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REVUE ROUMAINE DE BIOLOGIE

SÉRIE DE BIOLOGIE VÉGÉTALE

TOME 26

1981

N° 2

juillet — décembre

SOMMAIRE

V. SANDA, A. POPESCU, Contributions à l'étude de la structure de la végétation du bassin moyen de Jiu	103
NATALIA MITROIU-RĂDULESCU, MARIANA MOROIANU, Pollenmorphologie und Embryogenese bei <i>Digitalis purpurea</i> L. und <i>D. thapsi</i> L.	117
ROZALIA VINTILĂ, M. KEUL, AL. POLIZU, The effect of lindane on the growth of wheat seedlings (<i>Triticum vulgare</i> L.).	123
ANA FABIAN, FR. NAGY-TÓTH, ADRIANA BARNĂ, ELISA- BETA RÁCZ-BÉLTEKI, About the morphological and physiological characteristics of the blue-green alga <i>Phormi- dium viscosum</i> Lemm.	133
MARGARETA IORDAN, AURELIA BREZEANU, ANA ROȘU, The micropropagation of <i>Vitis vinifera</i> L. II. Aspects of morphoge- nesis in callus culture	141
ALEXANDRINA PĂTRAȘCU, Regeneration of potato plants by <i>in vitro</i> culture of stem segments	151
G. I. GHIORGIȚĂ, ECATERINA T. TÓTH, ELVIRA V. GILLE, The effects of gamma rays on <i>Vinca minor</i> L. and <i>Atropa belladonna</i> L.	157
ALEXANDRU S. BOLOGA, The influence of some chemical com- pounds on the planktonic primary productivity in the lake Izvoru Muntelui — Bicaz	165
ALEXANDRU MANOLIU, MIHAI MITITIUC, MIRCEA RUSAN, The parasitic and saprophytic mycoflora on <i>Poa pratensis</i> L. in the conditions of using variable nitrogen fertilizer doses	171
VIE SCIENTIFIQUE	177
COMPTES RENDUS	181
INDEX ALPHABÉTIQUE	185

CONTRIBUTIONS À L'ÉTUDE DE LA STRUCTURE DE LA VÉGÉTATION DU BASSIN MOYEN DE JIU

par

V. SANDA et A. POPESCU

A number of 11 associations—among them 7 are wooden phytocenoses and 4 herbaceous ones—are analysed; in the wooden vegetation *Festuco heterophyllae-Quercetum* R. et Z. Neuh. 64 is indicated as a new association for our country, and in the herbaceous phytocenoses *Rumici(acetosellae)-Agrostietum tenuis* is described as a new association for science.

Le plaine de Jiu, comme d'ailleurs l'entier territoire de l'Olténie, se remarque par la présence de nombreux et intéressants éléments de flore et de végétation, [2], [8], [9], [10]; on y rencontre certaines espèces de trèfle, rares dans la flore de notre pays, par exemple : *Trifolium pallidum*, *T. resupinatum* et *T. michelianum*.

Le territoire que nous avons étudié, à savoir les collines de l'entourage du secteur sylvicole de Peșteana-Jiu (village de Cocoreni, commune de Bîlteni) et l'unité de forêt II — Valea cu Apă, à l'altitude d'environ 250 m, située dans le même secteur, fait partie de la même région du Piemont-Gétique, le district montueux discontinu de chênaies de : rouvre, chêne chevelu, *Quercus farnetto* et des hêtraies de limite inférieure. Les hêtraies sont localisées sur les versants inférieurs; sur ceux ombragés peuvent surgir des mélanges de forêts de chêne rouvre et hêtre et sur ceux ensoleillés ou sur les cimes, on rencontre d'habitude *Quercetum farnetto-cerris*. Le relief entier est fragmenté et en fonction de l'exposition, sur de petites portions alternent les forêts de chêne rouvre, chêne chevelu, *Quercus farnetto*, ou hêtre.

Parmi les éléments floristiques les plus intéressants rencontrés dans le territoire étudié, nous mentionnons : *Helleborus odorus* fréquent dans tous les forêts, *Ruscus hypoglossum* qui végète dans les mélanges de rouvre et hêtre, *Epilobium lanceolatum*, *Carex umbrosa* et *Galium pseudoaristatum*.

LE CONSPECTUS DES ASSOCIATIONS

QUERCO-FAGETEA Br.-Bl. et Vlieger 37

FAGETALIA SILVATICA E Pawl. 28

Fagion dacicum Soó 64

subal. Carpinion dacicum Soó 64

1. Festuco heterophyllae-Quercetum R. et Z. Neuh. 64

2. Quercus petraeae-Carpinetum Soó et Pócs 57

3. Quercus robori-Carpinetum (Borza 41) Soó et Pócs (31) 57

4. Carpinio-Fagetum Paucă 41

Deschampsio-Fagion Soó 62 (Luzulo-Fagion Lohm. et Tx. 54 p.p.)

5. Petraeo-Fagetum Scam. (56) 59

QUERCETEA PUBESCENTI-PETRAEAE (Oberd. 48) Jakucs 60

ORNO-COTINETALIA Jakucs 60*Quercion farnetto* I. Horvat 546. *Quercetum farnetto-cerris* Georgescu 45**QUERCETALIA PUBESCENTIS** Br.-Bl. 31*Quercion pubescenti-petraeae* Br.-Bl. 31 em. Tx. 317. *Quercetum petraeae-cerris* Soó 57**SEDO-SCLERANTHETEA** Br.-Bl. 55 em. Moravec 67**SEDO-SCLERANTHETALIA** Br.-Bl. 55*Thero-Airion* Oberd. 578. *Filagini-Vulpium* Oberd. 38 (*Vulpio-Airetum* Borhidi 56)**ARRHENATHERETEA** Br.-Bl. 47**ARRHENATHERETALIA** Pawl. 28*Cynosurion cristati* Tx. 479. *Rumici (acetosellae)-Agrostietum tenuis* ass. nova**PLANTAGINETEA MAJORIS** Tx. et Prsg. 50**PLANTAGINETALIA MAJORIS** Tx. (47) 50*Agropyro-Rumicion crispi* Nordh. 4010. As. *Rumex crispus-Alopecurus geniculatus* Tx. (37) 5011. *Juncetum effusi* (Eggler 33) Soó 40**A. LA VÉGÉTATION LIGNEUSE**1. *Festuco heterophyllae-Quercetum* R. et Z. Neuh. 64 (tableau 1)

La forêt de chêne rouvre occupe les versants ensoleillés ou semi-
 ensoleillés, ayant une inclinaison modérée, des sols brun-acides, moyen
 podzoliques, argileux-sablonneux et modérément humifères. Ces sols ont
 un régime d'humidité estival sec-humide et les printemps sont caractérisés
 par un surplus d'humidité.

Fig. 1.—Aspect des phytocénoses de *Festuco heterophyllae-Quercetum*.

Les phytocénoses sont dominées par *Quercus petraea*, mais on y
 rencontre aussi des exemplaires rares de *Quercus polycarpa* et *Q. dale-*
champii (fig. 1).

Tableau 1

Festuco heterophyllae-Quercetum R. et Z. Neuh. 64

Forme biologique	Elément floristique	Nombre du relevé	1	2	3	4	5	
		Superficie (m²)	500	500	500	500	500	
		Hauteur de la végéta- tion	— Arbres (m)	25	30	20	32	30
			— Arbustes (m)	1	2	1,5	—	—
			— Herbes (cm)	40	45	40	45	40
		Couverture (%)	— Arbres	90	75	50	90	85
			— Arbustes	1	1	1	—	—
			— Herbes	15	40	70	25	25
Exposition	—	—	—	NV	NV			
Inclinaison (degré)	—	—	—	10	15			

		Carpinion					
MM	Ec(Md)	<i>Quercus petraea</i>	4—5	4—5	3—4	4	4—5
H	Ec	<i>Festuca heterophylla</i>		+		+	+
M	Ec	<i>Carpinus betulus</i> (plantule)					+
H	Atl-Md	<i>Luzula forsteri</i>	+	+			
H	Ec-Md	<i>Melica uniflora</i>				+	+
H	Ec	<i>Dactylis polygama</i>				+	+
H	Ec	<i>Carex umbrosa</i>	+	+			
		Fagion + Fagetalia					
G	E	<i>Dentaria bulbifera</i>	+	+1	+	1—2	1—2
MM	Ec	<i>Fagus silvatica</i>	+	+	+	+	+
H—Ch	E(Md)	<i>Ajuga reptans</i>		+		+	
H	Eua	<i>Scrophularia nodosa</i>	+	+	+	+	
H(Ch)	Ec(Md)	<i>Galeobdolon luteum</i>		+		+	+
H	Sm(Ec)	<i>Festuca drymeia</i>					+1
Ch	Ec(Md)	<i>Euphorbia amygdaloides</i>				+	+
H	Eua(Md)	<i>Campanula rapunculoides</i>	+	+			
H	Atl-Md	<i>Epilobium lanceolatum</i>	+	+			
G—H	Ec	<i>Carex brizoides</i>		+1	3		
		Quercio-Fagetea					
G	E	<i>Polygonatum latifolium</i>	+	+	+	+	+
M	E	<i>Crataegus curvisepala</i>	+	+	+	+	+
H	Eua(Md)	<i>Trifolium medium</i>	+		+		+
H	E(Md)	<i>Mycelis muralis</i>	+	+			+
G	E	<i>Cephalanthera longifolia</i>	+				
H	Cp	<i>Fragaria vesca</i>	+				+
H	Cp	<i>Prunella vulgaris</i>	+	+			
H	Sm(Ec)	<i>Potentilla micrantha</i>	+	+			
H	Eua	<i>Poa nemoralis</i>	+			+	+
H	Eua(Md)	<i>Viola silvestris</i>	+				+
Ch	E	<i>Veronica officinalis</i>	+	+	+	+	+
Th	Ec	<i>Galeopsis pubescens</i>	+	+			
H	Eua(Md)	<i>Carex pairaei</i>	+	+	+	+	+
Th-TH	Eua(Md)	<i>Moehringia trinervia</i>	+	+		+	
H	Eua(Md)	<i>Cruciata glabra</i>	+	+		+	+
H	Eua(Md)	<i>Astragalus glycyphyllos</i>		+			+
H	Cp(Md)	<i>Clinopodium vulgare</i>	+				+

Les espèces les plus rencontrées dans la couche herbacée sont : *Melica uniflora*, *Asperula odorata*, *Festuca drymeia*, *Polystichum setiferum*, *Geranium robertianum*, *Milium effusum*, *Melittis melissophyllum*, *Hieracium murorum*, *Lathyrus vernus*, etc.

5. *Petraeo-Fagetum* Scam. (56)59 occupe la troisième partie supérieure des versants ayant une inclinaison moyenne de 20° et une exposition nordique ; ce sont des populations de transition entre la forêt de chêne rouvre de plateau et le *Carpino-Fagetum* qui s'installent de règle à la base des versants.

La couche herbacée a un recouvrement moyen de 15%. Elle est formée de : *Melica uniflora*, *Dentaria bulbifera*, *Hieracium bauhini*, *Mycelis muralis*, *Polygonatum multiflorum*, *Galium aparine*, *G. schultesii*, *Ruscus hypoglossum*, *Festuca drymeia*, *Epilobium montanum*, *Scutellaria altissima*, *Geranium robertianum*, etc.

6. *Quercetum farnetto-cerris* Georgescu 45 (tableau 2, relevés 1—5). Ce sont les phytocénoses les plus répandues dans la région, occupant des stations situées sur les versants ensoleillés, isolément sur la cime, aux sols bruns, acides, faiblement squelettiques, mi-profonds, faiblement humiphères, ayant d'habitude un régime d'humidité estivale sec-humide, oligobasiques et oligotrophiques (fig. 3).



Fig. 3. — Phytocénoses adultes de *Quercus cerris* et *Q. farnetto* sur les terrasses de Jiu.

Dans la couche herbacée prédomine une flore acidophylle formée de : *Poa nemoralis*, *Carex pairaei*, *Festuca heterophylla*, *Genista elata*, *Luzula forsteri*, *Lathyrus niger* et en général plusieurs espèces caractéristiques à la classe *Quercetea pubescenti-petraeae*.

7. *Quercetum petraeae-cerris* Soó 57 (tableau 2, relevés 6 et 7) L'association se trouve répandue sur les plateaux ou les versants sudiques ayant une inclinaison faible (5°) en constituant une bande de passage

Tableau 2

Quercetum farnetto-cerris (relevés 1—5) et *Quercetum petraeae-cerris* (relevés 6 et 7)

Forme biologique	Elément floristique	Nombre du relevé	1	2	3	4	5	6	7
			500	500	500	500	500	500	500
		Superficie (m²)	500	500	500	500	500	500	500
		Hauteur — Arbres (m)	20	18	18	18	15	30	35
		de la — Arbustes (m)	2	3	1,5	4	2	—	2
		végétation — Herbes (cm)	40	40	40	45	30	45	40
		Couver- — Arbres	90	90	90	80	90	80	90
		ture (%) — Arbustes	15	20	25	30	5	—	5
		— Herbes	20	18	18	18	15	30	35
		Exposition	S	—	—	NV	—	S	—
		Inclinaison (degré)	15	—	—	25	—	5	—
		Localité	Coccoreni					Valea cu Apă	
		Quercion farnetto + Quercion pubescenti-petraeae							
MM	Sm	<i>Quercus cerris</i>	4	2	2	3	1	3	3
MM	Sm	<i>Quercus farnetto</i>	1	3	3	2	4	+	+
MM	E(Md)	<i>Quercus petraea</i>						2	2
H	Sm(Ec)	<i>Potentilla micrantha</i>	+	+	+	+	+	+	+
TH-H	Blc	<i>Verbascum glabratum</i>					+		+
Th-TH	Sm	<i>Sedum cepaea</i>	+	+					+
		Orno-Cotinetalia							
H	Alp-Blc	<i>Helleborus odoratus</i>	+	+	+	+		+	+
G	Pt-Md	<i>Asparagus tenuifolius</i>		+			+		
		Quercetalia pubescentis							
H	Ec(Md)	<i>Lathyrus niger</i>	+	+	+	+			+
H	Ct(Eua)	<i>Hieracium bauhini</i>	+		+				
H	Eua(Md)	<i>Trifolium medium</i>		+	+	+			
H	Sm	<i>Lychis coronaria</i>				+	+		
H	Ec	<i>Campanula persicifolia</i>	+	+	+	+	+		
		Quercetea pubescenti-petraeae							
H	Eua	<i>Poa nemoralis</i>	+1	1	1	+	1		
H	Eua(Md)	<i>Carex pairaei</i>	+	+	+		1—2	+	1—2
H	Dc	<i>Galium pseudoaristatum</i>	+	+			+		
Ch-N	E(Md)	<i>Genista elata</i>	+	+	+	+	+	+	+
H	Ec	<i>Festuca heterophylla</i>	+	+	+1	1	+	+	+
N	Ec(Md)	<i>Chamaecytisus hirsutus</i>		+	+	+			
M	E	<i>Ligustrum vulgare</i>			+		+		
		Pino-Quercetalia							
H	Eua	<i>Lychnis viscaria</i>	+	+	+	+			
H	Cp	<i>Hieracium umbellatum</i>	+			+			
Ch	E	<i>Veronica officinalis</i>		+	+	+			

(Tableau 2—suite)

Forme biologique	Elément floristique	Nombre du relevé						
		1	2	3	4	5	6	7
		Superficie (m ²)	500	500	500	500	500	500
		Hauteur — Arbres (m)	20	18	18	18	15	30
		de la — Arbustes (m)	2	3	1,5	4	2	—
		végétation — Herbes (cm)	10	40	40	45	30	45
		Couver- — Arbres	90	00	90	80	90	80
		ture (%) — Arbustes	15	30	25	30	5	—
		— Herbes	20	18	18	18	15	30
		Exposition	S	—	—	NV	—	S
		Inclinaison (degré)	15	—	—	25	—	5
		Localité	Cocoreni			Valea cu Apă		
MM	Ec	Carpinion + Fagion						
MM	Ec	<i>Carpinus betulus</i>	+	+	+	+		
MM—M	Eua(Md)	<i>Fagus sylvatica</i>					+	+
H	Atl-Md	<i>Cerasus avium</i>	+	+				
		<i>Lu. ulu forsteri</i>	+		+			
H	Eua	Fagetalia						
H	Eua	<i>Lathyrus vernus</i>	+	+				
H(Ch)	Ec(Md)	<i>Myosotis silvatica</i>		+				
		<i>Galeobdolon luteum</i>						+
M	E	Quercio-Fagetea						
MM—M	Ec	<i>Crataegus curvisepala</i>	+	+	+	+		+
H	Ec-Md	<i>Acer campestre</i>	+			+		
H	Eua(Md)	<i>Melica uniflora</i>	+	+		+		
G	Ec	<i>Cruciata glabra</i>		+		+		+
Th-TH	Eua(Md)	<i>Galium schultesii</i>				+		
H	Eua(Md)	<i>Moehringia trinervia</i>		+			+	+
Th-TH	Euh(Md)	<i>Viola silvestris</i>	+		+			+
H	Cp	<i>Lapsana communis</i>				+		+
		<i>Clinopodium vulgare</i>				+		
H—Ch	Eua(Md)	Compagnes						
Th	Eua(Md)	<i>Veronica chamaedrys</i>	+	+		+		
H	E(Md)	<i>Bilderdykia convolvulus</i>	+	+	+	+	+	+
TH—H	E	<i>Hieracium pilosella</i>					+	+
Th-TH	Cs	<i>Verbascum chaixii</i>	+	+				
H	Cp	<i>Stellaria media</i>	+	+				
M	Bic-Anat	<i>Poa pratensis</i>		+		+	2	+
Th	Cp(Md)	<i>Juglans regia</i>		+		+		
		<i>Galium aparine</i>			+	+		

Espèces dans un seul relevé : *Astragalus glycyphyllos* (7), *Ajuga genevensis* (1), *Achillea millefolium* (7), *Arenaria agrimonoides* (3), *Brachypodium silvaticum* (1), *Coronilla varia* (1), *Cardamine impatiens* (7), *Carex pallescens* (7), *Calamagrostis epigeios* (5), *Dactylis polygama* (7), *Fragaria vesca* (7), *Glechoma hirsuta* (4), *Galeopsis tetrahit* (2), *Geum urbanum* (4), *Hypericum perforatum* (6), *Lysimachia nummularia* (4), *Linaria vulgaris* (2), *Myelis muralis* (6), *Rubus candicans* (1), *Rosa canina* (4), *Stellaria holostea* (4), *Silene viridiflora* (2), *Sedum maximum* (1).
Date : 1.VII.1980
Altitude : 250 m

entre les forêts de chêne rouvre de plateau, et le *Quercetum farnetto-cerris* de la troisième partie supérieure des versants (fig. 4).

Les espèces de la couche herbacée souvent rencontrées sont : *Poa pratensis*, *Potentilla micrantha*, *Carex pairaei*, *Helleborus odorus*, *Genista elata*, *Festuca heterophylla*, *Bilderdykia convolvulus*, *Myelis muralis*, etc.



Fig. 4. — *Quercetum petraea-cerris* installé à la limite supérieure des versants de sud-est.

LA VÉGÉTATION HERBACÉE

8. *Filagini-Vulpium* Oberd. 38 (*Vulpio-Airetum* Borhidi 56)

Forme des phytocénoses au recouvrement moyen de 60—70%, rencontrées sur les pentes ayant une exposition sud ou sud-ouest, fortement podzoliques de l'immédiat voisinage de *Quercetum farnetto-cerris*.

D'habitude y prédominent : *Vulpia myuros* (AD = 3—4); à côté de celui-ci se rencontrent encore *Filago minima*, *Plantago lanceolata*, *Hypericum perforatum*, *Ventenata dubia*, *Agrostis tenuis*, *Poa bulbosa*, *Filago arvensis*, *Trifolium arvense*, *Prunella laciniata*, *Trifolium ochroleucum*, *Dorycnium herbaceum*, *Clinopodium vulgare*, *Lotus corniculatus* et *Galium tenuissimum*.

9. *Rumici (acetosellae)-Agrostietum tenuis* ass. nova (tableau 3)

Les prairies dominées par *Agrostis tenuis* végètent sur des sols différents du point de vue de la structure, à savoir : brun-jaunâtre podzolique, brun-blanc glaiseux, des sols podzoliques argileux-illuviaux et le sol brun-jaunâtre acide montagnard. Leur structure floristique a été étudiée par M. Păun et coll. [9] dans la zone subcarpatique de l'Olténie, intégrée dans *Agrostietum tenuis* Szafer, Pawl. et Kulcz. 23, en décrivant aussi une nouvelle sous-association-trifolietosum patens.

Dans une récente étude concernant les plaines d'*Agrostis tenuis* et *Festuca rubra* d'Olténie, Gh. Popescu [10] encadre les phytocénoses pures d'*Agrostis tenuis* aussi dans l'*Agrostietum tenuis* Szafer, Pawl. et Kulcz. 23.

Tableau 3

Rumici (*acetosellae*)-*Agrostietum tenuis* ass. nova

Forme biologique	Elément floristique	Nombre du relevé	1	2	3	4	5
		Superficie (m ²)	100	100	100	100	100
		Hauteur de la végétation (cm)	45	40	50	40	45
		Couverture (%)	95	95	100	95	95
		Exposition	—	—	S	—	S
		Inclinaison (degré)	—	—	15	—	5
		Localité	Cocoreni		Valea cu Apă		
H	Cp	Arrhenatheretea + Arrhenatheretalia					
H(G)	Cp	<i>Agrostis tenuis</i>	5	4—5	3—4	3	4—5
Th	E	<i>Rumex acetosella</i>		+	+	+	+
Th	Eua(Md)	<i>Bromus commutatus</i>	+				+
H	E-Md	<i>Trifolium arvense</i>	+		+	+	
Th—TH	E(Md)	<i>Lolium perenne</i>	+		+		
H	E(Md)	<i>Trifolium campestre</i>	+	+	+	+	+
H	E(Md)	<i>Hieracium pilosella</i>		+	+	+	
H	E-Md	<i>Luzula campestris</i>		+	+	+	
TH	(E(Md)	<i>Campanula patula</i>		+	+	+	
		Molinio-Arrhenatheretea + Molinieta					
H	Eua	<i>Plantago lanceolata</i>	+		+		+
H	Eua(Md)	<i>Trifolium repens</i>	+			+	
H	E(Md)	<i>Hypochoeris radicata</i>	+	+	+	+	
H—G	Cs	<i>Convolvulus arvensis</i>	+	+			+
H(G)	Eua	<i>Calamagrostis epigeios</i>	+	+	+	+	+
Th	Eua	<i>Centaureum umbellatum</i>	+	+			+
H	Eua(Md)	<i>Chrysanthemum leucanthemum</i>	+	+			+
TH—H	Eua(Md)	<i>Trifolium pratense</i>	+			+	
H	Eua(Md)	<i>Lotus corniculatus</i>	+	+	+		+
H	Eua(Md)	<i>Festuca arundinacea</i>				+	+
H	Cp	<i>Prunella vulgaris</i>	+			+	+
H	Eua	<i>Achillea millefolium</i>		+	+	+	+
		Festuco-Brometea + Festucion rupicolae					
H	Eua	<i>Hypericum perforatum</i>	+	+	+	+	+
Th	Eua(Md)	<i>Crepis rheadifolia</i>	+		+		
H	Md-Ec	<i>Prunella laciniata</i>	+		+		+
H	Eua(Md)	<i>Potentilla argentea</i>	+	+	+		+
H—Ch	Ec(Md)	<i>Dorycnium herbaceum</i>		+	1—2	1—2	
TH—H	Eua(Md)	<i>Carlina vulgaris</i>		+	+		
H	E(Md)	<i>Agrimonia eupatoria</i>		+	+	+	
H	Ec(Md)	<i>Coronilla varia</i>			+	+	
		Festucetalia valesiacae					
N	Pt-Pn-Blc	<i>Chamaecytisus albus</i>		1		1	
TH—H	E(Md)	<i>Centaurea micranthos</i>		+	+		
		Compagnes					
Th	Adv	<i>Stenactis annua</i>	+	+			

(Tableau 3—suite)

Forme biologique	Elément floristique	Nombre du relevé	1	2	3	4	5
		Superficie (m ²)	100	100	100	100	100
		Hauteur de la végétation (cm)	45	40	50	40	45
		Couverture (%)	95	95	100	95	95
		Exposition	—	—	S	—	S
		Inclinaison (degré)	—	—	15	—	5
		Localité	Cocoreni		Valea cu apă		
H	Ct(Eua)	<i>Hieracium bauhini</i>	+	+		+	
Th	Eua(Md)	<i>Vulpia myuros</i>	+	+			
H	Eua	<i>Chondrilla juncea</i>		+	+	+	
Th	Atl-Md	<i>Aira caryophylla</i>		+	+	+	
N	Ec	<i>Rubus sulcatus</i>		+	+	+	
H	Cp(Md)	<i>Clinopodium vulgare</i>		+	+	+	+
TH—H	Eua	<i>Verbascum nigrum</i>			+	+	
Th	Eua	<i>Filago minima</i>			+	+	
Th	Eua(Md)	<i>Vicia tetrasperma</i>		+	+	+	

Espèces dans un seul relevé : *Cichorium intybus* (2), *Cynoglossum officinale* (3), *Cruciata glabra* (4 : +1), *Dianthus armeria* (5), *Daucus carota* (2), *Eryngium campestre* (3), *Erigeron canadensis* (1), *Festuca rubra* (5), *Filago arvensis* (2), *Fragaria vesca* (5), *Genista elata* (4), *Galium mollugo* (3), *Prunus spinosa* (4), *Plantago media* (1), *Senecio jacobea* (4), *Scleranthus perennis* (3), *Tanacetum vulgare* (3), *Taraxacum officinale* (1), *Thymus pulegioides* (4), *Vicia sepium* (5), *V. cracca* (4), *Xeranthemum foetidum* (4).

Date : Relevés 1—4,3 VII. 1980 ; relevé 5 : 5.VII.1980.

En calculant l'indice de similitude d'après J. Jaccard entre les phytocénoses décrites par Gh. Popescu [10] et celles signalées par W. Szafer, B. Pawlowski et S. Kulczynski [14], dans les Tatras, sous le nom d'*Agrostidetum vulgaris* à une altitude qui varie entre 930—1100 m et qui comprend plusieurs espèces montagneuses, on constate qu'il est de 10,37%, ce qui montre une faible similitude. Le même calcul, fait selon la formule de Sørensen, montre aussi une faible similitude (18,78%) entre les phytocénoses d'Olténie décrites par Gh. Popescu et celles de Pologne.

En appliquant le coefficient de similitude de Jaccard au noyau central des phytocénoses d'Olténie, décrites par Gh. Popescu [10] comme *Agrostietum tenuis* Szafer, Pawl. et Kulcz. 23 et *Festuco (rubrae)-Agrostietum* Csürös-Káptalan 64, on observe une plus grande similitude (61,65%) et non pas une petite similitude ainsi que l'auteur nous indique selon la formule qu'il nous donne (p. 205).

Entre nos phytocénoses encadrées dans le *Rumici (acetosellae)-Agrostietum tenuis* et celles décrites par Gh. Popescu [10] en tant qu'*Agrostietum tenuis* Szafer, Pawl. et Kulcz. 23, l'indice de similitude calculé selon la formule indiquée par Gh. Popescu (p. 205) (coefficient de similitude = 0,53) montre une grande ressemblance.

St. Csűrös et A. Kovács [3] intègrent ces phytocénoses mésophylles des collines des alentours de Sighişoara et d'Agnita dans l'association *Agrostis tenuis-Dorycnium herbaceum* qu'ils ont décrite pour la première fois.

Dorycnium herbaceum, présente abondamment aussi dans nos relevés, prouve que ces prairies se sont installées à la suite du défrichement des forêts, l'espèce étant rencontrée seulement dans les relevés effectués dans l'immédiat voisinage de *Quercetum farnetto-ceris*, s'absentant généralement des autres.

Nous considérons ces agrostètes collinaires largement répandues dans notre pays, comme une nouvelle association, *Rumici (acetosellae)-Agrostietum tenuis*, différente de celles de la région montagnarde qui sont en général mêlées à *Festuca rubra*.

R. Soó [13] intègre l'association *Agrostietum tenuis* Szafer, Pawl. et Kulcz. 23 dans la classe *Nardo-Callunetea*, d'où l'on peut déduire qu'elle présente dans sa composition plusieurs espèces montagnardes, pendant que les agrostètes de la région collinaire sont intégrées dans la classe *Arrhenatheretea*, l'alliance *Cynosurion cristati*, dominées par des espèces mésophiles, avec la participation considérable des nombreuses xéro-mésophiles ou xérophiles de la classe *Festuco-Brometea* (tableau 3).

Dans les phytocénoses avec *Agrostis tenuis* des Tatras participe un grand nombre d'espèces montagneuses parmi lesquelles nous mentionnons : *Euphrasia montana*, *Phleum alpinum*, *Senecio subalpinus*, *Arabis halleri*, *Potentilla aurea*, *Festuca rubra*, *Phyteuma spicatum*, *Poa alpina*, *Rumex arifolius*, *Campanula kladniana*, *Rumex alpinus*, espèces qu'on rencontre aussi chez nous, mais dans les agrostètes des plaines l'altitude.

Les plaines avec *Agrostis tenuis* représentent un stade avancé en friche du substrat, labouré ou défriché. C'est ainsi qu'on explique la présence d'un nombre remarquable d'espèces de mauvaises herbes, maintenues même dans les phytocénoses dans lesquelles domine l'*Agrostis tenuis*. À la mesure de l'évolution des phytocénoses avec *Agrostis tenuis*, les espèces végétales caractéristiques aux terrains labourés deviennent de plus en plus rares, en voie de disparition là où l'influence de l'homme est réduite.

10. As. *Rumex crispus-Alopecurus geniculatus* Tx. (37)50 (Syn : *Alopecuretum aequalis* Soó 47, 49) est rencontré dans les microdépressions des chênaies de Valea cu Apă, en occupant d'habitude les bords. À côté de *Alopecurus aequalis* et *Rumex crispus* on rencontre : *Lysimachia nummularia*, *Mentha pulegium*, *Ranunculus repens*, *Hypericum hirsutum*, *Alisma plantago-aquatica*, *Agrostis stolonifera*, etc.

11. *Juncetum effusi* (Eggler 33) Soó 40 occupe le centre des microdépressions des chênaies de Valea cu Apă, ayant les mêmes espèces accompagnatrices que l'As. *Rumex crispus-Alopecurus geniculatus* Tx. (37)50.

BIBLIOGRAPHIE

1. Borza Al., Biológia, Bratislava, 1963, 18, 11, 856—864.
2. Buia Al., Păun M., Safta I., Pop M., Lucr. Inst. Agron. Craiova, Bucureşti (1959), 1960, 93—182.

3. Csűrös St., Kovács A., Contrib. bot., Cluj, 1962, 205—232.
4. Horvat I., Glavač V., Ellenberg H., Vegetation Südosteuropas, Jena, 1974.
5. Ivan Doina, Fitocenologie şi vegetaţia Republicii Socialiste România, Edit. didactică şi ped., Bucureşti, 1979.
6. Neuhäusl R., Neuhäuslová-Novotná Zdenka, Contrib. bot., Cluj, 1967, 247—261.
7. Passarge H., Hofmann G., Pflanzengesellschaften des nordostdeutschen Flachlandes II. Jena, 1968.
8. Păun M., Popescu Gh., Zaharia I., St. şi cerc. Tg. Jiu, 1972, 185—192.
9. Păun M., Zaharia I., Popescu Gh., St. şi cerc. Tg. Jiu, 1972, 193—200.
10. Popescu Gh., Contrib. bot. Cluj-Napoca, 1979, 199—206.
11. Resmeriţă I., Rev. roum. Biol., 1975, 20, 1, 19—26.
12. Soó R., Rev. roum. Biol., 14, 1, 65—72.
- 13.—, Synopsis systematico-geobotanica florum vegetationis Hungariae. Akadémiai Kiadó, Budapest, 1964—1973, 1—5.
14. Szafer W., Pawlowski B., Kulczyński S., Bull. Intern. de l'Acad. Polon. des Sci. et des lettres. Sér. Sc. nat., Cracoviae, 1927, Supl. II, 13—78.

Reçu le 13 août 1980

Institut de sciences biologiques
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POLLENMORPHOLOGIE UND EMBRYOGENESE BEI *DIGITALIS PURPUREA* L. und *D. THAPSI* L.

VON

NATALIA MITROIU-RĂDULESCU und MARIANA MOROIANU

From the analysis of pollen-grain morphology of *Digitalis purpurea* and *D. thapsi*, there results a high degree of resemblance of the pollen-grains of these two species; as a distinctive feature, the reticulation of the exine is much finer in *D. purpurea*.

Embryogenesis proceeds after the *Onagrad*-type, corresponding to *Veronica*-variety. The endosperm generates the two polar haustoria, while the micropylar one is tetracellular in *D. purpurea* and bicellular in *D. thapsi*, a feature characterizing the *Scrophulariaceae*.

Die Gattung *Digitalis* ist eine der wichtigsten der zahllosen Pflanzen, die überall auf der Erde als Heilpflanzen und Zierpflanzen angebaut werden. In der Heilkunde wird *Digitalis purpurea* L., als wichtigste Quelle herzwirksamer Glukoside, in großer Menge verwendet. Die in neuerer Zeit durchgeführten pflanzenchemischen Untersuchungen, welche auch andere Arten der Gattung *Digitalis* betreffen, haben gezeigt, daß ein vielseitiges Studium der Arten notwendig ist; zu diesen gehört auch *D. thapsi*. Abgesehen von älteren monographischen Schriften über die Gattung, gibt es auch neuere Arbeiten über die Biologie der *Digitalis*-Arten (8, 2).

Im Fachschrifttum findet man auch Angaben über den Pollentypus dieser Gattung (7, 5, 3, 4). Was die Embryogenese anbelangt, beziehen sich die Daten ganz allgemein auf die Familie der *Scrophulariaceae* (10, 6, 9) sowie auf gewisse zytologische Studien über den Embryosack von *Digitalis purpurea* (1).

Die hohe Bedeutung dieser Gattung war die Veranlassung für die vorliegende Arbeit, welche den Zweck verfolgt, die Daten über die Palynologie und die Embryogenese der beiden *Digitalis*-Arten zu bereichern.

Für die Untersuchungen wurde *Digitalis purpurea*- und *D. thapsi*-Material aus dem Botanischen Garten in Bukarest verwendet.

Der Pollen wurde sowohl in Wasser als auch in Chloralhydrat untersucht.

Die Beobachtungen über die Embryogenese wurden an Mikrotom-Längsschnitten (Dicke 10μ) durch das Gynäzeum in verschiedenen Entwicklungsstadien durchgeführt; die Schnitte wurden mit Ehrlich's Hämatoxylin gefärbt.

ERGEBNISSE UND DISKUSSION

Der Pollen beider Arten erscheint in Wasser und in Chloralhydrat hellgelb gefärbt.

Die Gestalt der Pollenkörner ist subprolat-prolat bei *D. purpurea* und prolat-kugelig-subprolat bei *D. thapsi*. Auch der Größe nach ähneln sich die Pollenkörner beider Arten, wobei die Abmessungen zwischen 28,8–31,2 μ $\varnothing$ bei *D. purpurea* und 28,8–33,6 μ $\varnothing$ bei *D. thapsi* schwanken. Bei letzterer ist die Sporodermis etwas dicker (1,6–2,4 μ) und weist bei beiden Arten denselben Typus der Struktur und der Ornamentation der Exine auf, nämlich tectat-baculat mit einem Netz auf der Außenfläche, welches bei *D. purpurea* viel feinmaschiger ist (Abb. 1, 2). Auch die Kolpen sind bei *D. purpurea* schmaler und feinwarziger.

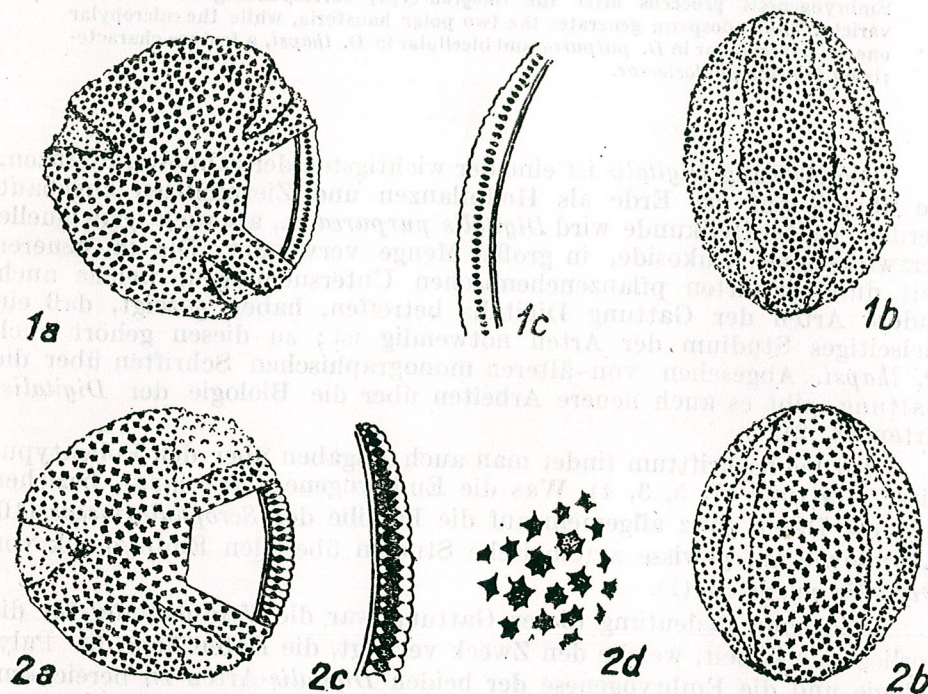


Abbildung 1: *Digitalis purpurea* L.; Scheitelansicht und Ausschnitt der Sporodermis eines Pollenkornes; b, Seitenansicht eines Pollenkornes mit langen Colpi; c, Struktur der Sporodermis (a, b = 1120 $\times$; c, = 2400 $\times$. Original.)

Abbildung 2: *Digitalis thapsi* L.; a, Scheitelansicht und Ausschnitt der Sporodermis; b, Seitenansicht mit langen Colpi; c, Struktur der Sporodermis; d, Vergrößerung eines Teiles der Außenansicht (a, b = 1120 $\times$; c, = 2400 $\times$; d, Handzeichnung. Original).

Bei keiner der beiden untersuchten Arten konnte die Anwesenheit von Pollenkitt auf der Oberfläche der Pollenkörner festgestellt werden, entgegen der Bemerkung von E. Daumann (2).

Die anatrop-apotropen Samenanlagen besitzen zwei Integumente und sind crassinucellat. Der Embryosack ist länglich und im vorderen Drittel erweitert, wobei die Erweiterung bei *D. thapsi* stärker zum Ausdruck kommt.

Bei *D. purpurea* ist der Übergang vom verlängerten zum erweiterten Teil allmählich, während er bei *D. thapsi* plötzlich erfolgt (Taf. I Abb. 3). Der Übergang besteht eigentlich in einer Einschnürung des Embryosacks, worin sich der Sekundärkern befindet. Der Embryosack besteht bei beiden Arten aus den gleichen Bestandteilen (Taf. I Abb. 4). Das Eindringen des Pollenschlauches in den Embryosack geschieht bei den untersuchten Spezies auf gleiche Weise, wobei eine Synergide durchdrungen wird (Taf. I Abb. 5). Die Befruchtung erfolgt sowohl bei *Digitalis purpurea* als auch bei *D. thapsi* auf die gleiche Art, wobei zuerst die Nebenzygote und danach die Hauptzygote gebildet wird (Taf. I, Abb. 6); das Volumen des Embryosacks nimmt dabei stark zu.

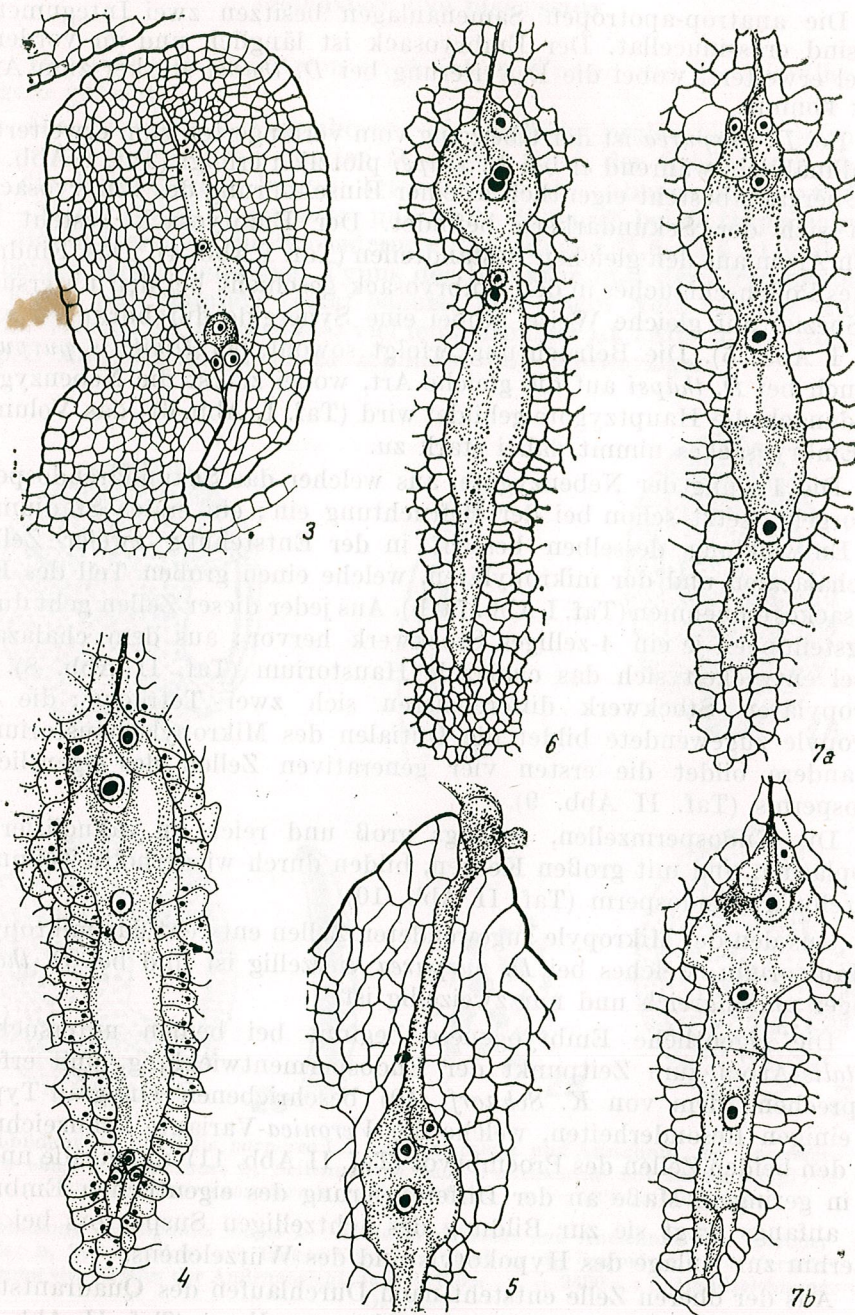
Die Teilung der Nebenzygote, aus welcher das Sekundärendosperm hervorgeht, setzt schon bei der Befruchtung ein; ein erstes Stadium in der Entwicklung desselben besteht in der Entstehung zweier Zellen, des chalazalen und der mikropylären, welche einen großen Teil des Embryosackes einnehmen (Taf. I Abb. 7a, b). Aus jeder dieser Zellen geht durch Längsteilungen je ein 4-zelliges Stockwerk hervor; aus dem chalazalen Stapel entwickelt sich das chalazale Haustorium (Taf. II Abb. 8). Im mikropylären Stockwerk differenzieren sich zwei Tetraden; die der Mikropyle zugewendete bildet die Initialen des Mikropylarhaustoriums; die andere bildet die ersten vier generativen Zellen des eigentlichen Endosperms (Taf. II Abb. 9).

Die Endospermzellen, anfangs groß und reich an vakuolisiertem Zytoplasma, und mit großen Kernen, bilden durch wiederholte Teilungen das zelluläre Endosperm (Taf. II Abb. 10).

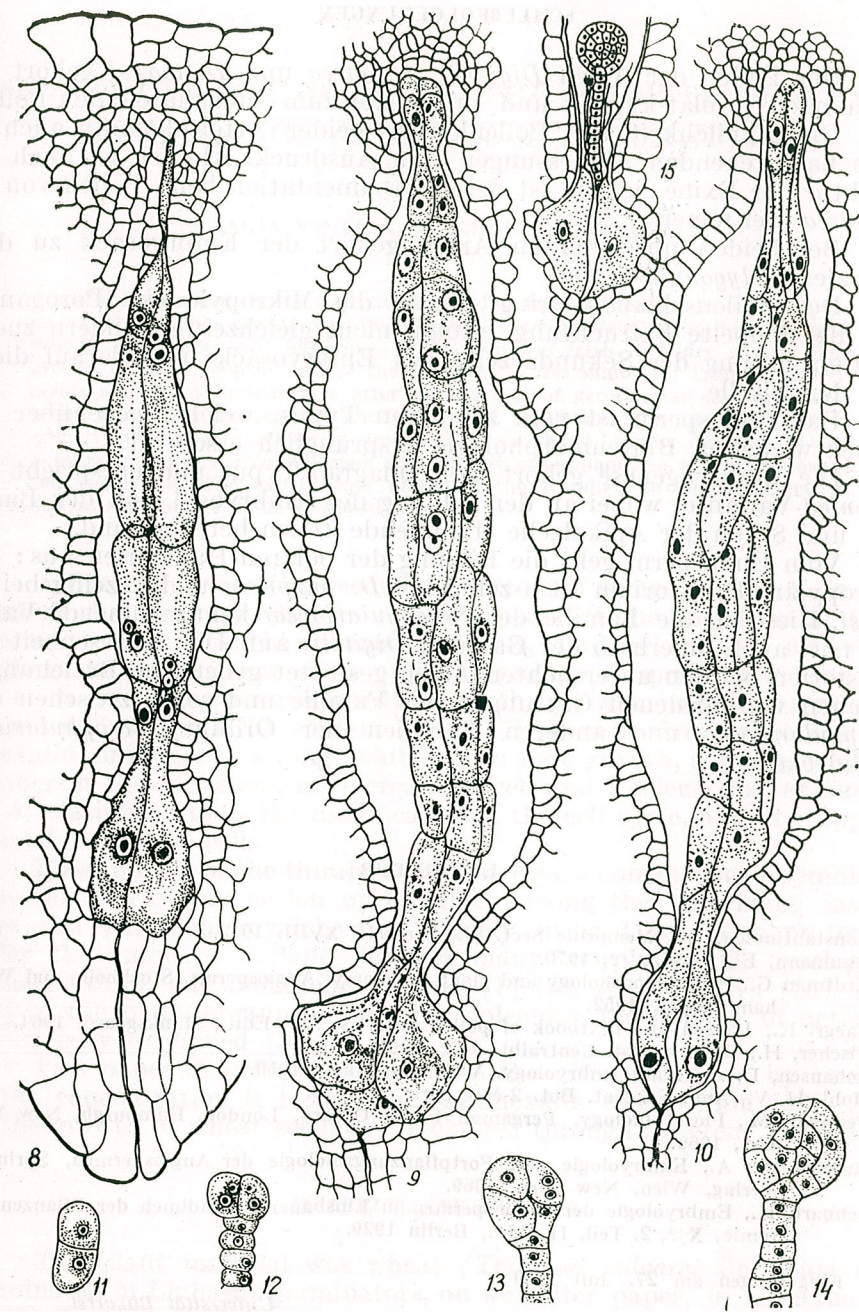
Aus den der Mikropyle zugewendeten Zellen entsteht das mikropyläre Haustorium, welches bei *D. purpurea* vierzellig ist und bei *D. thapsi* weniger umfangreich und nur zweizellig ist.

Die eigentliche Embryogenese beginnt bei beiden untersuchten *Digitalis*-Arten zum Zeitpunkt der Endospermentwicklung, und erfolgt entsprechend dem von K. Schnarf (10) beschriebenen Onagrad-Typus, mit einigen Besonderheiten, welche die *Veronica*-Variante kennzeichnen. Von den beiden Zellen des Proembryos (Taf. II Abb. 11) nimmt die untere nur in geringem Maße an der Differenzierung des eigentlichen Embryos teil; anfangs trägt sie zur Bildung des achtzelligen Suspensors bei und späterhin zur Anlage des Hypokotyls und des Würzelchens.

Aus der oberen Zelle entsteht nach Durchlaufen des Quadrantstadiums (Taf. II Abb. 12) und dann des Oktantstadiums (Taf. II Abb. 13) der kugelige Embryo (Taf. II Abb. 14, 15) aus dem sich im weiteren Verlauf die Initialen der Keimblätter, des Epikotyls, der Hypophyse mit den Initialen des Stamm-Zentralzylinders differenzieren.



Tafel I. Abbildung 3: *Digitalis thapsi* L., Embryosack in crassinucellaten anatropen-orthotropen Ovulum; 4, Reifer Embryosack von *D. purpurea*; 5, Eindringen des Pollenschlauches in eine Synergide von *D. purpurea*; 6, Befruchtung derselben; 7a, b, Erstes Stadium der Nebenzgotenteilung und Bildung des sekundären Endosperms und Entstehung einer mikropylären und chalazalen Zelle; 7a, bei *Digitalis purpurea*; 7b, bei *D. thapsi*; (3—7 = 560 $\times$. Original).



Tafel II. Abbildung 8: Differenzierung der chalazalen und mikropylären Stufe und des Endosperms bei *Digitalis purpurea*; 9, Endospermatische Haustorien und Endosperm bei *Digitalis purpurea*; 10, Dasselbe Stadium bei *D. thapsi*; 11—14, Verschiedene Entwicklungsstadien des Embryos bei *Digitalis purpurea* (11, zweizelliges Proembryo; 12, Quadrant-Stadium; 13, Octant-Stadium; 14, kugeliges Embryo; 15, kugeliges Embryo und ein Teil des mikropylären Haustoriums; 8—10, 15 = 560 $\times$; 11—14, Handzeichnungen) (Original).

SCHLUSFOLGERUNGEN

Der Pollen der Arten *Digitalis purpurea* und *D. thapsi* gehört der Form nach (prolat-kugelig und subprolat) zum subsphäroidalen Pollen.

Die Ähnlichkeit der Pollenkörner beider Arten kommt auch in ihren nahestehenden Abmessungen zum Ausdruck. Ähnlich ist auch die Struktur der Exine, jedoch ist deren Ornamentation beim Pollen von *D. purpurea* viel feiner.

Bei beiden untersuchten Arten gehört der Embryosack zu dem normalen *Polygonum*-Typus.

Der Pollenschlauch dringt durch die Mikropyle ein (Porogamie) und die doppelte Befruchtung erfolgt nicht gleichzeitig, sondern zuerst die Befruchtung der Sekundärzelle des Embryosacks und darauf diejenige der Eizelle.

Das Endosperm ist vom zellulären Typus, welcher gegenüber der hochentwickelten Blütenmorphologie ursprünglich erscheint.

Die Embryogenese gehört zum Onagrad-Typus und entspricht der *Veronica*-Variante, wobei an der Bildung des Embryos 4 von der Basalzelle und 8 von der Apikalzelle stammende Zellen beteiligt sind.

Vom Endosperm geht die Bildung der polaren Haustorien aus: das mikropyläre Haustorium ist 4-zellig bei *D. purpurea* und 2-zellig bei *D. thapsi*. Diese für die Familie der *Scrophulariaceae* kennzeichnende Variation tritt auch innerhalb der Gattung *Digitalis* auf. Die Anwesenheit der Haustorien bei den untersuchten Arten gestattet genetische Beziehungen zwischen verschiedenen Gattungen der Familie und sogar zwischen den *Scrophulariaceae* und anderen Familien der Ordnung *Scrophulariales* aufzudecken.

LITERATUR

1. Constantinescu, Gr., *Memoriile Sect. Șt., Ser. III, XVIII*, 1942.
2. Daumann, E., *Preslia*, 42, 1970.
3. Erdtman G., *Pollen morphology and plant taxonomy. Angiosperms*. Stockholm and Waltham, Mass., 1952.
4. Faegri K., Iversen, J., *Textbook of pollen analysis*, Sec. Edit., Munksgaard, 1964.
5. Fischer, H., *Beih. z. Bot. Centralbl.*, I, 2, 1891.
6. Johansen, D. A., *Plant embryology*, Waltham, Mass., 1950.
7. Mohl, H. V., *Ann. Sc. Nat. Bot.* 2-ème Série, 3, 1835.
8. Percival, M., *Floral biology*, Pergamon Press, Oxford, London, Edinburgh, New York etc., 1969.
9. Rutishauser, A., *Embryologie und Fortpflanzungsbiologie der Angiospermen*, Springer-Verlag, Wien, New York, 1969.
10. Schnarf, K., *Embryologie der Angiospermen in Linsbauer's Handbuch der Pflanzenanatomie*, X/2, 2. Teil, II. Abt., Berlin 1929.

Eingegangen am 27. Juli 1980

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THE EFFECT OF LINDANE ON THE GROWTH OF WHEAT SEEDLINGS (*TRITICUM VULGARE* L.)

BY

ROZALIA VINTILĂ, M. KEUL and AL. POLIZU

The effect of lindane, in the concentration it has in the thirahexalin composition, on wheat seedlings growth was investigated. Lindane strongly inhibits the root growth in length and determines the lowering of fresh and dry weight. The growth of the coleoptile and of the first leaf is less inhibited. Transfer experiments show that lindane acts after the starting of germination and organogenesis. The effects induced by lindane are not reversible. The inhibition of root growth induced by lindane is due, on the one hand, to the decrease of cell number in the meristematic zone and, on the other hand, to the inhibition of cell elongation. The experiments underline the thirahexalin phytotoxicity as due to the lindane in its composition.

Aiming to know the secondary effects of some pesticides, in previous works we have shown certain aspects of the thirahexalin (50% thiuram + 10% hexachlorobenzene + 20% lindane) action on wheat seedlings in an incipient stage of development. A special attention was given to the quantitative analysis of the growth and differentiation process and of the DNA content [16], [17]. It was shown that the thirahexalin produces a strong inhibition in root growth, a decrease of cell number in the meristem, an increase of cell and nucleus size, of nuclear DNA relative content, the modification of the cell cycle, and the appearance of polyploid cells.

These effects of the thirahexalin underline a conspicuous resemblance with the phytotoxic action of lindane. Among the chlorinate insecticides the γ -hexachlorocyclohexane, widely utilized in plant protection under the name of lindane, induces many morphological, cytological, ultrastructural, and metabolic changes [1] [2], [5], [7], [8], [10], [11], [15] in the plant organism, but the molecular mechanism of action is not entirely explained [3].

This paper describes and analyses the effects induced by lindane, at the concentration it has in the thirahexalin composition [16], [17], on the growth of wheat seedlings, reckoned through different parameters.

MATERIAL AND METHOD

The plant material was wheat (*Triticum vulgare*, Bezostaia sort) germinated in Linhardt germinators, on wet filter paper, in a thermostat at 22° C, under dark.

The treatment with lindane was applied taking into account the percentage ratio in the thirahexalin [16], the equivalent doses being of

25, 50, and 100 g lindane for 100 kg caryopsides. The lindane quantities in the above mentioned concentrations were recalculated for 20 caryopsides/ germinator, dissolved in 3 ml acetone, evenly spread on filter paper support. When caryopsides were set to spring, 25 ml water have been added. The control seedlings had an equivalent quantity of acetone in the medium. The necessary humidity was daily assured by adding distilled water during the germination and growth.

The growth dynamics in the root system, in the coleoptile, and in the first leaf respectively, was prosecuted to the 6th day, including the germination setting up; the growth in length was daily measured. The absolute length and the growth rate in 24 hours have been observed. For each variant, two repetitions for three parallel samples of 20 seedlings each have been made and the data statistically reckoned.

The growth of wheat seedlings treated with pesticide was also estimated by determining the fresh and dry weight at the end of the experiment. The effect of lindane on the root growth was determined through a cytologic parameter: the cell number [12] on successive root segments of 1 mm, 4 days after the experiment was set up, according to the described method [16].

RESULTS

Growth in length. The measurements effectuated each 24 hours on the root length, 48 hours after the experiment starting, are given in Fig. 1. The root system in the control seedlings shows a linear growth, directly proportional to the seedling age.

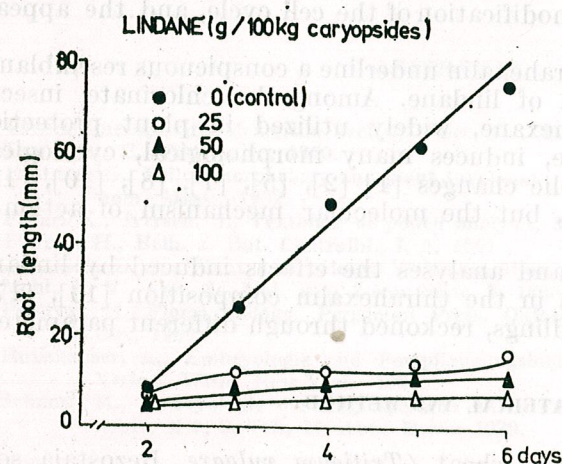


Fig. 1. — The effect of lindane on the root growth.

The treatment with lindane brings about a deviation from the normal pattern of root growth. Forty-eight hours after the experiment had been started, the length of the roots in the control seedlings and in those grown in lindane medium have resembling values (Fig. 1), suggesting that the action of the pesticide is not immediately manifest. After this

interval, the action of lindane is drastic, the root growth in length is strongly reduced in the following 24 hours, and then it practically ceases. It may be observed that the effects induced by the experimented lindane concentrations are almost identical, suggesting that the biologically active component exceeds in all cases the threshold of toxic concentration. The external signs of the effects of lindane are the much more short roots, stiff and club-like (subapical swelling), with branched out root hairs.

To appreciate the effect induced by lindane, supplementary information may also be obtained out of the root growth rate analysis (Fig. 2).

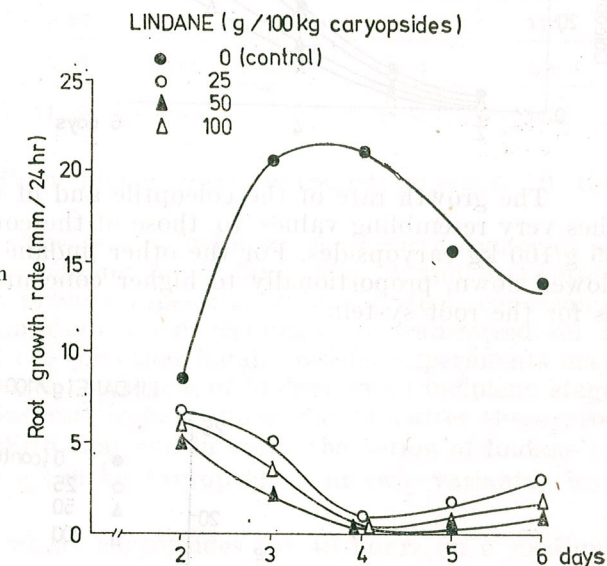


Fig. 2. — The effect of lindane on the root growth rate.

Thus, while with the control a first superior limit of the root growth is observed on the 4th day, the growth rate evaluates much under the control values, it being annulled on the 4th day (Fig. 2). The growth rate cancellation is produced by the stopping of growth in length and the appearance of new adventive roots (lindane does not influence the new roots, but their subsequent development). After the 4th day, the growth resumption, pointed out by the growth rate (Fig. 2), has extremely minute values (some mm), indicating that growth in length actually reached an end, as seen in Fig. 1.

The growth in length of the coleoptile and of the first leaf in the control and in treated plants develops according to certain exponential curves, represented in Fig. 3. Depending on the concentration, lindane also inhibits the coleoptile and the first leaf growth in length. However, the more moderate action of the pesticide on the development of the green part could be explained by the lack of its direct contact with lindane, unlike the case of the root, though it is known that lindane translocates in the green parts of plants too [14].

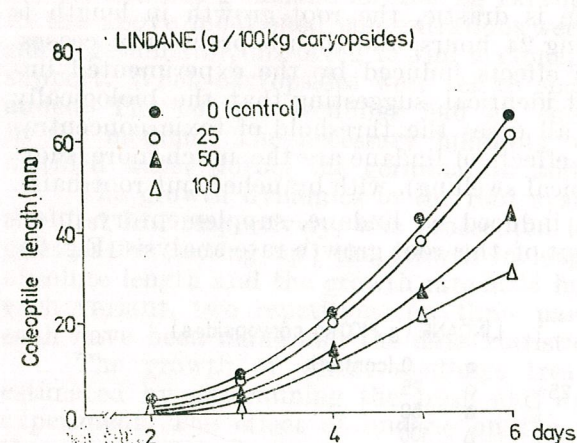


Fig. 3. — The effect of lindane on the coleoptile growth.

The growth rate of the coleoptile and of the first leaf (Fig. 4) reaches very resembling values to those of the control only for the dose of 25 g/100 kg caryopsides. For the other lindane doses, the growth rate is slowed down, proportionally to higher concentrations, without a ceasing as for the root system.

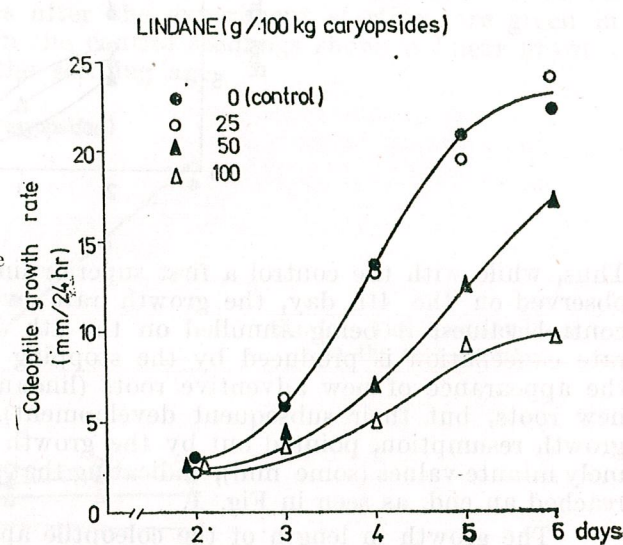


Fig. 4. — The effect of lindane on the coleoptile growth rate.

Fresh and dry weight. The determination of fresh and dry weight (Table 1) at the end of the experiment shows a drastic decrease of the fresh weight as compared to the increase of lindane dose for the root system. The fresh weight of the green part also decreases, but with a much more reduced effect. A similar evolution is to be seen with the dry absolute weight too. However, the relative values of the dry weight

Table 1

Effect of lindane on the fresh weight (f wt) and on the dry weight (dry wt) of wheat seedlings

Lindane (g/100 kg caryopsides)	Root			Coleoptile + 1 th leaf			Number of seedlings
	f wt	dry wt		f wt	dry wt		
	g	g	g/100 g f wt	g	g	g/100 g f wt	
0	7.09	0.43	6.14	6.53	0.63	9.62	112
25	4.11	0.32	7.97	7.06	0.69	9.77	114
50	2.30	0.22	9.82	5.65	0.57	10.11	109
100	1.51	0.17	11.15	5.55	0.58	10.45	113

underline an increase with a higher dose, extremely obvious in the root system.

Transfer experiments. The results including the growth dynamics of wheat seedlings on lindane medium for 6 days (Figs. 1 and 3) aroused the interest for the growth process, especially in root system, concerning the possibility of its resumption when seedlings are transferred on a medium without pesticide. On the other hand, transfer experiments may give the possibility to separate the action of lindane in an incipient stage of germination and organogenesis from its further action, after these processes have been started. With that end in view, the action of lindane in a single concentration (50 g/100 kg caryopsides), in two variants, was investigated:

A — germination of wheat caryopsides for 48 hours on a medium without lindane, transferred then on a medium with lindane for 24 and 48 hours, and again on a medium lacking the pesticide.

B — germination of wheat caryopsides for 48 hours in the presence of lindane, followed by seedlings transfer on a medium without lindane.

The data synthesized in Fig. 5 underline that in transfer experiments with A variant, the growth of roots and coleoptiles in length and that of the first leaf (fig. 5) are similar (in the limits of the biological variability) to a continuous treatment with lindane only (Fig. 1). These results render evident the lindane action as drastic after the starting of germination and organogenesis (in the first 48 hours the caryopsides being on a medium without lindane). The seedlings transfer after 24 or 48 hours under lindane action brings about identical results concerning the root length. Slight differences are observed only with the length of the coleoptile and first leaf, depending on the time after which the transfer is made (Fig. 5). The results are the same with a simple transfer of the seedlings from a medium with lindane to a medium missing the pesticide or if, subsequent to the transfer, a two hours washing in continuous water jet is made.

The results of variant B point out that lindane does not influence the root growth in length in the first 48 hours, the values being very close to the control. After this interval, the seedlings transfer on pesticide missing medium indicates a growth continuing on process but with very low values in the following 48 hours. Subsequently, the lengthening of the root becomes more intensive (Fig. 5).

Determination of the cell number. The drastic action of lindane in hindering the root growth in length determined us to investigate the influence of the treatment with pesticide on the proliferation capacity

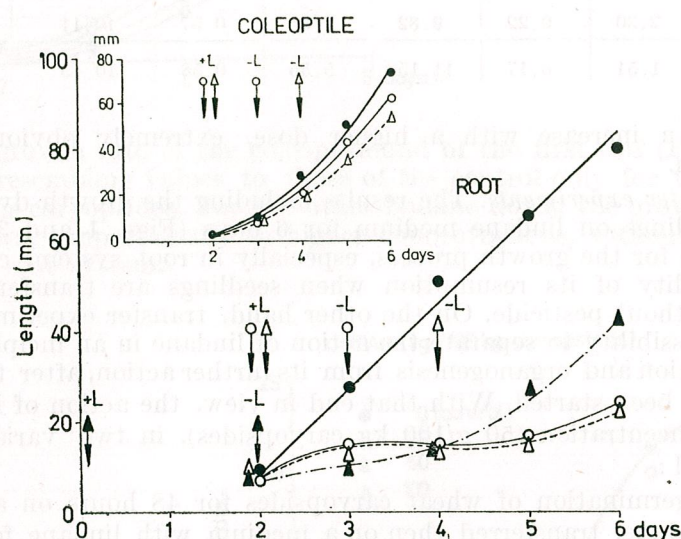
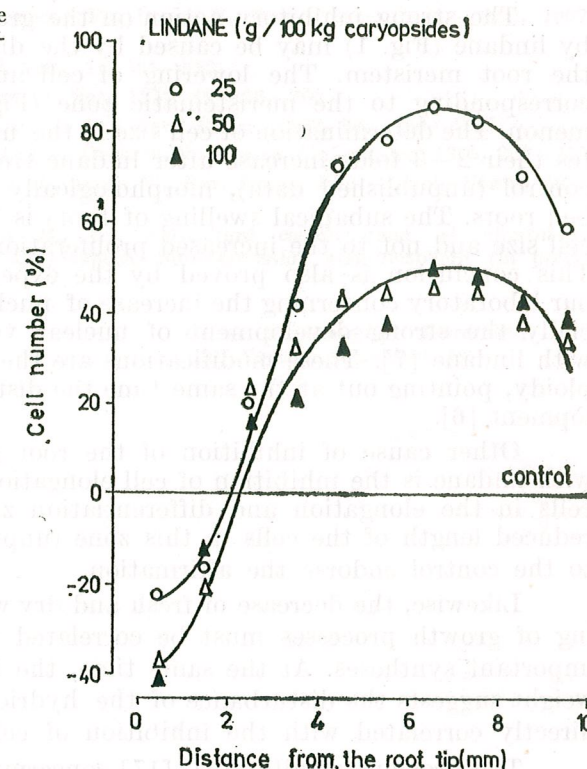


Fig. 5. — Short-term effect of lindane (transfer experiments) on the root and coleoptile growth.

of the meristematic tissue. It is known that the factors influencing the proliferation activity of the meristem determine growth modifications in the elongation zone too [4], [9]. The growth is manifest mostly during the differentiation process through the elongation of cells formed in the meristem.

The results of determinations (Fig. 6) show that the treatment with lindane induces the lowering of cells number in the first and second root segment, corresponding to the meristematic zone in wheat [13]. The cell lowering number is correlated between certain limits, with a higher dose. In the following root segments corresponding to the elongation and differentiation zone, the cells number increases as a result of the treatment with lindane, while the cells length (unpublished data) is reduced.

Fig. 6. — The effect of lindane on the cell number distribution in successive root segments.



DISCUSSIONS

Out of the results it is obvious that lindane has a drastic action on the development of the root system, and partially, depending on the dose, it actions on the coleoptile and the first leaf of wheat seedlings, as a consequence of the continuous growth on medium with pesticide (Figs. 1—4). The inhibition of the plants development, resulting from the different treatments with lindane or with preparations having lindane in their composition, was pointed out by other authors and with different vegetal tests [2], [8], [10], [11], [16]. In minute doses, lindane may have a stimulating effect in plant development [2].

The reversibility of the effects induced by lindane in lower plants, [1] could not be confirmed in higher plants. Our transfer experiments (A variant, Fig. 5) show that a 24 hours action of the pesticide is sufficient for inducing irreversible effects, when it takes place after the germination and organogenesis. Transfer experiments with variant B (Fig. 5) underline that in the first 48 hours the lindane has no influence on the root length growth, but they stress that the reversibility of effects is out of the question. The growth in length continues after a latent period and the reversibility of the effect is only partial (Fig. 5).

The strong inhibitory action on the growth of root length induced by lindane (Fig. 1) may be caused by the disturbance of the activity in the root meristem. The lowering of cell number in the root segments corresponding to the meristematic zone (Fig. 6) points out this phenomenon. The determination of cell size in the meristematic zone demonstrates their 2—3 folds increase after lindane treatment as compared to the control (unpublished data), morphologically rendered evident by clubbed roots. The subapical swelling of roots is hence due to the growth of cell size and not to the increased proliferation capacity of the meristem. This conclusion is also proved by the experimental data obtained in our laboratory concerning the increase of nuclear DNA content and especially the strong development of nuclear volumes after the treatment with lindane [7]. These modifications are the result of the induced polyploidy, pointing out at the same time the disturbance of cell cycle development [6].

Other cause of inhibition of the root growth after the treatment with lindane is the inhibition of cell elongation. The increased number of cells in the elongation and differentiation zone (Fig. 6) as well as the reduced length of the cells in this zone (unpublished data) as compared to the control endorse the affirmation.

Likewise, the decrease of fresh and dry weight shows that the blocking of growth processes must be correlated to the inhibition of some important syntheses. At the same time, the increase of the relative dry weight suggests the disturbance of the hydric metabolism, which may be directly correlated with the inhibition of cell elongation.

The previous results [16], [17] concerning the phytotoxic action of thirahexalin compared with the results in this paper show obvious resemblances. This similarity of the effects brings about the conclusion that the thirahexalin phytotoxicity is due to the lindane phytotoxicity.

REFERENCES

1. Borghi H., Puiseux-Dao S., Bonotto S., Hoursiangou-Neurbrun D., *Protoplasma*, 1973, **78**, 99—112.
2. Charnetski W. A., Lichtenstein E. P., Evert R. F., *Can. J. Bot.*, 1973, **51**, 2111—2117.
3. Corbett J. R., *The biochemical mode of action of pesticides*. Academic Press, London—New York, 1974.
4. Gudkov I. N., Grodzinski D. M., *Radiobiologia*, 1972, **12**, 566—572.
5. Lazăr-Keul G., Crăciun C., Soran V., *Biochem. Physiol. Pflanzen (BPP)*, 1978, **172**, 191—194.
6. Lazăr-Keul G., Soran V., Keul M., Polizu Al., *Rev. Roum. Biol.—Biol. Végét.*, 1981, **26**, 19—27.
7. Lazăr-Keul G., Soran V., Vintilă R., Polizu Al., Keul M., *Rev. Roum. Biol.—Biol. Végét.*, 1979, **24**, 69—75.
8. Lichtenstein E. P., Millington W. F., Cowley G. T., *J. Agr. Food Chem.*, 1962, **10**, 251—256.
9. Luxova M., *Biol. Plant.*, 1979, **21**, 355—360.
10. Manolache Fl., Morlova I., Ivaşcu V., *Analele I.C.C.A.*, 1964, **2**, 213—224.

11. Manolache C., Morlova I., Manolache Fl., Ivaşcu V., *Rev. Roum. Biol.—Bot.*, 1967, **12**, 273—279.
12. Obruceva N. V., *Fiziol. rastenii*, 1964, **11**, 551—552.
13. Ste-Marie G., Weinberger P., *Can. J. Bot.*, 1971, **49**, 195—200.
14. Talekar N. S., Lichtenstein E. P., *J. Agr. Food Chem.*, 1972, **20**, 1260—1263.
15. Trifu M., Cachița-Cozma D., Szalma I., *Contribuții botanice (Cluj-Napoca)* 1976, 251—256.
16. Vintilă R., Lazăr-Keul G., Keul M., Soran V., *Rev. Roum. Biol.—Biol. Végét.*, 1979, **24**, 133—141.
17. Vintilă R., Polizu Al., Keul M., in: *Agricultură contemporană și alimentație*, Acad. R.S.R., Filiala Cluj-Napoca, Subcommission Man-biosphere (in press).

Received April 26, 1980

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ABOUT THE MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF THE BLUE-GREEN ALGA *PHORMIDIUM VISCOSUM* LEMM.

BY

ANA FABIAN, FR. NAGY-TÓTH, ADRIANA BARNA, ELISABETA RÁCZ-BÉLTEKI

Phormidium viscosum Lemm. (filamentous blue-green alga) occurred on wet concrete blocks in a ditch of a thermoelectric work was selected in pure unialgal culture and grown in 10 artificial media, thermostated and thermoalternated, under static run and intense (bubbled) conditions, in order to establish its optimal nutritional requirements.

The form of thalli varied widely in the initial enriched culture, but it became more proportionated in the subsequent unialgal cultures on selected media, which besides the consequence of the simplified laboratory conditions, is considered as an adequacy of the nutrient media. In this respect the growth was optimal and the dimensions of the cells were more proportionated in Knop-Pringsheim-Felföldy's and Watanabe's media.

Extracts of thalli (prepared with distilled water and pure nutrient solutions) and liquids in which the algae have grown were with the *Lepidium* test bioassayed for their physiological effects. More inhibiting proved to be the liquids of Knop-Pringsheim-Felföldy's and Watanabe's culture media.

The physiological processes of organisms vary, more or less, according to the factors which act on them. The modifications brought about show up in the corresponding structures and processes of thalli and cells. It is believed that this correlation could be more explicit in blue-green algae, and a consequence is that their abundance and, accordingly, their importance in the environment increases with the human impact. The eagerness to study them manysidedly is therefore not surprising.

MATERIALS AND METHODS

The alga used in these experiences was found (Oct. 9, 1974) on the wet surface of concrete blocks in the outflow ditch of a thermopower station (temperature: water, 16°C; air, 10°C; pH, 7.5—8, water flow speed: about 35 m.s⁻¹).

The artificial medium from which the alga was collected had a simple composition (Benecke) suitable for green algae growth (due to its polytropy which characterizes many blue-green algae), so it could be separated afterwards and kept in pure unialgal culture.

Several static runs and intense (bubbled) batch cultures were performed at different temperatures (thermoalternated 20—22/18—20 and thermostated at 30°C) and light intensities (cool white fluorescent lamps

giving 2,500 and 5,500 luxs, respectively) in ten (liquid or agarized) artificial media (i.e. Benecke- K_2HPO_4 (BK₂), Benecke - KH_2PO_4 (BK), Witsch (W), Knop-Pringsheim (KP), Knop-Pringsheim-Felföldy (KPF), Allen-Arnon (AA), Sălăgeanu (SAL), Watanabe (WAT), Moyse (MO), Gorham (GO)). These media evidently differ both in respect of composition and of concentration of nutrients (Table 1). Some of them (AA, SAL, WAT, MO, GO, KPF) are complete media containing the microelements considered nowadays indispensable. "Any generalization concern-

Table 1

Composition of the artificial nutrient media used to cultivate *Phormidium viscosum* (g.l⁻¹, ml.l⁻¹)

Media	AA	SAL	BK	BK ₂	KP	KPF	WAT	MO	GO	W
Substances										
NaCl	0.233	—	—	—	—	—	—	—	—	—
MgSO ₄ ·7H ₂ O	0.246	—	0.02	0.02	0.10	0.10	0.20	0.125	0.075	—
CaCl ₂	0.055	—	—	—	—	—	0.05	—	0.036	—
Ca(NO ₃) ₂ ·4H ₂ O	—	—	0.12	0.12	0.10	0.10	—	—	—	—
K ₂ HPO ₄	0.174	—	—	0.05	0.20	0.20	0.50	0.50	0.36	—
KH ₂ PO ₄	—	0.01	0.05	—	—	—	—	—	—	0.05
K ₂ SO ₄	—	0.047	—	—	—	—	—	—	—	—
KNO ₃	—	—	—	—	1.00	1.00	0.50	0.25	—	0.10
NaNO ₃	—	—	—	—	—	—	—	0.25	1.50	—
NH ₄ NO ₃	—	0.20	—	—	—	—	—	—	—	—
FeCl ₃ ·7H ₂ O	—	0.001	0.001	0.001	0.01	—	—	—	—	—
FeSO ₄	—	—	—	—	—	—	0.05	0.005	—	—
Fe-citrate	—	—	—	—	—	0.01	—	—	0.006	0.006
Na ₂ CO ₃	—	—	—	—	—	—	—	—	0.02	—
Citric acid	—	—	—	—	—	0.01	—	—	0.006	—
EDTA	—	—	—	—	—	—	—	0.032	0.001	—
Acetic acid	—	—	—	—	—	1.00ml	—	—	—	—
Soil extract	—	—	—	—	—	100 „	—	—	—	100.ml
H ₃ BO ₃	0.008	0.005	—	—	—	0.004	0.114	0.005	0.005	—
MnSO ₄ ·4H ₂ O	0.020	—	—	—	—	—	—	—	—	—
MnCl ₂ ·4H ₂ O	—	5.10 ⁻⁴	—	—	—	0.002	0.014	5.10 ⁻⁴	5.10 ⁻⁴	—
MoO ₃	0.143	1.10 ⁻⁴	—	—	—	—	0.007	1.10 ⁻⁴	1.10 ⁻⁴	—
ZnSO ₄ ·7H ₂ O	0.221	5.10 ⁻⁴	—	—	—	0.004	0.084	5.10 ⁻⁴	5.10 ⁻⁴	—
CuSO ₄ ·5H ₂ O	79.10 ⁻⁴	2.10 ⁻⁴	—	—	—	0.004	0.016	2.10 ⁻⁴	2.10 ⁻⁴	—
Co(NO ₃) ₂ ·6H ₂ O	49.10 ⁻⁴	1.10 ⁻⁴	—	—	—	0.004	0.005	1.10 ⁻⁴	1.10 ⁻⁴	—
NH ₄ VO ₃	23.10 ⁻⁴	1.10 ⁻⁴	—	—	—	—	—	1.10 ⁻⁴	1.10 ⁻⁴	—
NiSO ₄ ·6H ₂ O	26.10 ⁻⁴	1.10 ⁻⁴	—	—	—	—	—	1.10 ⁻⁴	1.10 ⁻⁴	—
Cr(SO ₄) ₃	—	—	—	—	—	—	—	—	—	—
K ₂ SO ₄ ·24H ₂ O	98.10 ⁻⁴	1.10 ⁻⁴	—	—	—	—	—	1.10 ⁻⁴	1.10 ⁻⁴	—
Na ₂ WO ₄ ·2H ₂ O	16.10 ⁻⁴	1.10 ⁻⁴	—	—	—	—	—	1.10 ⁻⁴	1.10 ⁻⁴	—
TiO(C ₂ O ₄) _x ·yH ₂ O	8.10 ⁻⁵	—	—	—	—	—	—	—	—	—
K ₂ TiF ₆	—	2.10 ⁻⁴	—	—	—	—	—	2.10 ⁻⁴	2.10 ⁻⁴	—
LiCl	—	—	—	—	—	4.10 ⁻⁴	—	—	—	—
Al ₂ (SO ₄) ₃	—	—	—	—	—	4.10 ⁻³	—	—	—	—
SnCl ₂ ·2H ₂ O	—	—	—	—	—	2.10 ⁻⁴	—	—	—	—
NiSO ₄ ·7H ₂ O	—	—	—	—	—	4.10 ⁻³	—	—	—	—
TiO ₂	—	—	—	—	—	4.10 ⁻³	—	—	—	—
KI	—	—	—	—	—	2.10 ⁻⁴	—	—	—	—
KBr	—	—	—	—	—	2.10 ⁻⁴	—	—	—	—

ing the nutrition of algae is risky..., because of their tremendous heterogeneity, ... whatever attempt at generalizing the artificial nutrient media would be doubtful" [16].

In order to gather some informations about the ecophysiological effects which this species could exert on its surroundings and about the trends of the metabolism in different culture circumstances, the liquids/or the supernatant of cultures with *Lepidium*-test were bioassayed [1], [10].

RESULTS AND DISCUSSIONS

Phormidium viscosum occurs perhaps rather rarely. In the first catalogue of algae in Romania [15] it is not inserted. Compared with descriptions given in some available monographs [2] [3], [4], [5] this alga shows certain differences, e.g. lack (virtual/or real) of the mucilaginous sheath strata, slighter constriction at the crosswall of filaments (especially in liquid media), wider range of dimensions and proportions (length/diameter). It seems to be related to *Ph. papyraceum*, but its sheath does not stain with chloro-iodide-zinc solution and its thermophilic habitat was also proved in favour of *Ph. viscosum*.

As expected, the alga grew differently in the various media used. In solid (agar containing) media the trichomes tended towards the surface, and in liquid media, too, they adhered preferentially to the wall of flasks. This tendency is perhaps a mark of its inherited aerophilic-periphitic property. In younger cultures the thalli were bright blue-green subsequently fading to a blackish green owing to the exhaustion of nutrients and the accumulation of nitrogen in the cells [8], [13]. Such alternations could be observed especially in poorer media (e.g. BK, BK₂, W) (Plate I, 1—6).

The cell dimensions varied widely in the initial enrichment culture, i.e. diameter: 0.5—8.5 μ, length: 0.5—60 μ (Fig. 1). Elenkin's (1949) [2] values are: 4—5.5 × 8—16 μ. The limits of variations reduced considerably in pure cultures and are more or less characteristic of the nutrient media. If the initial dimensions were considered as 100% to which the subsequent cultures were related in respect of size of thalli and cells as well, the reduction would attain 18—54% in width and 20—53% in length (Table 2).

Table 2

Size (μ) of *Phormidium viscosum* cells in different nutrient media, proving the reduction of limits (expressed in %) as related to the wider variation in the initial enriched culture (which is considered 100%)

Media	Diameter		Length	
	μ	%	μ	%
Init. cult.	0.50—8.50	100	0.50—60.00	100
BK	2.00—3.25	36	1.25—4.00	40
KP	2.00—3.00	27	3.00—5.00	46
KPF	2.00—3.75	45	0.50—4.25	53
WAT	1.75—4.75	54	0.75—4.50	53
MO	2.75—3.00	18	1.25—3.50	20
GO	2.25—3.00	18	1.25—2.50	20
SAL	2.00—3.00	27	1.00—3.00	26

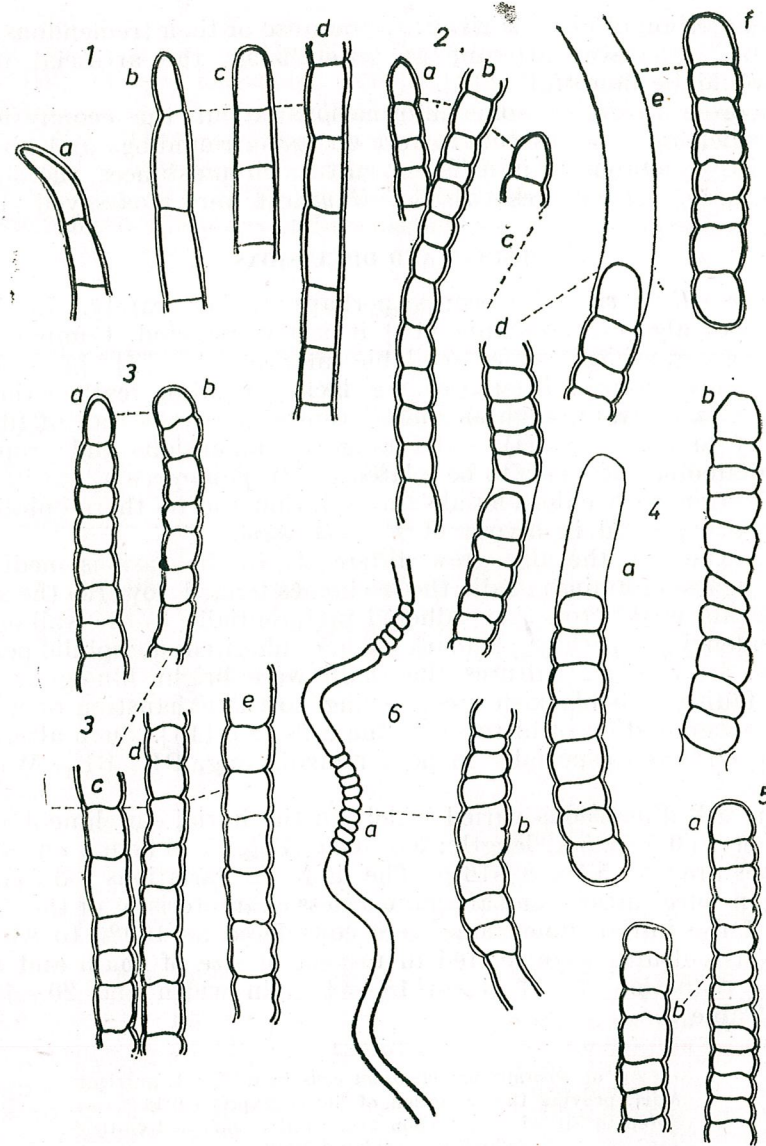


Plate I.—Forms of *Phormidium viscosum* developed in different life conditions. 1—Trichomes from the initial enriched culture with terminal (a—c) and middle (d) cells. 2—Trichomes formed in unialgal culture in Benecke's medium with terminal (a) and middle (b) cells from thermostated culture, and terminal cells (c) and formation (d) of hormogonium (f) inside the mucilaginous sheath (e) in thermoalternated culture. 3—Trichomes (a—e) formed in Knop-Pringsheim's medium. 4—6—Trichomes grown in bubbled (1.5 %CO₂ in sterilized air), thermoalternated cultures in Moyse's (4a, 4b), Gorham's (5a, 5b) and Sălăgeanu's (6a, 6b) media.

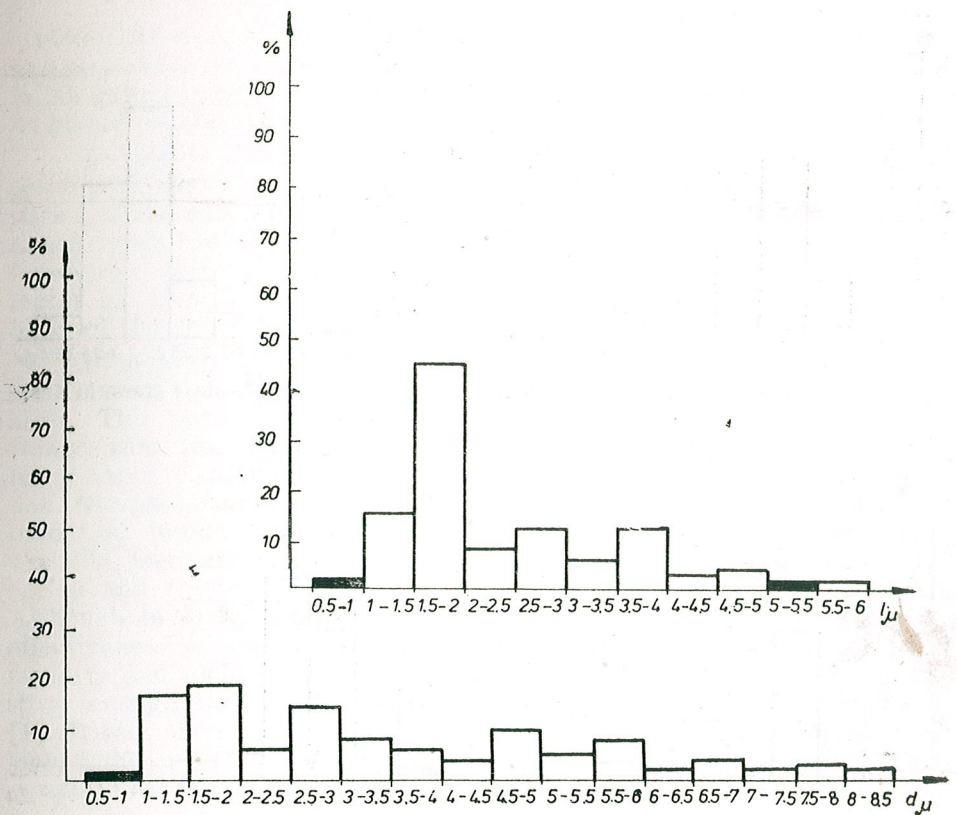


Fig. 1.—Distribution of cell size (in μ , l—length, d—diameter) of *Phormidium viscosum* in the initial enriched culture.

The most closely proportionated ("harmonious") filaments in respect of cell size were found in KPF's and WAT's media (Figs. 2—3). The distribution of cell size was similarly low in MO's and GO's media, too, but growth in this case was slower. According to the reduction of the limits of cell sizes the nutrient media used follow these series:

a) diameter: MO < KPF < BK < SAL < GO < WAT < KP < initial culture,

b) length: GO < SAL = KP < MO < BK < WAT = KPF < initial culture (Fig. 4).

The terminal cells were the more strikingly shortened and, as it is known, they are basically involved in the determination of the blue-green algae. It is noteworthy, both physiologically and taxonomically, that the proportioning of dimensions could be a mark of the quality of a medium [7], i.e. the most "uniform" filaments developed in the best media. Similar correlations established Komárek (1972) [9] based on ten strains of *Phormidium autumnale*, and Stam and Holleman

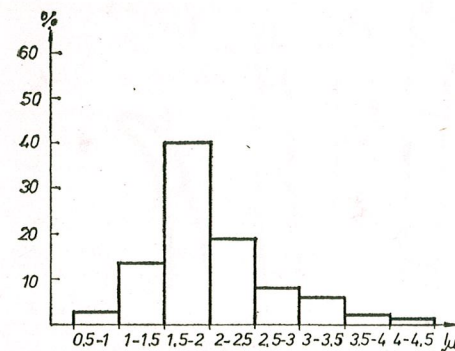


Fig. 2. — Distribution of cell size of *Phormidium viscosum* in unialgal culture grown in Knöp-Pringsheim-Felföldy's medium.

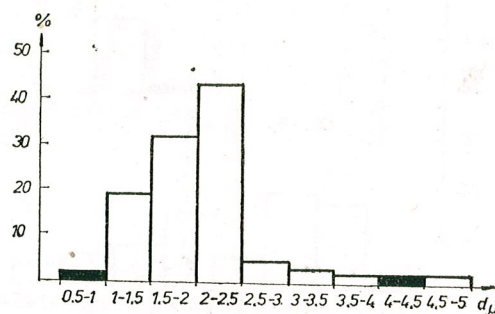
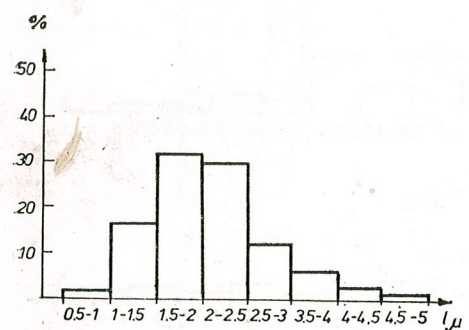


Fig. 3. — Distribution of cell size of *Phormidium viscosum* in unialgal culture in Watanabe's medium.

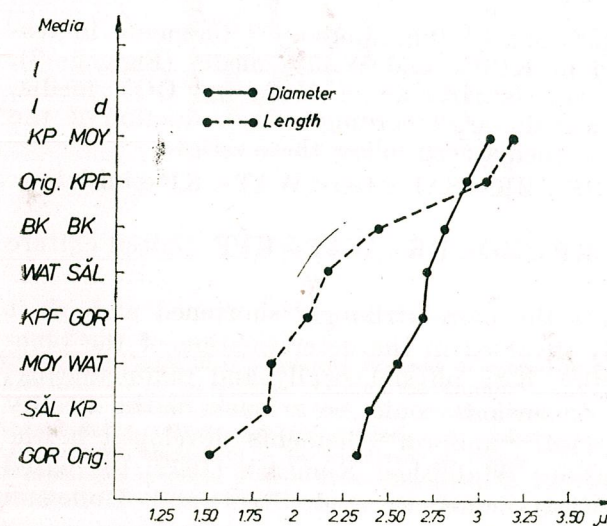


Fig. 4. — Succession of cell size frequency depending on nutrient media.

(1979) [14] studying some species of *Phormidium*, *Plectonema*, *Lyngbya* and *Synechococcus*. However, the morphological simplification of organisms in laboratory conditions is in fact a consequence of the existence of better stabilized factors than in the environment.

In intense batch cultures, growth was slow as compared with some green microalgae, so a set of experiments lasted 21 days. The characteristics of the cells and filaments depending on the available media (substrata) were less distinct than in static run cultures. Nevertheless, most favourable were WAT's and KPF's media. It seems that the key factor for growth improvement in these intense conditions is the bubbling of the cultures [16] which might be like the original lotic habitat.

Blue-green algae eliminate in their ambient generally more active substances, i.e. more dangerous (toxic) compounds than other green algae. This peculiarity together with their less favourable biochemical composition (for food), and better developed adaptability, frequently bring about "water blooms" (e.g. *Merismopedia*, *Anabaena*, *Aphanizomenon*, *Gomphosphaeria*, *Oscillatoria*) [8], [11] especially in fish ponds and oxidation ponds and ditches. The compounds eliminated are highly variable. Hermann's (1975) [6] study proves that their analysis, identification and tracing of biogenesis is a time consuming, tiresome task. Although bioassay could only give an overall indication concerning the effectiveness of released substances, it is nevertheless helpful due to its rapidity and easiness, can be helpful [10]. The *Lepidium* test being sensitive enough was and is still quite frequently applied to such detection [1]. It was, therefore, used in these experiments to find out some physiological effects of culture liquids and extracts of alga which would depend on growth conditions. The liquids were compared with pure nutrient solutions and distilled water as well (Table 3).

The strongest effect on the germination of *Lepidium* proved to have the supernatant of the cultures grown in WAT's medium and small effects

Table 3

The effect (after 5 days) of the culture liquids and extracts of *Phormidium viscosum* on germination and length of *Lepidium* root seedlings compared with that of pure nutrient solutions and distilled water

Variants	% of germination	Length of root	
		mm	%
Dist. water	72	11.3	100
KPF soln.	62	9.1	81
Extract of alga with H ₂ O	54	9.8	87
KPF's culture liquid therm.a.*	62	15.9	140
" " " " sta.	64	17.	150
WAT's " " " a.	56	12.4	110
" " " " sta.	42	15.3	135
MO's " " " a.	60	14.5	128

* therm. a. = thermoalternated; therm. sta. = thermostated cultures

being observed in those of KPF's whether the cultures grown thermos-tated or thermoalternated. Distilled water surprisingly delayed germination due probably to an osmotic imbalance.

CONCLUSIONS

Phormidium viscosum Lemm. collected from wet concrete blocks in the ditch of flowing warm water discharged by a thermopower station was isolated in pure culture and grown in ten types of artificial media (i.e. Benecke-K₂HPO₄, Benecke-KH₂PO₄, Witsch, Knop-Pringsheim, Knop-Pringsheim-Felföldy, Allen-Arnon, Sălăgeanu, Watanabe, Moyse, Gorham) in static runs and in intense bubbled batch conditions.

The most favourable media proved to be those of Watanabe's and Knop-Pringsheim-Felföldy's. The wide limits of cell size variations ascertained in the initial enriched culture significantly narrowed in the subsequent unialgal cultures. The succession in diameter reduction follows: MO < KPF < BK < SAL < GO < WAT < KP < initial culture, and length decrease: GO < SAL = KP < MO < BK < WAT = KPF < initial culture.

The physiological effectiveness of the compounds released by the cells in their media as well as cell extracts were bioassayed by the *Lepidium* test. Most effective proved to be Watanabe's medium while those obtained from Knop-Pringsheim-Felföldy's medium and the cells were lighter. The extract prepared with distilled water was stronger than the culture liquids.

REFERENCES

1. Brugovitzky E., Bosica I., Adorján M., *Studia Univ. Babeş-Bolyai, Ser. Biol.*, 1967, 2, 49–56.
2. Elenkin A. A., *Sinezelenye vodorosly SSSR Vyp. 2*, Izd. Akad. Nauk, Moscowa—Leningrad, 1949.
3. Felföldy L., *A kék algák kishatározója*, Víz. Dok. Tájé. Iroda, Budapest, 1972.
4. Geitler L., *Cyanophyceae*, Akad. Verl. Ges. Leipzig, 1932.
5. Gollerbach M. M., Poljanskii V. I., Savich V. P., *Sinezelenye vodorosly*, Izd. Sov. Nauka, Moscowa, 1953.
6. Hermann V., *Biogena Amine als Ausscheidungsprodukte einzelliger Süßwasseralgen*. Dissertation, Sprint-Druck GmbH, Stuttgart, 1975.
7. Jeeji-Bai N., *Arch. Protistenk.*, 1977, 119, 367–387.
8. Jüttner F., *Biochemie toxischer „Wasserblüten“ von Aphanizomenon flos-aquae (L.) Ralfs*. Dissertation, Fotodruck GmbH, Munich, 1971.
9. Komárek J., *Preslia*, 1972, 44, 293–307.
10. Linser H., Kiermayer O., *Methoden zur Bestimmung pflanzlicher Wuchsstoffe*, Springer-Verlag, Vienna, 1957.
11. McCoy L. F., Eng-Wilmot D. L., Dean M. F., *J. Agr. Food Chem.*, 1979, 27, 69–74.
12. Meffert M.-E., Krambeck H.-J., *Arch. Hydrobiol.* (Stuttgart), 1977, 79, 149–171.
13. Round F. E., *The Biology of Algae*, E. Arnold Publ., 1975.
14. Stam W. T., Holleman C. H., *Acta Bot. Neerl.*, 1979, 28, 45–66.
15. Tarnavski I. T., Olteanu M., *St. cerc. biol.*, 10, 269–290, 317–344.
16. Ţipa L., *St. cerc. biol., Ser. bot.*, 1969, 21, 443–452.

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THE MICROPROPAGATION OF *VITIS VINIFERA* L. II. ASPECTS OF MORPHOGENESIS IN CALLUS CULTURE

BY

MARGARETA IORDAN, AURELIA BREZEANU, ANA ROŞU

The main morphogenetic aspects remarked in callus cultures of *Vitis vinifera* are described and the factors which determine the expression of morphogenetic processes are discussed.

A wide range of *Vitis vinifera* cultivars were used as explant sources and the experiments were initiated in different phenological phases of the donor plants. 36 types of nutrient media were tested for callus proliferation and morphogenesis induction; the callus cells were examined on fresh material and in histological sections, in order to make evident the evolution of morphogenetic processes. The morphogenesis was frequently represented by vascular elements, rhysogenesis and sporadically by caulinar primordia and embryoids in incipient stages of differentiation.

The present stage of knowledge pointed out the possibility of *in vitro* spontaneous and controlled induction of the morphogenetic processes, represented mainly by tracheids, xylo-phloem nodules, organogenesis (rhysogenesis, caulogenesis) and somatic embryogenesis [1], [7], [12], [13], [14], [16], [17].

The organogenesis and somatic embryogenesis are especially important both from the fundamental point of view, for proving the plant cell totipotency and the factors that govern the cell differentiation and the morphogenetic phenomenon, and from the practical point of view, by offering efficient methods for clonal multiplication.

With regard to *Vitis vinifera* there are not many researches in the world concerned with the possibility of plant regeneration by organogenesis and somatic embryogenesis and positive results are recorded only in some laboratories and for a few cultivars [5], [6], [9], [10]. Favre [3], [4], in a series of papers described the obtaining of caulinar neofor-mations from *Vitis vinifera* and the dependence of this process on the physiological state of the plant and on the nature of the explant.

Mullins M. S. and Srinivasan [11] induced somatic embryoids and regenerated plantlets from nucellar callus of *Vitis*, Cabernet-Sauvignon cultivar but it seemed that the embryoids obtained failed to form adventitious embryos, an essential process for achieving a rapid and in mass micropropagation of the plant.

Hirabayashi [8] described the conditions which favoured the differentiation of bud structures and root primordia in the callus obtained from anthers of *V. thumbergii*, but the differentiated organs did not survive.

The present work intends to present the principal morphogenetic aspects remarked in the callus cultures from *V. vinifera* and to discuss the factors which determine the organogenesis and somatic embryogenesis induction, essential steps in regenerating complete new plants.

MATERIAL AND METHODS

The *V. vinifera* cultivars, Feteasca regală, Italian Riesling, Coarnă neagră, Chaslas, Perlette and the hybrid Dattier, were tested in our experiments.

The explants used in inoculation were represented by pieces of somatic tissues excised from very young organs of the plants grown in the field (elongating unignified shoots, petiols, mesophyll, tendrils, inflorescence axis and primordia, developing fruits) as well as from shoots obtained by forcing in the laboratory.

The inoculation of the explants excised from the plants grown in the field took place between April – October, in different phenological phases of the donor plants and in February – March in the case of shoots obtained by forcing in the laboratory.

The material was superficially sterilized by submerging in a 2–7% sodium hypochlorite solution or 5% calcium hypochlorite in a 1% bromocet solution, after the method described before [2]. The explants were aseptically inoculated on a number of 36 variants of solid or semisolid complex media which, besides the mineral and organic standard substances, contained plant hormones in various concentrations (Table 1).

The callus cells were examined both in fresh material and also in histological sections. In this respect small callus pieces (3–5 mm) were fixed in the Navasin fixative agent or in 3% glutaraldehyde, followed by embedding in paraffin, sectioning in slices of 10–15 μ m and staining with Erlich hematoxylin or toluidine blue.

RESULTS AND DISCUSSION

The accomplishment of callus growth and morphogenesis. Prior experiments specified the necessary conditions for callus initiation for a noticeable number of *V. vinifera* cultivars, pointing out the fact that the explants originated from different types of tissues from the same cultivars showed distinct capacities of callus initiation. But an essential condition for achieving the micropropagation by cell cloning stands in finding a correlation between the high potential for callus initiation and growth and its morphogenetic capacities; a high embryogenic potential creates the premise of the possibility for plant regeneration by these means. In the primary callus cultures we obtained the expression of morphogenesis usually associated with small calli, having a reduced or moderate speed of initiation, and it was represented only by rhysogenesis. The root promotion in these cases was especially remarked in the calli originating from internode axis, precisely from the cambial region of the explant, thus from cells of the secondary meristem. The dedifferentiated state neces-

Table 1
The nutrient culture media used for *Vitis vinifera* callus initiation and morphogenesis induction

Variants	Basal media	Macro- and micro-nutrients	Sugar source	Vitamins	Growth factors mg/l	The aim of utilisation	The effects
LS	LS	LS	30	LS	NAA-2	callus initiation growth and morphogenesis	+++ rhysogenesis
LS ₁	LS	LS	30	LS	2,4D-1	callus initiation and growth	++
LS ₂	LS	LS	30	LS	NAA-2 Kin-1	callus initiation, growth and morphogenesis	+++ meristemoides, xylem-phloem nodules, caulinar primordia, rhysogenesis
LS ₃	LS	LS	30	LS	NAA-2 Kin-4	callus growth morphogenesis	++
LS ₃ ²	LS	LS	50	LS	NAA-2 Kin-4	callus growth morphogenesis	+++ meristemoides, xylem nodules, radicle primordia
LS ₄	LS	LS	30	LS	NAA-2 Kin-2	callus growth morphogenesis	++
LS ₅	LS	LS	30	LS	NAA-1 Kin-1	callus growth morphogenesis	+++
MS	MS	MS	30	MS	—	morphogenesis	+++ embryogenic structures, groups of xylem-phloem elements, roots
K	MS (1962)	MS (1962)	30	MS (1962)	Kin-0.5 2,4D-1 IAA-10	callus initiation	+++

Table 1 (continued)

Variants	Basal media	Macro- and micro-nutrients	Sugar source	Vitamine	Growth factors mg/l	The aim of utilisation	The effects
K ₁	MS (1962)	MS (1962)	30	MS (1962)	Kin-0.5 2,4D-1.0 IAA-10 BAP-0.1	callus initiation callus growth	++ ++
K ₂	MS (1962)	MS (1962)	30	MS (1962)	Kin-0.5 2,4D-1.0 IAA-20 BAP-0.1	callus initiation callus growth	++ ++
K _{2m}	MS (1962)	MS (1962)	30	MS (1962)	Kin-0.5 NAA-2 IAA-20 BAP-0.1	callus initiation, growth and morphogenesis	+++ +++ +++ rhyso-genesis
K ₃	MS (1962)	MS (1962)	30	MS (1962)	Kin-5 BAP-0.1	callus growth and morphogenesis	++ —
K ₄	MS (1962)	MS (1962)	30	MS (1962)	Kin-10 BAP-0.1	callus growth and morphogenesis	+
K ₅	MS (1962)	MS (1962)	20	—	—	morphogenesis	isolated xylematic elements, or groups of branched xylematic elements
K ₆	MS (1962)	MS (1962)	30	MS (1962)	Kin-6 BAP-0.1 NAA-2 IAA-1.1	callus growth and morphogenesis	++ —
1	MS (1962)	MS (1962)	30	MS (1962)	BAP-2 NAA-0.5	callus growth and morphogenesis	++ —
2	MS (1962)	MS (1962)	30	MS (1962)	ABA-0.1	callus growth and morphogenesis	++ rhyso-genesis

Table 1 (continued)

2a	MS (1962)	MS (1962)	30	MS (1962)	ABA-0.6	callus growth and morphogenesis	++ rhyso-genesis
8	MS (1962)	Macro-MS (1962) micro 1/2 MS (1962)	20	MS (1962)	ABA-0.1 Ac. gib. 0.2	morphogenesis	intense rhyso-genesis
9	MS (1962)	MS (1962)	30	MS (1962)	BAP-0.5	morphogenesis	+++ rhyso-genesis
10	MS (1962)	MS (1962)	30	MS (1962)	BAP-2.5	morphogenesis	++ rhyso-genesis
11	MS (1962)	MS (1962)	30	MS (1962)	BAP-5.0	morphogenesis	senescence aspects
12	MS (1962)	MS (1962)	30	MS (1962)	BAP-10.0	morphogenesis	senescence aspects
13	MS (1962)	MS (1962)	30	MS (1962)	BAP-5.0	morphogenesis callus proliferation	— ++
14	MS (1962)	MS (1962)	30	MS (1962)	BAP-1.0 NAA-0.2	callus proliferation and morphogenesis	++ bundles of tracheids, xylem phloem nodules, roots
15	MS (1962)	MS (1962)	20	Staba	BAP-1.0 IBA-0.5	caulogenesis elongation	— +++

Table 1 (continued)

Variants	Basal media	Macro- and micro-nutrients	Sugar source	Vitamine	Growth factors mg/l	The aim of utilisation	The effects
16	De Fossard Mint and Lee (1974)	De Fossard	20.5	De Fossard	IAA-0.17 IBA-0.2 NAA-0.18 Kin-0.2	callus initiation, growth and morphogenesis	++ sporadic roots
17	De Fossard Cresswell and Nitsch	1/10 De Fossard 9/10 Cresswell and Nitsch	20.0	1/10 De Fossard 9/10 Cresswell and Nitsch	IBA-0.92 IAA-0.017 NAA-0.18 Kin-0.021	callus initiation, growth and morphogenesis	++ roots directly from the explant
18	"	9/10 De Fossard 1/10 Cresswell and Nitsch	20.0	9/10 De Fossard 1/10 Cresswell and Nitsch	IBA-0.19 IAA-0.15 NAA-0.16 Kin-0.19	callus initiation, growth and morphogenesis	++ roots directly from the explant
19	MS (1962)	MS (1962)	30	MS (1962)	BAP-0.5 IBA-0.1	callus initiation, morphogenesis, bud culture	+ - ++

sary for cell totipotency expression and the capacity for redifferentiation and morphogenesis were analysed in callus and cell suspension cultures both on the nutrient media used for callus initiation as well as on new formulas (Table 1).

We should mention as especially efficient for callus proliferation for the majority of the cultivars experimented the nutrient media, LS₂, LS₂z, K₂m, LS₄, 14, but the morphogenetic potential was reduced.

Some authors noticed for other plant species the fact that an active callus proliferation is not always associated with morphogenesis. It is probable the case of a superoptimal accumulation of some hormones in the cells, or even the callus cells are able at a certain time to elaborate their own synthesis mechanisms for the endogenous hormones they need and in these conditions, a supplementary addition of exogenous hormones has a negative effect on the differentiation and morphogenetic processes. Krul et al [9] obtained embryoid differentiation in calli which began to have a brownish color, grown in the absence of the auxin (2,4-D) on nutrient media with a reduced concentration of sugar.

It was evident that rhysogenesis was present especially on the media that contained kinetin in concentrations varying between 0.5–4.0 mg/l and NAA 0.2–2.0 mg/l. The abscisic acid used in concentrations between 0.1–0.5 mg/l also proved to be stimulative for rhysogenesis, as well as BAP in doses of 0.5–2.5 mg/l, alone or associated with NAA.

The nutrient medium LS₁ which contained only the auxin 2,4-D had a weak effect on callogenesis in comparison with the formulations that included other auxins, as IAA and NAA.

For the same auxin concentrations the cytokinins in doses as far as two times higher than their concentration in the basal medium had a distinctive stimulatory effect for callus induction and proliferation and morphogenesis was represented by meristemoids, xylo-phloem nodules, rhysogenesis and even caulinar-like structures.

The ratio auxin/cytokinin 1:1 had very good effects on callus initiation and proliferation, but weak effects on morphogenesis.

When the ratio auxin/cytokinin was 1:2 and the sugar quantity increased, a very good callus proliferation was recorded: this callus separated into globular structures which, examined histologically during the initial subcultures, revealed meristemoids and root primordia but after repeated subcultures the xylematic nodules were the only morphogenetic aspects present. The transfer of callus pieces on a medium without hormones (MS) or on media with diminished sugar concentrations and without vitamins (K₅), resulted in the formation of vigorous roots on MS medium and the histological sections revealed also bundles of vascular elements and some embryoid structures; the callus on K₅ medium, besides roots and numerous green granules evident macroscopically, presented in histological sections a lot of xylematic elements isolated or as groups of branched tracheids.

Using high concentrations of plant hormones in the stage of culture initiation and maintaining these concentrations during the subsequent subcultures ended in obtaining of calli with active proliferation. The rhysogenesis present during the first subcultures was inhibited later and the histological sections revealed only xylo-phloem nodules or vascular

elements arranged in bundles. We may make the assumption that high concentrations of hormones brought about the loss of the morphogenetic potential and at the same time the preferential selection of some cell type with rapid growth, apparently deprived of morphogenetic capacities. This supposition is in concordance with reports of some authors who recommended the proper regulation of hormone concentrations in an as early phase of culture as possible.

Media 17 and 18, which were modified basal media De Fossard and Cresswell and Nitsch, proved to be unsuitable for callus initiation and growth but favored the formation of vigorous roots directly on the explant.

The rhysogenesis represented the most important visible morphogenetic process in the case of vine cultivars we used in our experiments; this phenomenon was generally remarked for other woody plants as well, especially those that are easily propagated by the conventional techniques.

— *The analysis of the morphogenetic phenomenon on histological sections.* Morphologically, the calli from *V. vinifera* look in most cases like an achlorophyllous, glassy and friable mass of cells. But there are also peculiarities depending on the cultivar, regarding the pigmentation, the intensity of proliferation and the texture [2].

In the primary cultures, on the central part of the callus, corresponding to the zone of the initial explant, a lot of greenish-white or dark green nodular granulations with hard texture differentiated. The histological studies showed xylematic and sometimes xylo-phloem structures that we presume originated from the cambial region of the explant, which means they represent the result of the activity of some meristematic cells present in the initial inoculum. Such groups of cells may preserve their meristematic properties after many subsequent subcultures, continuing to generate vascular structures. If these granulations are isolated and cultivated directly on the nutrient medium they generate callus containing xylematic elements. This fact suggests that some cells continue to function as secondary meristems and the chance of reversing to the state of primary meristems, with totipotent cells is probably dependent on a multitude of still unknown factors. Because many calli differentiated also roots from the granular zones described above, we believe in the possibility of inducing organogenesis even though in this phase of our research the process cannot be achieved and controlled at will.

Caulogenesis represented a process difficult to induce. In all our experiments, dealing with a large number of variants, there were not recorded positive results in obtaining newly formed shoots from callus of somatic origin in order to regenerate complete plants by subsequent rooting of these shoots. In a few cases some shoot primordia were evident but they did not develop into viable shoots (fig. II, 8). Similar unsuccessful attempts in regenerating viable plants were mentioned by Hirabayashi [8], working with cultured anthers of *Vitis* sp. When the calli, freed from the influence of the initial explant after 2–3 subcultures are grown on media without hormones or with low hormonal concentrations, some groups of small meristematic cells, with dense plasmatic content, are differentiating, isolated from the parenchyma cells of the callus by a

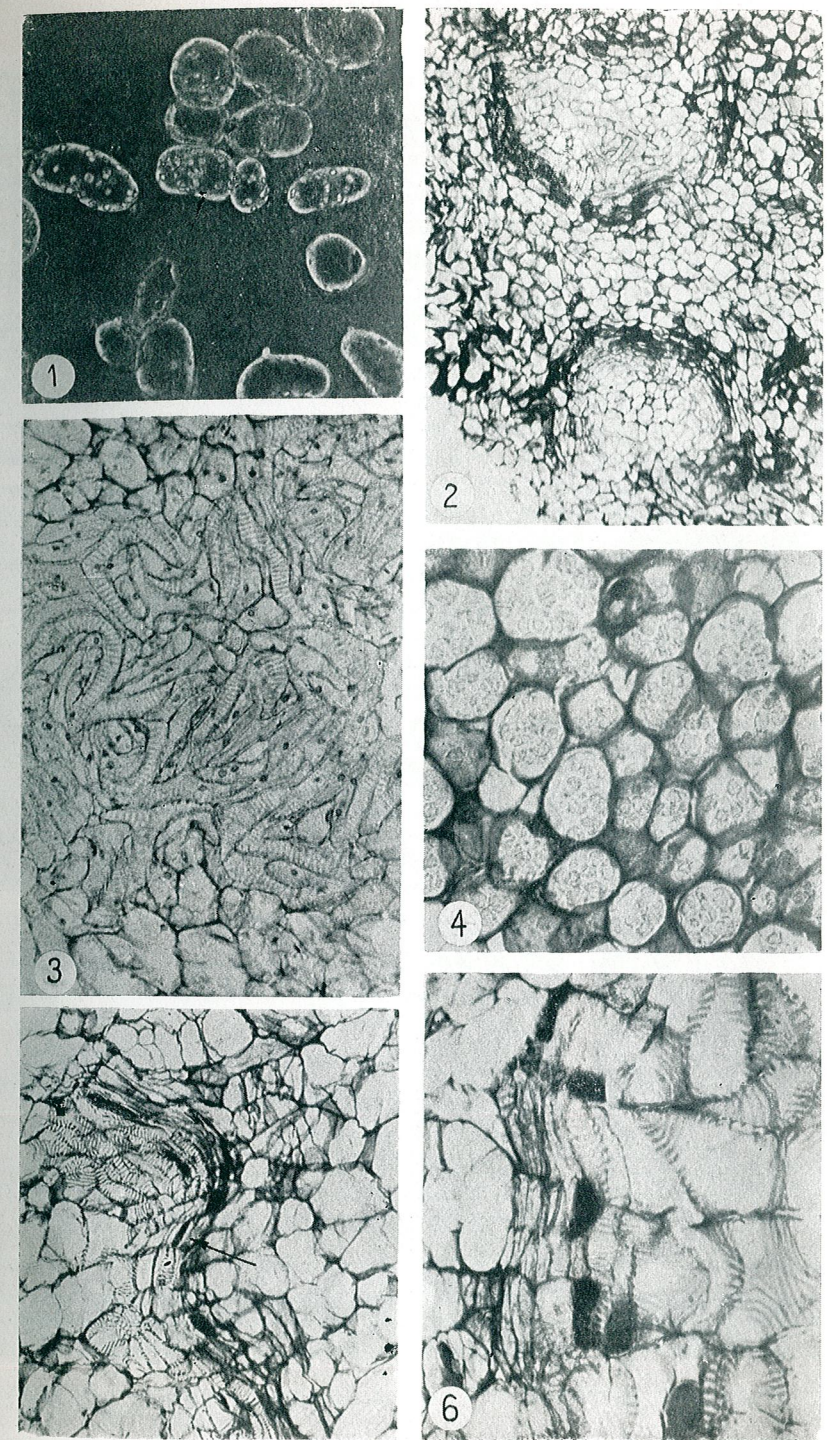


Fig. 1. — Morphogenetic aspects in the callus of *Vitis vinifera* L.

1. Young cells undergoing divisions in a morphogenetically callus (microscopical image in the phase contrast- 160 $\times$); 2. Meristematic globular (arrow) structures (meristemoids)— 50 $\times$); 3. Spiral vascular elements of tracheidal type randomly arranged (100 $\times$); 4. Parenchymatic cells loaded with starch grains (100 $\times$); 5. Arches of cambial cells (arrow) and differentiation of xylematic elements (50 $\times$); 6. Cambial zone on the way of differentiation in xylematic vascular elements (arrow) with intensely pigmented and lignified cells (100 $\times$);

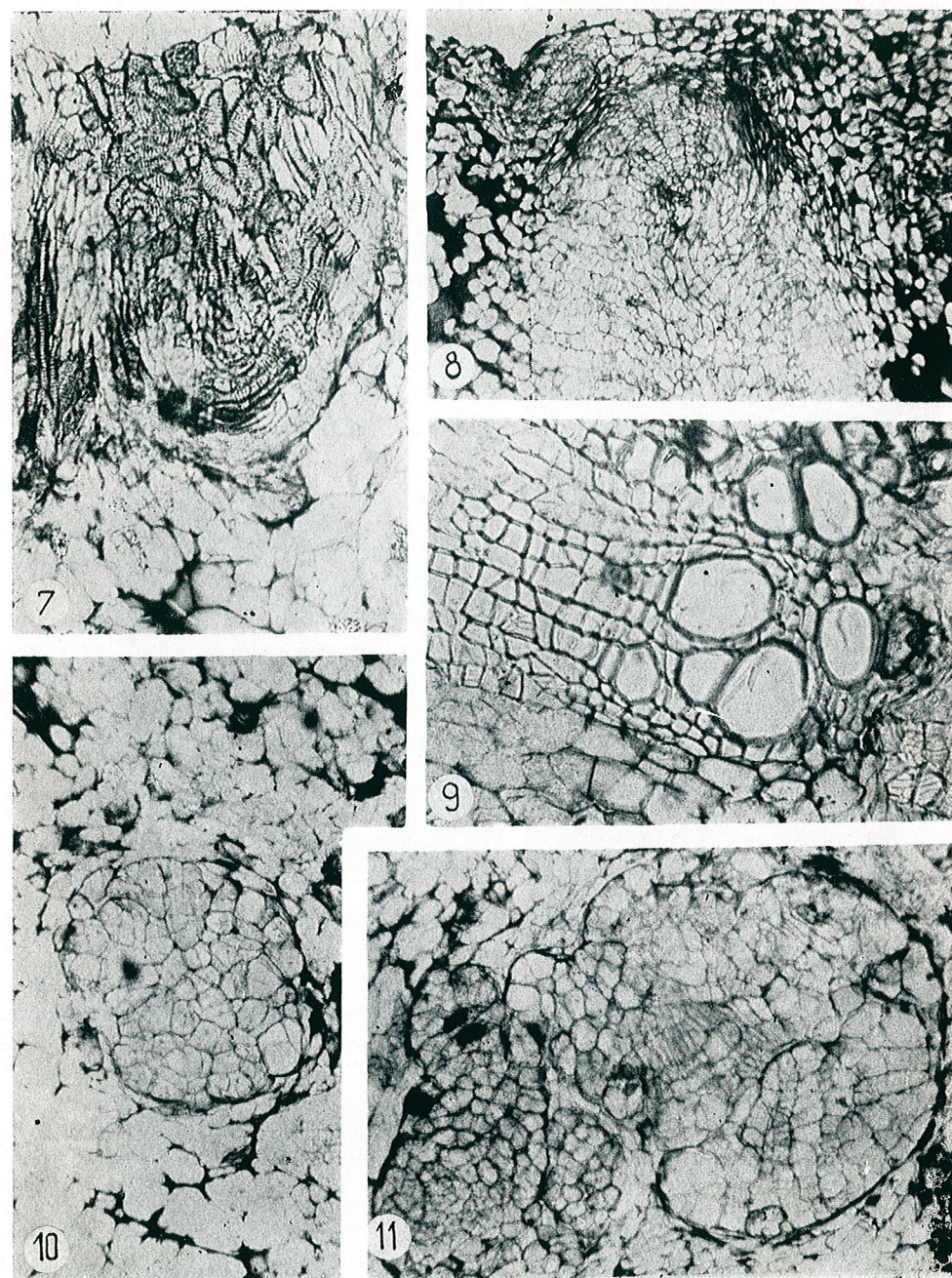


Fig. II. — Morphogenetic aspects in the callus of *Vitis vinifera* L.

7. Root primordia with xylematic vascular elements randomly distributed (100×); 8. Caulinar primordium (160×); 9. Xylem-phloem fascicle (structural detail, 100×); 10.—11.— Phases of embryogenesis: 10. The individualisation of a meristemoid (100×); 11. Embryoid in an incipient phase of differentiation (50×).

protoderm-like layer. These structures (meristemoids), depending on the culture conditions, may evolve further toward xylematic (Fig. I, 3, 5, 6) and xylem-phloem (Fig. II, 9) nodules or proembryogenic masses (Fig. II, 10) which subsequently develop into somatic embryoids. A high incidence of such embryogenic structures in callus culture represents the optimal time for isolating of some cell clones with a pronounced morphogenetic potential, able to generate new whole plants via somatic embryoids. Our studies correlated with reports of other authors [15] for other plant species, support the hypothesis that somatic embryoids originate in the nodular structures of the morphogenetic callus; these non-zygotic embryos arise from proembryogenic cell masses by repeated divisions, mainly in a very similar way to the development of zygotic embryos.

In some instances embryo or bud differentiation occurs directly in the initial explant, so the intermediary callus may be avoided; due to the low incidence of the embryogenic structures in callus of other plant species, some authors pay a special attention to this phenomenon; our future research will concentrate on studying this possibility which opens the way of overcoming some drawbacks like the lack of genetic stability that usually characterizes the tissue cultures.

From the morphogenetic aspects furnished by our light microscopy studies we can draw the conclusion that two different patterns of cyto-differentiation may be identified among callus cells: the differentiation of xylematic vascular elements, isolated or arranged in bundles, originating directly from cells that resulted from the activity of some cambial zones present in callus and, on the other hand, the differentiation of meristemoids which may develop in different ways: xylematic or xylem-phloematic nodules, root and shoot primordia or proembryogenic masses which develop in embryoids with a bipolar organisation.

Acknowledgements. The authors are grateful to the staff of the Experimental Vine Station Ștefănești and to eng. Ilie Popescu for their kindness to offer experimental plant material as well as to Mrs. Brinduş Mariana for her helpful technical assistance.

REFERENCES

1. * * * *Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture*. Ed. by J. Reinert, Y.P.S. Bajaj, Springer Verlag, Berlin, Heidelberg, New York, 1977, 343—349, 581—635.
2. Breazeanu Aurelia, Margareta Iordan, Ana Roşu, *The micropropagation of Vitis vinifera* L.: I. *The induction and characterization of callus from tissues of somatic origin*. Rev. Roum. Biol.—Biol., Végét., 1980, 2, 135—142.
3. Favre J. M., *Premiers résultats concernant l'obtention in vitro de néoformations caulinaires chez la vigne*. Ann. Amélior. Plants Fr., 1977, 27, 2, 151—169.
4. Favre J. M., *Influence de l'état physiologique de la plante et de la nature de l'organe prélevé sur l'obtention des néoformations caulinaires de la vigne*. In Congr. natl. soc. savantes. sect. sci. 101-Cri, Lille, 1976, fasc. I, 465—474.
5. Galzy R., *La culture in vitro des apex de Vitis rupestris*. C. R. Acad. Sci., 1972, 274, 210—213.
6. Gresshoff P. M., Doy C. H., *Derivation of a haploid cell line from V. vinifera and the importance of the stage of meiotic development of anthers for haploid culture of this and other genera*. Z. Pflanzenphysiol., 1974, 132—141.

7. Halperin W., *Morphogenesis in cell cultures*. Annu. Rev. Plant. Physiol., USA, 1969, **20**, 395—418.
8. Hirabayashi T., Kozaki I., Akihama T., *In vitro differentiation of shoot from anther callus in Vitis*, Hort. Science, 1976, **11**, 5, 511—512.
9. Krul, W. R., Worley F. J., *Formation of adventitious embryos in callus cultures of Seyval, a French hybrid grape*. J. Amer. Soc., Hort. Sci., 1977, **102**, 3, 360—363.
10. Mu S., Y. Kwei, S. Liu, Chang F., Lo. M. Yong Wang F., *Induction of callus from Vitis endosperm cultured in vitro*. Acta. Bot. Sin., 1977, **19**, 1, 93—94.
11. Mullins M. G., Srinivasan C., *Somatic embryos and plantlets from an ancient clone of the grape vine (cv. Cabernet-Sauvignon) by apomixis in vitro*. J. Exp. Bot., 1976, **27**, 1022—1030.
12. Murashige T., *Plant propagation through tissue culture*. Ann. Rev. Pl. Physiol., 1974, **25**, 135—166.
13. Murashige T., *Plant Cell and Organ Cultures as Horticultural Practices*. Acta Horticulturae, Symposium on Tissue Culture for Horticultural purposes, Belgium, 1977.
14. — *Plant tissue culture and its bio-technological application*. Ed. W. Barz, E. Reinhard, M. N. Zenk, Springer-Verlag, Berlin, Heidelberg, New York, 1977, 355—366, 392—403.
15. Reuther G., *Embryoid differentiation patterns in callus of the Iris and Asparagus*. BER DTSCH BOT GES, 1977, **90**, 3, 417—438.
16. Street H. E., *The Anatomy and Physiology of Morphogenesis; Studies involving tissue and cell cultures*: in Travaux de diés à la mémoire de G. Morel, 1977, 20—28.
17. Torrey J. G., *The initiation of organized development in plants*. Advan. Morphogenesis, Academic Press, New York, 1966, 5, 39—91.

Received November 3, 1980

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REGENERATION OF POTATO PLANTS BY *IN VITRO* CULTURE OF STEM SEGMENTS

BY

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The paper presents the succession of necessary stages in the regeneration of potato plants by *in vitro* culture of stem segments. The success of the *in vitro* culture is influenced by various factors: nature and the concentration of phytohormones from the culture medium, the concentration in sucrose, light, the age of the donor plant.

Among auxins, the dichlorophenoxyacetic acid (2,4 D) is the most favourable one both for the callus initiation and growth and among the cytokinins the zeatin has an essential role in the differentiation of shoots.

For some years plant tissue culture techniques have been used for the production of disease free and disease resistant plants, for the rapid multiplication of various genotypes and for maintaining stocks of valuable plants. More recently, several technical developments in plant tissue culture have made it possible that *in vitro* techniques should contribute considerably to plant breeding [21].

Plant cells have the enormous advantage of totipotency. From the genetic point of view, the capacity to regenerate fertile plants from cell cultures and tissue cultures means that genetic modifications at the cellular level can be evaluated in mature plants and possibly utilized in breeding programs.

By the culture *in vitro* of vegetal tissues we intended to extend the means of investigation and utilisation of genetic variability largely existing *in vivo* in culture plants, a variability referring to protein content, the equilibrium of amino acids in proteins, the resistance to pathogens etc.

There is a large number of plant species in which the potential for plant regeneration from callus has been achieved and it includes species of economic importance such as: wheat, rice, sugar cane, maize, barley, oats, coffee, potato, tomato [17].

Although, as compared with other plant species, potato is considered relatively recalcitrant in tissue cultures — the process of redifferentiation being hard to control — however, in recent years, good progress has been made as regards culture *in vitro*:

- rapid propagation and storage of valuable genotypes [4], [14], [15], [16], [24];
- regeneration of virus free plants by callus cultures [23];
- direct and indirect androgenesis [5], [19], [20];
- regeneration of plants from suspension cultures [7];

- doubling dihaploid potato clones by mesophyll cultures [1], [6];
- isolation of protoplasts and regeneration of plants from dihaploid and tetraploid genotypes [2], [3], [14], [18], [22];
- cellular hybridization by fusion of protoplasts [9], [10], [11].

We initiated some investigations regarding the potato tissue cultures, the potato being a species of high economic importance in this country. These investigations have in view both some fundamental aspects such as dedifferentiation and redifferentiation at a dihaploid and tetraploid level, and practical aspects such as the obtaining of high genetic variability useful for breeding.

MATERIAL AND METHOD

Potato (*Solanum tuberosum* L.) plants of the Mercur, Semenik, Măgura, Super, Ora, Mucel, Saphir, Colina, Procura, Jaerla cultivars were used as source of explants. Donor plants were grown both in the greenhouse and in the field.

The material was collected from the node regions and sterilized in a solution of mercury chloride 0.1% for 5 minutes then three times washed in sterile distilled water. After sterilization explants 2 mm thick and 3-5 mm in diameter were collected for inoculation.

In all experiments the modified Murashige and Skoog (MS) [12] nutritive medium was used by a supplementation with organic addenda of Nitsch and Nitsch [13], casein hydrolysate, D-mannitol and different growth regulators: with dichlorophenoxyacetic acid (2,4D) and 1-naphthaleneacetic acid (NAA) in concentrations of 2-10 mg/l for callus initiation and with 2,4D and kinetin for its growth. We tested 40 hormonal combinations of indole-3-acetic acid (IAA), 6-benzylaminopurine (BAP), zeatin, kinetin and gibberellic acid (GA) in concentrations of 0.1-15 mg/l for shoots morphogenesis. For shoots rooting the modified Murashige and Skoog medium without hormones was used. Fully differentiated plantlets were transferred for 10 days to a liquid Murashige and Skoog medium and then in pots with sterilized soil.

For callus induction the cultures were kept at the temperature of 27°C being exposed daily to 2,000 lux and for organs initiation to 5,000 lux. The plants that developed *in vitro* were exposed to 13,000 lux.

The culture media were sterilized by autoclaving at 121°C and vitamins and gibberellic acid by bacteriological filter G₅; the pH of media was adjusted with 1N NaOH to 5.8 before autoclaving.

RESULTS AND DISCUSSION

The initiation and growth of callus. The experiments carried out lead to the conclusion that it is possible to obtain callus from all tested cultivars on the modified nutritive medium Murashige and Skoog with 2,4D or NAA. Kinetin is not absolutely necessary for callus induction. We consider that the best medium variant is the one containing 2,4D 2 mg/l

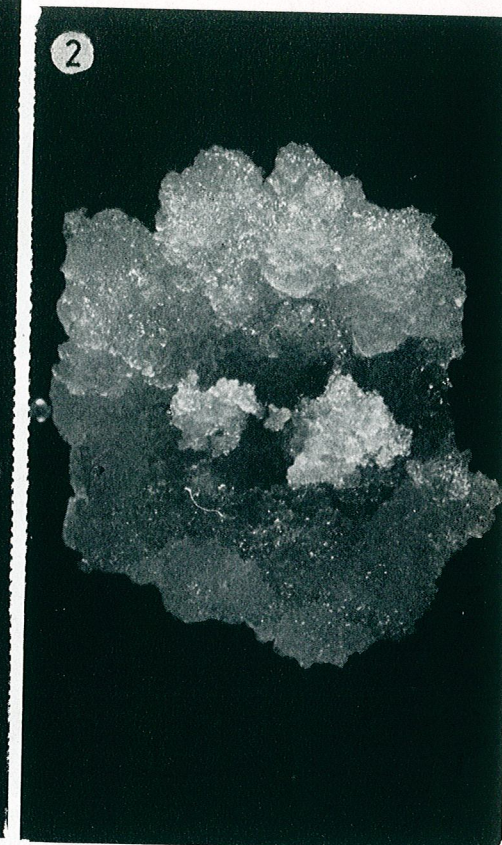


Fig. 1. — incipient stage of callus initiation — 7 days since inoculation Fig. 2. — intense proliferation of callus — one month since inoculation

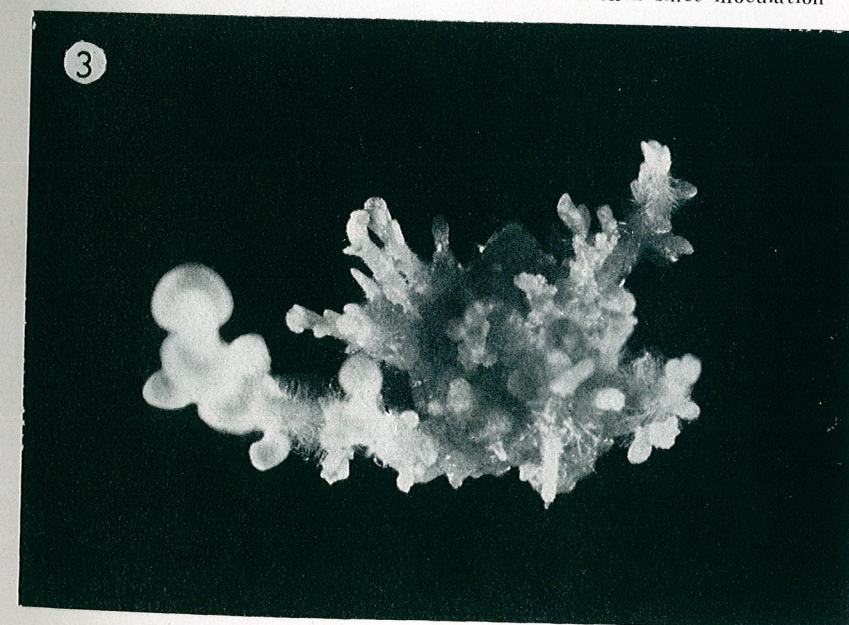


Fig. 3. — callus with multiple shoots — six months since inoculation



Fig. 4.—elongated shoots with incipient rooting—seven months since inoculation;

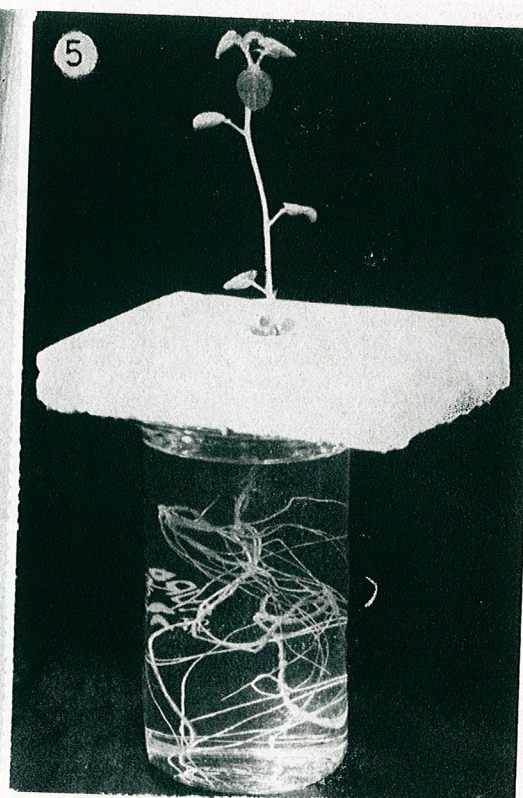


Fig. 5.—fully developed plant on the liquid Murashige and Skoog medium without hormones;



Fig. 6.—potato plant, 10 cm high, regenerated by in vitro culture of stem segments.

in the first stage of the sequence to obtain potato plants by culture of stem segments. At higher concentrations of 2,4D or in the presence of NAA no positive results are obtained in the next stages even if the other constituents of the medium are kept unchanged.

The callus appears approx. 7 days after the explant has been placed on the culture medium (Fig. 1) and it is generally friable. Differences among the cultivars have been noticed regarding the consistency, colour and growth rate.

The calli obtained were periodically transferred every two weeks on the same fresh medium and after several passages they were transferred on the modified Murashige and Skoog medium with different concentrations of 2,4D, kinetin and sucrose. Among all variants tested for potato callus growth the one with 2,4D 2 mg/l, kinetin 0.02 mg/l and sucrose 1.5% was the most favourable both for proliferation (Fig. 2) and for further differentiation of shoots in our experiments.

Regeneration of plants. From the 40 hormonal variants tested for shoots morphogenesis the best results were obtained on those containing zeatin. In the presence of this hormone, the calli become green and in direct contact with the culture medium, dark green nodules appear at the basis as shoot bud primordia. These nodules were noticed also on the variants with IAA 0.1 mg/l and BAP 0.5 mg/l as well as with IAA 0.1 mg/l and zeatin 0.5 mg/l but no shoots were differentiated. It is interesting to notice that this last variant was used by Shepard [18] for shoots differentiation in potato tuber culture and protoplasts from mesophyll culture but in our experiments the results were not the same. That was perhaps because of unknown endogene levels of hormones, that determine different conditions *in vitro* as regards the hormonal balance of the culture medium. For the material we used, the best variant for shoots differentiation was the one containing only zeatin 0.3 mg/l (table 1). Under these conditions, after repeated passages for 6 months multiple shoots

Table 1

Composition of nutrient media (mg/l) successfully used for regeneration of potato plants by *in vitro* culture of stem segments

Constituent	Initiation of callus	Culture of callus	Shoots differentiation	Roots differentiation
Inorganic salts				
Major elements	MS	MS	MS	MS
Minor elements	MS	MS	MS	MS
Organic constituents				
Organic addenda	N&N	N&N	N&N	N&N
Casein hydrolysate	—	—	1,000	1,000
Sucrose	3%	1.5%	2%	2%
D-mannitol	—	—	4%	4%
Agar	1%	1%	0.8%	0.8%
Growth regulators				
2,4D	2	2	—	—
Kinetin	—	0.02	—	—
Zeatin	—	—	0.3	—

developed (Fig. 3) which elongated, became pubescent and formed leaflets.

At about 2 cm the shoots were cut out of the calli and transferred on modified Murashige and Skoog medium, without hormones for roots differentiation (Fig. 4). Fully differentiated plantlets were kept for 10 days on a liquid Murashige and Skoog medium, without hormones (Fig. 5), therefore obtaining a better rooting and a more vigorous growth of plants before being placed in pots (Fig. 6). After their transfer in the greenhouse we noticed the tuber formation on these plants.

In the first stages of callus initiation and proliferation the light has not an essential role, but an intensity of 5,000 lux is absolutely necessary for the differentiation of shoots.

The age of the donor plant is also important for the success of the *in vitro* culture influencing both the callus growth and the organogenesis. Shoots differentiation was obtained only with explants from young plants of 4–5 weeks, grown in the greenhouse or in the field.

We may conclude that the success of *in vitro* culture of stem segments in potato depends not only on the constituents of the culture medium (suitable balance of phytohormones, concentration in sucrose) but also on some physical factors and some physiological peculiarities of the plant when explants are being collected.

Acknowledgements. The author wishes to thank Dr. T. Gorea and Dr. H. Groza from the Institute of Potato Research and Production Brașov, for their kindness to offer vegetal material for experiments and for efficacious discussions.

REFERENCES

1. Behnke M., Z. Pflanzenzüchtg., 1975, **75**, 262–265.
2. Binding H., Nehls R., Schieder O., Sopory S. K., Wenzel G., *Physiol. Plant.*, 1978, **43**, 52–54.
3. Butenko R. G., Kuchko A. A., Vitenko V. A., Avetisov V. A., *Fiziologhia rastenii*, 1977, **24**, 3, 660–665.
4. Denton I. R., Westcott R. J., Ford-Lloyd B. V., *Potato Res.*, 1977, **20**, 131–136.
5. Foroughi-Wehr B., Wilson H. M., Mix G., Gaul H. *Euphytica*, 1977, **26**, 261–267.
6. Jacobsen E., Z. Pflanzenzüchtg., 1978, **80**, 80–82.
7. Lam S. L., *Am. Potato Journal*, 1977, **54**, 12, 575–580.
8. Marani F., Pisi A., *Acta Horticulturae*, 1977, **78**, 415–424.
9. Melchers G., *Proceedings of Symposium on Plant Tissue Culture*, 1978, May 25–30, Beijing, 279–283.
10. Melchers G., *Proceedings of an International Symposium on Plant Cell Culture*, 1978, München, 306–311.
11. Melchers G., Sacristan D. M., Holder A. A., *Carlsberg Res. Commun.* 1978, **43**, 203–218.
12. Murashige T., Skoog F., *Physiol. Plant*, 1962, **15**, 474–497.
13. Nitsch J. P., Nitsch C., *Science*, 1969, **163**, 3862, 85–87.

14. Opatrny Z., Schumann U., Rakonsky S., Koblitz H., *Biologia Plantarum*, 1980, **22**, 107–116.
15. Roest S., Bokelmann G. S., *Potato Res.*, 1976, **19**, 173–178.
16. Roest S., *Acta Horticulturae*, 1977, **78**, 349–359.
17. Scowcroft W. R., *Advances in Agronomy*, 1977, **29**, 39–81.
18. Shepard J. F., Totten R. E., *Plant Physiol.*, 1977, **60**, 313–316.
19. Sopory S. K., Z. Pflanzenphysiol., 1977, **84**, 453–457.
20. Sopory S. K., Jacobsen E., Wenzel G., *Pl. Sci. Letters*, 1978, **12**, 47–54.
21. Thomas E., King P. J., Potrykus I., Z. Pflanzenzüchtg., 1979, **82**, 1–30.
22. Upadhy M. D., *Potato Res.*, 1975, **18**, 438–445.
23. Wang P. J., Huang L. C., *Can. J. Bot.*, 1975, **53**, 2565–2567.
24. Westcott R. J., Henshaw G. G., Roca W. M., *Pl. Sci. Letters*, 1977, **9**, 309–315.

Received April 11, 1981

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THE EFFECTS OF GAMMA RAYS ON *VINCA MINOR* L. AND *ATROPA BELLADONNA* L.*

BY

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This work presents some results on the behaviour of *Vinca minor* L. and *Atropa belladonna* L. grown from irradiated seeds with small doses of gamma rays in the first generation, and with doses of: 3, 6, 10, 15 and 20 kR in the second generation. The following aspects have been analysed: plant growth, contents of soluble sugars and of free amine nitrogen, the total content of nitrogen and alkaloids.

In the case of the two investigated species, plants showing a changed phenotypic aspect have been obtained. The leaves of these plants and the forms of peroxidases and esterases have been studied.

In the last years our main concern has been directed to the cultivation and melioration of the species *Vinca minor* L. and *Atropa belladonna* L. These two medicinal herbs are very important and highly used in pharmaceutics. Such interesting investigations, regarding the behaviour of cultured and spontaneous plants, have been made in *Vinca minor* [9-14], [16], [18], [21-23], but the results obtained till now are not the expected ones.

We proceed in two ways. On the one hand, we investigated local populations of these species [1-3], [5-7] and began cultivating (at "Stejarul" Research Station) some individuals to detect valuable forms. On the other hand, we treated the seeds of these species with physical and chemical mutagens to increase their variability and to select some promising forms.

MATERIAL AND METHODS

The irradiation of the seeds does not present difficulties in *Atropa belladonna*, because the plants give many seeds. But *Vinca minor* could not be irradiated, because this species has a vegetative reproduction. In the pedoclimatic conditions of our experimental field we could obtain a large quantity (1-2.5 kg per year) of *Vinca* seeds (from a field of 3,000 m²). The seeds were used in treatments with mutagens. Other difficulties were due to the reduced germinal power of the seeds, the reduced survival per cent of plants, their low resistance to diseases and cold. We have tried to cope with these difficulties.

Seeds of *Vinca minor* L. and *Atropa belladonna* L. gathered in the plants which, in the first generation, received small gamma ray doses were

again treated with 3, 6, 10, 15 and 20 kR, at a flow of 270 R/min. After irradiation, the seeds were sown in boxes with garden soil and put in the open field. The plants obtained were replanted in the experimental field in the middle of July 1978. At that moment we measured the length of the plants. For each variant, a large number of seeds was treated (50 g in *Vinca* and 10 g in *Atropa*); when we came to replant them, we had only 200–400 plants per variant. Only some tens survived, because of diseases and heavy winters.

The experimental field is pseudorendzine regosol, with alkaline reaction (pH), high content of carbonates, with bases saturated humus and good physical and hydrophysical properties. Multiannual climate data show generally a wet and cool weather.

We have studied the course of the total alkaloid content of the plants during 3 years. A first test was done two months after replanting, in the middle of September 1978. In 1979 (July for *Atropa* and September for *Vinca*) and 1980 (July for both plants) we determined, besides the alkaloid content, the content of soluble sugars, free amine nitrogen and total nitrogen. The analysis methods are given in [4]–[6]. The results of our investigations are presented in Table 1.

From our experimental field we identify changed individuals in both investigated plants. These individuals underwent the same analyses (Table 1); we also determined the forms of their peroxidase and esterase. Plant leaves were homogenized in cold 0.1 Tris-HCl buffer (pH=7.5), containing 0.5 M sucrose, 0.006 M ascorbic acid and 0.006 M cysteine hydrochloride. After centrifugation, 250 µl crude extract was used to determine the enzyme pattern by isoelectric focussing. The isoelectric focussing was performed in 0.5×7.0 cm polyacrylamide gels, using LKB Carrier Ampholine with a pH in the 3–10 range according to Wrighley (1968). Visualisation of isoesterases was achieved by Scandalios' method (1968) and the activity of isoperoxidases was detected using a technique adapted from McDonald (1972), at an incubation temperature of 37°C.

RESULTS AND DISCUSSIONS

In Table 1.1 we see that there are small growth differences between the control and the treated plants of *Vinca minor*. Two months after replanting, the total alkaloid content did not reveal a clear relation to the irradiation dose. Irradiation had a negative effect on the synthesis of alkaloids, except for the 6 kR dose, having increased the alkaloid content up to 140%. The alkaloids content is quite high in the first year, but in the 2nd and 3rd it decreases, and the irradiated plants record values under those of controls. As our pedoclimatic conditions are not favourable to *Vinca minor*, we could consider the results satisfactory. The same is also valid for individuals taken from other localities of Romania which, at "Stejarul" Station, registered lower values than the initial ones.

We have found low free amine nitrogen values and quite high ones for soluble sugars. Drought resistance of *Vinca minor* is increased due to its changed morphological and anatomical characters, but also to the

Table 1
The values of some physiological and biochemical parameters in gamma irradiated and modified plants of *Vinca minor* L. and *Atropa belladonna* L.

The variant	Length of plants (in mm)		Total sugars % d.s.		Red. sugars % d.s.		Free amine nitrogen % d.s.		Total nitrogen % d.s.		Total alkaloids content % d.s.		
	$\bar{x} \pm$	sx	1979	1980	1979	1980	1979	1980	1979	1980	1978	1979	1980
1. <i>Vinca minor</i> L.													
C	100.56	3.18	11.44	9.50	1.23	1.13	0.43	0.95	2.73	2.85	0.44	0.29	0.40
3 kR	107.74	2.20	11.38	11.05	1.22	1.08	0.45	0.84	2.57	2.79	0.36	0.30	0.37
6 kR	98.45	2.46	13.23	—	1.16	—	0.53	—	2.33	—	0.62	0.31	0.32
10 kR	97.46	2.15	12.53	12.90	1.13	1.21	0.43	0.73	2.55	2.84	0.33	0.26	0.30
15 kR	101.36	1.86	12.51	7.78	1.02	1.24	0.30	0.72	2.67	3.04	0.28	0.21	0.27
20 kR	107.90	2.75	—	10.14	—	1.00	—	1.00	—	2.97	0.41	0.34	0.32
PV-1	—	—	—	8.09	—	1.31	—	0.64	—	2.53	—	—	0.29
PV-2	—	—	—	8.12	—	1.08	—	0.74	—	2.48	—	—	0.18
2. <i>Atropa belladonna</i> L.													
C	115.66	3.19	6.40	6.30	1.40	1.06	1.81	1.37	3.61	3.99	0.25	0.36	0.52
3 kR	148.50	2.82	7.99	11.00	1.59	1.43	1.76	1.81	3.11	4.07	0.22	0.38	0.48
6 kR	115.73	2.35	8.50	8.15	1.54	1.38	1.75	1.59	3.76	3.70	0.20	0.41	0.40
10 kR	126.86	2.53	9.26	6.32	1.72	0.77	1.76	1.44	3.51	3.83	0.23	0.46	0.38
15 kR	126.40	3.54	8.89	7.54	1.13	1.61	1.97	1.41	3.40	3.86	0.34	0.46	0.46
20 kR	118.55	3.22	10.73	7.18	1.90	2.15	1.90	1.85	3.46	4.13	0.16	0.38	0.54
PA-1	—	—	—	10.95	—	1.10	—	1.42	—	4.10	—	—	0.46
PA-2	—	—	—	8.39	—	1.16	—	1.86	—	4.81	—	—	0.79
PA-3	—	—	—	6.85	—	1.59	—	1.67	—	4.45	—	—	0.74

increased values of sugars, determining a higher concentration of vacuolar sap. We could not find a sure relation between the alkaloid content and the other analysed indices.

Investigations of *Atropa belladonna* (Table 1.2) showed that almost all the doses stimulated the growth of surviving plants. Plants treated with 3 kR were 28% taller than the control ones (at planting time). Higher doses had a lower growth stimulating effect. After two months from replanting, when the plants were 50–70 cm high, their alkaloid content was reduced. The highest value (0.34%) was found in plants treated with 15 kR. In the second year the values were higher than in the first and in the irradiated plants they were higher than in controls (particularly in those irradiated with 6–15 kR). In the third year, the total alkaloid content in all variants was high, but in the irradiated plants it was lower than in controls.

In general, in *Atropa belladonna*, we could not find a relation between the total alkaloid content and the other parameters as a function of irradiation dose. Although, higher values of alkaloids are correlated to higher values of amine and total nitrogen, the values of these two indices are moderate and do not change significantly with irradiation. On the contrary, soluble sugars have high values in irradiated plants rising with the dose.

As we have shown at the beginning, some plants (denoted PV₁) of *Vinca minor* are changed, having larger leaves and higher disease and cold resistance. Others (denoted PV₂) showed narrower leaves, flowers with narrow petals, low growth and reduced resistance to external agents. The latter are of no practical interest, having also a reduced alkaloid content, but the former (PV₁) seems valuable, having also quite a good alkaloid content (Table 1.1).

Peroxidase isoenzyme patterns of *Vinca minor* leaves (Fig. 1) show evident differences between the changed plants and the control ones. Altogether, 5 bands with peroxidase activity have been identified in the control and PV₂, and 8 in PV₁ plants. In the modified plants we have found bands with peroxidase activity in the pH-range 6.8–7.5, which were absent in the control. Bands between pH 6.3–6.6, noticed in the control and PV₁ plants, do not appear in PV₂ plants, but the isoperoxidase at pH = 7.35 is highly evident. It is possible that the higher variability of peroxidase forms in PV₁ plants should contribute to their vigour and resistance to external factors.

Vinca minor has also a great number of isoesterases (Fig. 2), PV₂ plants being richer in isoesterases (14 bands). In the PV₁ plants there appeared a new and very clear band at pH = 7.7; in PV₂ plants we found 5 bands at pH 7.8–8.1, which are absent in PV₁, with only slight traces occurring in controls.

Changed plants have also appeared in *Atropa belladonna*, for example one with yellow flowers, fruit and seeds in plants irradiated with 3 kR (designated PA₁). According to "The Flora in Romania", such forms seem to exist in natural populations, too. From the plants irradiated with 20 kR, in general more vigorous, we isolated 2 smaller plants, with better developed stems, a richer ramification, smaller, thicker and leaves of a dark green colour. These plants have been denoted PA₂ and PA₃.

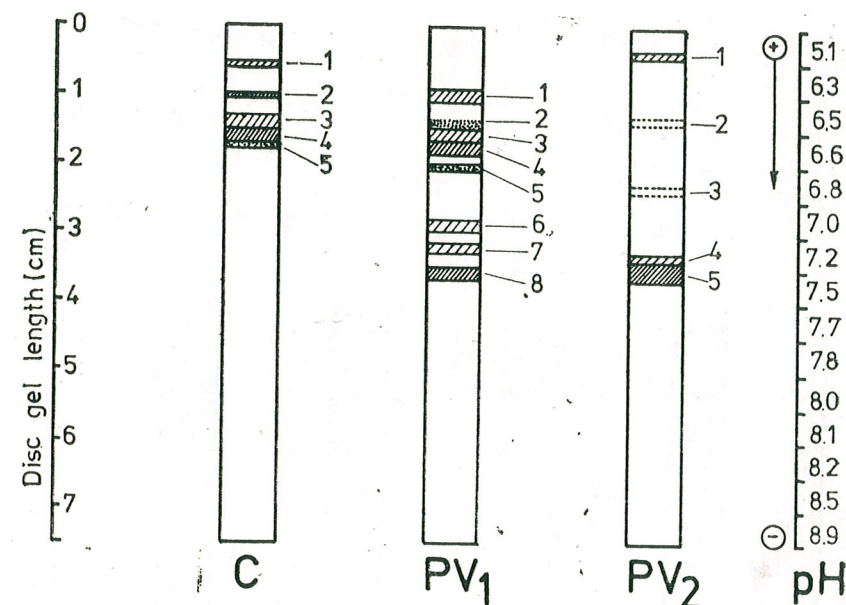


Fig. 1. — Schematic zymograms of isoperoxidases from leaves of *Vinca minor* L.

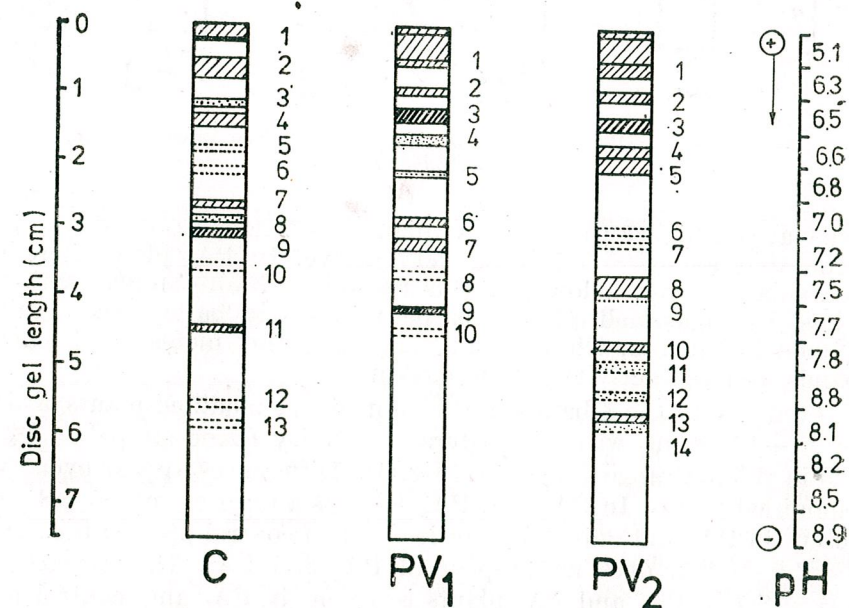


Fig. 2. — Schematic zymograms of isoesterases from leaves of *Vinca minor* L.

PA₁ plants had a similar alkaloid content as the controls. In exchange, the alkaloid content of PA₂ and PA₃ was significantly superior (50 % and 42%, respectively, more than in controls). At the same time, the total nitrogen and the free amine nitrogen content are high (Table 1.2).

Isoelectrophoreses show that *A. belladonna* is quite rich in multiple peroxidases in the pH range 5.7–7.7 (Fig. 3).

We have also identified quantitative and qualitative differences between modified and control plants. So, PA₁ plant has no peroxidase

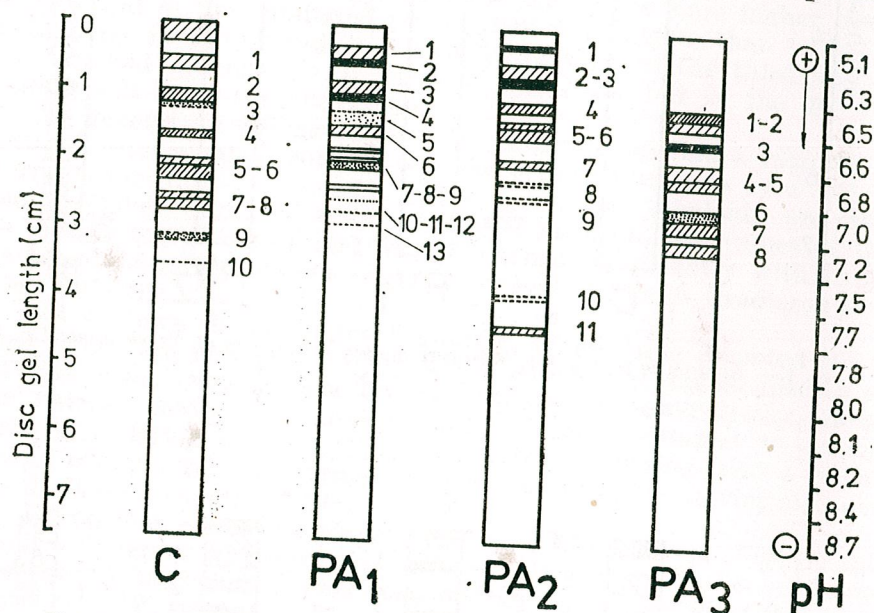


Fig. 3. — Schematic zymograms of isoperoxidases from leaves of *Atropa belladonna* L.

activity at pH higher than 7; PA₂ plant has 2 new forms, absent in the control, that at pH = 7.7 being very active. In PA₃ plant there is no isoperoxidase at pH below 6.4. Our results are quite similar with those obtained by Giacomelli (1967) for peroxidases in barley and Smith et al. (1969) in tobacco. They found that irradiation increased the number of bands and the activity of the enzyme.

Contrary to the 8 bands in the control, the modified plants of *Atropa* have 11–12 forms with the esterase activity (band at pH = 6.2 and bands in the pH range 7.4–8.1), (Fig. 4). Differences appear even among the modified plants. In PA₂ and PA₃ there is a form at pH = 6.8 which is absent in PA₁ and only traces of it occur in control plants; the form at pH = 7.9 in PA₁ does not appear in PA₂ and PA₃. The esterase band at pH = 8.1 in PA₂ and PA₃ plants is absent in PA₁ and control plants. We must also point out that the forms with pH = 7.1 and 8.1 in PA₃ plants are the most evident.

The quantitative and qualitative differences of the investigated enzymes in the modified plants of *Vinca minor* and *Atropa belladonna* reinforce the idea that they are not mere modifications (phenocopies), but reflect a changed genetic nature in these plants.

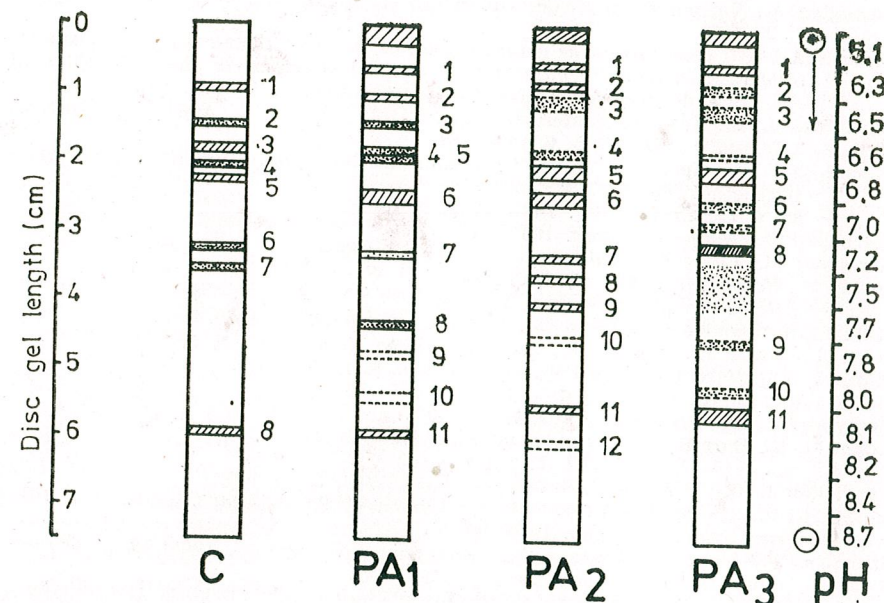


Fig. 4. — Schematic zymograms of isoesterases from leaves of *Atropa belladonna* L.

CONCLUSIONS

— A gamma-ray treatment has induced changes in growth, content of soluble sugars, free amine nitrogen, total nitrogen and alkaloids in *Vinca minor* L. and *Atropa belladonna* L.

— We have not identified a clear relation between the irradiation dose and the analysed parameters.

— Generally, the irradiated plants have a low alkaloid content in comparison with the control ones. In *Vinca minor* the alkaloid content decreases in time (as the culture gets older), while in *Atropa belladonna* it increases, reaching maximum values in the 3rd vegetation year.

— By means of gamma-ray we have obtained, in both species, forms with changed phenotype, which differ from the control plants in respect of morphology, alkaloid content, isoenzymes with peroxidase and esterase activity. They seem to be stable forms and some of these plants are promising for agriculture.

REFERENCES

1. Gheorghiu A., Ionescu-Matiu Elena, Lupulescu Victoria, Ghiorghita I. G., Gavrilescu G., *St. cerc. biol.*, Ser. bot. 1973, **26**, 6, 537—543.
2. Gheorghiu A., Ionescu-Matiu Elena, Ghiorghita I. G., Lupulescu Victoria, *Practica farmaceutică*, 1974, **4**, 153—161.
3. Gheorghiu A., Ionescu-Matiu Elena, Ghiorghita I. G. *St. cerc. biol.* Ser. bot., 1975, **27**, 1, 23—27.
4. Ghiorghita I. G., *Repercusiuni de natură fiziologică ale unor procese genetice declanșate de tratamente cu radiații ionizante și în câmpuri magnetice la plante de ploidii diferite*. Teză de doctorat, Universitatea „Al. I. Cuza” — Iași, 1975.
5. Ghiorghita I. G., Tóth T. Ecaterina, Lupăscu Gh., Băra I. I., Artenie V., *Lucr. Staf. „Stejarul”*, 1974—1975, **6**, 183—189.
6. Ghiorghita I. G., Apetroaiei Maria, Gheorghiu A., *Lucr. Staf. „Stejarul”*, Ser. Ecol. ter. și genet., 1976—1977, **7**, 203—208.
7. — — — *St. și Cerc. Biol.*, Ser. Bot., 1981, in press.
8. Giacomelli M., *Nature* (London), 1967, **213**, 1265.
9. Gorunović M., Lukić P., *Arhiv za farm.*, 1969, **3**, 139—141.
10. Hubay R., *Herba Hung.* 1966, **5**, 5, 17—24.
11. — — — *Herba Hung.*, 1967, **6**, 3, 27—38.
12. Márk G., Jovanovics K., Lörincz Cs., Szász K., *Herba Hung.*, 1969, **8**, 3.
13. Márk G., Gracza L., Bársony A., Szász K., *Herba Hung.*, 1969, **8**, 3, 19—32.
14. Máthé I., Vágújtálvi D., Kovács M., *Herba Hung.*, **6**, 1, 39—47.
15. McDonald T., Smith T. H., *Genetics*, 1972, **72**, 77—86.
16. Neczypor W., *Die Pharmazie*, 1969, **5**, 273—274.
17. Neslon O. E., Burr B., *Ann. Rev. Plant Physiol.*, 1973, **24**, 493—518.
18. Sárkány S., *Herba Hung.*, 1962, **1**, 2, 157—165.
19. Scandalios J. G., *A Review, Biochem. Genetics.*, 1968, **3**, 37—39.
20. Smith H. H., Hirona J., Conklin M. E., Lyman J. T., *Induced Mutation in Plants*. Proc. Symp. Pullmann. 1969, Wien.
21. Stromberg A. Ia., Djorbenadze A. M., *Soobšcenia AN GruzSSR*, 1968, **52**, 3, 683—688.
22. Sváb J., Földesi D., *Herba Hung.*, 1966, **5**, 254—267.
23. Szász K., Márk G., *The Commercial Cultivation of Vinca minor. The Vinca Alkaloids-Botany, Chemistry and Pharmacology*. Edited by W. Taylor, Marcel Dekker Inc. New York, 1973.
24. Wrigley C., *Sci. Tools*, 1968, **15**, 17—32.

* The paper was presented at the XIth ESNA, Meeting Debrecen-Hungary, 25—30 August, 1980.

Received November 13, 1980

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THE INFLUENCE OF SOME CHEMICAL COMPOUNDS ON THE PLANKTONIC PRIMARY PRODUCTIVITY IN THE LAKE IZVORU MUNTELUI-BICAZ

BY

ALEXANDRU S. BOLOGA

The effect of some chemical compounds on the planktonic primary productivity in the Lake Izvoru Muntelui-Bicaz was determined with a view to test the possibilities for eutrophization control. The ^{14}C *in situ* method was used. The results after having added the compounds were between 15.8—113.8 mg C m⁻³ day⁻¹ at 2.5 m depth, after 24 hours (in July) and between 1.3—21.3 mg C m⁻³ day⁻¹ at 0—5 m depth, after 82 days (in September), showing an inhibition between 2.4—85.4% after 24 hours and between 71.3—93.6% after 82 days, as compared to the control.

Planktonic production or productivity is one of the main indicators for the trophicity of an aquatic basin. Determination of planktonic productive potentiality aims especially at the evaluation of the biomass with a role of nutrient substrate for the consumers. Net primary production, the respiration value being excluded, is interesting as an energetic potential at the sequent level of the trophic chain.

The research on the primary productivity as an indicator for the trophicity of the Lake Izvoru Muntelui-Bicaz [1] was performed within the context of testing the possibilities of fighting against the eutrophization of the lake by chemical compounds. Eutrophization is a process which may be natural or may occur as a consequence of water quality deterioration, with a lengthy development, appearing conspicuously in natural lakes, less evident in artificial ones and rare in running waters. In this respect, besides other consequences of the intensifying human activity, there is a considerable increase in the quantity of nutrient matter drained into the water (from the polluted air, the fertilized soil and from domestic and industrial waste water effluents); this increase in food availabilities determines acceleration of the natural eutrophization rate with serious consequences for the water quality.

The eutrophization process, which can be detected by some physico-chemical indicators, is also apparent in the intensive development of the phytoplankton. As it was previously shown [13], the autotrophic capacity of a lake is an ideal criterion for establishing a scale of trophicity, which should be completed with some more parameters in order to be of use in all practical cases. The examination of the data on the primary production in numerous lakes has shown that it is not possible to obtain an undoubtful simple correlation between the productive capacity and the character of trophicity because of several impediments, such as: the methods used, the reference values chosen, etc.

Among the rather varied methods for the determination of the planktonic primary production and productivity [6], [8], [12], the ^{14}C method acquired international recognition. Although it was conceived to investigate the assimilatory capacity of the marine phytoplankton [11], the ^{14}C method proved its applicability in the research on the primary production of any aquatic environment, whether marine, brackish or fresh. Consequently, lacustrine primary production has been investigated by this procedure in different types of lakes.

After the introduction of the ^{14}C method in the marine planktonologic research along the Romanian coast [2], the radiobiological method was applied in the indigenous limnologic research in 1978, by the present experiments.

MATERIAL AND METHOD

In order to determine the influence of several chemical compounds upon the planktonic primary producers in the Lake Izvoru Muntelui-Bicaz (Fig. 1) it was made use of a pontoon built by I.C.P.G.A.¹, made up of a floatable frame supporting transparent plastic sacks filled with

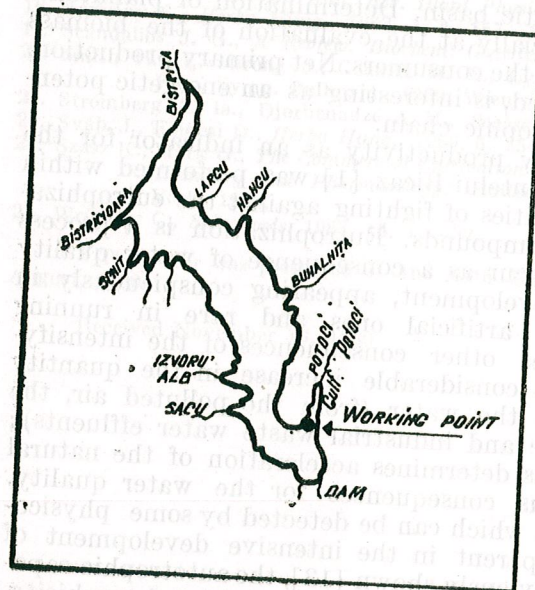


Fig. 1. — Chart of the Lake Izvoru Muntelui-Bicaz with the working point.

lake water: various chemical compounds were added into the sacks, as such or in mixture, in the following experimental variants:

1. control (lake water);
2. igran (0.07 mg l^{-1});
3. CuSO_4 (1 mg l^{-1});

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4. $\text{Al}_2(\text{SO}_4)_3$ (30 mg l^{-1}) + additions of N ($0.3 \text{ mg l}^{-1} \text{ NaNO}_3$) and P ($0.03 \text{ mg l}^{-1} \text{ NaH}_2\text{PO}_4$);
5. FeSO_4 (60 mg l^{-1}) + CaO (30 mg l^{-1}) + additions of N ($0.3 \text{ mg l}^{-1} \text{ NaNO}_3$) and P ($0.03 \text{ mg l}^{-1} \text{ NaH}_2\text{PO}_4$);
6. CuSO_4 (1 mg l^{-1}) + additions of N ($0.3 \text{ mg l}^{-1} \text{ NaNO}_3$) and P ($0.03 \text{ mg l}^{-1} \text{ NaH}_2\text{PO}_4$);
7. $\text{Al}_2(\text{SO}_4)_3$ (30 mg l^{-1});
8. control (lake water);
9. CaO (60 mg l^{-1}) + FeSO_4 (30 mg l^{-1});
10. control (directly from the lake, without the "sack effect").

The phytoplankton samples were collected from the sacks at 2.5 m depth after 24 hours since the addition of the chemical compounds, in July, and from those at 0, 2.5 and 5 m depth after 82 days, in September.

The ^{14}C method [11] *in situ* (cf. [10]) was used, by the liquid scintillation procedure [5]. Each of the (two) transparent and (one) dark bottles containing the phytoplankton to be exposed to the action of the chemical compounds and the control samples as well was inoculated with $1 \text{ ml NaH}^{14}\text{CO}_3$ solution and then sunk again at the initial depth, under natural photic, thermal and stirring conditions. The duration of the photosynthetic assimilation was 24 hours. At the end of the exposure period, the contents of the bottles were rapidly filtered, with an *in vacuum* device, through Millipore membrane filters 04700 HA. ($\phi = 0.45/\mu\text{m}$). The activity of the filters with the phytoplankton residuum was measured in standard polyethylene vials (with previously determined background) by means of a Packard Tri-Carb spectrometer model 3385 (at $+5^\circ\text{C}$) at I.F.I.N.²

As distinct from the measurement of filter radioactivity with a Geiger-Müller counter, the utilization of the liquid scintillation procedure allowed the diminution of errors due to self-absorption of the material on the filter and assured a measuring efficiency of 85%. The following receipt was used for the measurements: 3 ml dioxan (for filter solubilization) and 5 ml Unisolve³ liquid scintillator. Adequate background and sample extinction corrections were applied to the results; each sample was measured for 1 minute, three times.

The calculation of net primary productivity values on the basis of ^{14}C measurements was carried out after Burkaltseva's formula [4]. The value of total inorganic carbon content of the lake water was determined by analysis of total carbonic alkalinity (cf. [14]).

According to the specification of the modalities and the significance of ^{14}C fixation in darkness [3], [7], [9], the values obtained from dark bottles were neither subtracted, nor calculated as percentage of the photosynthetic assimilation values, but were used to verify the experimental proceedings (cf. [10]).

² Institute for Physics and Nuclear Engineering, Bucharest-Măgurele.

³ Unisolve (Koch Light Lab. Ltd.), a universal liquid scintillator, patented, ready prepared, a cocktail especially used for aqueous samples.

RESULTS AND DISCUSSION

The results concerning the influence of some chemical compounds on the primary productivity level in the Lake Izvoru Muntelui-Bicaz point out different values in the two seasons — July and September — due to both the changes of the environmental agents and the effect of the tested chemical compounds in time. Thus, the values are generally greater in July — between 15.8 and 123.3 mg C m⁻³ day⁻¹ — as compared to those in September — between 1.3 and 56.8 mg C m⁻³ day⁻¹ — although the water transparency was lower in July (Tables 1 and 2); during the above seasons, the main primary producers were *Dinobryon sociale* Ehr., *Cyclotella comta* (Ehr.) Kütz., *Asterionella formosa* Hass. and *Micractinium* sp. (Cărauş, personal communication).

During the summer season, the primary productivity reached maximum values at 2.5 m in control samples 8 (123.3 mg C m⁻³ day⁻¹) and 10 (108.5 mg C m⁻³ day⁻¹) from the sacks (Table 1); control 8 showed 13.6% stimulation as compared to control 10 from the lake. Values of the same order were found in the experimental variants 4 and 6, though the latter showed a 48.0% stimulation in comparison with control 10. The lowest values, due to the strongest inhibitory effect of the chemical compounds added, were found in experimental variants 2, 3, 7 and 9, showing 85.4%, 83.8%, 28.9% and respectively, 38.1% inhibition as compared to control 10.

During the autumn, after 82 days since the addition of the chemical compounds, the primary productivity values were considerably smaller; the maximum value of 56.8 mg C m⁻³ day⁻¹ was reached in the control sample 10 on the surface (0 m) (Table 2) and a decrease of the values from the surface to higher depths (2.5–5 m) was also found. The inhibitory effect of all tested compounds ranged between 71.3–93.6% as compared to control 10.

The experimental data show the immediate inhibitory effect of igran, CuSO₄, FeSO₄ + CaO with additions of N and P, Al₂(SO₄)₃ and

Table 1

Values of *in situ* (2.5 m) planktonic primary productivity in the Lake Izvoru Muntelui-Bicaz in July, 1978

Experimental variant	Horizon (m)	Transparency (m)	Luminescence (1×)	Temperature (°C)	O ₂ (mg l ⁻¹)	Mineral C (mg l ⁻¹)	Assimilated C (mgm ⁻³ day ⁻¹)
1	2.5	1.20	84	20.5	10.24	21.3	48.3
2	2.5	1.35	134	20.5	9.46	18.0	15.8
3	2.5	1.10	156	20.5	10.16	16.9	17.5
4	2.5	1.30	144	20.5	10.24	17.8	105.8
5	2.5	1.55	112	20.5	9.46	12.6	41.5
6	2.5	1.20	128	20.5	10.09	15.7	113.8
7	2.5	1.45	105	20.5	10.95	18.3	77.1
8	2.5	1.35	96	20.5	10.40	19.3	123.3
9	2.5	1.00	108	20.5	5.80	6.0	67.1
10	2.5	1.45	240	20.5	9.85	13.8	108.5

Table 2

Values of *in situ* (0–5 m) planktonic primary productivity in the Lake Izvoru Muntelui-Bicaz in September, 1978

Experimental variant	Horizon (m)	Transparency (m)	Temperature (°C)	O ₂ (mg l ⁻¹)	Mineral C (mg l ⁻¹)	Assimilated C (mg m ⁻³ day ⁻¹)
1	0	2.70	18.5	8.37	18.02	6.3
	2.5		17.8	8.06	17.42	3.5
	5		17.0	7.59	17.42	2.8
2	0	3.30	18.5	8.52	18.60	1.3
	2.5		17.8	8.06	19.76	2.2
	5		17.0	7.90	18.58	2.7
3	0	1.90	18.5	9.61	19.74	1.6
	2.5		17.8	8.68	19.16	4.0
	5		17.0	8.37	19.16	5.9
4	0	2.30	18.5	9.92	15.19	14.1
	2.5		17.8	8.68	14.59	3.6
	5		17.0	8.21	14.59	3.0
5	0	2.20	18.5	8.37	5.92	12.4
	2.5		17.8	8.68	6.49	7.2
	5		17.0	8.21	8.59	7.8
6	0	2.30	18.5	8.21	19.74	2.0
	2.5		17.8	8.52	19.16	2.5
	5		17.0	8.06	19.72	1.7
7	0	2.80	18.5	9.14	16.28	4.4
	2.5		17.8	8.52	16.84	4.8
	5		17.0	8.06	16.84	3.1
8	0	2.90	18.5	8.68	17.48	14.1
	2.5		17.8	8.06	17.48	5.7
	5		17.0	7.75	17.87	2.3
9	0	2.40	18.5	8.37	7.43	21.3
	2.5		17.8	8.68	8.01	13.2
	5		17.0	9.30	8.59	6.2
10	0	2.10	18.0	8.06	19.80	56.8
	2.5		17.5	8.06	19.02	30.9
	5		16.7	8.68	18.44	7.4

CuSO₄ + FeSO₄, as well as the inhibitory effect of all compounds in time — especially that of igran and CuSO₄. In the course of time Al₂(SO₄)₃ with additions of N and P, FeSO₄ with the same additions and CaO + FeSO₄ determined an increase in the assimilatory capacity as compared to controls 1 and/or 8; the presence of the major nutrients N and P, with an important role in the photosynthetic assimilation of the planktonic primary producers, could be an explanation for this stimulating effect, which is however annihilated in the presence of CuSO₄.

The values obtained simultaneously after the Winkler method, which was applied comparatively, proved to be uncertain; thus, e.g. in July, in variant 1, O₂ concentration was 0.79 mg l⁻¹ at 0 m and 0.00

mg l⁻¹ at 5 m depth; in variants 2, 3 and 9, O₂ concentration was also zero; at the same time, in control 10, it took smaller values than in the experimental variants with toxic compounds, and not farther than 2.5 m depth: 0.11 mg l⁻¹ at 0 m, 0.16 mg l⁻¹ at 2.5 m and 0.00 mg l⁻¹ at 5 m!

CONCLUSIONS

1. The ¹⁴C method by liquid scintillation counting was introduced in the Romanian limnological research, using NaH¹⁴CO₃ as indigenous source.
2. Under the given experimental conditions, the ¹⁴C method gave better results, as compared with the Winkler method.
3. The planktonic primary productivity of the Lake Izvoru Muntelui-Bicaz during 1978 was 108.5 mg C m⁻³ day⁻¹, in July (0 m depth), and between 7.4 — 56.8 mg C m⁻³ day⁻¹, in September (0—5 m), indicating the mesotrophic feature of this dammed lake.
4. From the chemical compounds tested, igran, CuSO₄ and Al₂(SO₄)₃ showed the strongest inhibitory effect on the assimilatory capacity of the planktonic primary producers.
5. The inhibitory effect of the tested chemical compounds ranged within 2.4 — 85.4% after 24 hours and within 71.3 — 93.6% after 82 days since their addition, as compared to the control sample.

Acknowledgements. The author is sincerely grateful to Dr. G. Ivancea and M. Florescu of I.C.P.G.A., Bucharest, and to Dr. eng. P. T. Frangopol of I.F.I.N., Bucharest-Măgurele, for their permanent cooperation.

REFERENCES

1. Bologa A. S., *Lucr. st. cerc. "Stejarul"*, in press.
2. —, Frangopol P. T., *Cerc. mar.-Rech. mar.*, 1979, **12**, 81—86.
3. Brouardel J., *Vie et Milieu*, 1973, B-22, 2, 247—256.
4. Burkaltseva M. A., in *Sovremennye metody rybohosiastvennyh morskikh gydrohimicheskikh issledovaniy*, M. V. Fedosova (ed.), Isd. Pisc. promysl., Moskva, 1973, p. 146—169.
5. Frangopol P. T., Bologa A. S., *Preprint CSEN/ICEFIZ*, 1979, RB-1, May, Bucharest, 1—39.
6. Gessner F., in *Hydrobotanik*, 2, Deutsch. Verl. Wiss., Berlin, 1959, 608—626.
7. Morris I., Yentsch C. M., Yentsch C. S., *Limnol. Oceanogr.*, 1972, **16**, 6, p. 854—858.
8. Schlieper C., *Methoden der meeresbiologischen Forschung*, VEB G. Fischer Verl., Jena, 1971.
9. Sournia A., in *L'énergie nucléaire et ses applications biologiques à Madagascar*, 1971, **12**, Tananarive, 251—267.
10. —, *Bull. et. comm. Médit.*, 1973, Num. spéc. 5, Monaco, 1—128.
11. Steemann Nielsen E., *J. Cons. perm. int. Explor. Mer*, 1952, **18**, 117—140.
12. Vollenweider R. A., *A Manual on Methods for Measuring Primary Production in Aquatic Environments*, I. B. Handb. **12**, Blackwell Sci. Publ., Oxford and Edinburgh, 1969.
13. —, *La techn. d'eutroph. et de l'assainiss.*, 1971, 295—296.
14. * * * *Protecția calității apelor (Protection of the Waters Quality)*, Bibl. Stand., ser. tehn. A, 1967, **62**, 137—138.

Received December 14, 1980

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THE PARASITIC AND SAPROPHYTIC MYCOFLORA ON *POA PRATENSIS* L. IN THE CONDITIONS OF USING VARIABLE NITROGEN FERTILIZER DOSES

BY

ALEXANDRU MANOLIU, MIHAI MITITIUC, MIRCEA RUSAN

The authors present the results concerning the influence of different nitrogen fertilizer doses on the appearance and frequency of the parasitic and saprophytic mycoflora on *Poa pratensis* L. The application of some high nitrogen fertilizer doses determines the sensibility of *Poa pratensis* plant to some pathogenic agents and the increase of the number of saprophyte fungi species.

In the two papers already published [3], [4], were presented the results of observations concerning the influence of some variable nitrogen fertilizer doses on the mycoflora of two fodder Gramineae, which were placed [9] in the following groups: plants with a raised fodder value (*Agrostis tenuis*) and good fodder plants (*Festuca rubra*). As a result, it was concluded that the one-sided application of a high amount of nitrogen fertilizers diminishes the resistance of *Agrostis tenuis* and *Festuca rubra* to some diseases, especially mildew, rust and septoriose. Our results confirm the literature data on this matter: Wingard, 1941, Coons, 1953, Stakman and Harrar, 1957 (mentioned after [1]), Tr. Săvulescu [10], [11], E. Rădulescu [7], [8].

This paper presents the parasitic and saprophytic mycoflora on *Poa pratensis* L., which belongs to a group of very good fodder plants.

MATERIAL AND METHOD

Our researches were made in 1974—1978 in the same conditions as for the Gramineae previously studied. The experiment having 18 variants, arranged in Latin rectangular, was conducted in Bălăceana village, Suceava county. All variants — except for the control — were fertilised with P 60 + K 60 kg/ha, every autumn as superphosphate and potassium salt. The nitrogen was applied as ammonium nitrate in spring and then it was fractionated after every harvesting.

During our experiments the nitrogen fertilizer doses were used as follows:

- 1974 — April 10, June 22, August 8;
- 1975 — April 2, June 1, July 15;
- 1976 — April 10, June 2, July 20;
- 1977 — March 28, May 25, July 22;
- 1978 — April 5, June 1, July 20;

Out of the 18 variants, the following ones were chosen for our studies: $V_{12} - N \ 53.3 + 53.3 + 53.3 \text{ kg/ha}$, $V_{13} - N \ 107 + 107 + 107 \text{ kg/ha}$, $V_{14} - N \ 160 + 160 + 160 \text{ kg/ha}$. With each variant studied, having an area of 100 m^2 ($10 \text{ m} \times 10 \text{ m}$) the *Poa pratensis* samples were minutely studied so that the pathogenic agents and the degree of plant affection could be estimated. The presence of fungi, especially of pathogenic ones, was estimated by observing the characteristic symptoms of illness and microscopically counting the fructifications on the affected organs by a stereomicroscopic lens.

For an easier interpretation of our results and considering the group of plants studied, we used a simplified scale of notation for fungi presence (for parasite and saprophyte) made of three qualifying marks:

+ = occasional fungus incidence

++ = average fungus incidence

+++ = high fungus incidence.

RESULTS AND DISCUSSIONS

The doses of nitrogen fertilizer applied had also influenced (as in the other two cases of fodder Graminaeae species already studied) the frequency of the parasite and saprophyte fungi and the degree of illness of *Poa pratensis*. In 1974 - 1978, 21 species of fungi were identified on *Poa pratensis* (Ascomycetes = 2 species, Basidiomycetes = 1 species, Deuteromycetes = 18 species), being distributed every year, as follows: 1974 - 21 species, 1975 - 13 species, 1976 - 10 species, 1977 - 12 species, 1978 - 20 species (Table 1). One can notice from this table that the increase of the applied nitrogen fertilizer dose produced also the increase of the number of fungi species which were identified in the respective variant. The greatest number of fungi species was identified in variant no. 14, to which the maximum quantity of nitrogen fertilizer was applied (480 kg/ha of active substance), in 1974 and 1978. In these two years, the climate conditions were propitious for the appearance of pathogenic agents, too (1974 - 776 mm precipitations; 1978 - 663 mm precipitations). The rust produced by *Puccinia poarum* appeared every year, in all the variants, noted with ++ and +++, the most sensitive being the *Poa pratensis* from variant no. 14.

In contrast with *Agrostis tenuis* and *Festuca rubra* which were parasitized by 7 species and, respectively 5 species of fungi belonging to *Septoria*, on *Poa pratensis* only one species of this genus was found, whose presence was noted, all these years, with +++ in variant no. 14. *Poa pratensis* became sensitive to septoriosis attack due to the high doses of nitrogen fertilizer, our observations refuting some literature data on the importance of nitrogen fertilizer in septoriosis. Thus, Pritchard and Porte (mentioned after [8]) studied the fertilizer's influence on the intensity of the *Septoria lycopersici* attack on tomato cultures; they concluded that the foliage increased and the fungus attack was lower.

We must also say that in the variants treated with nitrogen fertilizer the number of saprophytic fungi grew, having a negative role in

Table 1

The parasitic and saprophytic mycoflora on *Poa pratensis* in the conditions of variable nitrogen fertilizer doses

Species of fungi	Year	Host					
		V_{12}		V_{13}		V_{14}	
		L	S	L	S	L	S
1	2	3	4	5	6	7	8
<i>Erysiphe graminis</i>	1974	—	—	—	—	—	—
	1975	—	—	—	—	—	—
	1976	—	—	—	—	+	—
	1977	—	—	++	+	—	—
	1978	—	—	—	—	+++	+
<i>Mycosphaerella longissima</i>	1974	—	—	—	—	—	+
	1975	—	—	—	—	—	—
	1976	—	+	—	—	—	—
	1977	—	—	—	—	—	—
	1978	—	—	—	—	—	++
<i>Puccinia poarum</i>	1974	—	—	++	—	+++	—
	1975	—	—	++	—	+++	—
	1976	—	—	++	—	+++	—
	1977	++	—	+++	—	+++	—
	1978	++	—	+++	—	+++	—
<i>Phyllosticta crastophylla</i>	1974	+	—	+	—	+++	—
	1975	—	—	—	—	—	—
	1976	—	—	—	—	—	—
	1977	—	—	—	—	—	—
	1978	—	—	—	—	+	—
<i>Phoma graminis</i>	1974	—	—	—	+	—	—
	1975	—	—	—	+	—	++
	1976	—	—	—	—	—	—
	1977	—	—	—	—	—	—
	1978	—	+	—	—	+	++
<i>Pyrenochaeta leptospora</i>	1974	—	+	—	+	++	—
	1975	—	+	+	+	—	++
	1976	—	—	—	—	—	—
	1977	—	—	—	++	+++	—
	1978	—	—	—	—	++	++
<i>Coniothyrium graminum</i>	1974	—	—	—	++	—	+
	1975	—	—	—	—	—	—
	1976	—	—	—	—	—	—
	1977	—	+	—	—	—	—
	1978	—	—	—	—	—	++
<i>Ascochyta graminicola</i>	1974	—	—	+++	—	+++	—
	1975	—	++	—	—	—	—
	1976	—	—	—	—	—	—
	1977	++	—	+++	—	++	—
	1978	++	—	+++	—	—	—
<i>Stagonospora curvula</i>	1974	—	—	+	++	—	—
	1975	—	—	—	—	—	—
	1976	—	—	—	—	+	++
	1977	+	—	—	—	—	—
	1978	—	—	—	—	—	+
<i>Stagonospora graminella</i>	1974	—	—	—	+	++	—
	1975	—	—	—	—	—	—
	1976	—	—	—	—	—	—
	1977	—	—	—	—	—	—
	1978	—	—	—	—	—	+

Table 1 (continued)

1	2	3	4	5	6	7	8
<i>Septoria fusispora</i>	1974	+	—	++	—	+++	—
	1975	—	—	—	—	+++	—
	1976	—	—	+	—	+++	+
	1977	—	—	—	—	+++	—
	1978	—	—	+++	—	+++	—
<i>Dinemasporium graminum</i>	1974	—	++	—	—	—	+
	1975	—	—	—	++	—	—
	1976	—	—	—	—	—	—
	1977	—	—	—	—	—	—
	1978	—	—	—	—	—	++
<i>Vermicularia graminicola</i>	1974	—	—	++	+	—	++
	1975	++	—	++	—	+++	—
	1976	—	—	—	—	—	—
	1977	—	+	—	+	+++	++
	1978	—	—	+	++	+++	+++
<i>Leptostroma herbarum</i>	1974	—	—	+	—	—	++
	1975	+	—	—	—	—	—
	1976	—	—	—	+	—	—
	1977	—	—	—	—	—	—
	1978	—	—	—	—	++	—
<i>Cladosporium graminum</i>	1974	+	++	+	+	+	++
	1975	+	+	+	++	—	++
	1976	—	—	—	—	—	—
	1977	—	—	++	—	—	—
	1978	—	+	—	—	—	+
<i>Cladosporium herbarum</i>	1974	++	+	++	++	++	+
	1975	++	—	++	—	++	+
	1976	+	+	++	—	++	—
	1977	++	—	++	—	++	+
	1978	+	+	+	—	+++	+
<i>Epicoccum neglectum</i>	1974	+	+	—	—	+	+
	1975	—	—	—	—	—	—
	1976	—	—	—	—	—	+
	1977	—	—	—	—	—	—
	1978	—	—	+	+	—	+
<i>Epicoccum purpurascens</i>	1974	—	—	+	—	—	—
	1975	—	—	—	—	—	+
	1976	—	—	—	—	—	—
	1977	—	—	—	—	+	—
	1978	—	—	—	—	—	+
<i>Drechslera stage of Cochliobolus sativus</i>	1974	—	—	—	—	—	++
	1975	+	—	—	—	+	—
	1976	—	—	—	—	—	—
	1977	—	—	—	—	—	—
	1978	—	—	—	—	—	+
<i>Periconia pyrenospora</i>	1974	++	—	—	—	—	—
	1975	—	—	+	—	—	—
	1976	—	—	—	+	—	—
	1977	—	—	—	—	—	++
	1978	—	—	—	—	—	—
<i>Alternaria alternata</i>	1974	—	—	+	—	+	—
	1975	—	—	—	—	—	—
	1976	—	—	—	—	—	++
	1977	—	—	—	—	—	—
	1978	—	—	++	—	+++	—

L = leaves
S = stems

the preservation of the hay, determining its degradation. Before the saprophytic stage, some of these fungi may be pathogenic, causing different diseases, such as the "cladosporiose" of Gramineae, induced by different species of *Cladosporium* genus, especially *C. herbarum*. Also, toxicity of *Cladosporium herbarum* extracts for the animals was proved [5].

In Table 2 there are comparatively presented the genus of parasitic and saprophytic fungi on the three species of Gramineae which were

Table 2

The distribution of parasitic and saprophytic fungus genera by studied Gramineae

Gramineae	<i>Agrostis tenuis</i>	<i>Festuca rubra</i>	<i>Poa pratensis</i>
Fungus genera			
Erysiphe	—	1	1
Leptosphaeria	2	1	—
Mycosphaerella	—	—	1
Puccinia	1	1	1
Phyllosticta	1	1	1
Phoma	1	1	1
Pyrenochaeta	1	1	1
Coniothyrium	—	—	1
Hymenopsis	—	1	—
Ascochyta	1	1	1
Diplodia	1	—	—
Septoria	7	5	1
Stagonospora	1	2	2
Phaeoseptoria	1	—	—
Hendersonia	3	—	—
Leptostroma	1	—	1
Dinemasporium	1	1	1
Vermicularia	2	4	1
Cladosporium	2	2	2
Drechslera	1	—	1
Epicoccum	1	1	2
Periconia	—	—	1
Alternaria	—	1	1
Total	28	24	21

studied. From this table we notice that most of the fungi species were found on *Agrostis tenuis* (28 species) and the fewest on *Poa pratensis* (21 species). *Agrostis tenuis* seems to be the most sensible to the fungi attack in the conditions of the applications of variable doses of nitrogen fertilizer. This was also established from the analysis of this plant sensitivity to the attack of rust and septoriose, which were manifest with maximum intensity.

The genera having the greatest number of species were: *Septoria*, *Vermicularia*, *Hendersonia*, *Stagonospora*, *Epicoccum*. From this table we also notice that some genera (*Coniothyrium*, *Mycosphaerella*, *Diplodia*, *Phaeoseptoria*) were identified only in one of the studied Gramineae, while other genera (*Phyllosticta*, *Septoria*, *Ascochyta*, *Stagonospora* etc) were identified in all the three species of fodder Gramineae.

CONCLUSIONS

1. The one-sided applications of some high doses of nitrogen fertilizer led to a sensitization of *Poa pratensis* plants to the rust, mildew and septoriose. There is also an increase in the number of saprophytic fungus species.
2. The effect of the application of some nitrogen fertilizer doses on the appearance of some parasitic and saprophytic fungus species was more reduced when these fertilizers were given fractionated, than when applied in a single dose.

REFERENCES

1. Horsfall J. G. and Dimond A. E., *Plant pathology*, Academic Press, New York and London, I, 1959, II, III, 1960.
2. Manoliu Al., *Lucr. şt. S. E. A. Suceava* (dedicated volume) Red. rev. agr., Bucureşti, 327—335, 1973.
3. Manoliu Al., Mititiuc M., *Cercetări agronomice în Moldova*, Iaşi, I, 79—82, 1976.
4. Mititiuc M., Manoliu Al., *Analele şt. Univ. "Al. I. Cuza" Iaşi*, (New series), Sect. II, Biol. XXV, 4—8, 1979.
5. Pidopliciko N. M., *Gribnaia flora grubyh kormov*, Kiev, 1953.
6. Rădulescu E., *Probleme agricole*, 5, 46—55, 1967.
7. Rădulescu E., Resmeriţă I., *Probleme agricole*, 9, 15—20, 1964.
8. Rădulescu E., Docea E., Negru Al., *Septoriozele din România*, Ed. Academiei, Bucureşti, 1973.
9. Resmeriţă I., *Flora, vegetaţia şi potenţialul agroproductiv pe masivul Vlădeasa*, Ed. Academiei, Bucureşti, 1970.
10. Săvulescu Tr., *Monografia Uredinalelor din Republica Populară Română*, I, Ed. Academiei, Bucureşti, 1953.
11. Săvulescu Tr., Săvulescu Olga, *Tratat de patologie vegetală*, I, Ed. Academiei, Bucureşti, 1959.

Received January 15, 1981

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Zum 80. Geburtstag von Dr. Margit Dracinschi



Die von Prof. Dr. Mihail Guşuleac an der Universität von Cernăuţi zu Beginn dieses Jahrhunderts begründete botanische Schule hat Lehrkräfte ausgebildet und gefördert, welche sich in verschiedenen Zweigen der Botanik ausgezeichnet haben. Außer den Studien über Floristik, Taxonomie, Abstammungs- und Entwicklungslehre (Thallophyten und Cormophyten), Ökologie, Geobotanik und Phytosoziologie, Anatomie und Karyologie u.a. haben schon damals die Untersuchungen über die Zytologie mancher Pflanzengruppen nicht gefehlt.

Unter den ersten bei uns unternommenen Untersuchungen über die Spermatogenese bei gewissen Bryophyten, wie diejenigen von P. Eftimiu an *Bucegia romanica* (1933), von A. Mühlendorf bei *Sphagnum* (1930) und Lebermoosen (1931), sowie bei Pflanzen im allgemeinen (1931), nehmen die Untersuchungen über Pteridophyten von Dr. M. Dracinschi (Abb. 1), welche in den Jahren 1930—1932 veröffentlicht wurden, einen besonderen Platz ein.

In Suceava am 17.III.1900 geboren, besucht M. Dracinschi die Grundschule (1906—1911) und die Oberrealschule in Cernăuţi (1911—1918). Bei der Reifeprüfung erhielt sie die Note „Sehr gut, mit Auszeichnung“. In den Jahren 1919—1923 studiert sie an der Abteilung für Naturwissenschaften der Wissenschaftlichen Fakultät der Universität von Cernăuţi und legt auch die Prüfung in Philosophie und Pädagogik ab (1922).

Im akademischen Lehrbetrieb bekleidet sie die Stellung eines Assistenten am Botanischen Institut unter der Leitung von Prof. Dr. M. Guşuleac, Wissenschaftliche Fakultät. Im Jahre 1925 erwirbt sie den Titel eines Lizenziaten der Naturwissenschaften und besteht die Eignungsprüfung als Assistentin (1928). Darauf erfolgt ihre Ernennung zum Leiter des Praktikums (1929) und wirkt als solcher bis zum Jahre 1940. Mit ihrer Dissertation über ein viel mühselige Kleinarbeit erforderndes Thema der Zytologie usw. über die Spermatozoiden der Pteridophyten, erwarb sie am 11.Febr. 1933 die Doktorwürde im Hauptfach Botanik.

★

Ihre wissenschaftliche Tätigkeit zerfällt in zwei Perioden.

Die erste, tschernowitzer Periode (1923—1941), während welcher die Vervollkommenung und Behauptung auf einem grundlegenden Gebiet der Zytologie, dem Studium der Spermatozoiden bei den Pteridophyten erfolgt, findet ihren Niederschlag in Veröffentlichungen, welche heute als klassisch gelten und im Fachschrifttum vielfach zitiert und anerkannt worden sind.

Ihre Forschungstätigkeit betreffend die Spermatozoiden der Pteridophyten ist wohlbekannt. Sie entfaltet sich sowohl am Mikroskop als auch im Gewächshaus (Kultur von Vorkeimen und Keimpflanzen), wobei eine sinnvolle Methodik und Technik zur Anwendung gelangt, um die Entwicklung, die Morphologie, die Zytologie und Physiologie der Spermien dieser Gruppe niederer Cormophyten aufzuklären. In diesen Arbeiten werden die Fortbewegungsorgane der Spermien (Stamm, Geißeln) eingehend analysiert, sowie gleichfalls die Kerne, das Plasma und der Plasmarest in den Spermien zahlreicher Gattungen und Arten der Farnpflanzen; die Ergebnisse dieser Untersuchungen finden sich in drei Arbeiten (1930—1932) (s. die Literatur).

Die erste Arbeit (1930) bezieht sich auf das Studium der reifen Spermien bei den *Filicales* und bei *Pilularia globulifera* (*Marsileales*). In der nächsten Arbeit (1931) werden die reifen Spermien dreier Arten der Gattung *Equisetum* (*E. telmateja*, *E. silvaticum*, *E. arvense*) analysiert, wobei das Hauptaugenmerk auf deren Aufbau und Struktur, sowie auf die wesentlichen Ähnlichkeiten bei den untersuchten Arten gerichtet ist. Die dritte Arbeit (die Dissertation, 1932) stellt — mit neuen Untersuchungen und Ergebnissen — eine Synthese der Studien über die reifen Spermien verschiedener Gattungen und Arten der Pteridophyten dar (*Selaginella*, *Equisetum*, *Isoetes* und Gattungen der *Filicinae leptosporangiateae*: *Pilularia*, *Savinia*). Auf Grund taxonomischer Richtlinien werden dichotomische Bestimmungsschlüssel aufgestellt, wobei entweder die Anzahl der Geißeln (2, 25, 25–30, 40–50) oder deren Ansatzweise (einreihig, zweireihig, dreireihig im vorderen Teil der Spermatozoiden) verwendet wird, sowie auch andere Eigentümlichkeiten, wie die Körperform (walzenförmig, bandförmig–ellipsoidisch usw.). Es wurden dabei auch bei gewissen Vertretern dieser Pflanzengruppe die Ergebnisse anderer Autoren zum Vergleich herangezogen.

Die Besprechungen ihrer Arbeiten in den Fachzeitschriften sind voll des Lobes: Berichte über die wiss. Biologie (16, 1931, S. 777; 26, 1932, S. 300; 26, 1933, S. 498); Zeitschr. f. wiss. Mikroskopie (48, S. 261); Bot. Zentralblatt (161, Neue Reihe, 19, 1931, S. 41; 163, Neue Reihe, 21, 1932, S. 45); Fortschritte der Botanik I, 47–48 (Geitler, Morph., 1932).

Die Arbeiten von M. Dracinschi werden in zahlreichen botanischen Werken und Lehrbüchern angeführt, wobei manche Ergebnisse in den Text aufgenommen und Zeichnungen wiedergegeben wurden, wie z.B. in E. Strasburger (1939), in R. Wettstein (4. Aufl., 1933, S. 394, 401, 414 u.a.). Ihre dritte Arbeit (1933) wird auch in dem Handbuch von K. Schnarf „Monographie zur vergleichenden Zytologie I, Zyt. des Geschlechtsapp. der Cormophyten“, Sammlg. Borntraeger, Berlin (1941) angeführt; auch in manchen Lehrbüchern unseres Landes fanden die Arbeiten von M. Dracinschi Aufnahme.

In der zweiten Periode ihrer wissenschaftlichen Tätigkeit (1941–1962) arbeitete sie zunächst (1940–1941) als wissenschaftliche Hilfskraft am Botanischen Institut der Martin Luther-Universität in Halle/Saale unter der Leitung von Prof. Dr. W. Troll. In den nächsten 20 Jahren wirkte sie zuerst als wiss. Hilfskraft (1941–1943) und dann als wiss. Angestellte (1943–1962) am Botanischen Institut der Versuchs- und Forschungsanstalt für Wein-Obst- und Gartenbau in Geisenheim/Rhein, wo sie sich unter der Leitung von Prof. Dr. H. Schanderl mit gewissen Problemen aus dem Aufgabenkreis der angewandten Botanik usw. mit mikrobiologischen Studien befaßte. 1962 trat sie in den Ruhestand. In diesem Zeitraum veröffentlicht sie (allein oder in Zusammenarbeit) 7 Arbeiten auf diesem Gebiet.

Aus ihrer Forschungstätigkeit an diesem Institut gingen folgende eigene Arbeiten hervor: Über die bakterizide Wirkung von Wein und Traubenmost mit *Bacterium coli* als Test (1950); Über die Bereitung von FruchtdeSSERTweinen mit und ohne Hefenährmittel (1956); Der zeitliche Verlauf der Pollendifferenzierung bei Mandel, Pfirsich und Aprikose, und der Einfluß der Knospentemperatur auf diese Vorgänge (1958); Über die Blattspur der Weinrebe (1962).

Als Mitarbeiterin von Prof. Dr. H. Schanderl erscheint sie in folgenden Arbeiten: *Brettanomyces*, eine lästige Hefegattung in flaschenvergorenen Schaumweinen (1952); Über die Aufgärung von Naturapfelweinen zu Apfeldessertweinen; Über das Vorkommen von Flagellaten in frisch gekeltertem Apfelmot und Apfelfrestern.

Die Ergebnisse dieser Studien wurden aufgenommen ins Handbuch der Kellerwirtschaft II, Hugo Schanderl: „Mikrobiologie des Mostes und Weines“ (Stuttgart, 1959).



In ihrer Lehrtätigkeit wirkte Dr. Dracinschi als Assistent (1923–1928) und als Leiter des Botanischen Praktikums (1929–1940) über die Pflanzentaxonomie; zu ihren damaligen Studenten zählte auch der Verfasser vorliegenden Artikels (1931/32 – 1932/33). Wir Studenten schätzten ihr pädagogisches Feingefühl und ihre wissenschaftlichen Fähigkeiten sehr, die sie auf unsere Unterweisung und bei der Überwachung im Labor und im Gelände verwendete. Später wurde ich ihr jüngster Kollege und bewahrte ihr gegenüber weiterhin Respekt und Anerkennung. Sie hat uns noch in späteren Jahren liebenswürdigerweise durch die Zusendung von Fachschriften ausgeholfen.

Am Institut von Geisenheim/Rhein bestand ihre Lehrtätigkeit in der Leitung des Praktikums und in Vorlesungen über systematische Botanik.

Durch ihre wissenschaftliche Arbeit und die verdienstvollen Ergebnisse ihrer Forschungstätigkeit hat sich Dr. M. Dracinschi einen hervorragenden Platz gesichert mit der mühsamen Analyse des Aufbaus der Spermien bei Pteridophyten, welche sie erfolgreich unternommen hatte.

Ihr Verhalten gegenüber Kollegen, Studenten und den Menschen ihrer Umgebung ist von Bescheidenheit und wissenschaftlichem Ernst geprägt, von Menschlichkeit und Achtung für Alles, was würdevoll und hehr ist im akademischen Geschehen und in der Gesellschaft.

Zur Feier des 80. Geburtstages und fast 40 Jahren theoretischer und angewandter botanischer Forschungstätigkeit wünschen die Generationen der Studenten und Forscher der wissenschaftlichen Anstalten an denen sie gewirkt hat, ihrer hochgeehrten Kollegin Dr. M. Dracinschi Gesundheit und ein langes Leben, bei voller Würdigung ihrer wissenschaftlichen Leistungen auf dem Gebiete der Botanik.

Traian Ștefureac

NICHIFOR CEAPOIU, *Evoluția speciilor (Die Entwicklung der Arten)*
Edit. Academiei, București, 1980, 288 S.

Das Buch des Herrn Nichifor Ceapoiu ist eine Synthese unserer Kenntnisse über den Ursprung der heute bekannten Organismen. Der Verfasser bemüht sich besonders alle Angaben in Einklang mit seinem eigenen Denken zu bringen, mit der Vervollkommenheit einiger originellen Ideen als Ergebnis seiner anerkannten Tätigkeit als Genetiker, der so manche neue systematische Einheit aus der Pflanzenwelt beschrieben hat, zu geben. An vielen Stellen gelang es ihm den Dingen eine solche Auslegung, ganz besonders von einigen Phänomenen, als Ansichten über die Evolution im allgemeinen, zu geben. Indem er eine gediegene Kenntnis für die wissenschaftliche Beweisstellung verschiedener Gesichtspunkte gebraucht, hat er verschiedene spekulative Hypothesen umgangen und hat manche fälschliche Theorien, mit gutem Ergebnis bekämpft. Es war ihm so möglich auch einige neue Ansichten zu formulieren.

Die organische Welt mit ihrer großen Vielfältigkeit hat immer im Geiste der Menschheit die Frage gestellt, wie sich diese fortwährend verändert hat und im Laufe der Zeiten entwickelt hat. Es gelang dem Verfasser auch zu erklären, wie groß die Fähigkeit der Organismen ist die schweren und stiefmütterlichen Bedingungen in ihrem Leben zu überwinden und ihnen zu überleben. Zu all diesen Fragen, auch die zahlreichen Beiträge und Untersuchungen der damaligen wissenschaftlichen Möglichkeiten berücksichtigend, hat der Verfasser versucht all diesen gestellten Ansprüchen zu antworten, indem er zu den Worten Ch. Darwin's in seiner „Entstehung der Arten“ (1859) griff, der hier, dem entsprechenden Zustande der Wissenschaften, so zur Klärung einiger schwierigen Probleme beitrug. Die Fortschritte, welche auf verschiedenen Untersuchungsgebieten gemacht wurden und welche ganz besonders in letzter Zeit gewaltig vor sich gegangen sind, haben so manche Aspekte die Evolution betreffend eine gediegene und komplexe Antwort erhalten, ohne jedoch den wirklichen Bildungsvorgang der Lebewesen auf unserer Erde zu kennen, wie deren intime Mechanismen der Entfaltung der Art-Entwicklung in der Vergangenheit, sowie auch heute, welche trotz aller Errungenschaften unserer Kenntnis, fortwährend aktuell sind.

Der Verfasser äußert die Hoffnung, daß die Fortschritte welche im Rahmen der biologischen Wissenschaften erzielt werden, zu einer Lösung der Evolutionsbiologie in solchem Maße beitragen wird, daß einige noch unklaren Aspekte allmählich geklärt werden.

In den 14 Kapiteln welche der Verfasser in seiner „Evolution der Arten“ vorbringt und für die Aufklärung der Probleme vorteilhaft sind, betont er besonders im 8. Abschnitt die Grundzüge der Genetik über die Bildung der Arten und setzt seine Äußerungen im 9. Abschnitt (vielleicht der bedeutendste) die Mechanismen der Artbildung oder Speziation fort, bei welcher Gelegenheit er auseinanderlegt was er unter „Speciation“ versteht und wie die Vielfalt der Entstehung der Organismen vor sich ging und welche die Faktoren sind die zur Erscheinung und Entwicklung der Lebewesen führten. Er mißt bei dieser Gelegenheit den Chromosomen, in deren charakterischem Zustande, eine große und wichtige Bedeutung zu, die zur Bildung der Biologie als umfangreiche Einheit beitrugen.

Desgleichen geht er auf die Art und Weise der Evolution näher ein, sich besonders auf die phyletische Evolution beziehend und auf deren Züge mit charakteristischen Eigentümlichkeiten und endet mit einem Abschnitt welcher seinen eigenen Gesichtspunkt über die darwinistische Evolution vorstellt dabei auch über den modernen Neo-Darwinismus, sowie auch über die neodarwinistische Evolution sprechend. Er gibt Erklärungen über die adaptative Strahlung in der geologischen Vergangenheit, sowie über die Gegenwärtige und über die Faktoren die sie bedingen. Er bespricht die Richtungen der Evolution und die Kräfte näher welche diese bedingen, und auch die Geschwindigkeit der Entwicklung in der Natur. Er beendet seine Äußerungen über den „Schlüssel“ der transspezifischen Evolution, übergeht aber nicht die Mikro- und Makroevolution, sowohl die intraspezifische als auch die transspezifische, die letztere und deren genetischen Grundlagen betonend. Er bespricht auch die höheren Kategorien von Organismen.

Die Arbeit im allgemeinen betrachtend, kann man sagen, daß in unserer Literatur auf diese Weise ein Gesichtspunkt des Verfassers erschienen ist, der die Mehrzahl der sich im Umlange befindenden Daten der Biologie, die ganz besonders mit der Evolution in Verbin-

dung sind berücksichtigt hat. Das Werk entspricht den Anforderungen der wissenschaftlichen Forscher der Biologie, speziell jener der Genetik, sowie auch allen denen die sich mit der Existenz und mit dem Leben der Organismen, sowie deren Entwicklung in der Vergangenheit, beschäftigen haben. Die gründliche Kenntnis des vom Verfasser Vorgelegten kann als wichtiges Ergebnis eines großen Biologen angesehen werden, der in seinem Leben diesem Thema lange Zeit gewidmet hat, bis es ihm letztthin gelungen ist eine zugängliche, bezeugende und gut dokumentierte Synthese, welche eine persönliche und originale Note trägt, zu geben.

Ion T. Tarnavski

I. ANGHEL, AURELIA BREZEANU, N. TOMA, *Ultrastructura celulei vegetale (Die Ultrastruktur der Pflanzenzelle)*, Edit. Academiei, București, 1981, 206 S.

Das von den obigen Verfassern veröffentlichte Buch ist ein bedeutender Beitrag, vorläufig einzig in der rumänischen Literatur und wenig bekannt in der des Auslandes.

In 114 Tafeln mit 176 Teilfiguren auf 206 Seiten, wobei ein sehr gutes Papier verwendet wird, wiedergibt die Arbeit besonders genaue Abbildungen aus der Pflanzenwelt und zugleich auch deren Funktion, den Chemismus und den Metabolismus der Pflanzenzelle, die aus eigenen Untersuchungen entnommen, den Mitarbeitern nicht nur Kriterien für die Selektion von Angaben boten, sondern auch die Möglichkeit gaben einige kritische Analysen zu machen. Dies wurde in den letzten Jahren möglich durch die Einführung und Verallgemeinerung der Elektronenmikroskopie in den gegenwärtigen Untersuchungen der Zellbiologie, und auch durch die Verbesserung der zytochemischen Technik und Autohistographie usw., welche die strukturellen und funktionellen Zusammensetzungen der Pflanzenzelle der Prokaryoten und Eukaryoten angaben.

Auf diese Weise wurde den Forschern eine Unmenge von Ergebnissen zur Verfügung gestellt, welche mit besonderem Erfolge verwendet wurden. Ein origineller Beitrag der Verfasser ist besonders die Methode der Kriodecapage und die negative Färbung, die die Beschreibung einiger komplexer Aspekte der Zellbiologie möglich machen. Die Neuheit dieser Arbeit besteht in der genauen Mitteilung des gegenwärtigen Standes der Forschung der Zellstruktur der Prokaryoten und Eukaryoten, als auch besonders der Pflanzenzelle betreffend.

In dieser Arbeit werden auch die Zellformationen, deren Struktur und besonders die Funktionalität weniger bekannter Tatsachen besprochen und auch gelegentlich widersprochen.

Es wurden die Glioxysomen, Peroxysomen, Lysosomen und Lomasomen besonders gewürdigt und zugleich zur besseren Kenntnis derselben in der Pflanzenzelle beigetragen. Vieles wurde „in vivo“ und „in vitro“, vorzüglich die Phloemzellen, gemacht und die verschiedenen bedeutenden Einschlüsse für eine bessere Erkenntnis der Translokation und des inter- und intrazellulären Transportes beigetragen.

In diesem Atlas, mit kurzen und vollständigen Erklärungen im Texte, ist mit gutem Ergebnis auch die Erscheinung des natürlichen Alterszustandes wiedergegeben (hauptsächlich in Gewebekulturen), welche gegenwärtig auch im Auslande untersucht wird.

Nachdem die Verfasser kurzgefaßt und besonders reich illustriert auch die Vielfältigkeit der Pflanzenzelle vorstellen, wobei unterschiedlich, submikroskopisch, über die Eigentümlichkeiten der prokaryoten und eukaryoten Zellen gesprochen wird, geht man zur Ultrastruktur und zur molekulären Organisation der biologischen Membran, zum endoplasmatischen Retikulum und zur Infrastruktur der Zelleinschlüsse über, wobei auch die Mikrokörper (betreffend deren Ursprung und Entwicklung) und das lysosomale System beschrieben werden, sowie deren physiologische Bedeutung. Weiterhin werden die Mikrotubuli, Zilien und Flagellen diskutiert, der Kern und dessen mitotischer und meiotischer Ablauf wird eingehend beschrieben, es werden auch die paramuralen Körper, und die Zellwand erwähnt, bei welcher Gelegenheit auch der Ursprung, die chemische Zusammensetzung und deren Ultrastruktur aufgezeichnet wird und schließlich die Verbindung der Zellen durch die Punktationen und selbstverständlich der Plasmodesmen erwähnt, sowie deren Infrastruktur allgemein in dem ganzen vorgebrachten Material verwirklicht.

Diese Art von Beschreibung der Struktur der Pflanzenzelle mit Hilfe der Elektronenmikroskopie ist eine moderne Betrachtung, welche sich auf die genaue Kenntnis der Pflanzenzelle mit dem bekannten Lichtmikroskope gründet und bildet selbstverständlich als Pflanzenzytologie ein Grundgebiet der Biologie und ist in Verbindung mit der Erklärung der Lebenserscheinungen

und auch dieselbe zu ermöglichen und leichter zu erschließen und zu vertiefen, sowie die Vielfalt der biochemischen und physiologischen Prozesse zu erfassen.

Die in diesem Atlas vorhandenen Abbildungen berichten uns über die Fundamente des Pflanzenreiches und gestatten uns die bekannten Kenntnisse auszubauen und andere Gebiete der Pflanzenwelt zu erklären, indem wir uns auf diese Weise mehr der gegenwärtigen Wirklichkeit nähern.

Dieses Buch mit Aufmerksamkeit prüfend haben wir die Freude festzustellen, daß der Inhalt sehr nützlich und außerdem jedem der auf dem Gebiete der Biologie und besonders der Pflanzenphysiologie arbeitet notwendig ist und weil es im allgemeinen für die Orientierung sei es von Biologen, Agronomen, Silvikultoren oder Vertreter anderer Gebiete die mit der Biologie verwandt sind besonders wichtig ist, schlagen wir all diesen eine eingehende Kenntnis der Grundelemente unserer uns umgebenden Welt, vor.

Ion T. Tarnavski

REVUE ROUMAINE DE BIOLOGIE

SÉRIE DE BIOLOGIE VÉGÉTALE

TOME 26

1981

INDEX ALPHABÉTIQUE

	N°	Page
ATANASIU L., VOICA C., POLESCU LUCIA, SPIRESCU IOANA, Les acides aminés libres et protéiniques de <i>Spirulina platensis</i> (Gom.) Geitl . . .	1	37
BAICU T., L'action des pesticides aux différents niveaux d'organisation biologique	1	69
BĂRA I. ION, FLORIA FLORIN, GRIGORESCU CONSTANTIN, Caryotype de certaines espèces de plantes. VI. Étude des chromosomes mitotiques chez <i>Papaver somniferum</i> L. . .	1	87
BÎNDIU C., MIHALCIUC V., Modifications of the hydric state in green organs and stems after crown partial destruction by snow . . .	1	11
BOLOGA S. ALEXANDRU, The influence of some chemical compounds on the planktonic primary productivity in the lake Izvoru Muntelui-Bicaz . .	2	165
DINULESCU MARILENA, Micromycetes isolated from mines . . .	1	93
FABIAN ANA, NAGY-TÓTH FR., BARNA ADRIANA, RÁCZ-BÉLTEKI ELISABETA, About the morphological and physiological characteristics of the blue-green alga <i>Phormidium viscosum</i> Lemm . . .	2	133
GHIORGHITĂ I. GOGU, TÓTH T. ECATERINA, GILLE V. ELVIRA, The effects of gamma rays on <i>Vinca minor</i> L. and <i>Atropa belladonna</i> L. . .	2	157
IORDAN MARGARETA, BREZEANU AURELIA, ROȘU ANA, The micropropagation of <i>Vitis vinifera</i> L. II. Aspects of morphogenesis in callus culture	2	141
LAZĂR-KEUL GEORGETA, SORAN V., KEUL M., POLIZU AL., Die Beeinflussung der Tagesschwankungen des Mitoseindexes im Weizenwurzelmeristem (<i>Triticum vulgare</i> L.) durch Lindan . . .	1	19
MANOLIU ALEXANDRU, MITITIUC MIHAI, RUSAN MIRCEA, The parasitic and saprophytic mycoflora on <i>Poa pratensis</i> L. in the conditions of using variable nitrogen fertilizer doses . . .	2	171
MARTON AL., BUCUR N., KEUL M., CĂRĂUȘ I., The dependence of the plankton photosynthesis and respiration on certain physico-chemical factors in several eutrophic aquatic ecosystems . . .	1	3
MITROIU-RĂDULESCU NATALIA, MOROIANU MARIANA, Pollenmorphologie und Embryogenese bei <i>Digitalis purpurea</i> L. und <i>D. thapsi</i> L. . .	2	117
MORARIU IULIU, <i>Spiraea crenata</i> L., its ecology and protection in Brașov .	1	65
PĂTRAȘCU ALEXANDRINA, Regeneration of potato plants by <i>in vitro</i> culture of stem segments . . .	2	151
PAUCĂ-COMĂNESCU MIHAELA, TĂCINĂ AURICA, The quantity of mineral substances in plants of beech and common oak forests in the southern parts of Romania . . .	1	47
RAICU P., BADEA ELENA, STOIAN VERONICA, GREGORIAN L., Variation in chromosome number of tobacco plants differentiated from haploid and diploid calluses . . .	1	83
SANDA V., POPESCU A., Contributions à l'étude de la végétation du bassin moyen de Jiu . . .	2	103

SHIBER J. G., Trace metals in <i>Tamarix pentandra</i> and <i>Malva rotundifolia</i> from Beirut	1	55
STOICOVICI LUCIA, TUCRA I., The estimation of yield components in <i>Festuca vallesiaca</i> Schleich. and <i>Poa pratensis</i> L. perennial grasses as influenced by the nutrient treatment with NPK.	1	27
ȘTIRBAN M., VINTILĂ ROZALIA, KEUL M., BERCEA V., PRICĂ D., LAZĂR-KEUL GEORGETA, Die Auswirkungen der Anwendung von Caragard, Gesatop und Gramoxon zur Unkrautvertilgung in Obstgärten auf den Kohlenhydrat- und Pigmentgehalt in Apfelbaumblättern.	1	41
TARNAVSCHI T. ION, Die Chromosomenzahlen der Arten <i>Saxifraga demissa</i> Schott et Kotschy und <i>Saxifraga mutata</i> L. aus Rumänien	1	79
UNGUREAN LIVIA, ȘERBĂNESCU-JITARIU GABRIELA, MITROIU-RĂDULESCU NATALIA, Contributions à l'organogenèse florale et la microsporogenèse chez <i>Orobancha ramosa</i> L.	1	61
VINTILĂ ROZALIA, KEUL M., POLIZU AL., The effect of lindane on the growth of wheat seedlings (<i>Triticum vulgare</i> L).	2	123

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.

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