growth inhibition grades) and the DNA amount, performed in 10 species and one variety of *Nigella* genus, reveals the following :



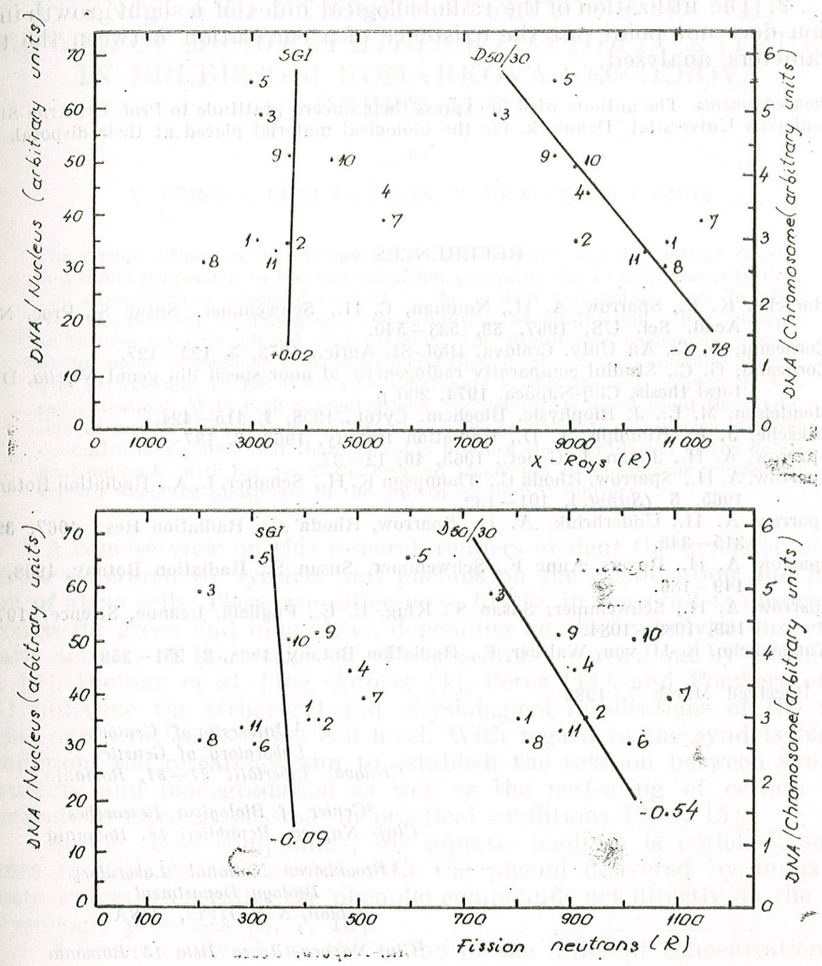


Fig. 2. — The correlation between the necessary exposure to produce slight stem growth inhibition or $D_{50/30}$ values and the DNA amount per nucleus or per chromosome for ten species and one variety of *Nigella*, submitted to acute irradiation with X-rays or fission neutrons. 1 — *N. arvensis aristata*; 2 — *N. arvensis arvensis*; 3 — *N. ciliaris*; 4 — *N. damascena* — single flower; 5 — *N. damascena* — double flower; 6 — *N. degenii jenny*; 7 — *N. doerfleri*; 8 — *N. hispanica*; 9 — *N. nigellastrum*; 10 — *N. orientalis*; 11 — *N. sativa*.

1. The existence of a negative correlation between the DNA amount and the $D_{50/30}$ value both in the case of X-rays irradiation ($r = - 0.78$), and in the case of fission neutrons irradiations ($r = - 0.54$). The fact that $r$ has a value different from — 1 points to the lack of a simple linear relation between the radiosensitivity and the DNA amount.

2. The utilization of the radiobiological index of a slight growth inhibition does not point out the existence of a correlation between the two parameters analysed.

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# PHENOL AND SYNDETS INFLUENCE ON THE GROWTH OF *MONORAPHIDIUM CONTORTUM* (THURET IN BRÉBISSON) KOMÁRKOVA-LEGNEROVÁ GREEN ALGA

BY

V. BERCEA, LENUȚA TICAN, N. DRAGOȘ and I. OROS

The phenol influences the increase of the growth rate and the optical density in a direct proportion to the concentration excepting the 10 mg/l concentration. The stimulation is rendered evident in the protein metabolism, too. In what concerns pigment accumulation, the inhibition or stimulation effect presents an inverse ratio to phenol concentration. The stimulation phenomera observed in some physiologic processes suggest that the alga could metabolize phenol. The TRIAL and DERO CRISTAL detergents favour the alga growth in low concentrations. With high concentrations, the detergents bring about the inhibition of growth and physiological processes. The growth rate stimulation at lower concentrations shows that the alga could produce the degradation of the detergent molecule, with the use of the resulted fragments as phosphorus sources for growth when the phosphate in the growth medium is limitary.

A concise view on this research renders evident the diversity of the effects generated by syndets and phenols on the organization and function of algae cells. There are differences, firstly, in the syndets absorption process for green and blue algae, depending on the hydrolytic and enzymatic activity of their cells [5]. The researches carried out by Doemel et al. [6], Hannan et al. [10], Aubert [1], Pérès [14], and Forsberg et al. [8] underline the structural and physiological implications of the syndets concentrations at alga cell level. With regard to the syndets biodegradation, the researches aim to establish the relation between syndets structure and biodegradation as well as the perfecting of certain test methods as close as possible to practical conditions [2, 4, 15].

Beside these compounds, the aquatic medium is enriched, sometimes in high concentrations, with the phenol delivered by industrial waste waters. Generally, the phenolic compounds act directly on the alga physiologic processes [3, 7, 13].

A recent study shows the action of the different concentrations of phenols and syndets on the growth of the *Monoraphidium contortum* green alga, based on the determination of some cell biochemical values.

## MATERIAL AND METHOD

As testing material, phenol and TRIAL, and DERO CRISTAL detergents were used on the experimental alga *Monoraphidium contortum* cultivated on Zehnder-Gorham medium [16]. The alga was grown in thermostat conditions at 25°C, under continuous illumination of 4500–5000

lx. The concentrations of phenol (mg/l) and of detergents (g/l) are shown in the graphs.

After 14 days the following parameters were determined: optical density (extinction), growth rate, content of assimilatory pigments and total (soluble) proteins. The optical density was colorimetrically determined in FEK photocolorimeter, using red filter and 5 cm³ absorption cell. The growth rate was approximated based on the cell number by haemocytometry. The determination of assimilatory pigments was performed by thin layer chromatography apud Hager and Bertenrath [9], using Mackinney's [12] reckoning formulas, and for proteins the Lowry method [11].

## RESULTS

Phenol increases the alga growth rate significantly and proportionally to the concentration le vel, excepting the 10 mg/l concentration in which the growth is slightly i nhibited (Fig. 1). The optical density evolution resembles the growth rate with significant stimulations at high concentrations of phenol.

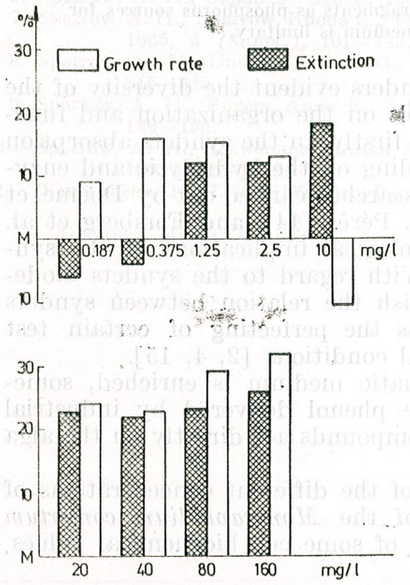


Fig. 1. — Influence of phenol on growth rate and optical density in the *Monoraphidium contortum* culture.

The values of assimilatory pigments content range on an undulating curve; it shows an inhibition of the pigments accumulation at low concentrations and a stimulating action at high concentrations of phenol (Fig. 2). The ratio between chlorophylls, carotenes, and xanthophylls varies with the phenol concentrations, rendering evident a slight inhibition of *a* and *b* chlorophyll accumulation at 0.187 mg/l, 1.25 mg/l concentrations. Phenol acts directly on the *a* and *b* chlorophyll biosynthesis; ts sti mulatory action is obvious in the lowering of the ratio in pigments



at concentrations of 10, 40, and 80 mg/l (Fig. 2). The process of protein biosynthesis continues to be stimulated by phenol, without a proportional dependence between effect and concentration (Fig. 2).

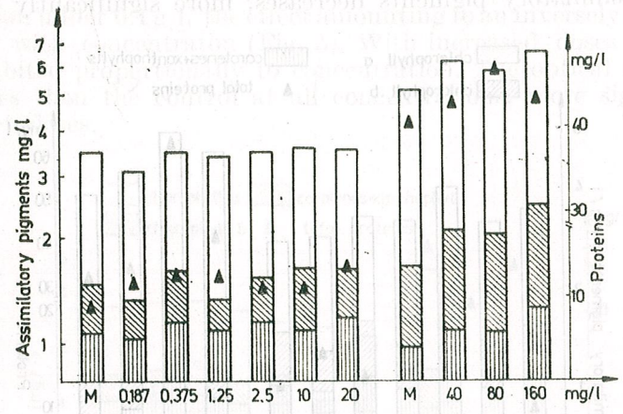


Fig. 2. — Action of phenol concentrations on assimilatory pigments and total (soluble) protein content.

In what concerns the syndets effects on *Monoraphidium contortum* cultures growth, both a chemical specificity and one depending on the detergent concentration are observed.

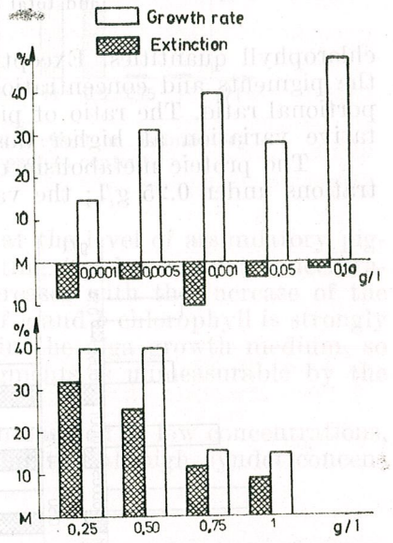


Fig. 3. — Modifications of growth rate and extinction as compared to the control induced by the TRIAL syndet.

The TRIAL detergent produces a significant stimulation of the growth rate at concentrations under 0.50 g/l, which decreases with the increase of concentration (Fig. 3). The optical density presents lower values as compared to the control, at concentrations under 0.10 g/l. With increased concentrations, the optical density values become higher as

compared to the control, and they are correlated with the growth rate.

The content of assimilatory pigments depends on the detergent concentration (Fig. 4). Little by little, by increasing the dose, the content of assimilatory pigments decreases, more significantly the *a* and *b*

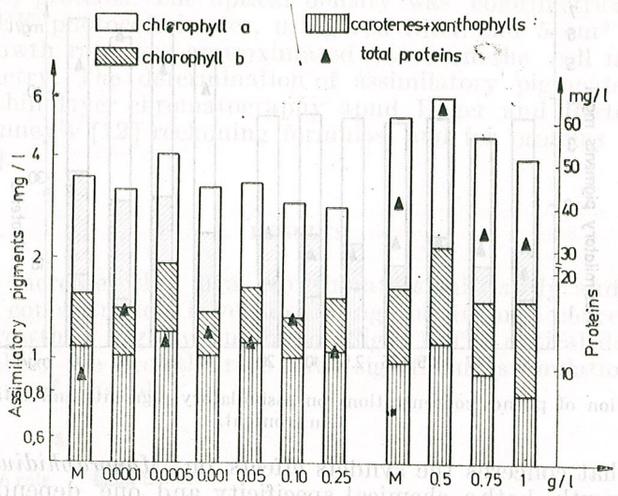


Fig. 4. — Effect of the TRIAL syndet on assimilatory pigments and total (soluble) protein content.

chlorophyll quantities. Excepting 0.0005 g/l and 0.5 g/l concentrations, the pigments and concentration stand in approximately a directly proportional ratio. The ratio of pigments renders evident a stronger quantitative variation at higher doses of detergents.

The proteic metabolism does not seem to be influenced by concentrations under 0.25 g/l; the values are higher than those in the control

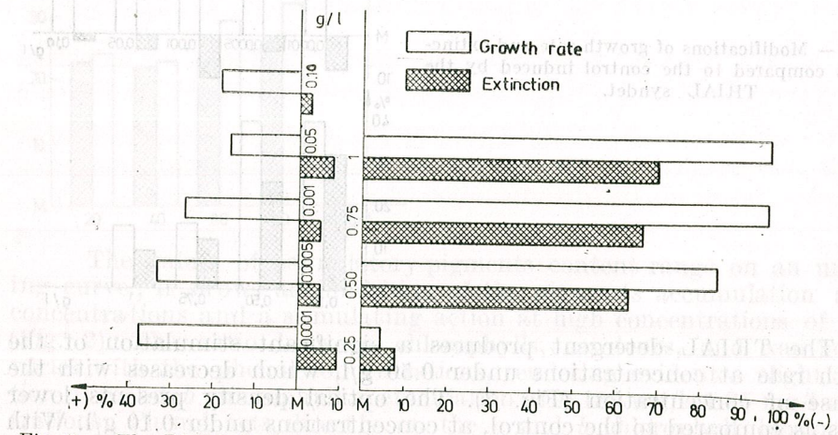


Fig. 5. — The DERO CRISTAL syndet influence on growth rate and cultures extinction.



(Fig. 4). With increased doses, the protein biosynthesis is directly influenced by the detergent, the values being more reduced than in the control.

The DERO CRISTAL syndet stimulates alga growth rate at a concentration under 0.1 g/l, the effect amounting to an inversely proportional relation with concentraton (Fig. 5). With increased doses the growth rate is inhibited proportionally to concentration. The optical density has lower values than the control at all concentrations, more significant at high syndet doses.

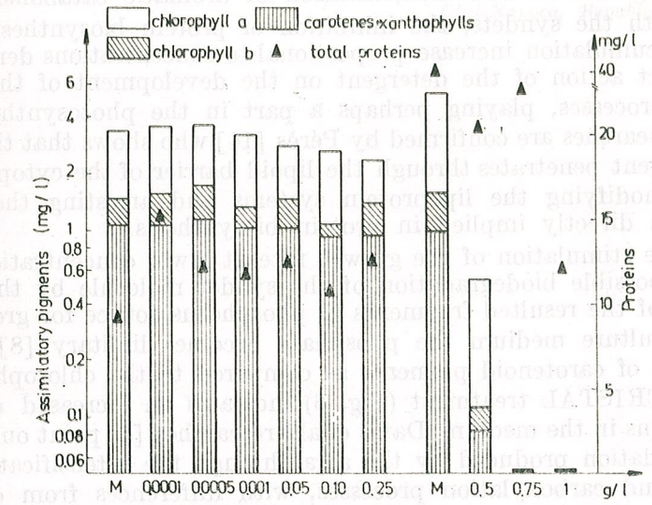


Fig. 6. — The DERO CRISTAL syndet effect on assimilatory pigments and total (soluble) protein content.

The toxic effect of the syndet lasts at the level of assimilatory pigments accumulation, too (Fig. 6). Excepting the low syndet concentrations, the pigment content gradually decreases with the increase of the syndet concentration. The accumulation of *a* and *b* chlorophyll is strongly influenced by the presence of detergent in the alga growth medium, so that with high doses the quantity of pigments is unmeasurable by the method used.

The protein biosynthesis process is intensified at low concentrations, less than 0.25 g/l, being significantly inhibited at high syndet concentrations.

## DISCUSSIONS

The fact that phenol intensifies the growth rate of the *Monoraphidium* alga confirms the research of Cairns et al. [3] on *Scenedesmus* and *Chlamydomonas* algae. The researches carried out by Mihnea et al. [13]

underline the stimulating effect of phenol on sea phytoplankton multiplication by the modification of the cell division rate and cell size, to the effect of their growth. The stimulating effect of phenol on growth processes and protein biosynthesis is probably caused by the decomposition of phenol by the alga and the use of carbon fragments as energy source ; it directly influences the pigments and the chlorophyll biosynthesis, increasing the photosynthetic capacity of the alga. As a matter of fact, Ellis' researches [7] show that the population of fresh water algae may remove the phenolic compounds from the aquatic medium by enzymatic degradation and intensification of aromatic catabolism.

With the syndets, the inhibition of protein biosynthesis and pigment accumulation increase proportional to concentrations demonstrating the direct action of the detergent on the development of these physiological processes, playing perhaps a part in the photosynthesis process. These researches are confirmed by Pérès [14] who shows that the molecule of detergent penetrates through the lipoid barrier of the cytoplasm membrane, modifying the lipoprotein systems and arresting the oxidative processes directly implied in protein biosynthesis.

The stimulation of the growth rate at lower concentrations underlines a possible biodegradation of the syndet molecule by the alga and the use of the resulted fragments as phosphorus source for growth, when in the culture medium the phosphate becomes limitary [8]. The high quantity of carotenoid pigments as compared to the chlorophylls in the DERO CRISTAL treatment (Fig. 6) indicates an increased quantity of phosphorus in the medium. Davis et al's researches [4] point out a possible biodegradation produced by the alga through the intensification of oxidation and carboxylation processes, with differences from one species to another. The higher concentrations of detergent cancels this capacity of the alga, so that the toxic effect on the growth and biosynthesis processes is intensely manifest.

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# L'EFFET DE L'HERBICIDE 2,4-D SUR LA STRUCTURE DES ORGANES VÉGÉTATIFS DE *XANTHIUM STRUMARIUM* L.

PAR

C. TOMA, RODICA RUGINĂ, MIHAELA NIȚĂ

The 2,4-D herbicide having the concentration of 200 mg/l brings about changes in the structure of all vegetative organs, particularly in the axial ones, changes that are equivalent to the hypertrophy and hyperplasia of the phloems as well as to the inhibition of tracheogenesis and of the lignification process. The maximum action of the herbicide is recorded 10 — 15 days after the treatment has been applied at the base of the root. The most sensitive organs are the phloems and of course the cambium. Correlating the histoanatomical data with the morphological ones and comparing them with the results obtained with other species, we conclude that *X. strumarium* belongs to the group of weeds that are moderately sensitive to the action of phenoxyacetic herbicides.

L'application du produit 2,4-D comme herbicide est depuis longtemps une méthode utilisée dans la pratique agricole, et pourtant on continue les recherches pour élucider certains aspects morpho-anatomiques, physiologiques et biochimiques à même d'expliquer le mécanisme d'action et les processus liés à l'apparition des anomalies de structure chez les mauvaises herbes et chez les plantes de culture.

De la littérature consultée [5, 6, 10, 13, 17] résultent la diversité des aspects étudiés jusqu'à présent, les différentes perturbations morpho-anatomiques qui peuvent apparaître sous l'action de l'herbicide 2,4-D, les organes et les tissus sur lesquels l'effet inhibiteur ou stimulateur est plus fort, la façon de se comporter surtout des tissus vasculaires et la direction dans laquelle est stimulée l'activité cambiale, le degré de sensibilité des différents organes et tissus, la concentration à laquelle 2,4-D est un inhibiteur ou un stimulateur de la croissance. En Roumanie, de telles recherches [2, 3, 4, 14, 15, 16] ont porté surtout sur des espèces de mauvaises herbes autres que *X. strumarium* et même sur certaines plantes de culture [18]. De la littérature étrangère il résulte qu'il n'y a que peu de chercheurs qui ont étudié des espèces du genre *Xanthium* soit séparément, soit dans le cadre des traits histo-anatomiques des plantes gammopétales [19] ou bien des dicotylédones en général [7, 11]. En ce qui concerne *X. strumarium*, dans un article antérieur [12] nous nous sommes occupés de certains aspects morphologiques chez des individus normaux et modifiés par le traitement avec 2,4-D ; dans ce qui suit, on va discuter l'influence de 2,4-D sur la structure des organes végétatifs en ontogenèse, en poursuivant la réaction des différents tissus, particulièrement de ceux méristématiques et vasculaires.

Rev. Roum. Biol.—Biol. Végét., Tome 29, N° 1, p. 53—60, Bucarest, 1984

## MATÉRIEL ET MÉTHODE DE TRAVAIL

Le matériel à étudier provient de graines récoltées de la flore spontanée et mises à germer le 20 avril sur le terrain expérimental du Jardin botanique de Iassy. L'herbicide, un sel de l'acide 2,4-dichlorophénoxyacétique en concentration de 200 mg/l, a été administré sous forme de solution après l'émergence (dans la phase de plantule âgée de 18 jours), par arrosement unique. Le matériel a été récolté en 6 étapes ontogéniques chez le témoin (à partir du 8 mai jusqu'au 8 juin, les plantules ayant 3, 9, 16, 22, 28 et 34 jours) et en 3 étapes ontogéniques dans la variante expérimentale (le matériel traité ayant 4, 10 et 16 jours depuis l'application de l'herbicide).

L'étude anatomique des organes végétatifs a été faite sur le matériel fixé, conservé, sectionné, coloré et monté d'après les procédés employés couramment dans les études d'anatomie végétale. Les coupes transversales (au microtome de paraffine Minot) ont été pratiquées dans la racine principale (au sommet et à la base), dans l'hypocotyle, l'épicotyle, la tige foliée et dans les feuilles. Les dessins, faits avec Projektionszeichenspiegel, au microscope L-Zeiss, sont groupés dans les planches I et II, et les photos, prises au microscope Amplival, sont groupées dans la planche III.

## RÉSULTATS

### 1. Témoin (Pl. I)

La *racine* a un endoderme de type primaire (avec les bandes de Caspary) et un cylindre central de type tétrarque. Le passage à la structure secondaire a lieu précocement, avec des scléréides et des cellules oxalifères se formant dans le parenchyme cortical et des canaux sécréteurs devant les faisceaux vasculaires, en même temps que des tissus conducteurs de type annulaire, leurs éléments constitutifs ayant les parois modérément sclérifiées et lignifiées. Dans la racine principale, la moelle persiste jusqu'à la maturation de la plante. Les radicelles ont un xylème compact (donc la moelle est absente) et dans le phloème se forment deux cordons opposés de fibres sclérenchymatiques collés au péricycle.

À la base, la racine a une structure de transition vers celle de la tige, avec un anneau xylémique très épais, traversé par de nombreux rayons médullaires larges, pluriséries et lignifiés qui le divisent en cordons ±distincts, la plupart groupés par couples, les pôles xylémiques primaires des deux cordons convergeant l'un vers l'autre. L'épiderme et l'écorce hypodermique (à cellules scléreuses), de même que les cordons de fibres péricycliques, sont des traits histologiques propres à la tige.

*L'hypocotyle* a une structure ± pareille à celle de l'épicotyle et de la tige foliée, à cette différence près que la disposition des vaisseaux de xylème rappelle celle de la région de transition. L'écorce externe représente un collenchyme angulaire de type méatique, et celle interne, parenchymatique, finit par un endoderme de type primaire. Le tissu conducteur est de type fasciculaire, le nombre des faisceaux vasculaires augmentant avec l'âge de la plante. À la périphérie des faisceaux vasculaires se trouvent des cordons de fibres sclérenchymatiques.



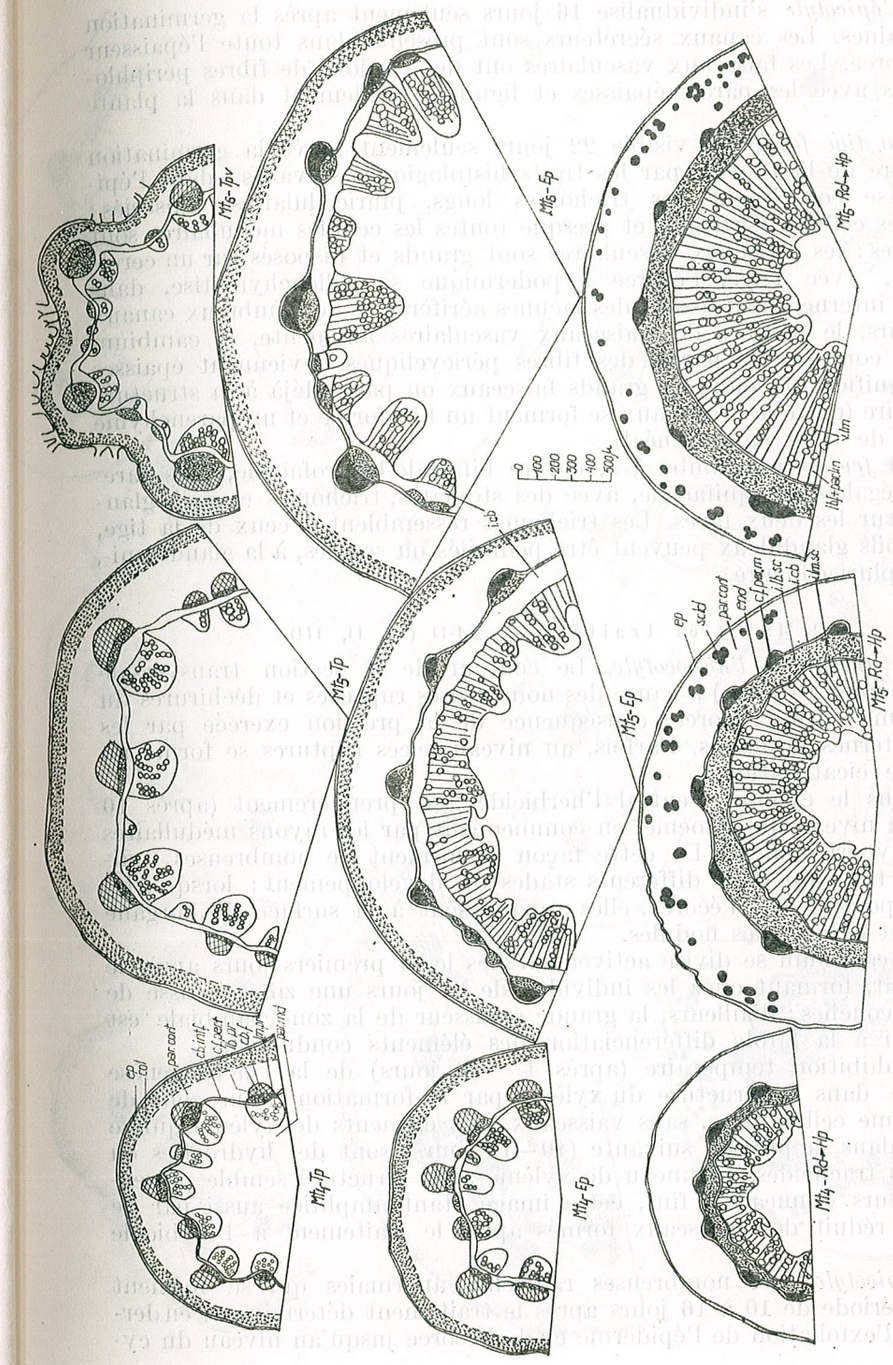


*Planche I.* — Schémas des sections transversales dans la tige foliée (Tp), dans l'épicotyle (Ep) et dans la région de transition (Rd, racine → Hp, hypocotyle) du témoin (Mt); 4,5,6, matériel de 22 jours (4), 28 jours (5) et 34 jours (6); cb, cambium (f, fasciculaire, inf, interfasciculaire); c.l.per, cordons de fibres péricycliques (t, jeunes, m, mûres); col, collenchyme; end, endoderme; ep, épiderme; i, anneau (cb, cambial, lb. sc, libérien secondaire, lm. sc, ligneux secondaire); lbf, libriforme; lm, ligneux, md, médullaire); scld, groupes de scléréides; v. lm, vaisseaux du bois.

*L'épicotyle* s'individualise 16 jours seulement après la germination des graines. Les canaux sécréteurs sont présents dans toute l'épaisseur de l'écorce. Les faisceaux vasculaires ont des cordons de fibres périphloémiques avec les parois épaisses et lignifiées seulement dans la plante adulte.

*La tige foliée* est visible 22 jours seulement après la germination et diffère de l'épicotyle par les traits histologiques suivants : dans l'épiderme se constituent des trichomes longs, pluricellulaires, unisériés ; certaines cellules corticales et presque toutes les cellules médullaires sont oxalifères ; les faisceaux vasculaires sont grands et disposés sur un cercle sinueux. Avec l'âge, l'écorce hypodermique se collenchymatise, dans l'écorce interne apparaissent des lacunes aérifères et de nombreux canaux sécréteurs, le nombre des faisceaux vasculaires augmente, le cambium devient continu, les parois des fibres péricycliques deviennent épaisses et se lignifient, et dans les grands faisceaux on passe déjà à la structure secondaire (entre les vaisseaux se forment un libriforme et un parenchyme ligneux de type paratrachéal).

*La feuille* a le limbe à structure bifaciale-hétérofaciale, plus rarement inégalement-équifaciale, avec des stomates, trichomes et poils glanduleux sur les deux faces. Les trichomes ressemblent à ceux de la tige, et les poils glanduleux peuvent être pédicélés ou sessiles, à la glande uni-, bi- ou pluricellulaire.

### 2. Matériel traité avec 2,4-D (Pl. II, III)

*La racine* et *l'hypocotyle*. Le contour de la section transversale change (après 16 jours) à cause des nombreuses ruptures et déchirures du rhizoderme et de l'écorce, conséquence de la pression exercée par les tissus internes proliférés. Parfois, au niveau de ces ruptures se forme un suber de cicatrisation.

Dans le cylindre central l'herbicide agit premièrement (après 10 jours) au niveau du phloème, en commençant par les rayons médullaires qui se hypertrophient. De cette façon se forment de nombreuses radicelles se trouvant dans différents stades de développement ; lorsqu'elles ne transpercent pas l'écorce, elles apparaissent à la surface de l'organe seulement comme des nodules.

Le cambium se divise activement dès les 4 premiers jours après le traitement, formant chez les individus de 34 jours une zone épaisse de 10 — 12 couches ; d'ailleurs, la grande épaisseur de la zone cambiale est due aussi à la faible différenciation des éléments conducteurs.

L'inhibition temporaire (après 4 — 10 jours) de la trachéogenèse se reflète dans la structure du xylème par la formation d'une zone de parenchyme cellulosique, sans vaisseaux. Les éléments de xylème qui se forment dans la période suivante (10—16 jours) sont des hydrocites ou des fibres trachéides. L'anneau de xylème ainsi structuré semble présenter plusieurs « anneaux » fins, cette image étant amplifiée aussi par le diamètre réduit des vaisseaux formés après le traitement à l'herbicide 2,4-D.

*L'épicotyle*. Les nombreuses radicelles anormales qui se forment dans la période de 10 à 16 jours après le traitement déterminent, en dernier lieu, l'exfoliation de l'épiderme et de l'écorce jusqu'au niveau du cy-



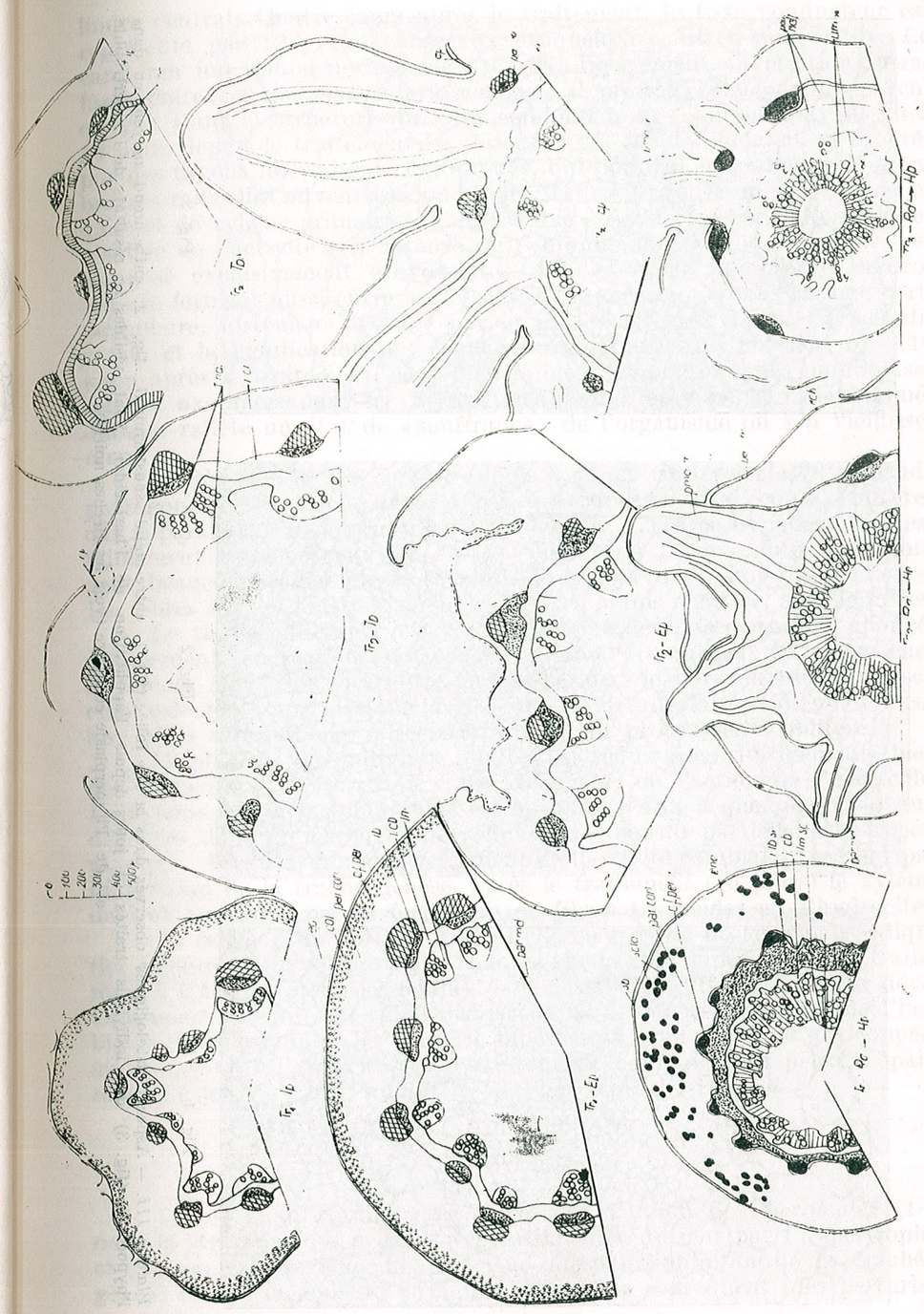

*Planche II.* — Schémas des sections transversales dans la tige foliée (Tp), dans l'épicotyle (Ep) et dans la région de transition (Rd, racine → Hp, hypocotyle) des individus traités (Tr) avec 2,4-D ; 1, 2, 3, matériel de 22 jours (1), 28 jours (2) et 34 jours (3) ; eol. c.f. per (t, m), end, ep, i (cb, lb. sc, lm. sc), lb, lm, par (cort, md), scld : voir Pl. I ; par. ndf, parenchyme non différencié, pmd. rd, primordium radicellaire ; pt, trichomes ; rp, rupture ; sb, suber ; t. lg, tissu lignifié ; t. ndf, tissu non différencié.

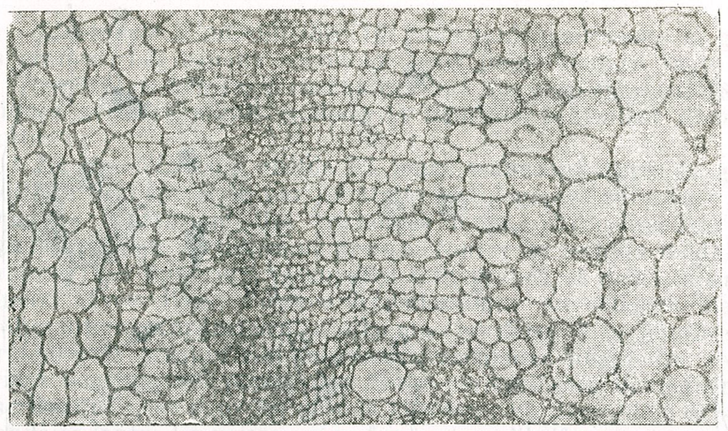

Fig. 3

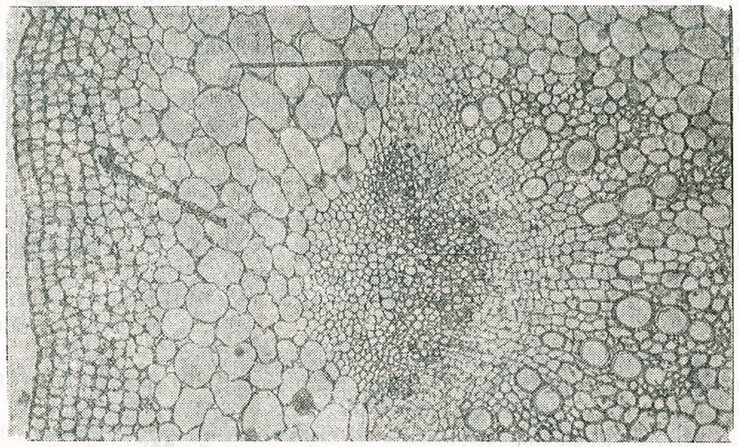

Fig. 2

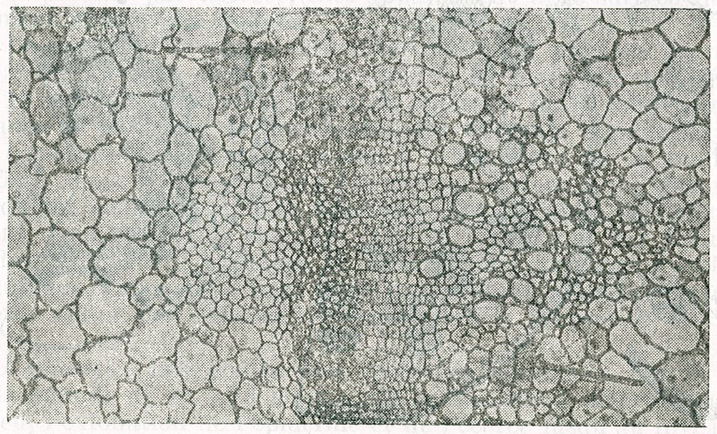

Fig. 1

*Planche III.* — Aspects histologiques observés dans la tige foliée (fig. 1, entre-nœud sousterminal; fig. 2, entre-nœud moyen) et dans l'hypocotyle (fig. 3) des individus traités (4 jours depuis l'application de l'herbicide). Sont visibles les résultats de l'hypertrophie et de l'hyperplasie cellulaire (voir les flèches noires).



lindre central. Quatre jours après le traitement, le tissu conducteur est représenté par 12 grands faisceaux et quelques autres plus petits. Le cambium fonctionne normalement et très activement en position intrafasciculaire; en position interfasciculaire il produit beaucoup de parenchyme. Dans la structure du tissu conducteur des individus ayant 10 à 16 jours depuis le traitement les faisceaux restent distincts, étant séparés par des rayons libéro-ligneux proliférés, qui donnent naissance à de nombreuses radicelles adventives anormales. Entre les zones de xylème secondaire et de xylème primaire s'interpose une épaisse zone parenchymatique, résultée de l'activité très intense du cambium. Le xylème secondaire est composé exclusivement d'hydrocites. D'ailleurs, de nombreuses hydrocites se forment aussi entre les faisceaux, de même que dans la zone périmédullaire, fortement affectée par le processus de division. La sclérification et la lignification des éléments xylémiques, qui ont lieu 10 — 16 jours après le traitement, sont bien faibles. La présence des nombreuses cellules oxalifères dans les rayons médullaires et dans la zone périmédullaire reflète un état de « souffrance » de l'organisme ou son vieillissement.

*La tige foliée.* Le contour de la section transversale (d'habitude pentagonal) est parfois modifié par des ruptures de l'écorce, ruptures qui apparaissent 10 jours après le traitement. L'écorce hypodermique est faiblement collenchymatisée, et les cellules de l'écorce interne s'allongent beaucoup radialement, se segmentant par des parois tangentielles. Les fibres des cordons péricycliques ont les parois épaisses, non lignifiées.

Le tissu conducteur est représenté par des faisceaux très allongés radialement (en section transversale) et moins nombreux (12) que chez le témoin (18). Dans les entre-nœuds basaux, le parenchyme interfasciculaire devient hyperplasique (à 10 jours), la manifestation s'étendant aussi aux pôles internes des faisceaux, autour du protoxylème oblitéré.

Le phloème des individus adultes est réduit quantitativement, bien des éléments se nécrosant à cause, bien sûr, de l'action de l'herbicide. Le xylème des mêmes individus est lui aussi réduit à quelques vaisseaux dispersés dans un parenchyme cellulosique devenu partiellement hyperplasique. A cause du bouleversement de l'activité cambiale, terminé par l'inhibition de la trachéogenèse, après le traitement (16 jours) le xylème devient atypique, par la formation de fibres trachéides et d'hydrocites.

*La feuille* a un limbe à mésophylle en totalité de type palissadique (les cellules étant pourtant plus hautes sur la face supérieure). Le traitement à 2,4-D surprend les feuilles dans des stades différents de leur développement, ce qui détermine des réactions différentes vis-à-vis de l'herbicide. Par exemple, les feuilles supérieures — encore non développées au moment de l'application du traitement — augmentent peu en épaisseur, à cause de l'effet inhibiteur de l'herbicide 2,4-D.

## CONCLUSIONS

1. Les modifications provoquées par l'action de l'herbicide 2,4-D dans la structure des organes végétatifs se traduisent par l'hypertrophie et l'hyperplasie du phloème, de même que par l'inhibition de la trachéogenèse et du phénomène de lignification. Par conséquent, des perturba-

tions apparaissent dans la circulation de la sève « brute » et de la sève « élaborée », qui — en corrélation avec celles manifestées par des ruptures et des déchirures de l'écorce (à cause de la prolifération des nombreuses radicelles adventives anormales) — mènent à la mort de la plante. C'est d'ailleurs ce qu'on poursuit par l'application de l'herbicide sur les mauvaises herbes de différentes cultures agricoles.

2. L'action la plus intense a lieu 10 à 16 jours après le traitement, à la base de la racine et de la tige ; les tissus les plus sensibles sont le phloème et, naturellement, le cambium.

3. L'herbicide 2,4-D agit sur la feuille en tant que défoliant et inhibiteur de la croissance de ses tissus.

4. En mettant en corrélation les données histo-anatomiques et celles morphologiques présentées antérieurement [12] et en les comparant à nos résultats ou à ceux d'autres auteurs pour différentes espèces de mauvaises herbes, on peut conclure que *Xanthium strumarium* L. fait partie du groupe de mauvaises herbes à sensibilité moyenne envers l'action des herbicides phénoxyacétiques. Nous parlons, bien sûr, de la concentration employée par nous (et recommandée par les spécialistes pour les cultures agricoles), qui n'est pas nuisible aux plantes utiles, surtout aux monocotylédones.

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# COMPARATIVE STUDY ON THE ULTRASTRUCTURE OF SOME 2n, 3n, 4n LINES OF *CITRULLUS LANATUS* (Thunb.) Mansf. I. ULTRASTRUCTURE OF NUCLEUS IN 2n AND 4n VEGETATIVE CELLS

BY

I. ANGHEL, N. TOMA, AURELIA BREZEANU

The comparative study of diploid and tetraploid plants of *Citrullus lanatus* revealed the existence of a tight correlation between the ploidy level and the morphoanatomical (macro and microscopical), biochemical and physiological features.

Modification of the nucleus / cytoplasm ratio, by increasing the chromosome number, produced strong modifications during cell division, fecundation and seed formation.

The electronomicroscopic study permitted us to reveal the changes at various cell structure levels, the modification of cell division mechanisms and in this way to elucidate, at least partly, the causes of low fertility of tetraploid plants and of total sterility of triploid ones.

At present, the nucleus is investigated by several well-known teams of research workers in the world and the interest in the field is even increasing. It is natural to be so if we consider the major implications of such a study in the fundamental and applicative biology ; elucidation of the mechanisms by which matter and energy are transformed within the living cell, the genuine transmission of genetic information, selfcontrol and self-preservation, cell differentiation and ageing, malignant transformation a.s.o., all these are closely interconnected with the information on the nucleus, as it is the essential component of the eukaryotic cell coordinating the whole vital activity.

There are several aspects of this important problem, the molecular ones being of course the most interesting. It is generally accepted today the idea that as a rule a convergent multidisciplinary study may assume the advancement in the field.

Starting from this assumption, our investigations on the ultrastructure of some *Citrullus lanatus* lines with various degrees of ploidy were first directed to the study of the nucleus, in order to reveal new aspects, especially those referring to the fine structure of 2n, 3n, 4n nuclei.

The present paper is just a first step to the elucidation of the ultrastructure of *Citrullus lanatus* nucleus, but at the same time an improved way of approaching the cytogenetic study initiated about two decades ago [1].

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## MATERIAL AND METHOD

Our investigations were carried out on some primary root and stem meristems. The apical fragments of young roots and stem buds were collected. The young roots were obtained from seeds after their germination in laboratory conditions, while the buds were collected from imature plants in the field.

The material — after a fine fragmentation — was fixed in glutaraldehyde 3.5% in phosphate buffer, pH = 7.2, at room temperature for two hours. After rinsing (5×—10×) the material was kept in phosphate buffer overnight — 16 hours in the refrigerator — then postfixed with a solution 1.0% of $OsO_4$ for two hours at room temperature and in the dark. The material dehydration was performed in alcohol and propylene oxide and afterwards included in Epon 812. The ultrasections obtained with a Tesla ultratom BS 490 A were stained with uranyl acetate and lead citrate Aceto-Reynolds and afterwards examined under a Philips 200 and Jem 7 electronmicroscope, at an acceleration speed of 80 kV.

## RESULTS AND DISCUSSION

The general ultrastructural characters of nuclei in meristematic cells of *Citrullus lanatus* 2n and 4n are similar to those in other organisms of higher plants [3]; [5]; [8]; [9]; after the analysis of a large number of electronomicroscopic images some aspects revealed by us come to emphasize the previously mentioned conclusions by means of light microscopy [1].

Therefore, in all cells, the nucleus is large, the nucleus/cytoplasm ratio being 1/3; when the value of this relationship has a tendency to be higher the cell starts dividing, reestablishing the normal balance.

At first sight the nucleus seems to be spherical or oval (Pl. I); during the differentiation process it becomes irregular in shape sometimes sinuously lobated, with deep enclaves, with a considerable larger nuclear metabolic surface, with a better contact between the cytoplasm and the nucleus and therefore a more rapid and efficient exchange of material and energy between the karyoplasm and the cytoplasm.

The ultrastructural elements, clearly revealed by all electronographs, are the nuclear envelope, chromonemata, karyoplasm and nucleolus.

The two elementary (unitary) membranes (of the nuclear envelope) following a sinuous line are of about the same thickness (about 7 nm) and separated by a perinuclear space of 12—18 nm in diameter.

Here and there, these two membranes fuse and circular perforations (pores) partly obturated by annules are formed. In spite of detailed studies on nuclear pores, the structure and function of a nuclear pore complex are little understood.

The data support the viewpoint according to which the pores are preferential places of material exchange between the cytoplasm and the nucleoplasm. The precise mechanisms of transport are still unclear. The

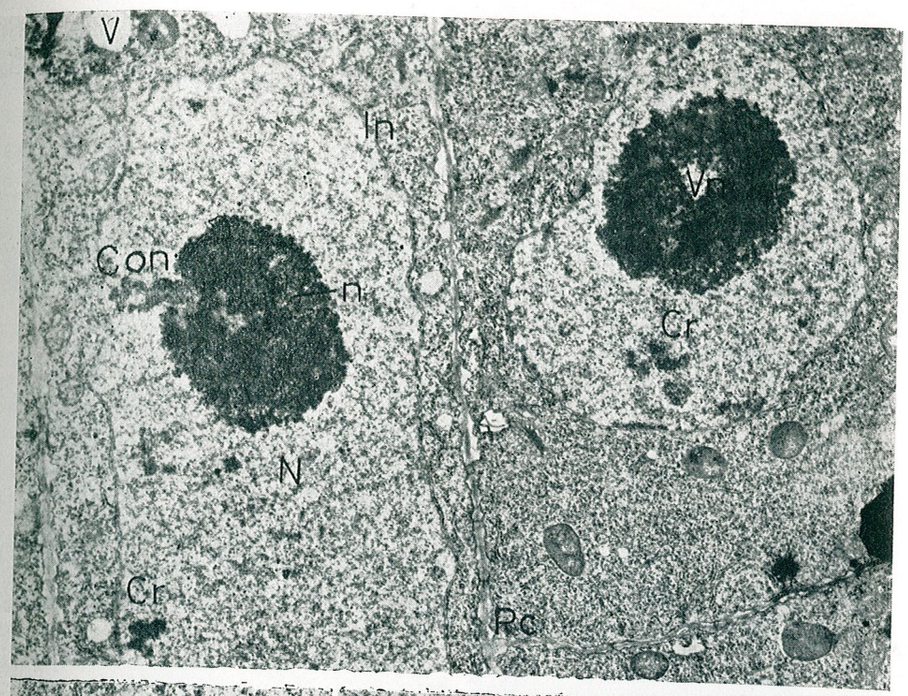


Plate I. — Ultrastructure of the root meristematic cell of *Citrullus lanatus* —Brăila variety (2n) — the nucleus shape, the nucleoplasmatic relationship, the structural elements of the nucleolus and the nucleolar organizer are evident (× 13,650). The inserts reveal the ultrastructure of the nuclear area with the nucleolus and nucleolar organizer in a cell of the stem meristem — Arad variety (4n) (× 12,500) and a detail of the nucleolus (× 45,000).
Cr — chromatine; In — nucleolar envelope; N — nucleus; N — nucleolus; Con — nucleolar organizer chromosome; Vn — nucleolar vacuole; Pc — cell wall; V — vacuole; zg — granular zone; zgf — granular fibrillar zone.

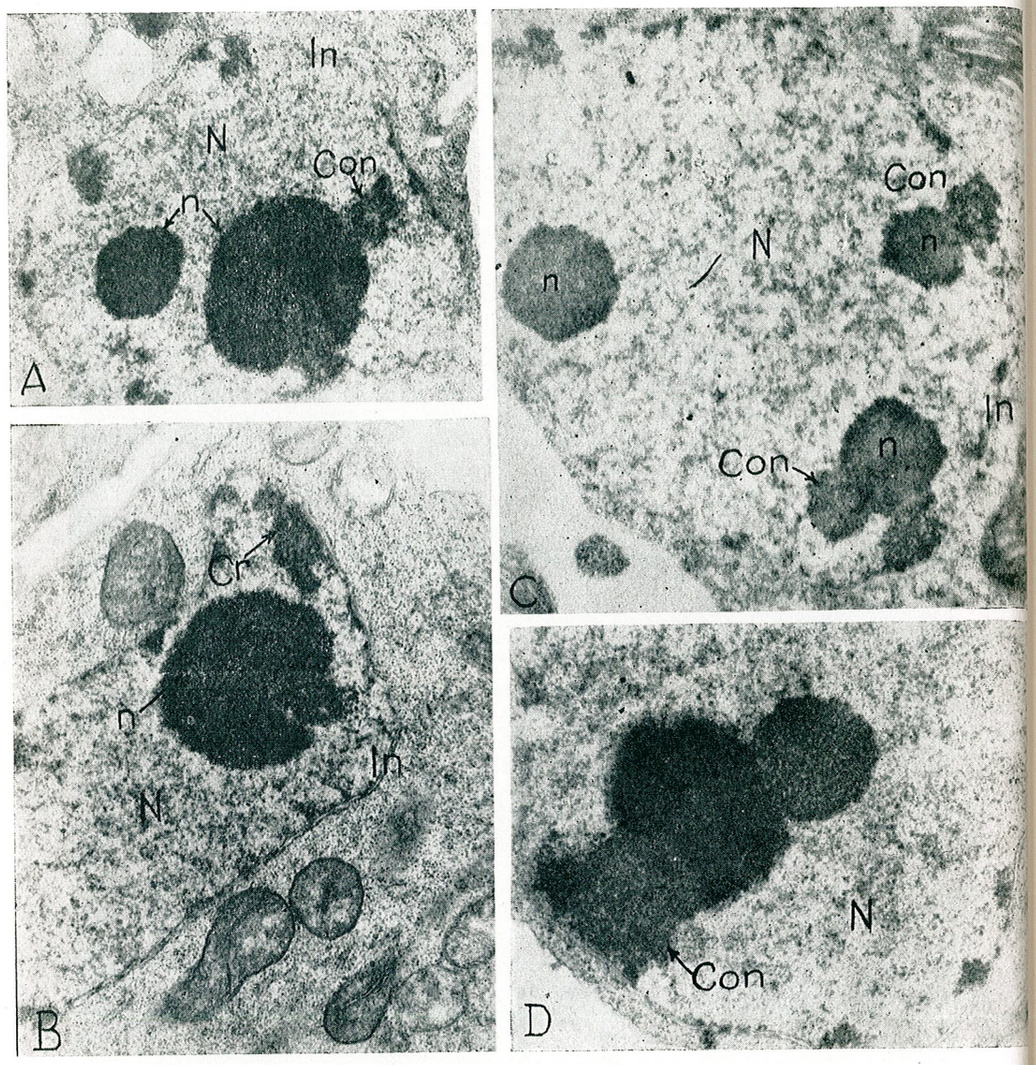


Plate II. — Ultrastructure of the root meristem cells nucleus of the Brăila variety (4n) (× 13,000). Con — nucleolar organizer chromosomes; Cr — chromatine; In — nuclear envelope; N — nucleus; n — nucleolus.



frequency of pores as well as their size vary considerably. We insist on the idea of a direct, positive correlation between the pores density and the intensity of cellular metabolic processes in general and nuclear in special. Variations regarding the diameter of nuclear pores appear even within the same nucleus. In some territories of the nuclear envelope the pores diameter is no larger than 120 nm, while in others is 180 nm.

We support a newer interpretation according to which the variation in the nuclear pores diameter is a result of a pulsatile activity evident in all pores, rather than statical differences between individual pores [11]. The karyoplasm of interkinetic nuclei is often smooth and granular-fibrillar. The granules (ribosomes) are dominant: they are collected in some areas in more or less compact masses or displaced along rows, suggesting the existence of some polysomal formations and implicitly the development of some proteosynthetic processes (Pl. II).

Chromonemata could rarely be differentiated in the interphase nuclei. Chromatic blocks of variable shape and size appear at the nucleus periphery, in close contact with the nuclear envelope; it demonstrates the morphological and functional connection between these two nuclear components: the nuclear envelope offers a support and protection to chromonemata being also implied in the replication and structuring of the genetic material (Pl. II). No chromatic masses were noticed to surround the pores. So, the hypothesis that chromatine is connected to the annule and that the cyclomere is part of a chromosome is not valid [2]; [3]; [7]; [10].

We consider that it is not the pores but any other part of the nuclear envelope which can be an insertion place of chromosomes. The form of chromatic blocks suggests that the attachment of chromosomes to the nuclear envelope is performed generally by the telomere but this is not the only possibility. The fact that the chromatic blocks appear more often at the periphery reveals another aspect: the chromosomes connection to the nuclear envelope would be preferentially performed if not exclusively by heterochromatine, the latter being the only condensed part and therefore easier to be revealed in the interphase.

Chromatic blocks are in close contact with nucleoli. These are interpreted as nucleolar organizer chromosomes. The frequency and clarity of their observation demonstrate that the nucleolar organizer area is in fact a heterochromatic area in which a large quantity of DNA concentrates, with highly repetitive sequences (DNAr) implied in the synthesis of RNAr, and implicitly in the ribosomes biogenesis and nucleolus organization. By means of this chromosome, the nucleolus is permanently connected to the nuclear envelope (Pl. I, II) [3]; [4]; [6]; [8].

It seems that there is a direct correlation between the number of chromatic blocks on an electron microscope in the interphase nuclei and the degree of ploidy.

The nucleolus is — for an incipient stage of investigation — the most spectacular nuclear component.

Several electronmicrographs come to support some previous observations according to which the number of nucleoli increases with the degree of ploidy, being a relationship of equality between the number

of nucleoli and the chromosomal sets (genomes), each of them characterized by the presence of a nuclear organizer chromosome (Pl. II).

The intense electron density of the nucleolus demonstrates that a large quantity of substance (organic and mineral) is concentrated at its level. The volume of the nucleolus as well as its shape are more often correlated with the physiological activity and not with the degree of ploidy. The nucleolus represents between a fifth and a third of the nuclear space. It generally has a spherical or oval form (just like the nucleus housing it), but the shape is constantly irregular, determined by expansions which are, as we suppose, related with the intensity of the physiological processes taking place at the level of this nuclear organite and implicitly with the translation speed of some nucleolar components to the karyoplasm and then to the plasmatic matrix. During the expulsion of the nucleolar material the intranuclear vacuoles can also be implied.

The fine structure of the nucleolus is granulary fibrillar ; the fibrils seem more abundant in the central area while the granules (ribosomes) in the peripheral region, where they are clearly evident. The areas resulting after a space distribution of the two nucleolonema components are not well outlined [3], [4], [5], [8].

In some figures (Pl. I) especially in the peripheral area of the nucleolus, the granules do not have a random distribution but an orderly one ; the linear rows of ribosomes resulted by their insertion on RNAm ; therefore they are polysomes, that is why it is suggested that in the nucleolus not only the RNAr synthesis but also the synthesis of proteins (especially the ribosomal ones) take place ; the connection of the two components to form ribonucleoprotein particles of 40 S′ and 60 S′ takes place at the same level.

Several electronmicrographs reveal clearly that in the interphase the nucleolar material, the proteins prevailing, is closely connected with the nucleolar organizer chromosome, but in the late prophase it detaches and disperses ; only the nucleoplasm is still attached to the nucleolar organizer region. The nucleoli are reorganised, enlarge and sometimes even fuse in the late telophase and early telophase. But if the nucleoli disorder coincides with the transition from prophase to metaphase, the occurrence of other stages of the nucleolar cycle varies from one cell type to another. In the diploid forms, it seems that the reorganization of nucleoli ends in the telophase, while in the tetraploid forms, the phenomenon occurs in the early interphase.

Other aspects related to the nucleus fine structure were also revealed by our investigations ; further studies will supply us data to be published later.

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# POSITIVE AND NEGATIVE SPECIES RELATIONSHIPS IN A COMMUNITY DOMINATED BY *FESTUCA VALESIACA* L. THE EFFECT OF MINERAL NUTRIENT TREATMENT

BY

LUCIA STOICOVICI and ȘT. GALLÓ

In an experimental field the change of herbage canopy structure with *Festuca valesiaca* * dominant species is in the untreated variant (the control) and in the treated variant (NPK in the highest dose) comparatively studied. In the hierarchical representation of the relationships between species it interferes with the $L^2$ coefficient, and thus the illustration of positive and negative correlations becomes possible. Two dendrograms comparatively constructed on the basis of numerous correlation coefficients between species in pairs show a deep influence of fertilizers on the number and the degree of association between species.

The aim of this communication is to examine by the correlation method, the way of species association in a xeric pasture under the influence of mineral nutrient treatment.

## METHODS

The experimental field (Juc-Bonțida, Cluj county) at 460 m altitude with a xeric vegetation dominated by *Festuca valesiaca* is made up of a randomized block system with some variants regularly fertilized with different levels of NPK mineral nutrients [4]. In this paper, the unfertilized variant (the control) and the fertilized variant with the highest level of NPK($N_{200}P_{100}K_{100}$) applied on a volume basis ($\equiv$ kg/ha) are examined. Each variant size was of 14 m². A 1 × 1 m quadrat subdivided into 25 quadrats with the 20 × 20 cm basal quadrat size was laid out successively over the entire variant surface. The occurrence of each species in the basal quadrat was recorded and the percentage frequency obtained on 1 m² was employed to calculate the correlation coefficients between species in pairs. Species which occurred less than six times in the total quadrats were eliminated. The correlation coefficients were then introduced in the 2 × 2 contingency tables for the remaining species. For the purpose of species relationships illustration it had been resorted to the hierarchical arrangement of these relations in dendrograms as suggested by Mountford [1]. Before the graphical representation, we considered necessary to transform the correlation coefficients so that all of them, irrespective of their size, sign or the degree of significance should participate in the statistical processing. We had in view the basic notion that the algebraic transformation of the coefficients should be achieved

* Species nomenclature follows Flora Europaea [3].

in order to : 1) represent the same reality concerning the species relationships like the correlation coefficients, namely :

$$r \to +1 \qquad L \to 0$$
$$r \to -1 \qquad L \to \infty$$

2) after the transformations, the new coefficients (L) should have positive values ($0 \to \infty$). If between the species the degree of association (the correlation) is slight, it is advisable, in dendrogram processing, to use $L$ or

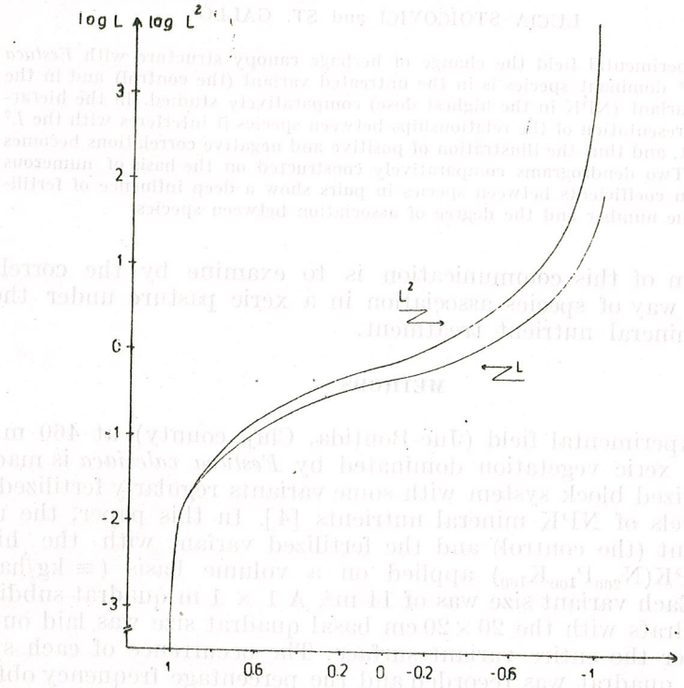


Fig. 1. — The relationship between the correlation coefficient $r$ and the $L$, $L^2$, respectively coefficients.

better $L^2$ coefficients (Fig. 1). For the transformation of each correlation coefficient the following formula is applied : $L = \frac{1}{1+r} - 0.50$ or $L^2 = \left[\frac{1}{1+r}\right]^2 - 0.25$.

## RESULTS

The *Festuca valesiaca* xeric community, which belongs to the ordinary communities of sunny slopes in the Transylvanian Plain, includes a great number of species (about 124) recorded inside and outside the expe-



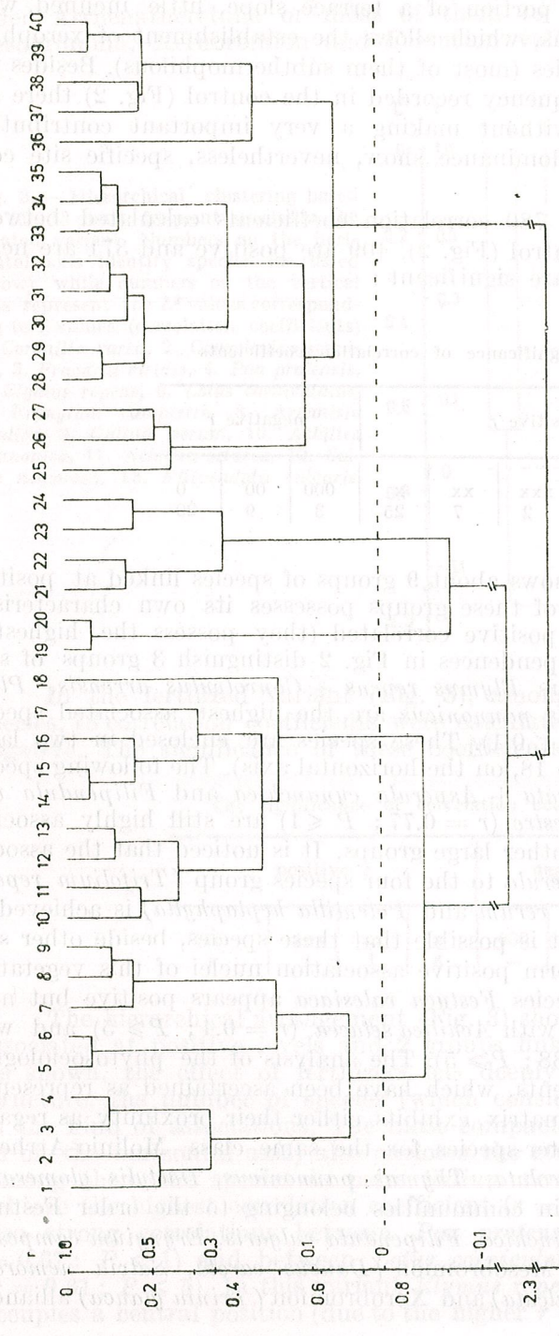


Fig. 2. — Hierarchical clustering based on the 40 most frequent species in the control. Numbers on the horizontal axis identify species (as listed below), while numbers on the vertical axis represent the $L^2$ values corresponding to $r$ values (correlation coefficients) 1. *Poa pratensis*, 2. *Inula britannica*, 3. *Medicago falcata*, 4. *Trinia glauca*, 5. *Elymus repens*, 6. *Convolvulus arvensis*, 7. *Stahis recta*, 8. *Cardaria draba*, 9. *Viola hirta*, 10. *Plantago media*, 11. *Veronica prostata*, 12. *Fragaria viridis*, 13. *Coronilla varia*, 14. *Plantago lanceolata*, 15. *Thymus pannonicus*, 16. *Thesium linophyllon*, 17. *Euphorbia cyparissias*, 18. *Theucrium chamaedrys*, 19. *Dactylis glomerata*, 20. *Asperula cynanchica*, 21. *Trifolium repens*, 22. *Carex tomentosa*, 23. *Galium verum*, 24. *Potentilla heptaphylla*, 25. *Festuca valesiaca*, 26. *Achillea setacea*, 27. *Veronica spicata*, 28. *Daucus carola*, 29. *Salvia nemorosa*, 30. *Cerastium fontanum* ssp. *trivale*, 31. *Agrimonia eupatoria*, 32. *Veronica chamaedrys*, 33. *Koeleria macrantha*, 34. *Medicago lupulina*, 35. *Dorycnium pentaphyllum* ssp. *herbaceum*, 36. *Lotus corniculatus*, 37. *Achillea pannonica*, 38. *Tarazacum officinale*, 39. *Filipendula vulgaris*, 40. *Eryngium campestre*.

rimental field, with prevailing pontic and continental elements. The field is located in the upper portion of a terrace slope, little inclined, with reduced barren conditions, which allows the establishment of xerophyte and mesoxerophyte species (most of them subthermophilous). Besides the 40 species with high frequency recorded in the control (Fig. 2) there are several species which without making a very important contribution in point of abundance-dominance show, nevertheless, specific site conditions.

From the total of 780 correlation coefficients calculated between the 40 species in the control (Fig. 2), 409 are positive and 371 are negative, but few of them are significant :

The significance of correlation coefficients

| | positive $r$ | | | negative $r$ | | |
|---|---|---|---|---|---|---|
| No. | xxx | xx | x | 000 | 00 | 0 |
| $r$ | 2 | 7 | 25 | 3 | 9 | 20 |

The dendrogram shows about 9 groups of species linked at positive association levels. Each of these groups possesses its own characteristic species in pairs, highly positive correlated (they possess the highest $r$ values). The negative dependences in Fig. 2 distinguish 3 groups of species. The species in pairs *Elymus repens* + *Convolvulus arvensis*, *Plantago lanceolata* + *Thymus pannonicus* are the highest associated species ($r = 0.78$ ; $r = 0.79$ ; $P < 0.1$). These species are enclosed in two large groups (from species 1 to 18, on the horizontal axis). The following species in pairs *Dactylis glomerata* + *Asperula cynanchica* and *Filipendula vulgaris* + *Eryngium campestre* ($r = 0.77$ ; $P \leqslant 1$) are still highly associated being comprised in other large groups. It is noticed that the association of *Dactylis* and *Asperula* to the four species group *(Trifolium repens*, *Carex tomentosa*, *Galium verum* and *Potentilla heptaphylla)* is achieved at a steep negative level. It is possible that these species, beside other species in Fig. 2, should form positive association nuclei of this vegetation type. The dominant species *Festuca valesiaca* appears positive but non-significantly correlated with *Achillea setacea* ($r = 0.4$ ; $P \geqslant 5$) and with *Veronica spicata* ($r = 0.38$ ; $P \geqslant 5$). The analysis of the phytosociological affiliation of these elements, which have been ascertained as representative in the correlation matrix, exhibits either their proximity as regards their framing as character-species for the same class Molinio-Arrhenatheretea *(Plantago lanceolata*, *Thymus pannonicus*, *Dactylis glomerata)*, or they are recognized in communities belonging to the order Festuco-Brometea *(Asperula cynanchica*, *Filipendula vulgaris*, *Eryngium campestre*, *Elymus repens)* or to Mesobromion *(Daucus carota*, *Salvia nemorosa*, *Medicago lupulina*, *M. falcata)* and Xerobromion *(Trinia glauca)* alliances.



Other species in the dendrogram belong either to communities within the order Arrhenatheretalia or most of them to communities within the Mesobromion, Xerobromion and Festucion vallesiacae alliances [2].

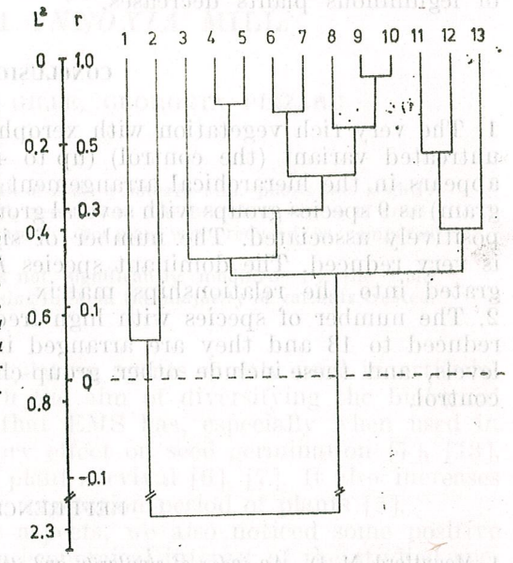


Fig. 3. — Hierarchical clustering based on the 13 most frequent species in the treated variant. Numbers on the horizontal axis identify species (as listed below), while numbers on the vertical axis represent the $L^2$ values corresponding to $r$ values (correlation coefficients) 1. *Coronilla varia*, 2. *Convolvulus arvensis*, 3. *Fragaria viridis*, 4. *Poa pratensis*, 5. *Elymus repens*, 6. *Lotus corniculatus*, 7. *Eryngium campestre*, 8. *Artemisia pontica*, 9. *Galium verum*, 10. *Achillea pannonica*, 11. *Achillea setacea*, 12. *Salvia nemorosa*, 13. *Filipendula vulgaris*.

In the fertilized variant (Fig. 3), among the 13 high frequency species, 78 correlation coefficients were calculated, 58 are positive and 20 negative. The significance of these coefficients is reduced :

The significance of correlation coefficients

| | positive $r$ | | | negative $r$ | | |
|---|---|---|---|---|---|---|
| No. | xxx | xx | x | 000 | 00 | 0 |
| $r$ | 1 | 1 | 6 | — | — | 1 |

The hierarchical arrangement (Fig. 3) shows about 3 species groups associated at positive levels and 2 groups linked at one negative level. As shown, the effect of fertilizers has deeply changed the community structure, the number of species (which considerably decreases) as well as the kind of associations. The most compact group (from species 4 to 10 on the horizontal axis) also encloses the strongest associated species. Among them the species in pairs *Galium verum* + *Achillea pannonica* achieve the highest correlation coefficient ($r = 0.81$ ; $P < 0.1$). There are also strong correlations between *Poa pratensis* + *Elymus repens* ($r = 0.67$ ; $P \leqslant 1$) and between *Lotus corniculatus* + *Eryngium campestre* ($r = 0.61$ ; $P \leqslant 5$). In this variant *Elymus repens*, as a dominant species, occupies a central position (due to the higher $r$ value). After fertilization

most of the species which remain are mesoxerophytes and only two are xerophytes. Regarding their nutritive requirements the oligotrophic and mesotrophic species as well as the nitrophilous eutrophic *Artemisia pontica* and *Elymus repens* are maintained. In the whole variant the number of leguminous plants decreases.

## CONCLUSIONS

1. The very rich vegetation with xerophytes and mesoxerophytes in the untreated variant (the control) (up to 40 species with high frequency) appears in the hierarchical arrangement between species (in the dendrogram) as 9 species groups with several group-characterizing species strongly positively associated. The number of significant correlation coefficients is very reduced. The dominant species *Festuca valesiaca* is slightly integrated into the relationships matrix.
2. The number of species with high frequency in the treated variant is reduced to 13 and they are arranged in 3 groups linked at significant levels, and these include other group-characterizing species versus the control.

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# EFFECTS OF THE TREATMENT WITH EMS IN *DATURA INNOXIA* MILL.

BY

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After the EMS treatment, the growth of *Datura innoxia* Mill. plants was slightly stimulated in $M_1$ and inhibited in $M_2$ generation. The branching degree of treated plants as well the number of capsules per plant were reduced as compared to the control.
The content of total alkaloids was not significantly modified by the EMS treatment in $M_1$ generation, increasing in $M_2$ in the majority of variants treated.

The EMS is one of the alkylating agents mostly used in artificial plant mutagenesis. Using it with the aim of diversifying the biological material led to the conclusion that EMS has, especially when used in great concentrations, an inhibitory effect on seed germination [7], [13], plant development [3], [6], and plant survival [6], [7]. It also increases sterility [3], [7] and extends the vegetation period of plants [5].

Along with these negative aspects, we also noticed some positive aspects which, depending on the economical interest of the studied species, present a series of advantages. There have been obtained dwarfish mutants and mutants with long straw in the case of wheat [11] and barley [8]; flax mutants with forked stems or supplementary stems [1]; precocious pea mutants [9]. There have also been selected mutants with an increased protein content in the case of barley [2], [4], wheat [10], [12], maize [14].

In the present paper we aim to observe the influence of the EMS treatment on some morphoanatomic aspects and on the content of total alkaloids in *Datura innoxia* Mill., along two successive generations — $M_1$ and $M_2$.

## MATERIAL AND METHODS

Seeds of *Datura innoxia* Mill. were treated for 24 hours, in $M_1$, with EMS (ethylmethanesulfonate) in concentrations of 0.019; 0.037; 0.075; 0.15; 0.30 and 0.60%. After treatment the seeds were sowed on the experimental field of S.C.A. Secuieni–Roman.

In $M_1$ and $M_2$ we analysed the height of plants, their branching, the number of capsules per plant and the content of total alkaloids in the leaves (rendered in scopolamine).

From variant no. 6 we isolated in $M_1$ an individual phenotypically modified, purple coloured (probably as a result of a marked accumulation

of anthocyans), which segregated thus : 50% normal plants from the point of view of their aerial colouring ; 16% purple coloured plants ; 34% plants having a colouring intermediary between the two categories.

## RESULTS AND DISCUSSIONS

The EMS, in concentrations between 0.019—0.15 % slightly stimulated the growing process of the *Datura innoxia* Mill. plants in $M_1$ (table 1). It is significant to show that when we used a maximum dose (0.60%) the growth rhythm of plants slightly diminished (with about 9%). The maximum registered growth was of 5% in the variant treated with 0.075% EMS.

Although we registered the existence of a positive relation between the size of the plant and its degree of branching ($r$ being between 0.60—0.77), the values of this parameter are — except variant 3 — somewhat reduced in two cases of the treated variant as compared to the control. As we have already mentioned, the majority of treated variants showed a slight stimulation of the growth in the height of plants. This situation, somehow paradoxical, suggests that the higher size of the plant was not associated to the increase of the number of branching nodes but to the lengthening of internodes. This phenomenon was really manifest in the case of variant 4 which registered the greatest height of plants. There is no doubt that, in $M_2$, the growth in the height of plants was slightly inhibited (table 1). It is worth mentioning the fact that this inhibition is more reduced in the case we used greater doses of mutagen (0.15—0.60%). We registered a situation different from that of $M_1$ when the growth of treated plants was a little better than that of the control.

The reduced size of treated plants is positively correlated with the degree of branching in $M_2$ too, the plants treated with EMS having, in general, one dichotomic branching node less than the treated plants (table 1).

The mutagen action of EMS was especially evident in the case of capsule production. It is generally known that every branching node produces a fruit but our experiment clearly proved that the number of capsules per plant decreased in the treated variants with over 50%, as compared to the control (table 1). Relating the number of capsules per plants to the degree of branching, which decreased in no case to 50% compared to the control (the greatest decrease was of 14% in variant 6), we may appreciate that the EMS had a negative influence which diminished the fertility of treated plants. This effect is well known in literature [3], [6], [7].

As from the point of view of the content of total alkaloids, the values of the experimental variants of *Datura innoxia*. Mill. range between the limits obtained by other authors too [15]. In $M_1$, this biochemical parameter was little affected by the mutagen action of EMS, the values oscillating around those registered by the control. We must though mention that in the case of some variants, namely 4 and 5, the content of alkaloids



*Table 1*

Effects of a 24 hours treatment with EMS in *Datura innoxia* Mill.

| No. | Variant | Generation | Height of the plant (cm) | | | Degree of branching | | | Number of capsules per plant | | Total aklaloids rendered in scopolamine (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $\bar{x} \pm s\bar{x}$ | $\bar{x}$% | $r$ | $\bar{x} \pm sx$ | $\bar{x}$% | $r$ | $\bar{x} \pm s\bar{x}$ | $\bar{x}$% | |
| 1 | Control | $M_1$ | 77.50 2.27 | 100.00 | 0.77 | 3.70 0.23 | 100.00 | — | — — | — | 0.21 |
| | | $M_2$ | 131.67 1.87 | 100.00 | 0.56 | 7.07 0.16 | 100.00 | 0.63 | 8.62 0.83 | 100.00 | 0.17 |
| 2 | 0.019% EMS | $M_1$ | 79.53 1.88 | 102.62 | 0.67 | 3.67 0.18 | 99.19 | — | — — | — | 0.24 |
| | | $M_2$ | 124.63 2.80 | 94.65 | 0.76 | 6.74 0.24 | 95.33 | 0.58 | 4.47 0.89 | 51.85 | 0.21 |
| 3 | 0.037% EMS | $M_1$ | 80.71 2.14 | 104.14 | 0.69 | 3.91 0.23 | 105.67 | — | — — | — | 0.19 |
| | | $M_2$ | 126.76 1.45 | 96.27 | 0.71 | 6.33 0.15 | 89.53 | 0.61 | 3.65 0.40 | 42.34 | 0.30 |
| 4 | 0.075% EMS | $M_1$ | 81.44 2.05 | 105.08 | 0.76 | 3.08 0.17 | 83.24 | — | — — | — | 0.10 |
| | | $M_2$ | 127.84 1.34 | 97.09 | 0.55 | 6.46 0.15 | 91.37 | 0.60 | 4.13 0.70 | 47.91 | 0.36 |
| 5 | 0.15% EMS | $M_1$ | 78.95 1.69 | 101.87 | 0.60 | 3.15 0.17 | 85.13 | — | — — | — | 0.11 |
| | | $M_2$ | 127.97 1.40 | 97.19 | 0.73 | 6.33 0.16 | 89.53 | 0.43 | 4.38 0.60 | 50.81 | 0.23 |
| 6 | 0.30% EMS | $M_1$ | 76.91 2.07 | 99.24 | 0.67 | 3.42 0.17 | 92.43 | — | — — | — | 0.22 |
| | | $M_2$ | 129.64 1.75 | 98.46 | 0.63 | 6.09 0.18 | 86.14 | 0.63 | 3.79 0.36 | 43.97 | 0.18 |
| 7 | 0.60% EMS | $M_1$ | 70.90 3.00 | 91.48 | 0.75 | 3.28 0.20 | 85.94 | — | — — | — | 0.23 |
| | | $M_2$ | 128.25 3.54 | 97.40 | 0.75 | 6.55 0.27 | 92.64 | 0.60 | 8.00 1.06 | 92.81 | 0.18 |
| 8 | a) plants with normal colouring | $M_2$ | 117.60 3.40 | — | 0.83 | 6.68 0.31 | — | 0.60 | 5.30 0.47 | — | 0.33 |
| | b) plants with purple colouring | $M_2$ | 118.13 7.55 | — | 0.85 | 6.00 0.50 | — | 0.20 | 3.70 0.66 | — | 0.38 |
| | c) plants with intermediary colouring | $M_2$ | 129.36 3.89 | — | 0.81 | 7.22 0.45 | — | 0.81 | 6.35 0.56 | — | — |

was reduced to half of that of the control (0.10 and 0.11 % as to 0.21 % characterizing the control). In $M_2$, the biosynthesis of alkaloids was not affected negatively in any of the variants treated. Even more, we noticed a stimulation of the metabolism of alkaloids, their content getting double as compared to the control in variant 4 (0.36 % as compared to 0.17 % in the control). But the greatest content of total alkaloids was registered by the mutant variant (purple coloured plants) —0.38 %. The normally coloured plants (variant 8), issued from the seeds of the $M_1$ modified plant, had an increased content of alkaloids (0.33 %).

The $M_2$ descendant of the $M_1$ modified plant (var. 8) appeared to be normal in the case of individual, that had an intermediary colouring (variant 8 c), the size of plants and the degree of branching being close to those of the untreated control. In the cases of the other two categories of individuals (var. 8 a and 8 b), the growth of plants was inhibited and the degree of branching and especially the number of capsules per plant diminished.

## CONCLUSIONS

1. In the treated $M_1$ generation, the growth process of the *Datura innoxia* Mill. plants was slightly stimulated by the EMS and, on the contrary, in $M_2$ generation they were inhibited.

2. The branching degree of treated plants, and especially the number of capsules per plant, are reduced by the EMS treatment both in $M_1$ and $M_2$.

3. The content of total alkaloids was not significantly modified by the EMS treatment in $M_1$, increasing in $M_2$ in the majority of treated variants.

4. The $M_2$ descendant similar to the modified $M_1$ genitor (the purple coloured plant) had a higher content of total alkaloids.

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# *In situ* DIFFERENT TERM $^{14}C$ BIOASSAYS FOR TESTING DAILY PRIMARY PRODUCTIVITY

BY

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Identical $^{14}C$-labelled phytoplankton samples were simultaneously *in situ* exposed for 4, 8 and 24 or 4 and 24 hours, respectively.
The results obtained after the mentioned exposure periods were differently computed for 24 h and the computation ways and values of daily primary productivity compared.
A 4 h exposure period for the natural phytoplankton communities proved to be adequate for purposes of primary productivity estimates.

The diversity of working procedures concerning the exposure periods within the $^{14}C$ method [11] has been recently exemplified in brief [4].

Thus, the analysis of references on the $^{14}C$ method proves the necessity for the explanation and standardization of its uses in order to allow a more realistic comparison of the data recorded in different marine areas.

The aim of the present paper is to check experimentally the differences of marine phytoplankton photosynthesis results after simultaneous different exposure periods of identical samples to natural illumination.

The experiments performed in the Constanța sector (44°10′N) in 1982 lasted, at the given latitude and in seasons with different durations of daylight, for 4, 8 and 24 or 4 and 24 hours, respectively.

Parallel experiments were carried out in the same place and under identical conditions for the mentioned durations in order to establish the necessary exposure duration of $^{14}C$-labelled phytoplankton samples for as accurate as possible daily primary productivity measurements; this desideratum originates in the fact, accepted nowadays, that too long exposures misrepresent the results due to the known negative effects which occur in the light and dark bottles [9] used in the $^{14}C$ method too.

## MATERIAL AND METHOD

Phytoplankton samples were collected in February, April, June, August, October and December, at one standard station situated 1.2 nautical miles offshore, from 0, 5 and 10 m depths (Table 1).

The $^{14}C$ method by liquid scintillation counting was used. 3 light and 1 dark (control) bottles containing phytoplankton were inoculated each with 25 μCi (925 kBq) aqueous $NaH^{14}CO_3$ solution and exposed

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*Table 1*

Experimental conditions for *in situ* daily primary productivity determinations in the Constanța sector, 1.2. n.m. offshore, in 1982

| Date of sampling | Date of determination | Depth (m) | Duration of natural illumination (h) | Time of exposure | Nebulosity | Temperature of air (°C) | $NaH^{14}CO_3$ activity (μci) | Date of sample measurement |
|---|---|---|---|---|---|---|---|---|
| Feb., 16 | Feb., 16/17 | 0,5,10 | 10 | 10—14= 4 | 10/10 | 1.5 | 25 | Mar., 22 |
| | | | | 10—18= 8 | 5/10 | 3.0 | 25 | ,, |
| | | | | 10—10=24 | 10/10 | 3.0 | 25 | ,, |
| Apr., 26 | Apr., 26/27 | 0,5,10 | 14 | 10—14= 4 | 0/10 | 13.0 | 25 | Oct., 11 |
| | | | | 10—18= 8 | 5/10 | 13.0 | 25 | ,, |
| | | | | 10—10=24 | 0/10 | 13.0 | 25 | ,, |
| June, 16 | June, 16/17 | 0,5,10 | 15 | 10—14= 4 | 0/10 | 20.0 | 25 | ,, |
| | | | | 10—18= 8 | 10/10 | 18.0 | 25 | ,, |
| | | | | 10—10=24 | 5/10 | 16.0 | 25 | ,, |
| Aug., 13 | Aug., 13/14 | 0,5,10 | 14 | 10—14= 4 | 0/10 | 28.0 | 25 | Dec., 14 |
| | | | | 10—18= 8 | 0/10 | 28.0 | 25 | ,, |
| | | | | 10—10=24 | 0/10 | 28.0 | 25 | ,, |
| Oct., 21 | Oct., 21/22 | 0,5,10 | 10 | 10—14= 4 | 0/10 | 9.0 | 25 | ,, |
| | | | | 10—10=24 | 0/10 | 17.5 | 25 | ,, |
| Dec., 06 | Dec., 06/07 | 0,5,10 | 9 | 10—14= 4 | 0/10 | 1.0 | 25 | ,, |
| | | | | 10—14=24 | 0/10 | 4.0 | 25 | ,, |





*Table 2*

Values of some physical-chemical factors of sea water in the Constanța sector, 1.2. n.m. offshore, in 1982

| Month | Depth (m) | Light (lx) | Temperature (°C) | | | | pH | $S^0/_{00}$ | Total inorganic carbon (mg $l^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| | | 10,00 (h) | 10,00 | 14,00 | 18,00 | 10,00 | | | |
| II | 0 | >5000 | 1.5 | 3.5 | 1.5 | 2.0 | 8.1 | 13.95 | 40.45 |
| | 5 | 150 | 2.0 | 2.0 | 2.0 | 2.0 | 8.3 | 14.33 | 40.45 |
| | 10 | 7 | 2.0 | 2.0 | 2.0 | 2.0 | 8.3 | 14.07 | 39.24 |
| IV | 0 | >5000 | 9.5 | 9.5 | 9.5 | 9.5 | 8.2 | 11.17 | 38.02 |
| | 5 | 500 | 9.5 | 9.0 | 9.5 | 9.5 | 8.4 | 11.42 | 38.02 |
| | 10 | 110 | 10.0 | 10.0 | 10.0 | 10.0 | 8.4 | 12.47 | 38.02 |
| VI | 0 | >5000 | 13.5 | 13.5 | 13.0 | 14.0 | 8.2 | 12.32 | 40.49 |
| | 5 | >5000 | 11.5 | 11.5 | 10.0 | 12.0 | 8.2 | 12.42 | 40.49 |
| | 10 | 3500 | 9.5 | 9.5 | 8.5 | 11.0 | 8.3 | 12.76 | 40.49 |
| VIII | 0 | >5000 | 25.0 | 25.0 | 25.0 | 25.0 | 8.5 | 14.15 | 33.78 |
| | 5 | 1000 | 24.0 | 24.0 | 24.0 | 24.5 | 8.5 | 14.24 | 33.78 |
| | 10 | 80 | 24.0 | 24.0 | 24.0 | 24.5 | 8.7 | 14.35 | 34.38 |
| X | 0 | >5000 | 16.0 | 16.0 | — | 16.5 | 8.2 | 16.92 | 38.01 |
| | 5 | 2700 | 17.0 | 17.0 | — | 17.0 | 8.3 | 17.34 | 38.01 |
| | 10 | 600 | 17.0 | 17.0 | — | 17.0 | 8.4 | 17.12 | 38.01 |
| XII | 0 | >5000 | 7.5 | 7.0 | — | 7.0 | 8.3 | 13.77 | 34.00 |
| | 5 | 340 | 7.0 | 7.0 | — | 7.0 | 8.4 | 14.24 | 35.58 |
| | 10 | 80 | 7.0 | 7.0 | — | 7.0 | 8.4 | 14.42 | 36.80 |

*in situ* at the mentioned depths. Identical samples from each depth were exposed for 4, 8 and 24 or 4 and 24 hours to natural illumination and fixed after the end of the exposure period with formaline (Table 1).

During the experiments the nebulosity and the temperature of the air were noted (Table 1). Light, temperature, pH, salinity and total inorganic carbon content of sea water were also measured (Table 2). Light measurements were made be means of an Ogawa Seiki underwater illuminometer type OSK 3174. Total inorganic carbon content of sea water was determined by total carbonic alkalinity analysis (cf. 14).

After the fixation the contents of the bottles were filtered with an in vacuum device, on Millipore membrane filters HA 04700 ($\varnothing = 0.45$ μm), and washed with a 2% HCl solution and water. The filters with the radioactive phytoplankton residues, preserved in Packard type vials (with a previously determined background) were counted (Table 1) with an automatic N.E. spectrometer type 8310/1/2 at + 5°C, using the following scintillation mixture : 3 ml dioxan (for filter solubilization) and 5 ml Unisolve scintillator. The samples were measured with an efficiency of 83%. Background and quenching corrections were applied to the results. Each sample was measured for 1 min three times.

Primary productivity values were computed according to the usual formula [6 ; 11 ; 12] and expressed per day in two ways (for the exposures of 4 and 8 hours) : a) by directly multiplying the result by 4 (4 hour experiment) or by 2 (8 h experiment), and b) by dividing the result by 4 or 8, respectively (value per hour) and multiplying by the daylight duration in hours [4]. The measurement values for the dark (control) bottles were neither subtracted from the photosynthetic assimilation values, nor calculated as percentage of the latter [10].

## RESULTS AND DISCUSSION

The comparison of daily primary productivity results on the 0—10 m water column, obtained during different exposure periods and using two computation ways, evinces higher values after exposures of 4 and 8 hours and much lower after 24 h (Table 3).

Generally, in experiments with short exposure periods, of 4 and 8 h, somewhat higher values occur also after shorter exposures, and vice versa. As compared with previous primary productivity data from the same Constanța sector [1 ; 2 ; 3], obtained after 1/2 day ( × 2) under *in situ* "simulated" conditions, the present results after 24 h are much lower and thus uncertain in point of their fidelity.

These experimental results confirm that an exposure of 24 h of phytoplankton samples, even *in situ*, is too long so that the obtained data do not indicate the real primary productivity level. The decrease of the assimilatory capacity of phytoplankton in such an interval can be attributed to the increase of the "bottle effects" (lack of turbulence, sedimentation of phytoplanktonic cells, increase of bacterial activity, and so on) ; it is also confirmed [8] that the primary productivity determined during a 24 h exposure is considerably lower than the sum of the rates measured in the same time interval in successive exposures of short duration.



*Table 3*

*In situ* daily planktonic primary productivity values (mg C $m^{-3}$ $day^{-1}$) after 4, 8 and 24 hours from $^{14}C$ inoculation, according to two computation methods (a, b)

| Month | Depth (m) | Duration of exposure period (hours) | | | | |
|---|---|---|---|---|---|---|
| | | 4 | | 8 | | 24 |
| | | a | b | a | b | |
| II | 0 | 81.2 | 51.0 | 44.6 | 27.9 | 3.8 |
| | 5 | 20.8 | 14.0 | 1.8 | 1.1 | 1.2 |
| | 10 | 17.6 | 13.5 | 1.6 | 1.0 | 0.4 |
| IV | 0 | 9.6 | 8.4 | 9.8 | 8.6 | 3.7 |
| | 5 | 8.4 | 7.4 | 8.8 | 7.7 | 5.3 |
| | 10 | 4.0 | 3.5 | 4.2 | 3.7 | 3.4 |
| VI | 0 | 6.8 | 6.8 | 7.6 | 7.1 | 0.8 |
| | 5 | 10.4 | 9.8 | 1.4 | 1.3 | 0.7 |
| | 10 | 8.8 | 8.3 | 2.2 | 2.1 | 0.3 |
| VIII | 0 | 107.6 | 94.2 | 47.0 | 41.1 | 74.4 |
| | 5 | 15.6 | 13.7 | 6.0 | 5.3 | 9.3 |
| | 10 | 24.8 | 21.7 | 3.4 | 3.0 | 9.8 |
| X | 0 | 60.8 | 38.8 | — | — | 6.2 |
| | 5 | 29.6 | 18.8 | — | — | 9.9 |
| | 10 | 15.4 | 3.5 | — | — | 1.1 |
| XII | 0 | 40.0 | 22.5 | — | — | 4.6 |
| | 5 | 37.6 | 21.2 | — | — | 6.2 |
| | 10 | 11.2 | 6.3 | — | — | 3.7 |

As to the computation possibilities used for daily primary productivity measurements in these experiments with identical phytoplankton samples, similar results by both computation methods (a and b) can be noted, with somewhat higher values in the first case (a) as compared to the second one (b) ; the differences between these two computation ways and results, respectively, are the consequence of the different approaches to the daylight period, as either constant (a) or real, depending on the given latitude and season (b). As concerns the higher results of primary productivity after shorter exposure periods, the single exception is represented by the 24 h experiment in August ; these high values could be associated with the effect of temperature (24.5°C) on the development of phytoplankton during such a long exposure.

According to the results from the Constanța sector in 1982, in shallow waters, the necessity of short exposures of the $^{14}C$-inoculated phytoplankton samples became evident in order to obtain more realistic primary productivity data. In contrast to most of the researches in this

field up to now, nowadays exposure periods even shorter than 4 h are also used [5 ; 7 ; 13]; on the basis of the present *in situ* results as well as of the previous *in situ* "simulated" ones [4] we suggest the 4h exposure of $^{14}C$-inoculated phytoplankton samples as necessary and sufficient.

## CONCLUSIONS

1. Identical phytoplankton samples simultaneously exposed for different periods render much lower the primary productivity values after a 24 hours exposure than those after shorter exposures.

2. The comparison of experimental results obtained after 4, 8 and 24 h emphasizes the necessity of 4 h exposures of $^{14}C$-inoculated phytoplankton samples in order to determine daily primary productivity.

3. The results obtained differ somehow between them according to the used computation method, especially when higher primary productivity levels occur, but are generally of the same order of magnitude.

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# SCIENTISTS AND PEACE

The progress of scientific and technological creation was essential in the hierarchy of values which determined the evolution of civilization during the history of human society, attaining its summit by the great performances of our times, that have extended the boundaries of knowledge to unexpected horizons.

The results of scientific research, oriented constructively, are fundamental in solving, in the interest of mankind, some stringent problems, both at national and world level, in order to fulfill the highest human aspirations of civilisation and welfare. The impact of contemporary technico-scientific revolution on society is felt in all fields of human existence. The human genius generated huge forces whose purpose should be the full, normal and well-balanced development of man, the solving of social-economic problems of humanity.

Thus, remarkable progress has been recorded in the last decades in the field of biology. Obtaining energy from nonconventional sources, guiding the transformation of plants by techniques of genetic engineering for increasing quality and yield, obtaining proteins and active biological substances, "reprogramming" some microorganisms for metabolic activities thereby producing important pharmaceutical products, are just a few examples to show that by knowing the inner cellular and molecular mechanisms wide perspectives for guiding nature to the benefit of economic and social progress are opened.

Unfortunately, at present, scientific research has triggered an unprecedented development of military technique, leading to a real change of modern war strategy and tactics. The natural course of knowledge was distorted by subordinating a series of investigations in the field of physics, chemistry, biology, to military projects. Therefore a reversal of values between scientific discovery and development of productive forces took place, since more and more scientific researches are directed to closed fields, to obtain and accumulate weapons with huge destructive capacities.

The development of secret investigations for military purposes, stimulated by the allocation of important funds from some national budgets, raises barriers to the circulation of scientific information and to the actual implementation of the right of nations to benefit from the new achievements of technico-scientific revolution for their social and economic progress. The increasing number of researchers working in the field of military investigations takes out from the scientific field some discoveries that might contribute to solving top problems in the domain of energy, agriculture, food, development of nonpolluting energies, health and maintenance of the proper environment for life on our planet.

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In this context, the high responsibility of the international scientific community for stopping the military programs, for safeguarding the past and present achievements of mankind, its destinies and hopes for the future are but obvious. Scientists knowing better the devastating consequences of military programs for the life on the earth are highly responsible to the society, for the activity of maintaining peace. They must be involved in the broad and ever more vigorous movements for peace that are taking place, at present, in the countries in the world.

The ideal of a peaceful world, based on knowledge and humanism has always characterized the existence and national conscience of the Romanian people. Romania, always faithful to the idea of peace, promotes an active external policy meant to orient the international life to a peaceful, constructive course of events. To fulfill this purpose, nations should have free access to the achievements of science and culture, so that all material and spiritual values, the fruit of humanity's centuries-long efforts, should not support the destruction of civilisation, but the cause of progress and peace. By stimulating the original creation of each nation, the universal patrimony of knowledge, free from the danger of war, will be enriched.

In socialist Romania, the scientists, researchers, technologists and other specialists act as important forces in favour of peace and progress, for making science, technology and culture a benefit to the whole world community, for supporting the emancipation of all nations.

The important task of scientists, as defenders of human dignity and of the future of human society is expressed by the authorized voice of our country's President, as follows : "The most noble duty of all scientists and researchers in all fields and all over the world is to put the whole potential of contemporary science and technology in the service of progress, welfare, freedom and independence of all nations, to assure the supreme right of man to life and peace".

Therefore, the scientists who, by their own profession, are supposed to dedicate their minds, knowledge and work to the achievement of the fundamental aspirations of mankind, have the social and moral obligation to make sure that the results of scientific research and technological creation be employed only for peaceful purposes, as the peace issue is inevitably linked to the destiny of human civilization.

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## G. ZARNEA, *Tratat de microbiologie generală* (Traité de microbiologie générale), I^er^ vol. Editura Academiei, Bucureşti, 1983, 426 p., 115 pl., 171 fig.

La parution toute récente du premier volume du Traité de microbiologie générale, conçu par son auteur, prof. dr. G. Zarnea, comme un ensemble de quatre volumes, incite à une présentation détaillée en raison des éminentes qualités de cet ouvrage. Celui-ci constitue une magistrale synthèse non seulement des complexes connaissances actuelles en microbiologie, mais aussi de leurs implications novatrices au profit de la biologie moléculaire, de l'ingéniérie génétique et de biotechnologies extrêmement fécondes.

C'est pour la première fois qu'un Traité de microbiologie d'une telle envergure vient enrichir la bibliographie scientifique roumaine. L'auteur y inscrit son originalité par l'ordonnance parfaite de l'énorme information accumulée, par la rigueur de ses interprétations qu'il rend accessibles au moyen d'une abondante illustration moderne, dont de nombreux schémas originaux. Tout vise à nous renseigner, la systématisation et la précision chronologique des données se ralliant à la clarté et à la sobriété du style.

Le livre retrace tout d'abord les principales étapes historiques de la microbiologie : découverte des microorganismes depuis Leeuwenhoek à Pasteur, les recherches de ce dernier sur les fermentations, la génération spontanée, l'origine microbienne des maladies infectieuses et l'immunologie. Suivent la découverte des virus, de l'enzymologie microbienne, de la chimiothérapie et des antibiotiques, l'avènement de la génétique des microorganismes et de la biologie moléculaire. Sont finalement envisagés l'apport de V. Babeş et le rôle de J. Cantacuzino et de son école dans le développement des sciences microbiologiques.

Plus de la moitié du volume est ensuite consacrée à la Virologie générale, non pas parce que l'auteur considère les virus comme les entités vivantes les plus primitives. Car il partage sans réserves le concept d'A. Lwoff selon lequel les particules virales infectieuses sont devenues autonomes à partir du génome de cellules procaryotes ou eucaryotes, c'est ce qui explique leur parasitisme intracellulaire obligatoire pour se répliquer. Par conséquent, l'analyse approfondie des virus permet une meilleure compréhension de la biologie moléculaire des bactéries et des eucaryotes.

Concernant l'anatomie des virus, on passe en revue leur génome soit à l'ADN, soit à l'ARN, la capside virale et son enveloppe. L'architecture moléculaire des virus révèle les modalités de leur symétrie qui aboutit à une grande diversification de groupes. Sont indiquées les méthodes de culture de virus dans des lignées cellulaires in vitro, les phases successives de la réplication des virus comportant la biosynthèse de protéines, la morphogenèse des virus et l'élimination des virions hors de leurs cellules-hôtes selon les différentes catégories virales.

Après avoir montré la complexité des relations entre les virus et leurs cellules-hôtes, les propriétés antivirales des interférons, on discute l'important problème de l'oncogenèse virale et on souligne l'intérêt théorique de la transcriptase inverse. L'état subviral des viroïdes se rattache aux virus des plantes.

La variété des bactériophages, la lysogénie et ses bases moléculaires sont amplement traitées vu, au-delà de leur importance bactériologique, les résultats fondamentaux que ces recherches expérimentales ont apportés à la génétique moléculaire. Suivent finalement des considérations générales sur la nature, l'origine, l'évolution et les critères de classification des virus.

La seconde partie du volume, intitulée Anatomie bactérienne, ramène le concept de bactérie à la cellule procaryote, expose minutieusement ses caractères différentiels vis-à-vis des cellules eucaryotes végétales et animales, s'efforce d'éclaircir leurs rapports phylogénétiques dans l'évolution du monde vivant.

La morphologie comparée des bactéries, leurs propriétés physiques précèdent l'étude approfondie de leur ultrastructure, analysée jusqu'à l'échelle moléculaire et concernant : la paroi cellulaire, protoplastes et sphéroplastes, l'espace périplasmique, la membrane plasmatique, les mézosomes, le filament nucléaire, les ribosomes, l'appareil de photosynthèse, inclusions métaboliques, vacuoles, spores et leur genèse, rhapidosomes, magnétosomes, capsule

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et couche muqueuse. Particulièrement intéressantes sont les données relatives au glycocalyx et au fonctionnement des flagelles. Suivent les caractéristiques des groupes de bactéries plus complexes : Rickettsies, Mycoplasmes, Chlumydies, Spirochètes, Myxobactéries, Actinomycètes. Les soi-disant « algues bleues » sont considérées comme étant des Cyanobactéries, douées d'une forte capacité de photosynthèse qui leur confère un important rôle écologique. En dernier lieu, sont examinés les aspects de différenciation cellulaire propres aux bactéries. Chacune des deux parties du livre s'achève par une bibliographie sélective.

Les sommaires des volumes qui paraîtront ultérieurement s'annoncent du plus haut intérêt. Le II$^{e}$ vol. traitera de la physiologie et la taxonomie évolutive des microorganismes. Le III$^{e}$ vol. aura pour objet les microorganismes eucaryotes et les problèmes de la génétique bactérienne et virale. Le IV$^{e}$ vol. va porter sur l'écologie des microorganismes et les multiples applications de la microbiologie.

Le succès immédiat du présent volume autorise les meilleures prévisions pour l'ensemble de l'ouvrage qui formera pour de nombreux spécialistes la pierre angulaire de leur participation efficace à la grandiose révolution scientifique et technique caractéristique de notre époque.

*Acad. prof. Radu Codreanu*

## 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 seront exécutés à l'encre de Chine noire, sur papier calque.

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

Les références bibliographiques, citées par ordre alphabétique, 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, en anglais. 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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