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REVUE ROUMAINE DE BIOLOGIE
SÉRIE DE BIOLOGIE VÉGÉTALE
Calea Victoriei 125
R-79717, București, România
téléphone 650 76 80

EDITURA ACADEMIEI ROMÂNE
Calea Victoriei 125
R-79717, București, România
téléphone 650 76 80

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L'ÉTUDE DES PRAIRIES MÉSO-HYGROPHILES DE L'ALLIANCE *AGROSTION STOLONIFERAE* SOÓ (33)71 DE ROUMANIE

A. POPESCU et V. SANDA

Le travail fait l'analyse de 16 associations des prairies méso-hygrophiles largement répandues tout le long des rivières et sur les terrains où l'eau s'accumule et stagne pour une bonne période de temps pendant la saison de la végétation. La multitude des conditions de stationnement a déterminé l'installation d'une végétation diversifiée, riche en espèces composantes, groupée jusqu'à présent en nombreuses unités cénotaxonomiques. Conformément à l'étude comparative des relevés ces phytocénoses ont été groupées en 16 associations, en faisant à cette occasion de nombreuses synonymies ou de nouvelles intégrations cénotaxonomiques.

Les associations des prairies mésophiles sont bien représentées dans la végétation de notre pays, comprenand un grand nombre de cénotaxa qui appartiennent à la classe *Molinio-Arrhenatheretea* Tx. 37.

Bien que dans certains travaux, la végétation des prairies, des terrasses et des terrains plus humides fût groupée dans la classe *Molinio-Juncetea* et celle des terrains frais, avec un contenu élevé en humus, dans la classe *Arrhenatheretea*, nous adoptons la classification de R. Tüxen (1937) par larquelle toutes les associations de prairies mésophiles sont réunies dans la classe *Molinio-Arrhenatheretea*.

Les espèces de reconnaissance de la classe sont celles qui caractérisent les prairies mésophiles, à savoir : *Poa pratensis*, *Alopecurus pratensis*, *Festuca pratensis*, *Holcus lanatus*, *Poa trivialis*, *Ononis arvensis*, *Phleum pratense*, *Ranunculus acris (acer)*, *Euphrasia rostkoviana*, *Rhinanthus minor*, *Vicia cracca*.

MOLINIETALIA W. Koch 26

L'ordre groupe les prairies mésotrophes et oligo-mésotrophes des sols excessivement humides. Les espèces caractéristiques : *Angelica sylvestris Cirsium oleraceum*, *Deschampsia caespitosa*, *Equisetum palustre*, *Filipendula ulmaria*, *Juncus effusus*, *J. conglomeratus*, *Lysimachia vulgaris*, *Lathyrus palustcr*, *Linum catharticum*, *Polygala amara*, *Silaum silaus*, *Trollius europaeus*, *Thalictrum lucidum*.

Agrostion(albae) = stoloniferae Soó(33)71

Les associations de cette alliance forment des prairies méso-hygrophiles tout le long des rivières, installées sur des sols humico-gléiques. Sur ces terrains, l'eau peut stationner pendant une assez longue période et il en résulte une fréquente apparition dans les associations de l'alliance *Agrostion stoloniferae* des espèces de nuance hygrophile, caractéristiques aux alliances *Phragmition*, *Bolboschoenion*, etc.

REV. ROUM. BIOL. — BIOL. VÉGÉT., TOME 38, Nº 1, P. 3 — 13, BUCAREST, 1993

Espèces de reconnaissance: *Agrostis stolonifera*, *Melilotus altissima*, *Oenanthe silaifolia*, *Juncus atratus*, *Silene multiflora*, *Senecio erucifolius*, *Trifolium hybridum*.

1. **Agrostetum caninae** Harg. 42

Agrostis canina est une espèce caractéristique aux paturages humides qui se développent sur les sols bruns ou bruns-podzolites situés dans un état d'embourbement progressif. Dans la composition des phytocénoses d'*Agrostis canina* on trove de nombreuses espèces de paturages caractéristiques pour d'autres associations de prairies, ce qui prouve la direction de leur èvolution. Les espèces dominantes dans l'association sont celle hygrophiles et mésophiles. Sur les terrains remués, dans les phytocénoses d'*Agrostis canina* des espèces ségétales et rudérales surgissent.

Parmi les espèces les plus fréquentes dans l'association, nous mentionnons : *Deschampsia caespitosa*, *Anthoxanthum odoratum*, *Festuca pratensis*, *Alopecurus pratensis*, *Galium palustre*, *Juncus effusus*, *J. conglomeratus*, *Luzula multiflora*, *Genista tinctoria*, *Trifolium repens*, *Lotus corniculatus*, *Trifolium pratense*, *Stellaria graminea*, *Ranunculus acris*, *Holcus lanatus*.

L'association est répandue dans la région sous-carpatique, étant connue jusqu'à présent dans Ţara Oaşului, du bassin supérieur et moyen de l'Olt, la dépression de Făgăraş, les monts Căpăţînei et Piteşti jusqu'à la forêt de Trivale.

La sous-association *jasionetosum montanae* Gergely et Raţiu 80 est décrite dans Ţara Oaşului grâce à une grande présence de l'espèce *Jasione montana* dans les phytocénoses d'*Agrostis canina*.

2. **Agrostetum stoloniferae** (Ujvárosi 41) Burduja et al. 56

(Syn. : *Agrostideto-Caricetum distantis* Soó 40, *Agrostis alba-Carex distans* ass. Soó 28, *Ranunculo-Agrostietum stoloniferae* Resmeriţă 77, *Agrostidetum pluritrifolietosum* Borza 53 n.n. conf. Borza 63, *Agrostidetum albae substepposum* Borza 62, *Lolio-Agrostetum stoloniferae* Dihoru (69) 70).

Agrostidetum stoloniferae se développe sur les terrains bas, soumis aux innondation périodiques surtout au cours du printemps. Elle préfère les sols lourds, argileux, fangeux et ceux dont l'eaux fréatique est à la surface.

C'est une des plus répandues associations de prairies, trouvée au long des rivières, dans les bocages, dans le Delta du Danube et autour des bassins à l'eau continue. Les conditions écologiques lui imprime un caractère méso-hygrophile accentué qui se reflète dans sa structure floristique. Presqu'une moitié du grand nombre d'espèces qui entrent dans la composition des phytocénoses d'*Agrostis stolonifera*, sont mésohygrophytes et hygrophytes. Parmi les espèces à peu près toujours présentes dans les phytocénoses d'*Agrostis stololonifera* nous mentionnons : *Alopecurus pratensis*, *Poa pratensis*, *Medicago lupulina*, *Trifolium fragiferum*, *T. pratense*, *T. repens*, *Potentilla reptans*, *Lotus corniculatus*, *Ranunculus repens*, *Lysimachia nummularia*, *Daucus carota*, *Rorippa sylvestris*, *Gratiola officinalis* (Tableau 1).

La participation d'un grand nombre d'espèces dans l'association d'*Agrostis stolonifera* et l'abondance de certaines d'elles imprime un aspect hétérogène, ce qui a déterminé la description de certaines situations particulières sous différents noms comme associations ou sous-associations. Les sous-associations les plus représentatives chez nous sont : *agrostetosum* Soó 64, *deschampsietosum* Soó 57, *eleocharetosum* Soó 64 (Syn. : *Agrostis alba-Eleocharis palustris* Soó 53), *caricetosum vulpinae* Soó 57, *poëtosum trivialis* Soó 57 (Syn : *Agrostis alba-Poa trivialis* Ubrizsy 43, Siroki 58), *ranunculetosum acris* Nagy apud Soó 57 et *narcissetosum stellaris* Mititelu et Dorca 87.

Comme faciès sont décrites avec : *Juncus conglomeratus*, *J. inflexus*, *J. effusus* Zaharia 72 et avec *Scirpus sylvaticus* Resmeriţă 72.

3. **Poëtum pratensis** Răv., Căzăc. et Turenschi 56

(Syn. : *Trifolio-Poëtum pratensis* (Răv. et al. 56) Resmeriţă 75)
Les prairies de *Poa pratensis* sont largement répandues dans la sylvosteppe de notre pays, en occupant surtout les alluvions sabloneuses, sabloneuse-luteuses bourbeuses, en leur imprimant un caractere mésophile. Sur les terrains tourbeux, auprès de *Poa pratensis* c'est *Alopecurus pratensis* qui se développe abondamment en remplacant *Poa pratensis* lorsque l'humidité du sol devient excessive.

Les phytocénoses de *Poa pratensis* présentent une composition floristique assez riche en espèces ; parimi les plus caractéristiques nous rappelons : *Agrostis stolonifera*, *Alopecurus pratensis*, *Trifolium repens*, *Ranunculus repens*, *Daucus carota*, *Lolium perenne*, *Dactylis glomerata*, *Taraxacum officinale*, *Lotus corniculatus*, *Agropyron repens*, *Trifolium pratense*, *Medicago lupulina*. La plupart des espèces qui composent les phytocénoes de *Poa pratensis* sont bonne et très bonnes fourragères.

La présence en grande quantité d'un grand nombre d'espèces dans l'association a déterminé la description de certaines unités subordonnées à savoir : *poëtosum pratensis*, *dactyletosum glomeratae*, *agropyretosum repentis*, *lolietosum perennis* Grigore 71, facies avec *Trifolium pallidum* Mariana Cîrţu 71, *Anthoxanthum odoratum* Chifu et Ştefan 82.

4. **Agrostetum pisidicae** Buia, Păun, Safta, Pop 59

Connue jusqu'à present seulement en Oltenia, *Agrostetum pisidicae* peuple les microdépressions avec un excédent d'humidité, issu des précipitations de printemps.

Les phytocénoses d'*Agrostis pisidica*, moins riches en espèces que les associations précédentes, ont dans leur composition des plantes mésohygrophyles et mésophiles, parmi lesquelles les plus caractéristiques sont : *Agrostis pisidica*, *Pholiurus pannonicus*, *Trifolium fragiferum*, *Ranunculus lateriflorus*, *Rorippa kerneri*, *Lythrum hyssopifolium*. L'association supporte bien l'embourbement de l'eau pendant une longue période de temps, ce qui explique la présence de certaines espèces hygrophiles dans ses phytocénoses.

5. **Poëtum silvicolae (oltenicum)** Buia, Păun, Safta, Pop 59

(Syn : *Poëto (silvicolae)—Agrostetum stoloniferae* Dobrescu et Kovács 74). Occupe les terrains bas, humide tout le long de l'année, cantonneé

dans des sols légers argileux-sablonneux, ayant l'eau phréatique à petite profondeur. La composition floristique reflète le caractère mésohygrophile des phytocénoses de *Poa silvicola*, la plupart des espèces étant mésohygrophiles et hygrophiles. Les espèces les plus représentatives de l'association sont : *Poa silvicola*, *P. pratensis*, *Alopecurus pratensis*, *Festuca pratensis*, *Agrostis stolonifera*, *Trifolium repens*, *T. resupinatum*, *Carex hirta*, *Juncus effusus*, *Rorippa austriaca*, *Lysimachia nummularia*, *Potentilla reptans*, *Oenanthe silaifolia*.

C'est une association avec beaucoup d'éléments très bons fourragers ayant des productions de 10—15.000 kg à l'hectare. L'association a été signalée en Banat, Oltenia, Moldova, Dobrogea (Babadag).

La sous-association *trifolietosum dubiae* Păun (64) 66, connue en Oltenia, a été identifiée grâce à la présence en grande quantité de l'espèce *Trifolium dubium*.

6. **Medicagini (lupulinae)—Agropyretum repentis** Popescu, Sanda, Doltu 80 (Syn. : *Agropyretum repentis* Burduja et al. 56 ; Răv. et al. 56 non Felföldy 42 ; Stadiu *Agropyro-Peucedanietum latifolii* Turenschi 66 ; *Agrostio-Agropyretum repentis* Dobrescu et Kovács 74).

L'espèce caractéristique a une grande amplitude écologique, en se développant autant sur les terrains en friche que sur ceux cultivés. Dans ce dernier cas, *Agropyron repens* se développe au bout des champs labourés ou sur les surfaces moins remuées en formant des phytocénoses dans lesquelles la plupart des espèces composantes sont ségétales. L'association est fréquente dans la plaine du Danube et des grandes rivières de Crişana, Banat, Oltenia, Muntenia, Moldova, Dobrudja et du Delta du Danube.

Les espèces les plus fréquentes des phytocénoses formées par *Agropyron repens* et *Medicago lupulina* sont : *Agrostis stolonifera*, *Poa pratensis*, *Trifolium fragiferum*, *T. repens*, *Potentilla reptans*, *Alopecurus pratensis*, *Rorippa sylvestris*.

7. **Trifolietum subteranei** Buia, Păun, Maloş, Olaru 63

L'association est connue en Banat et Oltenia où elle s'installe sur les terrains alluviaux, plans, mi-humides et mi-salés. Dans la structure de l'association on distingue trois couches. La première, haute de 65 cm est formée de : *Alopecurus pratensis*, *Poa pratensis*, *P. silvicola*, *Bromus commutatus*, toutes avec une petite dominance.

La deuxième couche, d'environ 15 cm en hauteur, est représentée par *Trifolium subteraneum*. La troisième couche, d'une hauteur de 10—15 cm, est formée de : *Hordeum hystrix*, *Trifolium parviflorum*, *T. striatum*, *T. ornithopodioides*, *T. repens*, *Veronica arvensis*.

L'association s'installe selon les groupements dominants de *Poa silvicola*, *Alopecurus pratensis*, etc., à la suite de l'augmentation du pourcentage de concentration des sels. A mesure que la concentration des sels du sol augmente, *Trifolium subteraneum* est remplacée par *Puccinellia distans* et d'autres espèces hallophiles, par exemple *Petrosimonia* triandra ou même *Salicornia europaea* et *Suaeda maritima*.

8. **Agrostideto-Festucetum pratensis** Soó 49

Les phytocénoses d'*Agrostis stolonifera* et *Festuca pratensis* se développent sur les terrains moites, dont l'humidité est accrue particulièrement au printemps. Grâce à l'humidité prononcée du substrat, dans cette association surgissent beaucoup d'espèces mésohygrophiles et même hygrophiles. Les espèces les plus fréquemment rencontrées dans l'association sont : *Poa pratensis*, *Trifolium repens*, *T. pratense*, *Poa palustris*, *Holcus lanatus*, etc. L'association est signalée en Transylvanie et Maramureş.

9. **Rumiceto-Trifolietum** Dihoru, Cristurean et Andrei 73

Est signalée dans le Defilé du Danube et dans la dépression de Dubova, où *Trifolium patens* pousse abondamment. Elle apparaît en groupes compactes dans lesquels *Trifolium patens* et accompagnée de *Rumex acetosa* qui a une taille plus grande, d'environ 90 cm.

On a observé dans l'association deux variantes : 1) avec *Rumex acetosa*, cantonnée dans les endroits plus humides où se développent abondamment *Rhinanthus rumelicus* et *Trifolium resupinatum* et 2) avec *Trifolium diffusum*, *Festuca valesiaca*, *Vulpia myuros* qui occupent les terrains plus secs.

10. **Lolietum perennis** Safta 43

(Incl. As. *Lolium perenne-Festuca sulcata* Gergely et Flavia Raţiu 65). Les prairies de *Lolium perenne* végètent sur les terrains riches en substances azoteuses ou foulés par les animaux prendant le pacage. L'espèce caractéristique a des nécessités écologiques assez larges de la sorte qu'elle puisse se développer sur des tchernozioms, des sols alluvionnaires, des sols bruns ou faiblement salés.

A la suite de l'appauvrissement du substrat en azote et à l'aridité, *Lolietum perennis* se transforme en *Festucetum rupicolae* ou *Festucetum pseudovinae cynodontetosum*.

La composition floristique des phytocénoses de *Lolium perenne* est particulièrement riche, comprenant dans la plupart des espèces mésophiles et moins méso-xérophiles. Parmi les plus représentatives nous rappelons : *Agrostis stolonifera*, *Agropyron repens*, *Poa pratensis*, *Coronilla varia*, *Festuca valesiaca*, *F. rupicola*, *Lotus corniculatus*, *Medicago lupulina*, *Trifolium repens*, *Polygonum aviculare*, *Plantago lanceolata*, *Daucus carota*.

Dans les phytocénoses de *Lolium perenne* des terrains faiblement salés participent un important nombre d'espèces supportant les hallophiles et les faiblement hallophiles, tels : *Juncus gerardi*, *Trifolium fragiferum*, *Hordeum hystrix*, *Festuca pseudovina*, etc.

Dans l'association on connaît les sous-associations ; *trifolietosum fragiferi* Grigore 71, *trifolietosum repentis* Grigore 71 (Syn. : faciès avec *Trifolium repens* Mariana Cîrţu 71) et *normale* Grigore 71.

L'association est répandue dans toutes les régions du pays dans les zones de plaine et de colline.

11. Poëtum trivialis Soó 40

(Syn. : *Agrosteto-Poëtum trivialis* Soó 38, *Trifolio-Poëtum trivialis* (Soran 62) Resmeriță et al. 71.

Poa trivialis espèce soushydrophile, s'installe surtout sur les terrains excessivement humides. Ses phytocénoses sont rencontrées le long des rivières, auprès des sources et des marais permanents. La composition floristique est riche en espèces, dans la plupart mésophiles et soshydrophiles. Les espèces les plus représentatives de l'association sont : *Alopecurus pratensis*, *Festuca pratensis*, *F. arundinacea*, *Lotus corniculatus*, *Agrostis stolonifera*, *Medicago lupulina*, *Trifolium pratense*, *Plantago lanceolata*, *Taraxacum officinale*. Parimi les espèces représentatives de l'association, hygrophiles et soushygrophiles nous citons : *Galium palustre*, *Potentilla reptans*, *Ranunculus repens*, *Symphytum officinale*, *Juncus inflexus*, *Ranunculus sardous*.

L'association est répandue en Crișana. Transylvanie, Banat, Oltenia, Muntenia et Moldova.

12. Lythro-Calamagrostidetum epigei I. Po 68

Les phytocénoses de *Calamagrostis epigeios* sont formées d'un grand nombre d'espèces mésophygrophiles rencontrées sur des sols légers, ameublis, humides, quelquefois faiblement salés dans la zone de plaine et de colline. L'espèce caractéristique préfère les sols alluviaux, riches en humus. Parmi les espèces caractéristiques pour l'association nous citons : *Lythrum virgatum*, *L. salicaria*, *Euphorbia lucida*, *Symphytum officinale*, *Lycopus exaltatus*, *Stachys palustris*, *Cirsium canum*.

La présence de certaines espèces caractéristiques à l'alliance *Agrostion stoloniferae* telles : *Agrostis stolonifera*, *Poa pratensis*, etc., justifient l'intégration de l'association dans cette alliance.

L'association est répandue en Crișana, Banat, Dobrogea et Moldova.

13. Lythro (salicariae)-Juncetum effusi-inflexi Todor et al. 71

Les cénoses de l'association sont installées sur les terrains excessivement humides provenus du colmatage des bassins aquatiques. L'eau phréatique se trouve à une petite profondeur, le sol est en permanence humide, son séchage se réalisant seulement à la surface.

L'association comprend des espèces à l'aide desquelles on peut reconnaître l'alliance *Agrostion stoloniferae*, et c'est pour cette raison qu'elle a pu etre intégrée dans ce groupement. Les associations caractéristiques de *Juncus effusus* et *J. inflexus* ont été intégrées d'une maniére différente. De la sorte, St. Horvatić (1930) intègre les phytocénoses de *Juncus effusus* en tant que sous-association à ***Deschampsietum caespitosae***.

Parmi les espèces représentatives de l'association nous rappelons : *Juncus effusus*, *J. inflexus*, *Lythrum salicaria*, *Festuca pratensis*, *Gratiola officinalis*, *Poa trivialis*, *Trifolium hybridum*, *Juncus conglomeratus*. *Holcus lanatus*, *Stellaria graminea*, *Galium mollugo*, *Carex hirta*.

L'association est connue en Banat, l'étang de Crișari, entre Măcești et Pojejena, dans le district Caraș-Severin.

Tableau 1

Agrosletum stoloniferae (Újvárosi 41) Burduja et al. 56

Nombre courant	1	2	3	4	5	6	7	8
N° des relevés	32	10	14	36	9	35	—	6
Agrostion stoloniferae								
Agrostis stolonifera	V	V	V	V	V	V	V	V
Alopecurus pratensis	III	II	V	III	IV	—	II	IV
Festuca pratensis	IV	III	III	I	—	—	II	—
Juncus inflexus	III	II	III	II	IV	—	I	IV
Carex distans	I	I	—	I	I	—	II	—
Juncus compressus	II	I	—	I	I	—	—	II
Taraxacum officinale	V	V	IV	V	V	I	—	V
Achillea asplenifolia	I	I	II	III	III	—	—	—
Poa silvicola	—	—	—	V	II	—	—	—
Agropyron repens	III	III	V	IV	V	—	—	—
Alopecurus ventricosus	—	—	II	I	II	—	—	—
Cardamine pratensis	II	III	I	I	I	—	—	—
Carex hirta	II	II	III	II	II	—	—	—
Carex melanostachya	I	I	II	II	II	—	—	—
Carex vulpina	—	II	II	II	II	I	—	V
Cirsium palustre	II	I	—	I	—	—	—	—
Deschampsia caespitosa	II	I	—	I	I	—	—	—
Euphorbia virgata	—	—	I	I	I	—	—	—
Festuca arundinacea	III	II	II	I	I	—	—	—
Fritillaria meleagris	—	—	I	I	I	—	—	—
Galega officinalis	—	I	I	I	I	—	—	II
Galium rubioides	II	I	I	I	I	—	—	—
Gratiola officinalis	—	—	—	I	I	II	—	IV
Lathyrus pratensis	IV	II	II	II	II	—	—	—
Lepidium latifolium	—	—	—	I	I	—	—	—
Lotus uliginosus	II	I	—	—	—	—	—	—
Lythrum virgatum	V	II	IV	IV	III	—	—	—
Oenanthe silaifolia	—	—	—	I	II	—	—	—
Plantago altissima	II	II	I	II	I	—	—	II
Poa trivialis	IV	III	II	II	II	I	—	—
Ranunculus sardous	II	II	III	II	II	I	—	—
Rorippa sylvestris	III	III	IV	III	III	III	II	V
Rumex conferlus	—	—	—	I	I	—	—	—
Scutellaria hastifolia	II	—	II	I	I	—	—	—
Serratula wolffii	—	—	—	I	I	—	—	—
Senecio erucifolius	I	—	I	I	I	—	—	—
Trifolium fragiferum	I	—	III	I	II	—	II	V
Trifolium hybridum	IV	III	IV	II	II	—	—	—
Veronica longifolia	I	I	—	I	I	—	—	—
Molinietalia								
Caltha laeta	II	III	I	I	I	—	—	—
Carex leporina	II	II	I	I	—	—	—	—
Cirsium canum	I	I	I	I	I	—	—	—
Cirsium rivulare	II	II	I	—	—	—	—	II
Equisetum palustre	IV	IV	III	III	III	—	—	—
Inula salicina	II	II	II	II	I	—	—	—
Juncus articulatus	IV	IV	II	II	II	—	—	—
Juncus atratus	I	II	—	—	—	—	—	—
Juncus effusus	IV	V	I	II	I	II	—	II

Tableau 1 (suite

Nombre courent	1	2	3	4	5	6	7	8
Linum catharticum	IV	IV	I	I	I	—	—	—
Lychnis flos-cuculi	V	IV	II	II	II	—	—	—
Lysimachia nummularia	V	V	III	III	V	II	—	V
Lysimachia vulgaris	II	II	I	I	I	—	—	—
Mentha aquatica	II	I	I	I	I	—	—	—
Mentha arvensis	I	I	I	I	I	—	—	IV
Mentha pulegium	III	II	II	II	II	—	II	V
Odontites rubra	II	I	—	—	—	—	—	—
Ononis arvensis	II	I	I	II	II	—	—	—
Orchis laxiflora	II	II	—	I	—	—	—	—
Potentilla erecta	IV	III	—	I	—	—	—	—
Polygonum bistorta	II	I	—	—	—	—	—	—
Ranunculus acris	IV	III	IV	II	III	—	—	—
Rumex crispus	IV	II	III	III	III	—	II	III
Sanguisorba officinalis	II	II	—	I	—	—	—	—
Senecio barbaraeifolius	—	I	—	I	I	—	—	IV
Serratula tinctoria	I	I	I	I	I	—	—	—
Stellaria graminea	V	V	II	I	II	—	—	—
Symphytum officinale	V	IV	III	III	V	—	II	—
Thalictrum lucidum	III	II	I	II	II	—	—	—
Molinio-Arrhenatheretea								
Trifolium repens	V	IV	IV	III	V	III	II	V
Medicago lupulina	—	—	—	—	—	—	—	V
Lotus corniculatus	—	—	—	—	—	—	II	V
Daucus carota	—	—	—	—	—	—	II	V
Gichorium intybus	—	—	—	—	—	—	II	IV
Teucrium scordium	—	—	—	—	—	—	—	IV
Poo pratensis	—	—	—	—	—	V	—	III
Centaurea pannonica	—	—	—	—	—	—	—	II
Trifolium pratense	—	—	—	—	—	IV	—	III
Leontodon autumnalis	II	II	II	I	I	—	—	IV
Potentilla reptans	—	—	—	—	—	III	—	V
Ranunculus sceleratus	—	—	—	—	—	II	—	—
Heleocharis palustris	—	—	—	—	—	II	—	IV
Cerastium caespitosum	—	—	—	—	—	I	—	—
Prunella vulgaris	IV	III	III	II	II	I	—	V
Ranunculus stevenii	—	—	—	—	—	—	—	II
Achillea millefolium	II	II	I	I	II	—	—	IV
Agrostis tenuis	III	III	I	I	—	—	—	—
Anthoxanthum odoratum	II	III	—	—	—	—	—	—
Bellis perennis	III	III	II	II	—	—	—	—
Briza media	IV	IV	I	I	—	—	—	—
Calamagrostis epigeios	II	II	I	I	II	—	—	—
Carex pallescens	II	II	—	—	—	—	—	—
Cerastium fontanum	I	III	I	II	II	—	—	—
Centaurea jacea	III	II	II	I	II	—	—	—
Colchicum autumnale	I	II	—	—	—	—	—	—
Cynosurus cristatus	II	III	—	I	—	—	—	—
Dactylis glomerata	III	II	II	II	II	—	—	—
Euphorbia villosa	I	I	I	I	II	—	—	—
Filipendula ulmaria	II	II	—	I	—	—	—	—
Geranium pratense	II	II	—	—	—	—	—	—
Rhinanthus minor	II	II	II	I	I	—	—	—
Rorippa austriaca	I	—	I	I	II	—	—	—
Valeriana officinalis	II	II	—	I	I	—	—	—

Tableau 1 (suite)

Nombre courent	1	2	3	4	5	6	7	8
Compagnes								
Lolium perenne	—	—	—	—	—	I	II	V
Verbena officinalis	—	—	—	—	—	—	—	IV
Inula britannica	—	—	—	—	—	—	II	IV
Plantago major	—	—	—	—	I	—	—	III
Bidens tripartita	—	—	—	—	—	—	II	III
Polygonum hydropiper	—	—	—	—	—	II	II	—
Lycopus exaltatus	—	—	—	—	—	—	II	—
Aster tripolium	—	—	—	—	—	—	II	—
Juncus gerardi	—	—	—	—	II	—	—	IV
Lythrum salicaria	—	—	—	—	I	—	—	III
Plantago media	—	—	—	—	II	—	—	V
Alopecurus aequalis	—	—	—	—	II	—	—	—
Veronica anagallis-aquatica	—	—	—	—	II	—	—	—
Galium palustre	—	—	—	—	I	—	—	V
Lycopus europaeus	—	—	—	—	—	I	—	—
Carex acutiformis	—	—	—	—	—	—	—	II
Carex contigua	—	—	—	—	—	—	—	V
Alisma lanceolatum	—	—	—	—	—	—	—	II
Equisetum ramosissimum	—	—	—	—	—	—	—	II
Lotus tenuis	—	—	—	—	—	—	—	V
Polygonum lapathifolium	—	—	—	—	—	—	—	V
Polygonum amphibium f. terrestre	—	—	—	—	—	—	—	III
Lythrum hyssopifolia	—	—	—	—	—	—	—	IV
Pastinaca sativa	—	—	—	—	—	—	—	II
Stachys annua	—	—	—	—	—	—	—	II
Plantago lanceolata	—	—	—	—	—	—	—	III
Matricaria inodora	—	—	—	—	—	—	—	III

Provenance des relevés : 1 — Th. Chifu et N. Ştefan, 1982, Bassin de la rivière Suceava ; 2 — Th. Chifu et N. Ştefan, 1973, Bassin de la rivière Nemţişor ; 3 — Gh. Mihai, 1971, Bassin de la rivière Başeu ; 4 — E. Turenchi, 1964, Bassin de la rivière Birlad ; 5 — D. Mititelu, 1971, Bassin de la rivière Elan ; 6 — Popescu A. et al., 1984, Plaine de Muntenia ; 7 — Evdochia Puşcaru et al., 1963, Banat ; 8 — I. Pop, 1968, Pleines des Criş.

14. Caricetum distantis-vulpinae Todor 47

Les phytocénoses de *Carex distans* et *Carex vulpina* font le passage de l'alliance *Magnocaricon* vers l'ordre *Arrhenatheretalia*. Elles s'installent sur les terrains humides en supportant pendant quelque temps l'embourbement de l'eau à la surface du sol. Les espèces caractéristiques à l'association sont : *Carex vulpina*, *C. distans*, *Agrostis stolonifera*, *Alopecurus pratensis*, *Galium palustre*, *Eleocharis palustris*. Dans ces phytocénoses surgissent fréquemment : *Poa trivialis*, *Festuca arundinacea*, *F. pratensis*, *Lycopus europaeus*, *Poa pratensis*, *Potentilla reptans*, *Lythrum salicaria*. Quelquefois les espèces *Alopecurus pratensis*, *Poa pratensis*, *Festuca pratensis*, *Agrostis stolonifera* présentent une grande dominance en formant des faciès. La grande présence de ces espèces indique la direction d'évolution des phytocénoses édifiées par *Carex distans* et *Carex vulpina*. L'association est connue en Transylvanie.

15. **Alopecuretum ventricosi** Turenschi 66

Se développent sur les sols fangeux faiblement salés en condition d'humidité accrue. Dans la composition floristique entrent des espèces hygrophiles, mésophiles et moins xéromésophiles. Auprès de l'espèce dominante, *Alopecurus ventricosus*, *Agropyron repens* participe dans l'association en tant que taxon caractéristique. Les espèces caractéristiques de l'alliance d'*Agrostion stoloniferae* rencontrées dans l'association sont : *Alopecurus pratensis*, *Agrostis stolonifera*, *Ranunculus repens*, *Potentilla reptans*, ainsi que des espèces hydrophiles comme *Glyceria maxima*, *Ranunculus sceleratus*, etc.

La direction d'évolution et l'association d'*Alopecuretum ventricosi* est semblable à celle d'*Alopecuretum pratensis* vers l'installation des phytocénoses mésophiles *(Agrostetum stoloniferae, Poëtum pratensis)* mais plus lente à cause de l'humidité en exces. L'association est connue en Moldova.

16. **Ranunculeto (strigulosi)-Equisetetum palustris** Gh. Popescu (74)

75, 79. Peuple les terrains excessivement humides surtout au cours du printemps lorsque l'eau peut atteindre 10—15 cm en profondeur, mais qui sèche en été. Les cénoses de l'association se trouvent dans l'aréal des prairies de la classe *Molinio-Juncetea* avec lesquelles s'interpénètrent. Le fond des espèces communes atteste l'appartenance à cette alliance.

Les deux espèces caractéristiques : *Ranunculus strigulosus* et *Equisetum palustre* ont une écologie ressemblante quoiqu'elles se trouvent aussi dans d'autres formation végétables herbeuses dans les endroits humides.

Les espèces les plus représentatives dans le cadre phytocénoses de cette association sont : *Trifolium pratense*, *T. repens*, *Lotus corniculatus*, *Carex distans*, *Equisetum arvense*, *Symphytum officinale*, *Lythrum salicaria*, *Eleocharis palustris*, *Juncus inflexus*, *Galium palustre*.

L'association est connue en Oltenia (Folești-Păușești, Otăsău).

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COENOTAXONOMY AND STRUCTURE OF PHYTOCOENOSES OF THE *CHENOPODIETEA* CLASS IN THE VEGETATION OF ROMANIA

V. SANDA, A. POPESCU

Several aspects are analyzed under multiple aspects (structure, dynamics, evolution, geographic distribution). A number of 26 associations of the *Chenopodietea* Br.-Bl. emend. Lohm., J. Tx. et Tx. 61 class framed in the orders *Polygono-Chenopodietalia* (Tx. et Lohm. 50) J. Tx. 61 and *Onopordetalia* Br.-Bl. et Tx. 43 emend. Görs 66. The *Sisymbrietalia* J. Tx. 61 order represented the object of a special study.

The *Chenopodietea* Br.-Bl.51 emend Lohm., J. Tx. et Tx. 61 class is grouping together the vegetation characteristic to waste lands on vacant grounds and road borders with soil rich in disintegrating organic matters. The species that make up the vegetation of this class are nitrophilous in their great majority, therophytes and thero-hemicryptophytes with adjustments that allow them to extend quickly on the lands left unworked.

The species characteristic to the class are : *Amaranthus albus*, *A. retroflexus*, *Chenopodium album*, *Ch. opulifolium*, *Ch. polyspermum*, *Cardaria draba*, *Capsella bursa-pastoris*, *Datura stramonium*, *Echinochloa crus-galli*, *Rumex conglomeratus*, *Sonchus oleraceus*, *Solanum nigrum*, *Stellaria media*, *Senecio vulgaris*, *Verbena officinalis*, *Xanthium spinosum*, *X. strumarium*, etc.

POLYGONO – CHENOPODIETALIA (Tx. et Lohm 50) J. Tx. 61

Gathers the associations in the cultures of maize, beet, vegetables and potatoes. The recognition species are : *Amaranthus lividus*, *Chenopodium hybridum*, *Ch. polyspermum*, *Euphorbia peplus*, *Fumaria officinalis*, *Geranium columbinum*, *Lamium amplexicaule*, *Veronica opaca*.

Veronico-Euphorbion Siss. 42

It characterizes the fallow ground vegetation and the cultivated lands but maintained in a bad condition. Recognition species : *Fumaria officinalis*, *Sonchus asper*, *Veronica agrestis*, *Euphorbia helioscopia*, *Chenopodium album*.

1. **Galeopsidetum speciosae** Krusem. et Vlieg. 39

It installs into the weeder out unirrigated cultures and especially in those of potatoe northern the country (Suceava district). The annual species growing in cornfields where *Galeopsis speciosa* and *Galeopsis tetrahit* play an important part, are comprised in the phytocoenotic species. Beside them, *Stellaria media*, *Galinsoga parviflora*, *Capsella bursa-pastoris*, *Senecio vulgaris*, *Sonchus asper*, *S. arvensis*, *Plantago major*, *Potentilla reptans*, etc., are also developing.

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The *Galeopsis speciosa* phytocoenoses develop with individuals extremely abundant that constitute a danger dor the cultures in the premontane areas. Spreading the Tarcău and Bistriţa Aurie basins.

2. **Lamio-Veronicetum politae** (Prodan 39) Kornaš 50

(Syn. : *Veroniceto-Lamietum banaticum* St. Grigore 68 subass. *lamietosum purpurei* Grigore 68 et subass. *lamietosum amplexicauli* Grigore 71).

The association appears frequently during spring time, in gardens, vineyards, cultures not well maintained and fallow grounds. În the flower composition, a great number of species enter, ruderal ones and those growing in cornfields mostly annual and biennial. Recognition species : *Lamium amplexicaule, L. purpureum, Veronica persica, V. hederifolia, V. polita V. opaca.* Along these, *Polygonum persicaria, Thlaspi arvense, Senecio vulgaris, Stellaria media, Sinapis arvensis, Capsella bursa-pastoris, Cardaria draba, Sonchus oleraceus, Lamium purpureum* are almost never missing.

Within the association, the facies with *Stellaria media* Burduja et Florița Diaconescu 76 is described.

D. Mititelu and collaborators (1968) mention the association *Setario-Veronicetum (politae)* Oberd. 57 (Syn. : *Panico-Mercurialietum* Tx. 50 p.p.) in the vegetation found in the surroundings of Bacău but in the more recent works referring to this territory, they do no longer cite it. It must be the *Lamio-Veronicetum politae* coenotaxon again.

Polygono-Chenopodion polyspermi W. Koch 26 ; Siss, 46

Comprises the pioneer associations on the lands cultivated, but badly maintained or on those left unworked for a year. Recognition species : ***Digitaria sanguinalis, Polygonum persicaria, Thlaspi arvense, Diplotaxis muralis, Euphorbia helioscopia, Sonchus asper, Senecio vernalis, Galinsoga parviflora.***

subal. Eu-Polygono-Chenopodion Siss. 46

3. **Galinsogo-Euphorbietum pepli** Mititelu 72

It develops on soils rather humid and shady rich in azotates and loose structure and neutral up to hardly acid reaction. It is to be met frequently in tree orchards, yards, around the buildings on shady layer. The two characteristic species *Galinsoga parviflora* and *Euphorbia peplus* develop well and fructify abundantly especially among the flower layers neglected and in the vegetable gardens.

In the flower composition of the association there frequently appear : *Veronica polita, Polygonum persicaria, Chenopodium hybridum, Euphorbia helioscopia, Sonchus arvensis, Lamium amplexicaule, L. purpureum, Chenopodium polyspermum, Stellaria media, Senecio vulgaris, Solanum nigrum, Artemisia annua, Polygonum aviculare,* etc.

The association resembles to *Oxalido-Euphorbietum pepli* Oberd. 49 described in Germany and has an ecology very much like the one we have here, known only in Moldavia up to the present.

4. **Soncho (arvensis)-Erigerontetum canadensis** Mititelu 71, Florița Diaconescu 78.

The phytocoenoses of *Sonchus arvensis* and *Erigeron canadensis* develop on fallow grounds, orchards, terraces and on unirrigated cultures, on clayey, damp soils. Owing to the vigorous characteristic species, they can have a great extent and stop the installment of other taxons.

During spring time within the association there can be distinguished a smaller layer made out of *Veronica polita, V. agrestis, Cardaria draba, Diplotaxis muralis, Lamium amplexicaule, Euphorbia helioscopia, Senecio vernalis, Sinapis arvensis.*

The association is signaled out in Moldovia only, but the two edifying species are extremely spread in all parts of the country which assumes a large extent of this coenotaxon.

5. **Chenopodio (glaucae)-Amaranthetum lividi** Dihoru 75

It vegetates near to the garbage piles, along the fences proving the nitrophily of the edifying species. Between the two characteristic species *Amaranthus lividus* is more devoted to the biotope, *Chenopodium glaucum* being able to appear in places poorer in azotates. Species more frequently met in the phytocoenoses of *Chenopodium glaucum* and *Amaranthus lividus* are : *Amaranthus hybridus, Galinsoga parviflora, Bidens tripartita, Rumex conglomeratus, Polygonum lapathifolium, P. hydropiper, Artemisia absinthium, Chenopodium album,* etc.

The association resembles *Chenopodietum glauci* (Wenzl 34) Raabe 50, but this last one lacks *Amaranthus lividus.* The association is known only from Siriu.

6. **Fumarietum officinalis** (Krusem. et Vlieg. 39) Tx. 50

(Syn. : *Veronico-Fumarietum* Tx. 55).

It develops in gardens and cultures kept in bad conditions on fields left unworked but with a spongy soil rich in azotates. It is a vernal association with lots of annual species, growing in corn-fields and ruderal ones. Recognition species : *Fumaria officinalis, Veronica polita, V. persica, Sonchus asper, Thlaspi arvense, Lamium purpureum, Euphorbia helioscopia, Polygonum persicaria, Sonchus arvensis, Chenopodium polyspermum, Senecio vernalis, Lamium amplexicaule, Stellaria media.* The edifying species is rather spread all over the country but the association is mentioned only in Wallachia (surroundings of Bucharest).

7. **Soncho-Veronicetum agrestis** Br.-Bl. 49

It is signaled out by Al. Borza (1959) in Sebeș Valley and near to Secaș. Together with the edifying species *Sonchus arvensis* and *Veronica agrestis* also vegetate : *Cirsium arvense, Convolvulus arvensis, Veronica polita, Euphorbia helioscopia, Setaria viridis, S. pumila, S. verticillata.* The lack of the species *Stachys palustris* within the phytocoenoses in the Sebeș Valley, determined Al. Borza to describe the subassociation *secasense* Borza 56, 65. This association has been signaled out before, in Turda and in the surroundings of Bucharest by Al. Borza, too.

8. **Galeopsido tetrahit-Stellarietum mediae** Pass. 75

The association has been identified in a culture of potatoes in Petreni, Panaci locality (D. Mititelu et al. 1987) having as species characteristic for the association : *Stellaria media, Galeopsis tetrahit, Sonchus oleraceus* and *Cirsium arvense* and among the companions most significant for the alliance and order are : *Chenopodium polyspermum, Ch. album, Lamium purpureum, Veronica persica, Setaria viridis, Armoracia rusticana, Euphorbia helioscopia.*

subal. Digitario (Panico)-Setarion Siss. 46 corr. hoc loco Groups together the associations in alliance *Polygono-Chenopodion* on the positive shape of relief.

9. **Digitario (sanguinalis)-Galinsogetum** Beck 41

(Syn. : *Setario-Galinsogetum* Tx.50, *Echinochloo-Galinsogetum parviflorae* Fl. Diaconescu 78, *Digitarietum ischaemi* Tx. et Prsg. (42)50).

The association develops on spongy grounds in cultures of weedings maintained in bad conditions. The characteristic species *Galinsoga parviflora, Digitaria sanguinalis* and *Setaria viridis* are annual, mesophyll plants growing in cornfields.

Within the structure of the association, there more frequently appear : *Senecio vernalis, Polygonum persicaria, Lamium amplexicaule, Chenopodium polyspermum, Veronica agrestis, Sonchus asper, Lamium purpureum, Chenopodium album, Stellaria media, Senecio vulgaris, Capsella bursa-pastoris, Convolvulus arvensis.* It is an association largely spread especially in the southern and eastern parts of Romania.

10. **Echinochloo-Veronicetum persicae** Pass. 75

It vegetates in Frătăuții Vechi (Suceava district) in the cultures of two-row barely (D. Mititelu et al. 1987) where the two characteristic species *Echinochloa crus-galli* and *Veronica persica* are accompanied more frequently by : *Polygonum lapathifolium, Stellaria media, Armoracia rusticana, Chenopodium album, Galinsoga parviflora, Setaria lutescens,* etc.

11. **Lapsano-Veronicetum persicae** Pass. 75

Like the previous one, it has been met in the cultures of wheat, sugar beet and turnip-rooted cabbage where *Lapsana communis* and *Veronica persica* are frequently accompanied by the common species, characteristic for the alliance and order.

12. **Abutilo-Solanetum nigri** Mititelu et Barabaș 87

It is described from Berezeni (Vaslui district) where the two characteristic species *Abutilon theophrasti* and *Solanum nigrum* vegetate in cultures of soya beans, potatoes, onions, and tomatoes together with : *Amaranthus retroflexus, Echinochloa crus-galli, Setaria viridis, S. glauca, Chenopodium album, Ch. hybridum,* etc.

ONOPORDETALIA Br.-Bl. et Tx. 43 emend. Görs 66

It comprises the weeds of rather tall size, usually species with thermophil nuance. These phytocoenoses develop towards those of steppe type of the class *Festuco-Brometea* and sometimes towards series of fruticant types of the orders *Prunetalia* or *Orno-Cotinetalia.*

The main species of recognition of the order are : *Anchusa officinalis A. procera, Anthemis austriaca, Berteroa incana, Centaurea diffusa, C' solstitialis, Cirsium boujarti, Cynoglossum officinale, Dipsacus fullonum, Hordelymus asper, Lappula squarrosa, Lavathera thuringiaca, Marrubium vulgare, Melilotus officinalis, Reseda lutea, R. luteola, Rumex patientia, Verbascum thapsiforme.*

Onopordion acanthii Br.-Bl. 26 s.str.

The phytocoenoses of this alliance are edified by weeds of tall size that vegetate on dunged grounds and well sun-lit.

Characteristic species : *Carduus acanthoides, C. nutans, Centaurea calcitrapa, C. iberica, Cirsium furiens, C. lanceolatum, C. serrulatum, Echium vulgare, Melilotus alba, Nicandra physaloides, Onopordon acanthium, O. tauricum, Xanthium spinosum, X. strumarium, Althaea cannabina, Cirsium vulgare, Cynoglossum officinale, Hyoscyamus niger, Torilis arvensis, Geranium molle, Centaurea solstitialis.*

13. **Cynoglosso (hungarici)-Carduetum candicantis** Roman 74

It installs in the glades of the forests where the phytocoenoses of the *Festucion rupicolae* alliance that were intensely grazed, develop towards a strong weeded surface closing with *Carduus candicans* and *Cynoglossum hungaricum.* The degree of these phytocoenoses is determined by the intensity of the grazing and by the period of animal stopping. After 3—6 years, these phytocoenoses pass gradually towards steppe groupings *(Festuco-Brometea)* or into fruticant types *(Prunetalia, Orno-Cotinetalia).*

Excepting the two characteristic and dominant species of the *Cynoglossum hungaricum* and *Carduus candicans* association that constitute this coenotaxon, *Anthemis tinctoria* and *Erysimum cuspidatum* are also noted.

14. **Cirsietum arvensi-lanceolati** Mititelu 72

It forms dense phytocoenoses on alluvial soils or alluvial deposits installing on the unfallowed and alluvialled grazes completely degraded because of the early grazing. It can also be met on wet soils where overflowing waters have stagnated. Among the constant species of the association mention must be made on : *Amaranthus retroflexus, Urtica dioica, Atriplex tatarica, Chenopodium album, Capsella bursa-pastoris, Echinochloa crus-galli, Crepis setosa, Erigeron canadensis,* etc.

15. As. **Xanthium spinosum-X. strumarium** Paucă 41

The recognition species of the association *Xanthium spinosum* and *Xanthium strumarium* vegetate together with : *Malva neglecta, Rumex pulcher, Carduus nutans, Verbena officinalis, Urtica dioica, Sambucus ebulus* that

sometimes form a facies. It is a ruderal association much influenced by grazing and the sinking by the animals.

16. **Argusio-Petasitetum spuriae** (Borza 31 n.n.) Dihoru et Negrean 76

The nitrophilous association described in Sulina is in a continuous expansion in the Danube Delta. In Sfîntu Gheorghe, *Petasites spurius* forms compact phytocoenoses on lands lacking crops near the lodgings, vegetating together with a series of nitrophilous species as : *Linaria vulgaris*, *Tanacetum vulgare*, *Artemisia vulgaris*, *Rumex dentatus* ssp. *halácsyi*, etc. At Ciotic it interposes among the phytocoenoses of *Carici (distantis)—Festucetum arundinaceae* that extend on the lower places, the microdepressions with more accentuated humidity and sand-loving vegetation represented by *Aperetum maritimae* and *Secaletum silvestre*. At the end of summer *Petasites spurius* transmit the characteristics of the sands surrounding the fishing ground, as the pastures made by *Apera maritima* are grazed and those made by *Secale silvestre* are distroyed especially by animals that trample on. The lack of the second species of diagnosing the association *Arguzia sibirica* in the phytocoenoses studied by us in Ciotic and Sfîntu Gheorghe, that install especially in microdepressions where excess of humidity is accumulating, justifies us to separate a new subassociation — *petasitetosum spuriae* — with the following characteristic species : *Petasites spurius*, *Gypsophila perfoliata*, *Apera maritima*, *Plantago indica*, *Secale silvestre*. The *Petasites spurius* phytocenoses from Ciotic represent a sand-loving variant of this association.

17. **Onopordetum tauricі** (Borza 31 n.n.) Morariu 57

The association installs around or on the pastoral establishments or sometimes at the brink of some cultures. The main edifier of the association is *Onopordon tauricum*, species of Taur-Balkan origin to which are added a group of differential Pontic-Balkans-Submediterranean species as : *Carthamus lanatus*, *Phleum paniculatum*, *Daucus guttatus* ssp. *zahariadi*, *Tribulus terrestris*, *Galium humifusum*, *Cephalaria transsilvanica*. The association is formed by a group of ruderal species often accompanied by characteristics of the *Festuco-Brometea* class as : *Salvia nemorosa*, ssp. *tesquicola*, *Artemisia austriaca*, *Achillea setacea*, *Agropyron cristatum* ssp. *pectinatum*.

18. **Onopordetum acanthii** Br.-Bl. (23)36

(Syn. : *Carduo-Onopordetum* Soó 47)

It develops on the grounds with disintegrating organic substances, on the place of old sheepfolds, being a typically nitrophilous association. Large phytocoenoses are met in the field, along the roads, the railways, on the grounds used as graze for sheep and especially on places where animals have stopped. The number of individuals of the edifying species is very large, hindering the development of other accompanying species ; that is why the *Onopordon acanthium* phytocenoses are characterized by annual, vernal species that end their vegetative cycle before being covered by the dominant species. The most frequent companies of the association are : *Carduus acanthoides*, *Hordeum murinum*, *Matricaria chamomilla*, *Lolium perenne*, *Polygonum aviculare*, etc. (Table 1) ruderal

Table 1

Onopordetum acanthii Br.-Bl. (23)36

Number of row	1	2	3	4	5	6	7
Number of survey	—	—	14	4	1	1	1
Onopordion+Onopordetum							
Onopordon acanthium	V	V	V	V	3—4	3	1
Carduus acanthoides	III	III	III	—	+	1	—
Hyoscyamus niger	—	—	I	II	—	—	+
Artemisia absinthium	—	—	—	—	+	+	—
Berteroa incana	V	III	—	—	—	—	—
Anchusa officinalis	II	—	—	III	—	—	—
Cynoglossum officinale	II	IV	—	—	—	—	+
Marrubium vulgare	III	V	—	—	—	—	—
Reseda lutea	IV	IV	—	—	—	—	—
Echium vulgare	III	V	I	—	—	+	—
Arction							
Ballota nigra	—	—	I	I	—	1	—
Conium maculatum	—	—	II	III	—	—	+
Arctium lappa	—	—	—	IV	—	—	+
Sisymbrion+Sisymbrietalia							
Malva sylvestris	—	—	I	—	+	—	+
Hordeum murinum	—	—	III	III	—	—	—
Descurainia sophia	—	—	III	IV	—	—	—
Bromus sterilis	—	—	I	—	—	+	—
Lappula echinata	V	—	II	—	—	—	—
Sisymbrium loeselii	—	—	II	I	—	2	—
Malva neglecta	—	—	I	I	+	+	+
Erigeron canadensis	II	II	I	—	—	—	—
Bromus tectorum	—	—	I	III	—	+	—
Lepidium ruderale	—	—	I	—	—	2	—
Sisymbrium officinale	—	—	—	I	+	—	—
Chenopodietea							
Anagallis arvensis	—	—	I	—	+	—	—
Atriplex patula	—	—	I	—	+	—	—
Matricaria chamomilla	I	—	IV	III	—	+	—
Capsella bursa-pastoris	III	—	IV	IV	+	—	—
Agropyron repens	—	—	II	II	—	+	—
Geranium pusillum	I	—	I	—	+	+	+
Atriplex tatarica	—	—	III	—	—	+	—
Cirsium lanceolatum	I	—	I	—	—	—	—
Stellaria media	—	—	I	III	+	—	—

I : *Anthemis arvensis* (3), *Verbascum densiflorum* (2), *Melilotus alba* (2).
II : *Torilis arvensis* (3), *Caduus nutans* (3), *Melilotus officinalis* (2), *Rumex patientia* (2), *Anchusa ochroleuca* (2).
V : Crepis foetida (2).

II : *Verbena officinalis* (1), *Artemisia vulgaris* (2).
IV : *Arctium tomentosum* (4)

II : *Cardaria draba* (3)
III : *Lappula squarrosa* (4)

Table 1 (suite)

Number of row	1	2	3	4	5	6	7
Medicago lupulina	–	–	II	+	–	+	–
Convolvulus arvensis	I	–	III	–	–	+	–
Chenopodium album	II	IV	II	III	–	+	+
Amaranthus retroflexus	II	–	II	–	–	–	–
Xanthium spinosum	–	–	III	II	–	–	+
Solanum nigrum	I	–	I	–	+	–	–
Xanthium strumarium	I	II	–	–	–	–	–
Lactuca serriola	–	–	I	–	–	+	+
Urtica dioica	III	V	–	IV	+	–	–
Crepis setosa	II	–	I	–	–	–	–

I: *Erodium cicutarium* (3), *Bromus hordeaceus* (3), *Taraxacum officinale* (3), *Thlaspi arvense* (3), *Echinochloa crus-galli* (3), *Kochia scoparia* (3), *Digitaria sanguinalis* (3), *Bromus japonicus* (3).

II: *Bromus arvensis* (3), *Matricaria inodora* (3), *Cynodon dactylon* (3), *Sonchus arvensis* (3), *Urtica urens* (4).

Accompanying species

Euphorbia cyparissias	–	–	–	–	–	+	+
Ranunculus repens	–	–	–	–	+	+	–
Lolium perenne	–	–	IV	–	+	–	–
Polygonum aviculare	–	–	III	IV	+	1	+
Poa annua	–	–	I	–	–	+	–
Trifolium repens	–	–	–	–	+	+	–
Poa pratensis	–	–	I	I	–	–	–
Plantago major	–	–	I	–	–	+	–
Rorippa sylvestris	–	–	I	–	+	+	–

I: *Ranunculus sardous* (3), *Cirsium arvense* (3).

II: *Papaver dubium* (3), *Veronica persica* (3), *Bromus commutatus* (3), *Cichorium intybus* (3), *Vicia grandiflora* var. *sordida* (3).

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1 survey: *Amaranthus hybridus* (3), *A. retroflexus* (4), *A. albus* (3), *Achillea neilreichii* (3), *A. millefolium* (5), *Arenaria serpyllifolia* (3), *Artemisia annua* (6), *A. absinthium* (5), *Aegilops cylindrica* (3), *Bromus commutatus* (3), *Bilderdykia convolvulus* (6), *Barbarea vulgaris* (5), *Cannabis sativa* (3), *Camelina microcarpa* (3), *Carduus hamulosus* (3), *Chenopodium bonus-henricus* (7), *Ch. polyspermum* (5), *Cerastium fontanum triviale* (5), *Daucus carota* (6), *Delphinium consolida* (3), *Dactylis glomerata* (5), *Erigeron canadensis* (6), *Euphorbia falcata* (3), *E. helioscopia* (3), *Geranium rotundifolium* (4), *Galeopsis angustifolia* (3), *Lotus corniculatus* (5), *Lactuca saligna* (6), *Lamium purpureum* (3), *Lithospermum arvense* (7), *Lepidium campestre* (4), *L. draba* (6), *Lamium amplexicaule* (3), *Mentha longifolia* (5), *Medicago falcata* (6), *Malva pusilla* (7), *Matricaria inodora* (6), *Polygonum persicaria* (3), *P. mite* (6), *Poa trivialis* (3), *Pharbitis purpurea* (3), *Pimpinella saxifraga* (7), *Potentilla supina* (3), *Plantago altissima* (6), *P. lanceolata* (5), *Ranunculus sardous* (3), *Rumex confertus* (3), *R. conglomeratus* (5), *R. crispus* (6), *Rorippa austriaca* (3), *Salvia nemorosa* (4), *Sonchus asper* (7), *S. oleraceus* (5), *Setaria glauca* (3), *Sambucus ebulus* (3), *Sclerochloa dura* (6), *Setaria viridis* (3), *Trifolium resupinatum* (3), *T. hybridum* (5), *T. fragiferum* (6), *Torilis anthriscus* (7), *Veronica arvensis* (3). *V. persica* (5), *Vicia striata* (3), *V. pannonica* (3), *V. sepium* (3), *V. hirsuta* (3), *Verbena officinalis* (6).

Place of surveys: 1 — D. Mititelu, N. Barabaș, 1975, Lunca Prutului; 2 — D. Mititelu et al. 1978, Roman; 3 — Popescu et al. 1984, Muntenia; 4 — Al. Borza, 1959, Valea Sebeșului; 5 — Gh. Dihoru, 1975, Siriu; 6 — I. Pop, 1969, Someșul Mare-Cluj; 7 — A. Paucă, 1941, Codru-Muma.

species, characteristic of the lands rich in azotate substances of organic nature. Within the association, the subassociations *(carduetosum acanthoidis* (All. 22) Soó 64 (Syn.: *Carduetum acanthoidis* Morariu 39), *centauretosum calcitrapae* Kárpáti I. apud Soó 61 and *xanthietosum* Soó 61, are described.

19. **Carduetum nutantis** Săvulescu 27, Paucă 41, Morariu 43

Being a thermophil species *Carduus nutans* grows especially in the south and east of the country, where it can constitute rather extended phytocenoses. It prefers spongy grounds, relatively rich in azotate substances as those remained without cultures, fallow grounds 2 or 3 years old, as well as those along the roads and earthworks on railways, lands that are used as graze for sheep. Within the phytocoenoses, there are numerous ruderal, annual species to be met such as: *Hordeum murinum*, *Lactuca serriola*, *Erigeron canadensis*, *Capsella bursa-pastoris*, but also species of annual or perennial grazings among which we may cite: *Poa pratensis*, *Trifolium pratense*, *Lolium perenne*, *Medicago lupulina*, that indicate the evolution of these phytocoenoses towards the installing of pasture vegetation.

20. **Carduetum hamulosi** Florița Diaconescu 78

There is a grouping where the participating species occupy the superground space in three distinct strata: a. *Cynodon dactylon*, *Lotus corniculatus*, *Medicago lupulina*, *Plantago major*, *Taraxacum officinale*; b. *Cynoglossum officinale*, *Euphorbia esula*, *Falcaria vulgaris*, *Reseda lutea*, *Trifolium pratense*, *Matricaria perforata*; c. *Artemisia absinthium*, *Carduus hamulosus*, *Cirsium arvense*, *C. vulgare*, *Daucus carota*, *Erigeron canadensis*.

The evolution of phytocoenoses along 4 years had the same direction with those of *Echio-Melilotetum* resulting their elimination by partial following with *Cynodon dactylon* and thus the association becomes rather ephemeral.

21. **Centaureetum calcitrapo-ibericae** Dobrescu et Kovács 72

(Syn.: *Centaureetum calcitrapae* Mititelu 70, *Onopordetum acanthii* Br.-Bl. 36 subass. *centauretosum calcitrapae* Kárpáti I. apud Soó 61 p.p.).

The devoted species of the association are *Centaurea calcitrapa* and *C. iberica* that colonize the alluvial deposits recently rich in nutritive substances. In the *Onopordion* alliance *Cardus acanthoides*, *C. nutans*, *Cirsium vulgare*, *Lapulla squarrosa*, *Onopordon acanthium*, take part in and from *Onopordetalia* order, the species *Bunias orientalis*, *Lamium purpureum*, *Marrubium vulgare*, *Urtica dioica*, *Verbascum thapsus*. Within the association, species in the classes *Plantaginetea*, *Chenopodietea* and *Festuco-Brometea* also participate.

Within the association, the subassociation *centauretosum ibericae* Dobrescu et Kovács 72 is described.

22. **Artemisietum scopariae** Borza et Lupșa 63

The phytocoenoses of this association described by Al. Borza and Viorica Lupșa on the walls of Alba Iulia fortress, vegetate on the slopes of the

terraces with deep erosions with a loessoid substratum at the surface from Cochirleni and Salygni (C. Burduja and Cl. Horeanu, 1976). In the Mehedinţi plateau (N. Roman, 1974) it installs in the abandoned yards. *Artemisia scoparia* vegetates together with *Erigeron canadensis*, *Amaranthus crispus*, *Cynoglossum officinale*, *Malva sylvestris*, *Verbena officinalis*, *Chenopodium album*, *Marrubium praecox*, *Xanthium spinosum*, *Ballota nigra*, *Polygonum aviculare*, *Verbascum thapsus*, *Achillea crithmifolia*, *Artemisia absinthium*, *Arctium lappa*. The preponderance of the therophytes as well as the Eurasiatic elements render evident the character of pioneer's work of these phytocoenoses.

Within the association, the subassociation *chenopodietosum botrys* Coste 75 is signaled and so is the regional variant *banaticum* Coste 75.

Brachyaction ciliatae I. Pop et Gh. Vițalariu 71

It groups the ruderal phytocoenoses in connection with soils slightly salined. Recognition species : *Brachyactis ciliata*, *Artemisia annua*, *Ambrosia artemisiifolia*, *Amaranthus albus*, *A. blitoides*, *Galinsoga parviflora*, *Xanthium strumarium*, *Iva xanthiifolia*, *Lepidium virginicum*.

23. **Erigero (canadensis)-Brachyactetum ciliatae** I. Pop et Gh. Vițalariu 71.

The characteristic species both for the association *(Brachyactes ciliata* and *Erigeron canadensis)* and the alliance are in their great majority adventitious plants. On the Bahlui Plain, where it was described, the association contains a suite of halophilous differential plants such as : *Aster tripolium*, *Crypsis schoenoides*, *Hordeum hystrix*, *Juncus gerardi*, *Lotus tenuis*, *Puccinellia distans*, *Trifolium fragiferum*, that mirror the soil of salined hole type with the phreatic water almost at the surface. The association vegetates on moderate salines, clayey soils, sandy, alluvial, at the border of rivers entering the structure of the waterside or swamp vegetation. In its evolution the association is replaced by *Lolio-Plantaginetum majoris*.

24. **Ambrosietum artemisiifoliae** Vițalariu 73

It forms phytocoenoses both on the sides of the railway in the Socola-Iași station as well as on the waste lands at the end of the platform of unloading. The association presents ecologically resemblances to the *Erigero (canadensis)-Brachyactetum ciliatae*.

The characteristic species *Ambrosia artemisiifolia* is accompanied by o few adventitious species of North-American origin as : *Amaranthus albus*, *Erigeron canadensis*, *Iva xanthifolia*, *Galinsoga parviflora*, *Xanthium strumarium*, *Amaranthus blitoides*, *Brachyactis ciliata Artemisia annua*, accompanied by some halophilous ones that mirror the nature of the soil of hole type slightly salined, such as : *Atriplex tatarica*, *Puccinellia distans*, *Trifolium fragiferum*.

In the analysis of the flower composition, the fact that this association develops towards *Lolio-Plantaginetum majoris*, may be intuited.

Dauco-Melition Görs 66

Recognition species : *Daucus carota*, *Melilotus officinalis*, *M. alba*, *Echium vulgare*, *Anchusa officinalis*, *Verbascum nigrum*, *Silene alba*, *Erigeron canadensis*.

25. **Echio-Melilotetum albi** Tx. 42

(Syn. : *Meliloto-Echietum* Soó 49, *Melilotetum albi* Pass. 64, *Melilotetum* Müller ex Oberd. 67). Occupies recently formed sunny grounds such as the grovels or sunny coasts where strata of organic matters are deposited. The recognition species of the association are : *Echium vulgare*, *Melilotus officinalis*, *M. alba*. In the order *Onopordetalia* and the alliance *Onopordion* also participate : *Carduus acanthoides*, *Berteroa incana*, *Reseda lutea*, *Cirsium lanceolatum*, *Cynoglossum officinale*, *Asperugo procumbens*, *Artemisia absinthium*, *Cirsium arvense*, *Leonurus cardiaca*, *Lappula squarrosa*, *Silene alba*, *Malva sylvestris*, *Torilis arvensis*.

Within the association two subassociations are described : *linarietosum vulgaris* Grigore 71 and *plantaginetosum arenariae* Popescu, Sanda, Doltu 80.

26. **Berteroëtum incanae** Siss. et Tideman 46 emend. Pass. 64

The association is characteristic of the sunny soils, rich in nitrates. It is frequently met, having a large spreading in the Romanian Plain, on road sides, especially on the slopes raised by digging ditches, on ruderalized commons on rather moist soils. It is very frequent in the glades of the forest steppes, there where the grazing is forbidden. The phytocoenoses of *Berteroa incana* comprise a great number of species both ruderal and especially coming out from pastures that existed on the respective lands. We consider that this association is secondary but installed, as a result of the change in the composition of the substratum and of intensive grazing.

More frequently signaled out species within the association are : *Poa pratensis*, *Botriochloa ischaemum*, *Bromus sterilis*, *Agropyron repens*, *Urtica dioica*, *Ballota nigra*, *Carduus nutans*, *Verbascum lychnitis*, *Anchusa officinalis*, *Echium vulgare*.

Two subassociations are signaled out within the association : *stipetosum cappilatae* Oprea 76 and *rorippetosum pyrenaici* Pop et Hodișan 70.

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

L-METHIONINE ENRICHED MUTANTS OBTAINED FROM THE METHYLOTROPHIC YEAST *CANDIDA BOIDINII* ICCF26

DORINA AVRAM*, RODICA STAN*, ADRIAN VAMANU**, FLORICA DAN**

L-Methionine enriched mutants were isolated from the methylotrophic yeast *Candida boidinii* ICCF26 in a sulphur deficient medium. The effect of carbon source as well as that of L-methionine addition on L-methionine accumulation and on growth were also investigated in the mutants and in the wild strain.

Methanol is an attractive, nontraditional carbon source which can be used by methylotrophic microorganisms for single cell protein and metabolites production. Biomass obtaining by yeasts from methanol presents some biotechnological advantages over the use of traditional carbohydrate raw materials. These are: low cost and water miscibility of methanol, reduced risks of culture medium contamination, low nucleic acid and high protein content of yeast biomass (2), (6). Similar to other types of biomasses obtained as a result of microorganism fermentation, yeast biomass has a low L-methionine content compared to animal and human requirements. For this reason, the use of enriched L-methionine mutants has been recommended (1), (3), (7).

This paper describes the isolation of L-methionine rich mutants from the methylotrophic yeast *C. boidinii* ICCF26 in a sulphur deficient medium and also investigates the influence of carbon source and of the L-methionine addition in growth medium on L-methionine pool and on strain growth.

MATERIAL AND METHODS

Microorganism : Candida boidinii ICCF26 was kindly supplied by Dr. Natalia Olărăscu from Chemical and Pharmaceutical Research Institute, Bucharest.

Isolation of L-methionine enriched mutants : Mutagenesis was carried out by standard methods (5), using ehtylmethane sulphonate (EMS) (60 µl/ml) and N–methyl–N–nitro–N–nitrosoguanidine (NG) (250 µg/ml). The treated cells were spread on plates containing Sulphur Deficient Medium (SDMedium) (4). Small colonies were selected and then cultivated in Optimal Synthetic Medium (OSMedium) at 28°C for 96 hours. This medium contained either 1.5% methanol or 1.5% glucose as carbon source. The cultivation was carried out in batch culture on a reciprocal shaker at 100 rpm.

L-Methionine pool was determined by paper and thin layer chromatography in the supernatant obtained after pelleting the biomass boiled in distilled water for 60 minutes.

Growth was appreciated turbidimetrically at 610 nm, referring to a standard DCW curve.

RESULTS AND DISCUSSION

1. ISOLATION OF L-METHIONINE ENRICHED MUTANTS

The principle of L-methionine enriched mutants selection used in this study was based on the fact that on SDMedium, strains which are supposed to be enriched in sulphur containing aminoacids had smaller colonies than common ones, due to their greater necessities in sulphur (4). On such a medium, containing only 2.5 μg $MgSO_4 \times 7H_2O$/ml, *C. boidinii* ICCF26 had a slow growth, giving smaller uniform colonies than on a sulphur sufficient medium. The cells treated by EMS and NG gave a ununiform growth on SDMedium and thereby it is possible to select the smallest colonies. The survival rate was 0.4%, when the cells were treated by EMS and 0.04%, in the case of NG treatment.

The frequency with which the small colonies were obtained was 6.8%—7%, comparable with that obtained by OKANISHI and GREGORY (4) in *Candida tropicalis*.

The smallest colonies obtained were grown in OSMedium and investigated for L-methionine pool. Several mutants accumulated higher levels of L-methionine than the wild strain (Fig. 1). One of these mutants, SN—78, had a five fold higher L-methionine pool than the wild strain. Another mutant, SE—57, had 2.2 times more L-methionine as compared with *C. boidinii* ICCF26. These two mutants were further investigated.

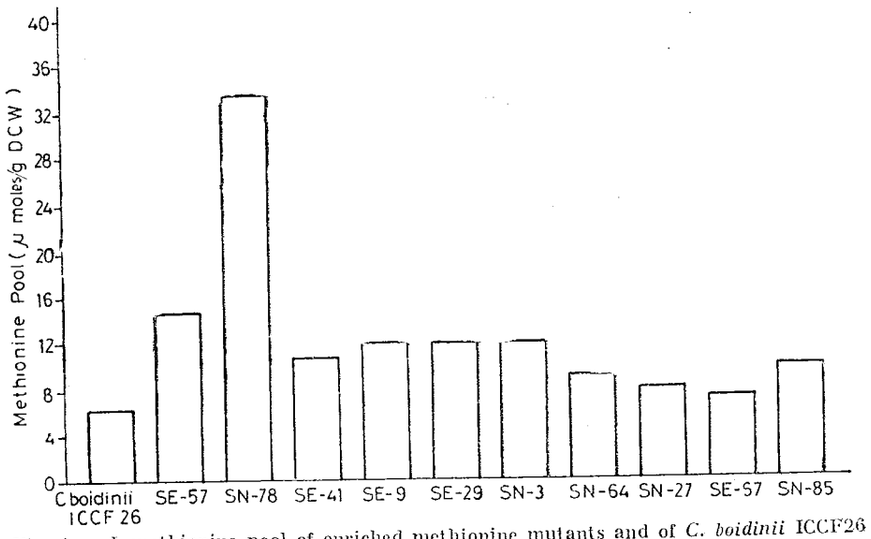

Fig. 1. — L-methionine pool of enriched methionine mutants and of *C. boidinii* ICCF26

2. EFFECT OF CARBON SOURCE AND L-METHIONINE ADDITION ON L-METHIONINE POOL AND ON GROWTH IN THE MUTANTS AND IN C. BOIDINII ICCF26

As could be seen in Fig. 2, the mutants SE—57 and SN—78 had similar patterns of L-methionine accumulation, to *C. boidinii* depending on carbon source and on L-methionine supplementation of the culture medium. The levels of L-methionine pool are however higher in the mutants than in the wild strain in all the situations.

In mutants and in *C. boidinii* ICCF26 methanol seemed to be superior to glucose as carbon source for L-methionine accumulation. These results are comparable with those obtained by TANI et al. (7) with an

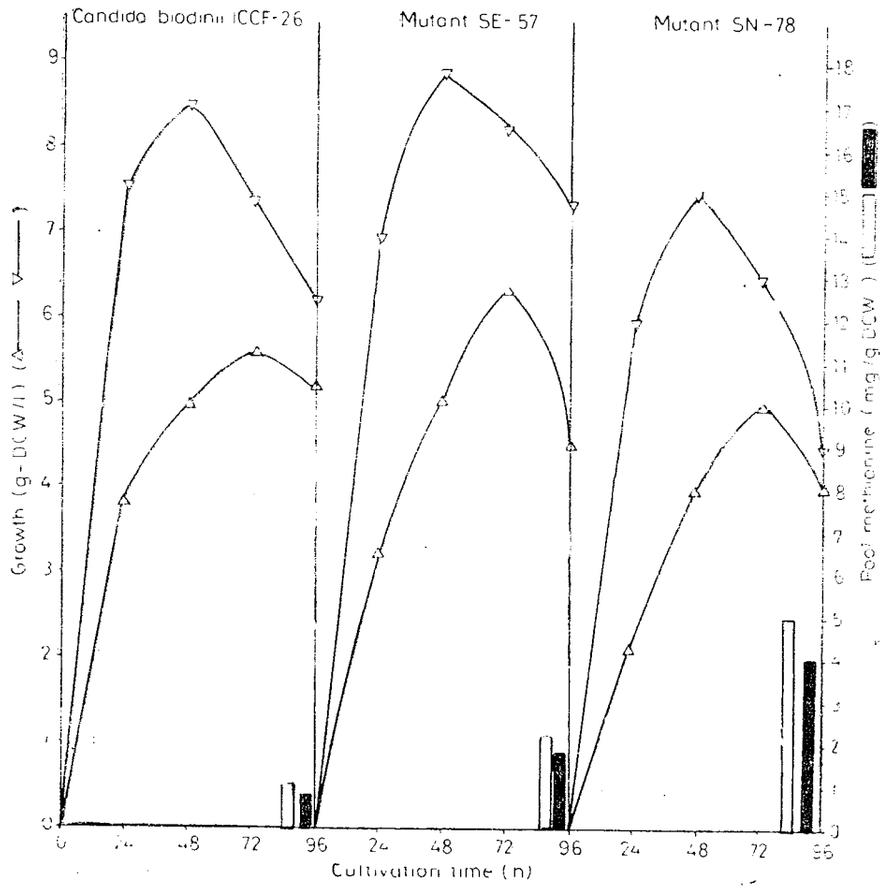

Fig. 2. — The effect of carbon source on L-methionine accumulation and on growth in mutants and in *C. boidinii* ICCF26

▽— growth in glucose OSMedium
△— growth in methanol OSMedium
▮ L-methionine pool in glucose OSMedium
▯ L-methionine pool in methanol OSMedium

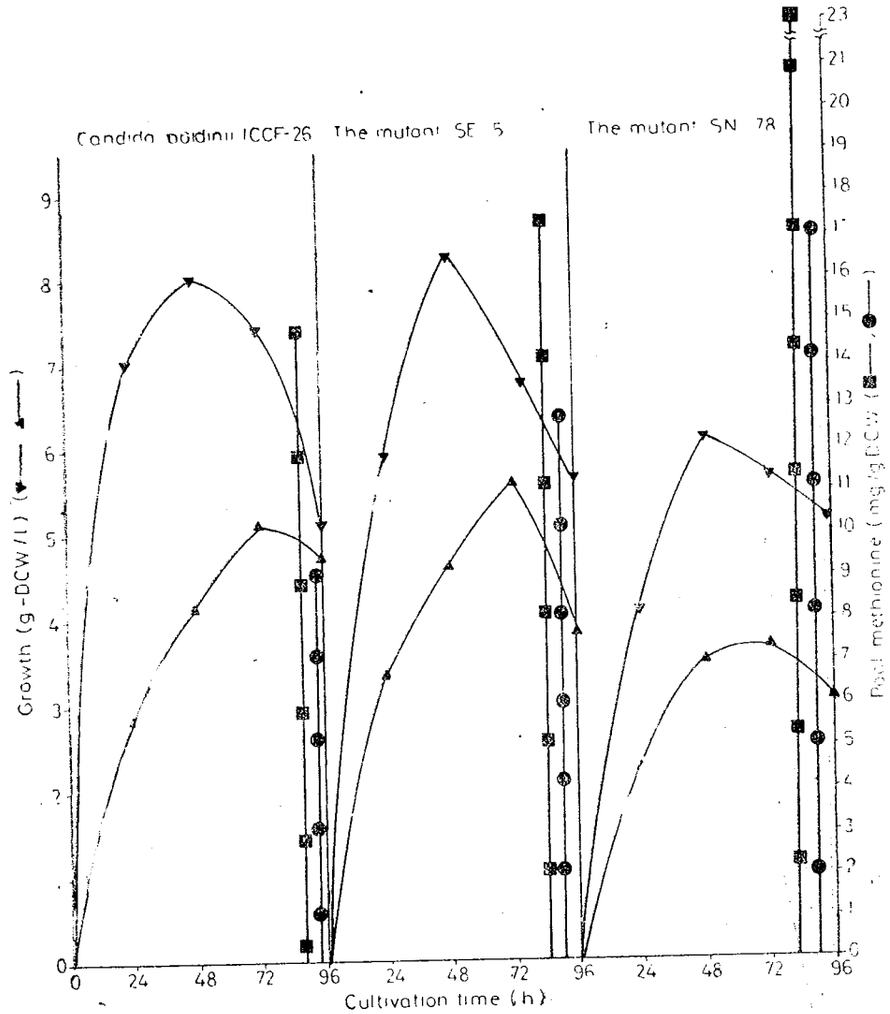

Fig. 3. — The effect of L-methionine supplementation of the culture medium upon L-methionine accumulation in mutants and in *C. boidinii* ICCF26

▼ — growth in glucose OSMedium supplemented with 20mM L-methionine
▲ — growth in methanol OSMedium supplemented with 20mM L-methionine
● — L-methionine pool in glucose OSMedium supplemented with 20 mM L-methionine
■ — L-methionine pool in methanol OSMedium supplemented with 20 mM L-methionine

ethionine resistant mutant. He appreciated that in the ethionine resistant mutant a high formation of methyl groups occurred when grown on methanol and this could be correlated with a high accumulation of L-methionine in methanol medium.

As could be expected the two L-methionine rich mutants and *C. boidinii* ICCF26 had higher growth capacity in glucose medium than in methanol medium (Figs. 2 and 3). Generally, the growth yield coefficients of the methylotrophic yeasts are lower in methanol medium than in glucose medium (6).

The growth capacity of the mutant SE—57 exceeded that of the wild strain by 2.58% in glucose medium and by 12.6% in methanol medium (Fig. 2).

The mutant SN—78 had only 88.94% of the wild strain growth capacity in glucose medium and 88.55% in methanol medium (Fig. 2). It is possible that the growth capacity of this mutant be affected by mutagenesis or such a high level of L-methionine accumulation (5mg/gDCW) have as a result the decrease of the growth capacity.

L-methionine addition (20mM) to the culture medium resulted in a slightly inhibition of the growth both in methanol and in glucose medium, in the mutants and in the wild strain. It is possible that the entrance of such a high quantity of L-methionine consumes a high amount of energy, resulting in the decrease of growth.

L-methionine supplementation of the culture medium led to a great increase of L-methionine pool in the wild strain while in the mutants the increase was less higher (Fig. 3). In the strain SN—78, which had the highest L-methionine pool, in the case of the lack of L-methionine from the culture medium, the increase was only 4.3 fold higher in methanol medium and 4.6 fold higher in glucose medium, after the supplementation of the culture medium with 20 mM L-methionine. In the mutant SE—57, which had about half of the L-methionine pool of the mutant SN—78, the increase was 7.9 and 7.16 fold higher when the culture medium was supplemented with L-methionine. In the wild strain, which had the lowest L-methionine pool, the highest increase of L-methionine level was registered: 14.92 and 13.14 times, in methanol and glucose medium respectively, in the case of L-methionine supplementation.

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* *Institute of Biology,*
Bucharest, Splaiul Independenței 296
** *Chemical-Pharmaceutical Research Institute*
Bucharest Șos. Vitan, 112,

METABOLICAL ADAPTATION TO INDUSTRIAL POLLUTION IN BEECH (*FAGUS SYLVATICA* L.) AND HORNBEAM (*CARPINUS BETULUS* L.) LEAVES

I. EVOLUTION OF DRY SUBSTANCE AND TOTAL PROTEINS

V. BERCEA, DANA BATHORY, ANCA RUSU, V. SORAN

The paper deals with the growth dynamics of the dry substance and total proteins in beech (*Fagus sylvatica* L.) and hornbeam (*Carpinus betulus* L.) leaves under the influence of polluting gases (sulphur oxides, nitrogen oxides) and fall-outs (combinations of the following heavy metals : Pb, Cu Zn, Cd), discharged by the polluting agent "Works for Processing Non-ferrous Metals" in Zlatna (Alba county, Romania). The dry substance (the ecological equivalent of the net primary production) has increased in control beech and hornbeam leaves, the prolonged lag-phase (9.05.—6.06.1991) being followed by a growth pattern either according to Chanter's curve (2) in beech or according to Gompertz's curve (3), (19) in hornbeam. Due to air pollution with toxic gases and fall-outs, the amount of dry substance has increased or lowered as compared to control, and this has brought about a change in growth curves, as stimulating and inhibitting processes have been recorded. The increase in total protein amounts, with relevant differences between polluted areas and controls, has evolved according to exponential curves of a clearly asymptotic tenedency during the last three months of the vegetation period (Aug., Sept. and Oct.), displaying a sigmoid pattern.

Few data are available on the toxic effect of pollutants, mainly sulphur dioxide, upon edifying and subedifying wood species in forest ecosystems (8), (10), (11), (12), (18), (20), and they refer more to North America than to Europe. The metabolical adapting reactions of trees and the ecological recovering of forests to pollution with toxic gases and heavy metal compounds are still very little known (6), (22). The information available so far has allowed William H. Smith (20) to classify trees in forest ecosystems into three ecophysiological groups, depending on their reaction to various polluting agents (O_3, SO_z, O_3+SO_z, acids) : a) tolerant trees ; b) mean-sensitive trees and c) highly sensitive trees. According to this classification, the two species studied here (*Fagus sylvatica* L. and *Carpinus betulus* L.) can be assigned to the ecophysiological group of trees tolerant to air pollution. The study was carried out on these two species because the investigated area (the town of Zlatna and Ampoi Valley, Alba county) is characterized by forests consisting mainly of beech mixed with other species, such as hornbeam. Some of the specimens making up the populations of the two species were found to be pollution-tolerant while others were highly sensitive to pollution with SO_z and heavy metals (9), (23).

REV. ROUM. BIOL.—BIOL. VÉGÉT., TOME 38, N° 1, P. 33—41, BUCAREST, 1993

MATERIAL AND METHOD

Investigations were focused on the Ampoi Valley and the town of Zlatna (Alba county), where the "Works for Processing non-Ferrous Metals" lie. The area is highly polluted with gases, such as sulphur dioxide and nitrogen oxides, and fall-outs of the chemical combinations of the following heavy metals : Pb, Cd, Cu, Zn and others. Experiments were carried out on beech *(Fagus sylvatica* L.) and hornbeam *(Carpinus betulus* L.) populations located in various parts of the Ampoi Valley, taking into account the damage degree in the leaves, the concentration of polluting elements in the air and their distribution over the investigated area due to air currents and other climatic conditions. The site for sampling vegetal material (beech and hornbeam leaves) were the following a) highly or severely polluted landscape lying in the close neighbourhood of the polluting source on the northern slope of one of the hills separating the Ampoi Valley from the Geoagiu Valley ; b) rather severely polluted landscape, with a sampling site about 15 km upstream from the polluting source on the Rosioara stream and another sampling site lying about 25 km downstream from the polluting source in an oak forest mixed with beech and hornbeam ; c) control landscape in an unpolluted area located near Baia de Aries not far from "Fagul imparatului", a nature monument. The three areas were selected according to the data regarding the degree of pollution correlated with the distance between the polluting agent and the damaged vegetation (4), (5), (22).

The investigations carried out regarded the contents in dry substance and proteins of the leaves from the two species mentioned. The vegetal material was sampled during the whole vegetation period in the first decade of May, June, July, August, September and October, 1991. Sampling was performed only from the south-eastern part of the tree at about 6 m above the ground. Thirty disc samples were obtained each time from the sampled leaves, one disc sample being 1.33 cm^2 wide. In order to estimate the dry substance (ecological equivalent for net primary production), the disc samples were first weighed when fresh, then dried at 105°C and weighed again. The resulting amount of dry substance estimated in mg was related to the unit of photosynthesizing foliar surface (mg dry substance/cm^2 of foliar surface).

Lowry's method (14) was used for estimating the amount of total proteins (mg total proteins existing per 1 cm^2 of foliar surface).

RESULTS AND DISCUSSION

1. **Evolution in the growth of dry substance.** In ecology and ecophysiology (13), the amount of dry substance present in various organisms or parts of organisms represents, besides is equivalent in calories, the simplest way of expressing the net primary production related to the unit of surface and time. This net primary production is usually rendered in grams of dry (generally organic) substance fixed on 1 m^2 during 1 year. Our researches carried out on beech and hornbeam foliar surfaces used for estimates mg of dry substance per cm^2 sampled each month.

a) **Increase in dry substance in beech leaves** *(Fagus sylvatica* L.). The dry substance recorded in control beech leaves during the vegetation period in 1991 ranged between 182—564 g/m^2 from May to October. This represented half the amount of dry substance produced by deciduous forests in the temperate zone (13), (15), (22), the other half accumulating yearly in roots, trunk, bark and branches (amount not estimated in our investigation).

The increase in dry substance followed a growth curve or function of the Chanter type (2), (3). It started with a prolongued lag-phase since the first decade of May (9.05.1991) until the first decade of June (6.06.1991), when the dry substances recorded in beech leaves ranged around 20 mg/$/cm^2$ of foliar surface. Having concluded its morphological and anatomical development in May, the leaf gradually increased its content in dry substance per surface unit according to the Chanter curve (2), which is well-known to be (1), (3) an intermediate or hybrid growth function between the Gompertz equation and the logistic one. The dynamics of the whole process is given in figure 1A, curve 1. It can be noticed that the amount of dry substance doubled in June, while in August-October it ranged around 54 mg/cm^2. This flattening of the growth curve is accounted for by a compensation of several metabolic reactions leading to the stabilization of the increase in net primary poduction during the second part and towards the end of the vegetation period.

The presence of polluting elements (Fig. 1A, curves 2—5) in the air, mainly SO_2 and heavy metals, has induced modifications in the Chanter growth curves.

The ecotype of tolerant beech in the highly polluted landscape and in the one lying upstream from the polluting source was characterized by the alteration of the Chanter curve into a Gompertz curve (Fig. 1A, curves 2 and 4), the latter revealing a slower increase in organic substance as compared to control, the increase reaching nevertheless the level of the control by the end of the vegetation period. This last characteristic was used, in fact, as argument for regarding the ecotype in the highly polluted landscape as tolerant. The ecotype in the upstream area seemed to be less subjected to pollution with toxic gases and heavy metals due to air currents along the Ampoi Valley.

As to the beech ecotype regarded as sensitive to pollution, it is noteworthy that the Chanter growth function tended to turn into a logistic one. The diagram of this function (as shown in fig. 1A, curves 3 and 5) revealed a spectacular increase in dry substance per cm^2 of foliar surface immediately after the leaf's full growth (June 1991), followed by a slowing down of the synthesis of organic substance. This behaviour proves that the sensitive ecotype cannot adapt to pollution because the metabolic and ecophysiologic processes deteriorate during the first part of the vegetation period and consequently the specimens are exhausted biologically. Noteworthy is the situation of the beech located upstream from the polluting source. It occurs in a region dominated by oak *(Quercus robur* L.), living outside its ecological optimum, and this fact increases its sensitivity to toxic gases present constantly or sporadically in the air.

b) **Increase in dry substance in hornbeam leaves** *(Carpinus betulus* L.). The dry substance recorded in control hornbeam leaves during

the vegetation period in 1991 ranged between 129—583 g per m^2 from May to October. Just like in beech, this amount of dry substance represents about of half the net primary production of hornbeam.

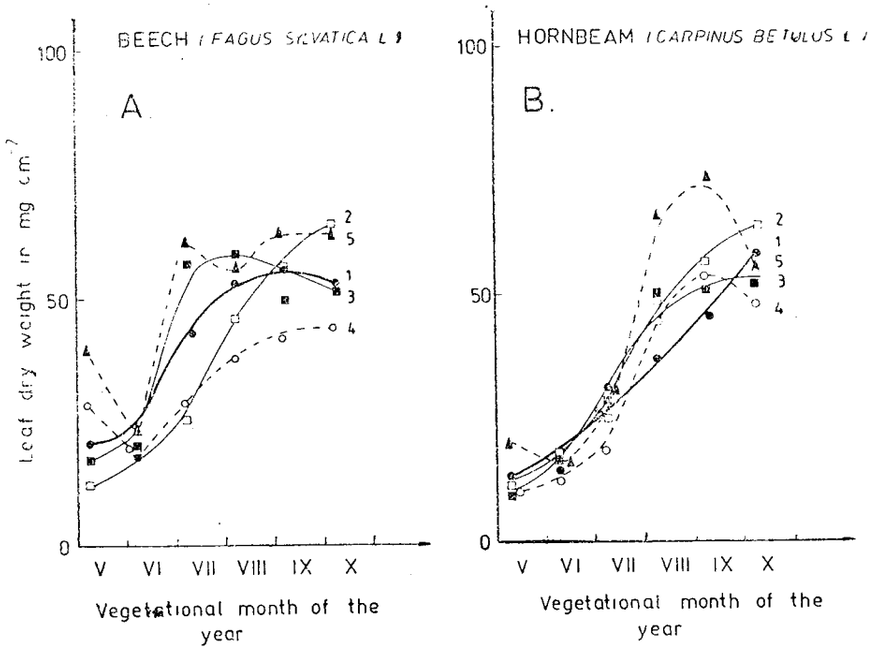

Fig. 1. — Evolution of the amounts of dry substance during the vegetation period of the year 1991 under the influence of air pollution:

A = in control (1) beech leaves (*Fagus sylvatica* L.), pollution-tolerant ecotype (2), pollution-sensitive ecotype (3), specimens upstream (4), and down-stream (5) from the polluting source; B = in control (1) hornbeam leaves (*Carpinus betulus* L.), pollution — tolerant ecotype (2), pollution sensitive ecotype (3), specimens upstream (4) and downstream (5) from the polluting source.

The increase in dry substance in hornbeam leaves followed the pattern of the Gompertz growth function (1), (3), (21). V. Sahleanu (19) pointed out that in growth phenomena regarding organisms or parts of organisms the Gompertz curves are always accompanied by an exponential decrease. It seems that this slow (Fig. 1B, curve 1) and constant growth of the dry substance is a species characteristic for hornbeam and possibly for the group, since beech and hornbeam belong to two different families (the first to the *Fagaceae* and the second to the *Betulaceae*). As the increase in dry substance in control hornbeam was differently accounted for, being expressed through another mathematic function, it also brought about a different reaction to pollution. All the cases affected by air pollution displayed an alteration of the Gompertz growth function that is typical for control.

In the hornbeam ecotype considered tolerant to air pollution, as well as in the more sensitive one, the increase in dry substance (mg/cm^2)

follows a simple exponential growth pattern until the beginning of August, but during the last three months of the vegetation period (August September, October) it drops severely. This reaction is less visible and closer to control values in the pollution — tolerant ecotype occurring in the highly polluted landscape (Fig. 1B, curve 2). On the other hand, the amount of dry substance in the pollution—sensitive ecotype increases more than the double of control values in August, and this difference, although smaller and smaller, is kept above control values untill the end of the vegetation period (Fig. 1B, curve 3).

The hornbeam specimens occurring in the rather severely polluted landscape upstream and downstream from the polluting source display another change in the exponential growth curve. It turns into a polynomic exponential curve (3), (21) due to the fact that the gradual accumulation of polluting elements, much slower than in specimens occurring closer to the polluting source, becomes obvious only towards the end of the vegetation period. This reaction was found to be stronger downstream from the polluting source (Fig. 1B, curves 4 and 5).

The careful investigation of diagrams in figures 1A and B reveals a few general ecophysiological data. During May the dry substance in beech and hornbeam leaves increases slowly, being supported by reserves accumulated by the trees during the vegetation period of the previous year. The level of this accumulation was influenced by the air pollution at that time interfering with the pollution during the present growth period. Therefore, the amounts of dry substance recorded at the end of the growth period in leaves are very close (insignificant statistical differences) to those recorded at the beginning of June. Differences between sampling sites (close to, downstream from or upstream from the polluting source) and ecotypes occur gradually, during the later vegetation period (July-October). In the highly polluted landscape, the difference between the pollution tolerant ecotype and the relatively sensitive one to SO_2 and fall-outs of heavy metals becomes relevant only towards the end of the vegetation period. The fact that the trees upstream from the polluting source proved to be suffering from pollution (figs. 1A and B and curve 4) as shown by the decrease in their dry substance, as compared to control, can be assigned first of all to the proximity of the polluting agent and only secondly to air currents (4), (5), (22).

2. **Evolution of the increase in total proteins.** It is considered that of all the biochemical components in a vegetal organism, proteins, glucids and lipids are worth a thorough investigation, besides the growth dynamics of the dry substance. The evolution of the amount of total proteins in beech and hornbeam leaves can provide data on the biochemical and physiological ways in which the living parts of vegetal organisms react to pollution during the vegetation period. We have taken up the investigation of proteins for several reasons: a) total proteins can amount up to 5—18% of the dry substance in leaves; b) they are, eventually, one of the final products of photosynthesis; c) the synthesis of proteins is brought about by the genetic information present both in the nuclear DNA and mostly in the chloroplastic DNA. The variations in the amount

of total proteins in leaves, related to the dry substance and to the foliar surface, can provide direct information on the possibility that the polluting gases present in leaves may have somehow affected photosynthesis and protein synthesis. In the latter case, the information available can show the degree in which the circulation of genetic information from DNA to ribosomes has been altered.

Figure 2A presents the evolution of total proteins in beech and hornbeam leaves sampled from the control landscape on the Aries Valley. It can be noticed that the maximum synthesis of total proteins (10 mg in beech and 7 mg in hornbeam per cm^2) is achieved only in September. The curve given by the growth function of total proteins is similar in both species, belonging to a polynomic exponential. The only difference between control species is the lower amount of total proteins synthesized in hornbeam during the last three months (August, September, October). The pattern of curve in Fig. 2A proves the fact that the intimate processes of protein synthesis and photosynthesis in the two species living under similar climatic conditions have led to the same final result.

What has pollution brought about? In the highly polluted landscape, the sensitive ecotypes have been found to synthesize an almost double amount of total proteins as compared to control (Fig. 2B, curves 3 and 4). On the other hand, the tolerant ecotypes (Fig. 2B, curves 1 and 2), although they produce a smaller amount ot total proteins per foliar surface (close to that in control), they display a change in the pattern of the growth curve, which means a change in the mathematical function of the evolution of total proteins. The new function corresponds to a logistic growth equation (1), (7), (16), (17) and it reflects, in the case of pollution, a slowing down in metabolic processes parallel to an accumulation in total proteins due to the inhibition of translocation processes to other parts of the plant. In the sampling site located upstream from the polluting source (Fig. 2C), the evolution of the amount of total proteins displayed the same pattern as in control trees, the only difference lying in the fact that the amounts of total proteins presents in beech leaves reached similar values to those in hornbeam leaves. In the site located downstream from the polluting source, the amounts of proteins present in horbeam leaves were somewhat larger than in control (Fig. 2D, curve 2), while in beech the logistic curve (Fig. 2D, curve 1) indicates protein accumulation towards the end of the vegetation period.

One of the problems to be solved by further researches is the possibility that SO_2 may influence the activity of enzymatic proteins by acidifying the cell sap and the cytoplasm (6).

The results regarding the evolution of the amounts of total proteins on beech and hornbeam leaves during the whole vegetation period of the year 1991 reveal that their biosynthesis has been deeply altered only in the trees from the higly polluted landscape, i.e. about 0.5—2 km from the polluting source. In the sampling sites further upstream or downstream, the amount of total proteins tends to follow the same pattern as that in control trees.

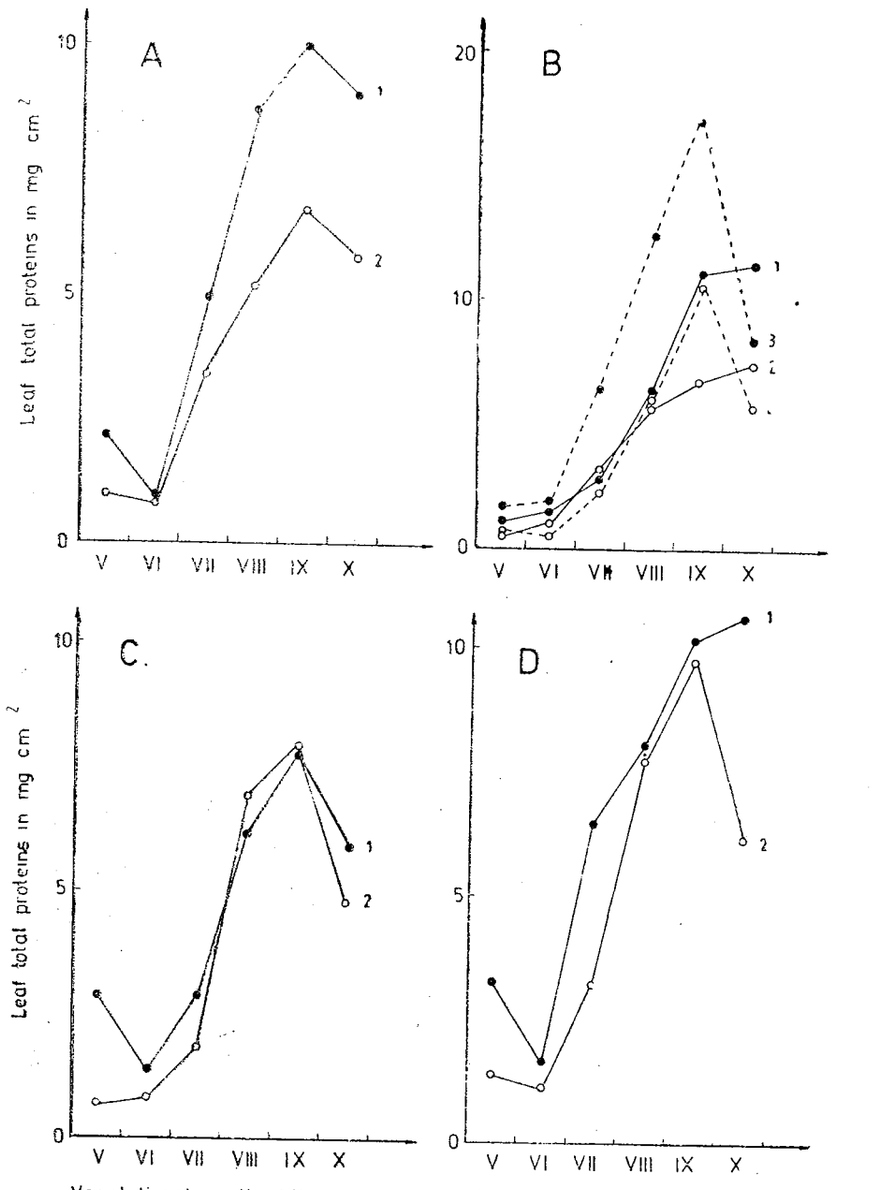

Fig. 2. — Evolution of the amounts of total proteins in beech (*Fagus sylvatica* L.) and hornbeam (*Carpinus betulus* L.) leaves during the vegetation period of the year 1991 under the influence of air pollution:

A = in control trees, 1 — beech, 2 — hornbeam; B = in tolerant ecotypes, 1 — beech, 2 — hornbeam and in sensitive ecotypes, 3 — beech, 4 — hornbeam; C = in specimens upstream from the polluting source, 1 — beech, 2 — hornbeam; D — in specimens downstream from the polluting source, 1 — beech, 2 — hornbeam.

CONCLUSIONS

The investigations aiming at revealing the effects of toxic gases (sulphur oxides and nitrogen oxides) and fall-outs of heavy metals discharged by the "Works for Processing non — Ferrous Metals" in Zlatna upon the evolution of the contents in dry substance and total proteins of beech and hornbeam leaves have led to the following conclusions :

1. Both beech and hornbeam have proved to be relatively tolerant species to pollution with SO_2, nitrogen oxides and fall-outs of the various compounds of heavy metals ;

2. In the highly polluted landscape, both beech and hornbeam have presented a tolerant ecotype and a more sensitive one to air pollution ;

3. Both the amount of dry substance and that of total proteins have been more significantly altered in the highly polluted landscape, while in the rather severely polluted landscape these changes have been less significant ;

4. The situations regarded as dangerous for the life of the trees are those characterized by a complete change in pattern of the synthesis and accumulation of the dry substance, and mainly of total proteins, as compared to control ;

5. A large part of the pollution-induced changes in the evolution of the accumulation of dry substance (net primary production) and total proteins in beech and hornbeam leaves can be regarded as metabolic adaptation in response to the stress caused by pollutants.

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Institute of Biological Researches
48 Republicii Street
3400 Cluj-Napoca, Romania

THE EFFECT OF STEROID HORMONES ON CYTODIFFERENTIATION AND MORPHOGENETIC PROCESSES IN PLANTS

II. INFLUENCE OF PROGESTERONE ON MORPHOGENETIC PROCESSES IN VIVO

GINA COGĂLNICEANU, AURELIA BREZEANU, COMAN ION

The effect of progesterone, a steroidic compound with hormonal significance in mammals, was tested on the developmental patterns of *Nicotiana tabacum* L. plants. The rate of germination as well as the average growth, total length of axis and the number of leaves were monitored. Concentrations between 0.5 and up to 25 mg progesterone/l were tested. A detrimental influence on seed germination, axillary growth and growth speed was observed. The moment when the treatment with progesterone was applied had a special significance. The inhibitory effect proved to be enduring when progesterone was introduced at germination and totally abolished in postcotyledonary stage.

Steroidic compounds are widely distributed in plants (5). Among these steroids with hormonal significance in mammals, the estrone, progesterone, testosterone, epitestosterone, androsterone have been isolated both in different kinds of vegetable tissues and in plant cell and tissue cultures (11, 15).

Progesterone (14-pregnen-3, 20 dione), one of the most frequent steroids, has been found in almost all species tested, at the level of leaf, stem, inflorescence, root and seed samples, more or less abundant (2, 11).

No correlation between steroid occurrence in plant species and tissue, organ, sex, age, season or metabolic specialization has been emphasized. Due to the paucity of data on steroids in plant tissues, the role of these compounds is speculative at present. Different hypotheses on steroids involvement in the physiology of plants have been forwarded : sex determination (6, 7, 8), tumorigenesis (10), synthesis of pheromone (3). Interesting results have been achieved in the study of the metabolism of progesterone (and other steroids) by plant tissue cultures (e.g. : cell suspension of *Nicotiana tabacum*, *N. rustica*, *Dioscorea deltoidea*, *Cheiranthus cheirii* (4, 13, 14). Androgens were detected in seed samples of all species tested and progesterone in most of them (11).

We presume that these compounds have a physiological role in the developmental patterns of vegetal organisms. In the light of these assumptions the involvement of progesterone on the modulation of some morphogenetic processes in somatic tissue culture of *Nicotiana tabaeum* cv. *Xanthi* was investigated in a previous article (1). The effect of exogenous progesterone on the early stages of plant development : seeds germination, epicotyl elongation and development of first leaves was analyzed in this paper. Several parameters were measured : percentage of germination, axis length, number of leaves, rhythm of growth.

The possible interaction between endogenous auxin and exogenous progesterone was also tested.

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MATERIALS AND METHODS

Aseptic germination of *Nicotiana tabacum* L. cv. *Xanthi* seeds was obtained. The surface sterilization was best acquired by soaking seeds in 1%. $HgCl_2$ for 15 min followed by three washes in sterile distilled water. The seeds were axenically cultured on the Murashige-Skoog (MS) medium (9) without growth hormones, in the presence or absence of progesterone. One hundred seeds were used in each germination experiment. Subsequent passages of seedlings on MS medium with or without progesterone were made. In another series of experiments seedlings with fully expanded two leaves were cultured on MS with progesterone. The average growth, total length of axis and the number of leaves were monitored for 10—20 seedlings per variant.

The progesterone pulvis was incorporated in the culture media both for the germination or postcotyledonary development, in concentrations of: (*1*) 0; (*2*) 0.25; (*3*) 1.25; (*4*) 2.5; (*5*) 5; (*6*) 10; (*7*) 25 mg/l.

In order to emphasize the possible correlations between the effects of progesterone and endogenous auxin the wheat coleoptile test was used (16). Coleoptile segments of 8 mm length, cut at 2 mm from the apex were preincubated for 60 min in natrium citrate buffer (TC), 1mM, pH 5.8. Coleoptiles (20—30/experiment) were grown in buffer supplemented with:

— (P_1) 0.25; (P_2) 2.5; (P_3) 25 mg progesterone/l;

— NNA 10^{-5} M;

— NAA 10^{-5} M and (P_1) 0.25; (P_2) 2.5; (P_3) 25 mg progesterone/l;

Control coleoptiles were grown in buffer citrate only. Coleoptile incubation was carried at 20 ± 2°C, in the dark for 20 hours. The results are presented as the mean value of coleoptile elongation (n=25—30) from three separate experiments.

RESULTS AND DISCUSSION

The germination was inhibited after the addition of progesterone in the culture medium depending on concentration. At low concentrations (1—4) germination was slightly inhibited. Higher concentrations (5—7)

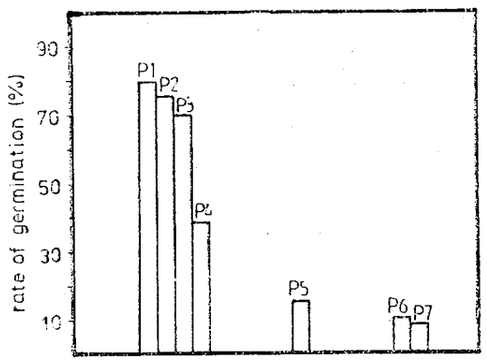

Fig. 1. —The effect of different concentrations of progesterone (P_1=0; P_2 = 0.25; P_3 = 1.25; P_4 = 2.5; P_5 = 5.0; P_6 =10.0; P_7=25 mg/l) on seed germination. P_1—P_4: ten days latency of germination; P_5: 14 days latency of germination; P_6—P_7: 20 days latency of germination.

caused a longer latency period and a severe reduction in the number of germinated seeds (figure 1 and 2 a, b, c). The process is reversible, seeds that did not germinate after 30 days on media with 25 mg progesterone/l

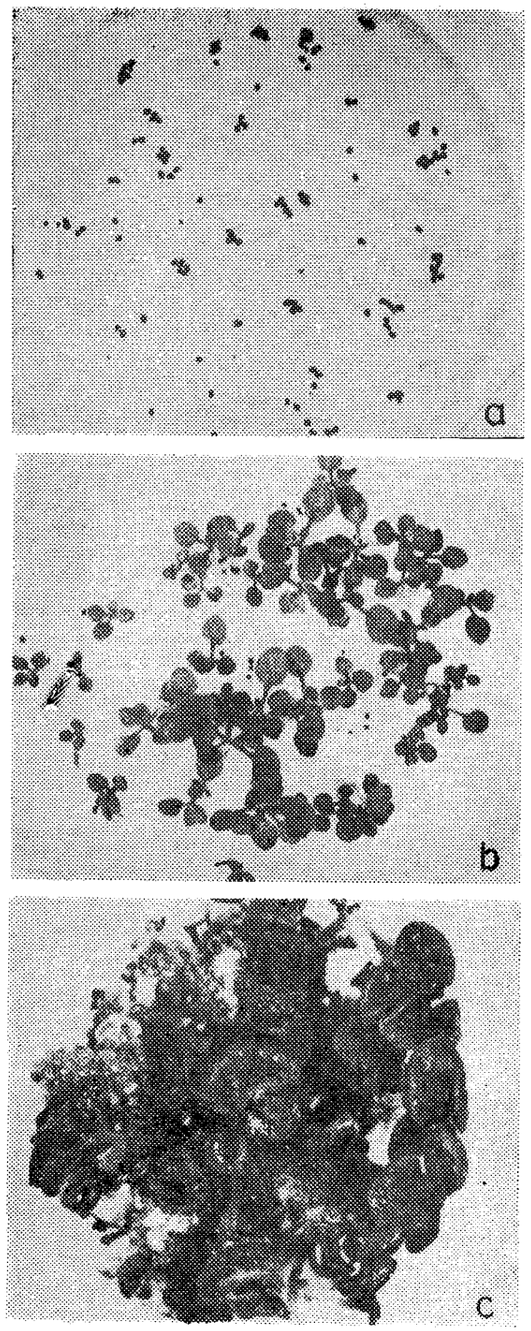

Fig. 2. — Germination of *Nicotiana tabacum* seeds on culture medium supplemented with different concentrations of progesterone: a. 25 mg/l
b. 5 mg/l
c. 0 mg/l

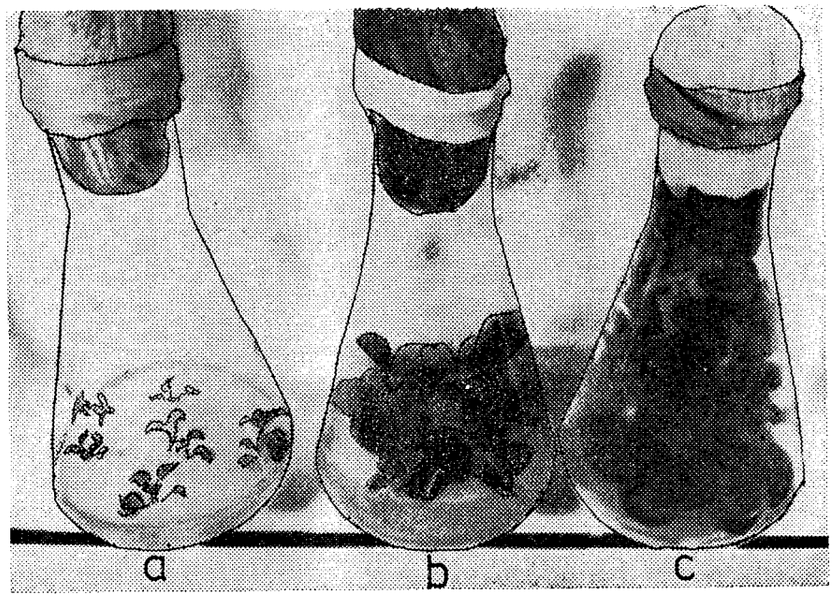

Fig. 3. — The postcotyledonary development of plants in the presence of different concentrations of progesterone in the culture medium : a. 25 mg/l b. 5 mg/l c. 0 mg/l.

germinate in proportion of 23% on media without progesterone after 20 days.

The presence of progesterone in seeds was demonstrated in the majority of the plant species investigated (11). Its inhibitory effects on germination might be caused by overdosing.

In another series of experiments, seedlings in postcotyledonary stage, cultivated in aseptic conditions on MS medium without progesterone, were subcultivated on MS medium with progesterone in different concentrations (1—7). The following parameters were measured : axillary growth, foliar buds growth and growth speed (figure 4). It was shown that axial growth of shoots is strongly influenced by progesterone, in direct correlation with its concentration. The development of the foliar system is not influenced significantly by progesterone, since foliar primordium develops very similarly to control plants. In the experiments containing 10 and 25 mg progesterone/l, dwarf plants appeared, with small leaves compared to normal plants, often etiolated (figure 3 a, b, c). Although the place where plant cells metabolize progesterone was considered to be the microsome (12), structural changes of the chloroplast system, especially thylakoids, might be present. Studies in progress will elucidate this aspect.

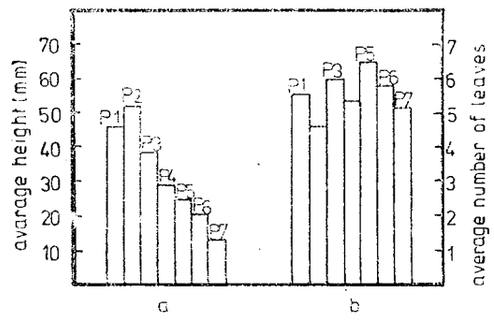

Fig. 4. — The influence of various concentrations of progesterone (P_1—P_7) on :
a. axial growth
b. the development of foliar system

In order to be saved from perishing, plants treated with maximum concentrations of progesterone were subcultivated on MS medium without progesterone and they continued their growth with swiftness, surpassing control plants both in average daily growth (2.64 mm/day compared to 1.62 mm/day). It can be concluded that progesterone does not have a permanent effect, only a selective inhibition, influencing mainly plant axillary growth. High concentrations (10—25 mg progesterone/l) are toxic, causing metabolic damages presented by the ultrastructure, mainly by chloroplast.

In order to locate the impact of the treatment with progesterone, plants germinated on medium with different concentrations were sub

cultivated on medium without progesterone and on medium containing the same concentration of progesterone. It was shown that during the experimental period (28 days), plants germinated on medium with progesterone, subcultivated also on medium with the same concentration of progesterone, displayed the same selective inhibition. A relative independent evolution of foliar buds was observed (figure 5). Plants germinated on medium with progesterone, subcultivated on medium without progesterone, displayed a lasting but diminishing effect of axial growth inhibition and a delayed evolution of foliar buds. These results suggest that germination represents a series of linked processes, small modifications being slowly repaired in the subsequent development of the plant. No adaptation in time of the growing plant was observed towards progesterone, high concentrations causing dwarf plants, a reduction of foliar surface and slow and progressive etiolation.

The significant and selective influence of progesterone on axillar growth and embowering allow the hypothesis of an interference with natural auxin. To prove it the wheat coleoptile test was used. This test

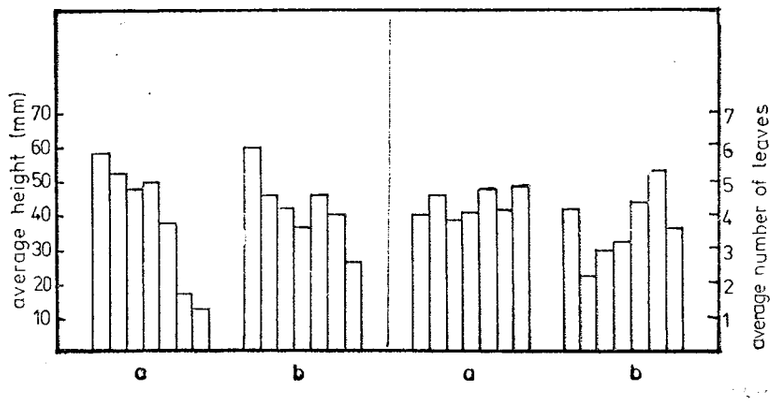

Fig. 5. — The effect of subcultivation of plants germinated on media with P_1—P_7 concentrations of progesterone on:
a. medium with the same concentration of progesterone;
b. medium without progesterone.

showed that progesterone in citrate buffer does not have the capacity of stimulating wheat coleoptile elongation (table 1). As the concentration of progesterone was increased, elongation was inhibited. In the tests where auxin (NAA) was added it was shown that the stimulating effect of exogenous auxin was constantly reduced, disappearing at a maximum concentration. It is difficult to assert based on this experiment only if the interference with auxin is informational, hormonal or if progesterone in high concentrations has only toxic effects, reducing metabolic processes, including the ones stimulated by auxin.

Seeds collected from plants treated during their growth with progesterone were used in a germination test, the differences obtained being not significant (Table 2).

Table 1

Wheat coleoptile elongation influenced by the presence in Na citrate buffer 1mM, pH = 5.8, of either NAA, P_{1-3} or both

Solutions for incubation	Wheat coleoptile elongation (mm)		Solutions for incubation
TC	12.05	14.37	NAA
TC + P_1	12.35	14.37	NAA + P_1
TC + P_2	11.46	12.57	NAA + P_2
TC + P_3	10.32	10.61	NAA + P_3

Table 2

Germination test for seeds harvested from plants treated with various concentrations of progesterone during their growth

Concentration of progesterone (mg/l)	Rate of germinatio (%)
0.00	98
0.25	98
1.25	98
2.50	90
5.00	95
10.00	93
25.00	90

CONCLUSIONS

1. In our experimental conditions, progesterone had a harmful influence on seed germination, axillary growth and growth speed.

2. A selective inhibition by progesterone was observed on axillary growth. The wheat coleoptile test showed a diminished effect of auxin at large concentrations of progesterone (25 mg/l).

3. The moment when the treatment with progesterone was done was important. The inhibitory effect was proved to be lasting when progesterone was introduced at germination and it was totally abolished when progesterone was introduced in postcotyledonary stage.

4. Leaf etiolation after treatment with 10 and 25 mg progesterone/l was caused by metabolic changes that could be reflected in the plastidial system ultrastructure.

5. Further studies are needed in order to test the influence of progesterone on various steps of plant growth and at lower concentrations than the ones used in the present study.

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Institute of Biology
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INTERSPECIFIC PROTOPLAST FUSION IN *STREPTOMYCES*

CĂLINA PETRUȚA CORNEA[1], I. VĂTAFU[1], ANETA POP[2], AURELIA BREZEANU[1], L. SAVU[1], SMARANDA DOBROTĂ[1]

Protoplasts were produced from two *Streptomyces* strains able to degrade microcrystalline cellulose. The greatest efficiency of protoplast formation (more than 90%) was achieved without precultivation of bacteria in the presence of glycine. The highest regeneration frequencies (20—23%) were obtained when the protoplasts were embedded in soft agarose upper layer. Electron microscopy observations showed the formation of large bodies as results of multiple fusions. Two recombinant strains resistant to Km and Tc were selected and analysed for cellulase production.

Protoplast fusion provides a mean of genetic recombination and is a promising technique for the improvement of industrial microorganisms. Stimulation of dihydrovanillin and lignin degradation (4, 13) and cellulase production (17) have already been achieved by these methods in different microorganisms. For cellulolytic microorganisms it seems particularly attractive because of the supposed multigenic nature of their polysaccharide degradation systems (cellulase and xylanase) (3, 14).

The possibility of protoplast formation, regeneration and fusion has been demonstrated in *Streptomyces* years ago (2, 8, 12, 15). However, the methods of protoplast isolation and regeneration used in different laboratories varied and only in few experiments cellulolytic *Streptomyces* strains were used.

In this paper studies in protoplast isolation, regeneration and fusion in two *Streptomyces* strains developed for cellulase production are presented.

MATERIALS AND METHODS

ORGANISMS AND METHODS

Streptomyces flavovirens SfG3 and *Streptomyces sp.* SD16, two locally isolated strains, were grown on basal medium described by Crawford and McCoy (6). For cellulase production cultures were grown in the same basal medium supplemented with 1% Avicell PH105 or with 1% carboxymethyl cellulose (Austranal). For protoplast isolation the bacteria were cultivated for 48 hours in YEME medium (9) but without glycine. Hypertonic medium for protoplast isolation contained: 0.01M $MgCl_2$, 0.02M $CaCl_2$, 0.02 M NaCl, 0.01 M phosphate buffer pH 7.3 and sucrose as osmotic stabilizer, supplemented with lysozyme 1—2 mg/ml. For the regeneration of cell wall two media were used: a lower layer represented by MRM (5) and an upper layer consisting of the same MRM medium but modified by replacing glucose with 1% CMC and agar with 0.4% agarose. The upper layer was supplemented (when necessary) with kanamycin (20 μg/ml) and tetracycline (10 μg/ml).

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PROTOPLAST ISOLATION, REGENERATION AND FUSION

The pellets resulted after centrifugation of 48 hours old cultures were resuspended in hypertonic medium with lysozyme and incubated for 3 hours at 30°C. The protoplast formation was monitored by microscopic observations and by osmotic shock. Fusion was performed by mixing 0.1 ml protoplast suspension from both strains with 0.8 ml of 40% PEG 6000 in hypertonic medium. After 2 minutes the suspension was diluted 10 fold with hypertonic medium and aliquots of 0.1 ml were included in 3 ml of regeneration medium (upper layer) and plated on the lower layer of regeneration medium. In another variant, the protoplasts were plated directly on the lower layer of regeneration medium. Fusion frequency was estimated as the ration of the number of colonies grown on the selective medium (with both antibiotics) to the number of protoplasts mixed from each strain (14).

ENZYME ASSAYS

The action of cultures supernatants on CMC and Whatman No. 1 filter paper (FP-ase) was assayed as described by MacKenzie et al. (*11*) and reducing sugar release was determined with DNS reagent (18). One unit of enzyme activity was defined as the amount of enzyme releasing 1 μM of reducing sugar (expressed as glucose) per minute. The protein concentrations of supernatants were determined by the method of Lowry et al. (*10*).

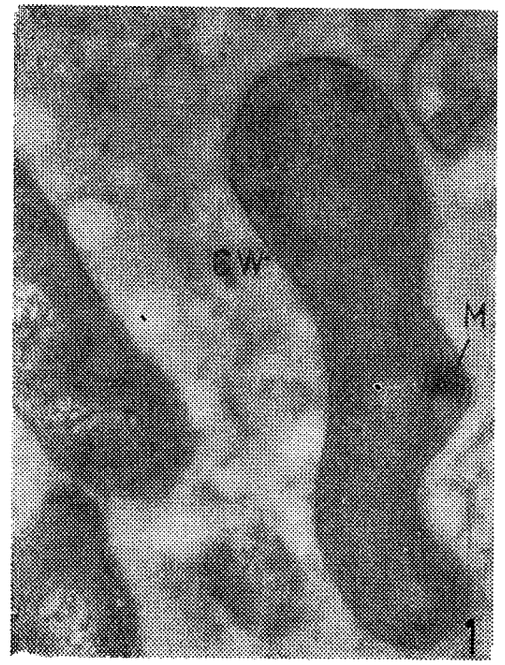
TRANSMISSION ELECTRON MICROSCOPY

Samples were fixed in 6% glutaraldehyde, processed by the classic procedure of electron microscopy and embedded in Epon 812 resin. The ultrathin sections obtained with a Tesla ultramicrotom were stained by uranyl acetate and lead citrate according to Hayat (7) and examined under a Phillips microscope.

RESULTS AND DISCUSSION

It is well known that *Streptomycetes* have a very complex cell wall so a treatment with glycine is usually necessary to convert mycellium to protoplasts (9, 13). Our strains were able to form protoplasts at a convenient efficiency (more than 90%) without pretreatment with glycine, their cell walls being sensitive to lysozyme attack. The hypertonic medium used seems to be good not only for these two strains but also for other streptomycetes tested in our laboratory (5).

Different ways to remove the cell wall of *Streptomyces* in the presence of lysozyme were observed (Plate I). The differences appeared could be due to the composition of the cell wall as well as to incubation period in the presence of lytic enzyme. A short time of incubation (10 minutes) caused some nicks instead of a prolonged one (for 30 minutes) which determined a fragmentation or a lysis of the cell wall on large areas (Figs 2and 3).



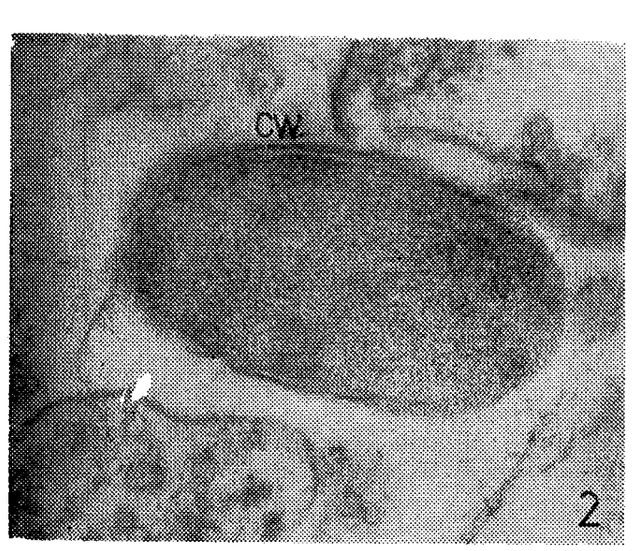
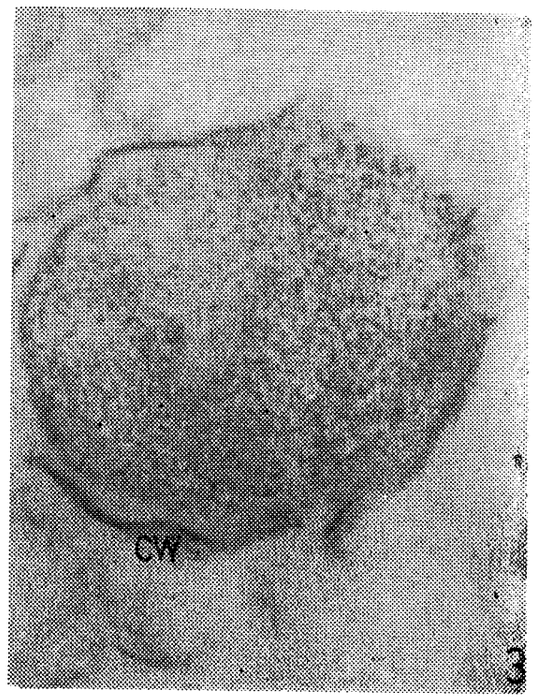
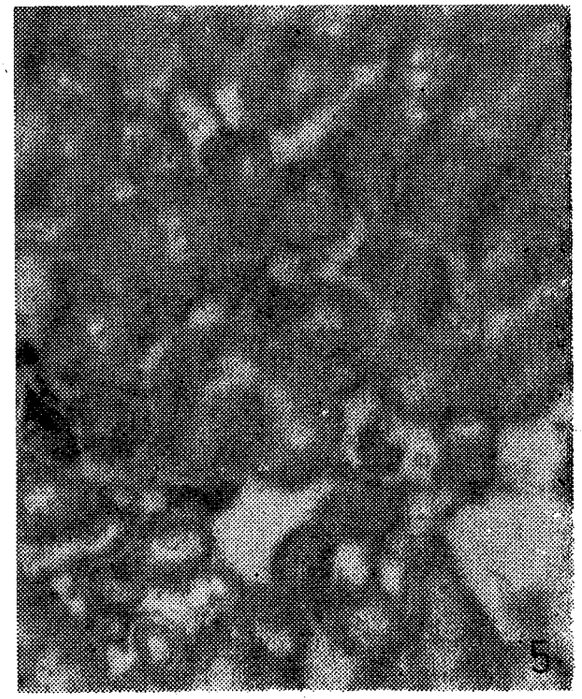


Plate I. Protoplast formation in *Streptomyces sp.* SD16 and *S. flavovirens*.

Fig. 1. — The ultrastructure of *S. flavovirens* SfG3 cells untreated with lysozyme.

Fig. 2. — Cell of *S. flavovirens* SfG3 after 10 minutes of action of lysozyme.





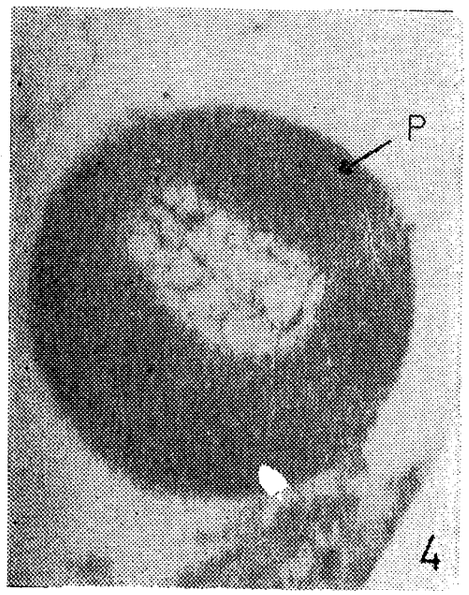


Fig. 3. — Cell of *Streptomyces sp.* SD16 after 30 minutes of lytic treatment.
Fig. 4. — Proper protoplast obtained after 30 minutes of lytic treatment.

Plate II. Steps of interspecific protoplast fusion in *Streptomyces.*

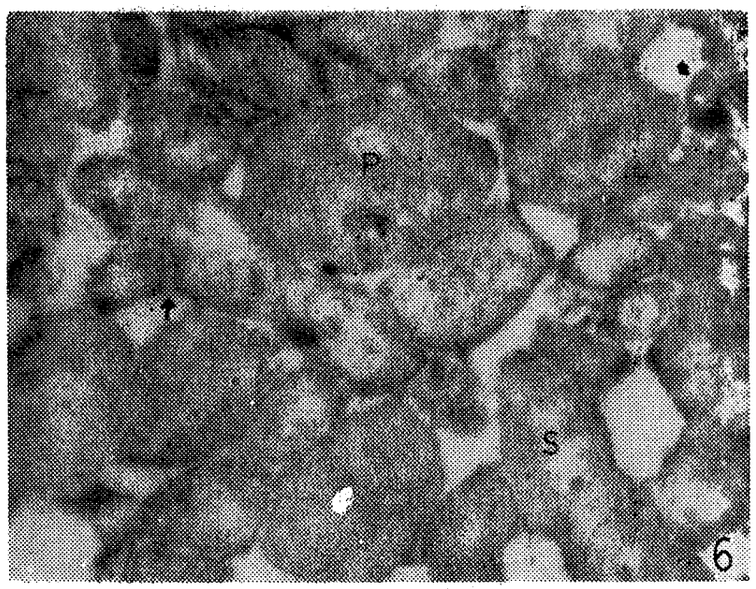


Fig. 5. — Agglutination of protoplasts (P) and spheroplasts (S) in the presence of PEG
Fig. 6. — Adherence between two or many protoplasts.

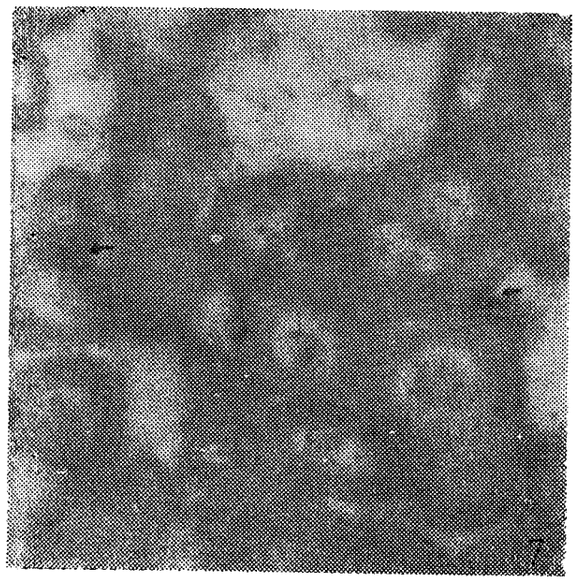


Fig. 7. — Enlargement of contact areas between protoplasts.
Fig. 8. — Final stage of fusion showing cytoplasm intermixing and formation of large cells.

It has been generally appreciated that the protoplasts have a spherical form (Fig. 4). But we could frequently notice protoplasts without any cell wall material having an irregular form. This could raise some problems concerning the forces which act for keeping the shape of the protoplasts in the absence of the cell wall.

Two ways of plating the protoplasts on the regeneration medium were tried : spreading on the medium surface and embedding the protoplasts in the soft agarose upper layer. The second method gave the best frequencies of regeneration : 23% for *S. flavovirens* SfG3 and 20% for *Streptomyces sp.* SD16.

Fusion between the kanamycin resistant strain of *Streptomyces sp.* SD16 and tetracycline resistant strain of *S. flavovirens* SFG3 gave rise to the formation of hybrid colonies on selective medium (containing 1% CMC as unique carbon source and both antibiotics), at a frequency of 0.35%.

The fusion process was also analysed by electron microscopy (Plate II). Generally, fusion process in *Streptomyces* strains experimented by us is similar with other experimental systems (1, 19). Three steps in fusion process were recorded. Agglutination of protoplasts or spheroplasts (Fig. 5) represents a prerequisite stage necessary for the fusion begining. It is determined by an activation of the plasma membrane depending on PEG concentration. The exact action of PEG is not well understood so far, but Arnold et al. (1) demonstrated the indirect action of PEG on the cell membrane by membrane polarity modification. At this stage, the adherence between two or many protoplasts is observed (Fig. 6). The adherence can appear in few areas of contact or on a large surface of the adjacent plasma membranes (Fig. 7).

In the second stage, so-called "bispherical shape", between agglutinated protoplasts an electronodense space appeared probably as results of the complex membrane processes appearance.

In the final stage the membrane boundaries between the fused protoplasts disappear leading to the cytoplasm intermixing (Fig. 8). Large bodies with irregular shape appear as a result of the treatment with PEG suggesting that to this process participate more than two protoplasts. This is in agreement with the observations of other authors (8, 12). We are not able to say at this stage which of these bodies are formed by intraspecific fusion and which by interspecific fusion, but the selection technique resolves this problem.

The products of interspecific fusion were first selected on the selective medium containing antibiotics (Km^r, Tc^r) and 1% CMC. The colonies showing a large area of CMC degradation (by use of the Congo red method of selection) (*16*) were submitted for segregation analysis and cellulase production. The stability of the Km^r Tc^r strains was tested by plating them on basal medium without antibiotics followed by the recheck of resistance to antibiotics. Among the colonies tested, two of them (F3 and F4) presented no segregation and a modified ability to degrade the cellulosic substrates. This fact could indicate that these strains are already genetically stable haploid recombinants.

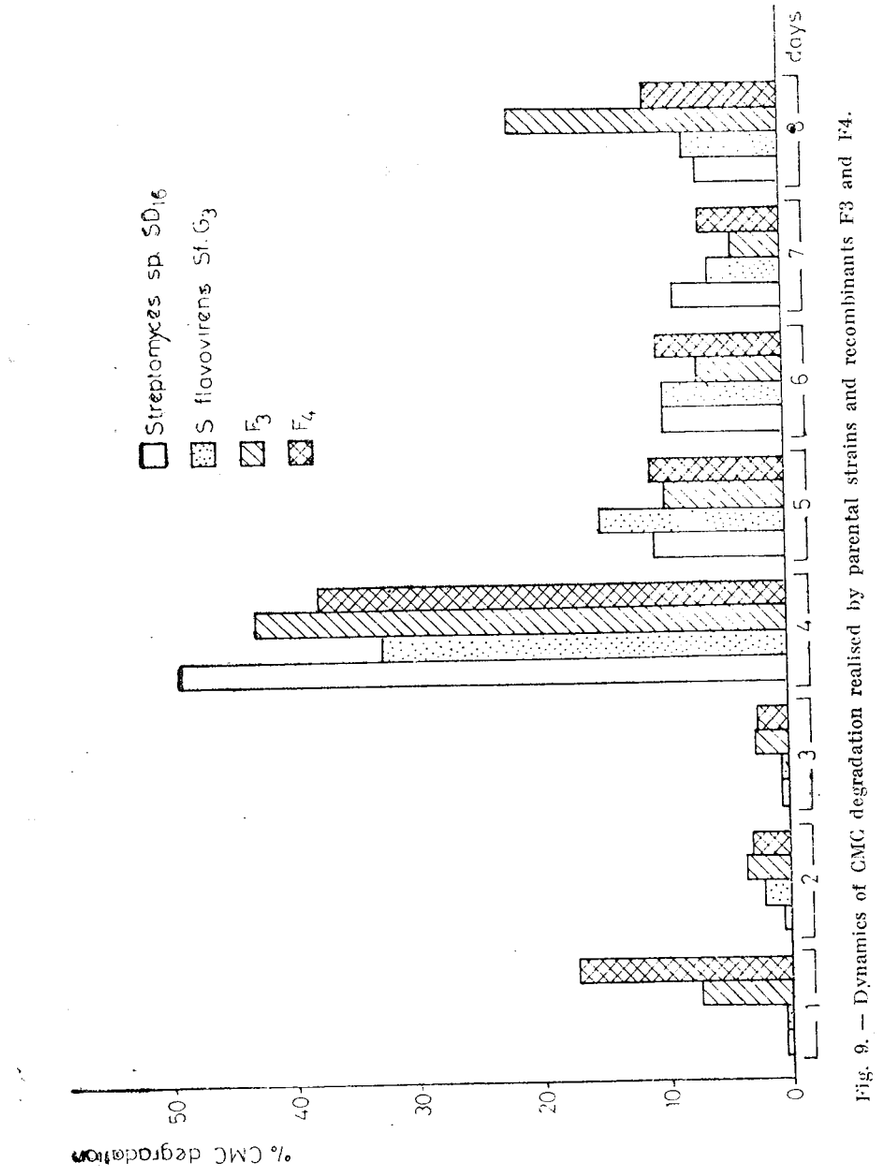


Fig. 9. — Dynamics of CMC degradation realised by parental strains and recombinants F3 and F4.

To study the rate of CMC degradation, the recombinants and the parental strains were cultivated in 10 ml of liquid basal medium with 1% CMC and activity was estimated daily. As shown in Fig. 9 the maximum rate of degradation is achieved after 4 days and is completed after 8 days only for recombinants. The parental strains were able to degrade, during the same period, only 73.8% of the substrate *(S. flavovirens* SfG3) or 88% respectively *(Streptomyces sp.* SD16).

Compared with parental strains, F3 and F4 showed a different dynamics of cellulase degradation, in fact a different pattern either in the cellulolytic enzymes biosynthesis or in these enzymes liberation in culture media. Despite the fact that the increasing of cellulase degradation was not spectacular, we might assume that recombination event(s) among the genes coding for cellulase took place.

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[1] *Institute og Biology, Bucharest*
[2] *Faculty of Veterinary Medicine, Bucharest*

LA VARIATION DES ÉLÉMENTS MINÉRAUX DANS LES SARMENTS, DURANT LA PÉRIODE DE REPOS

ANCA ANTOHE

In the present paper the researches represent a continuation of the previous ones (1, 2, 3, 4) carried in the north-east of Moldavia on *Vitis vinifera* types : Aligoté (type originated from France) and black Feteasca (autochthonous type). The amplitude of variation of the mineral elements is different depending on the amount of Nitrogen, it is big in all elements of the branches (bark and phloem, wead, bud and node) and diminishes in the forced repose.
The quantitative variation of anions is found especially within the conducting tissue.

La vigne dans un climat tempéré, passe par diverses périodes de repos, lorsque toutes ses activités physiologiques sont de beaucoup plus réduites, en vue de se protéger ainsi contre les actions défavorables de la température.

Le repos absolu (total) s'installe en même temps que la maturation des drageons et atteint l'intensité maximum. Le réveil après le repos a lieu en hiver, deux ou trois mois avant le bourgeonnement naturel de la vigne. Pendant cette période, le repos absolu (total) a disparu, mais c'est maintenant qu'apparait le repos forcé, imposé par les condition défavorables du milieu (9, 10).

Pendant le repos, a lieu une série de transformations biochimiques dans les sarments et les bourgeons (5, 6, 7, 8).

Dans ce travail on a poursuivi la variation quantitative des éléments minéraux pendant la période de repos pour deux sortes de vigne : Aligoté (espèce originaire de France) et Feteasca neagră (espèce autochtone).

MATÉRIEL ET MÉTHODE

On a effectué l'étude dans la Station Expérimentale Horti-Viticole de Iași, dans des conditions identiques d'expérimentation à celles rappelées dans les travaux antérieurs (1, 2, 3, 4).

Pendant la période de repos on a étudié les sarments d'une année provenant des deux espèces dont nous avons parlé.

Les sarments ont été divisés en plusieurs parties : la base (qui comprend les internoeuds 1—4), le milieu (internœuds 4—9) et le sommet (internœuds 10—18). On a analysé les internœuds divisés en écorce avec le liber et séparément le bois. Les bourgeons ont été analysés avec leur nœuds.

On a observé la variation des éléments minéraux pendant les mois de décembre (repos absolu), de février (repos forcé) et de mars (au début de la végétation).

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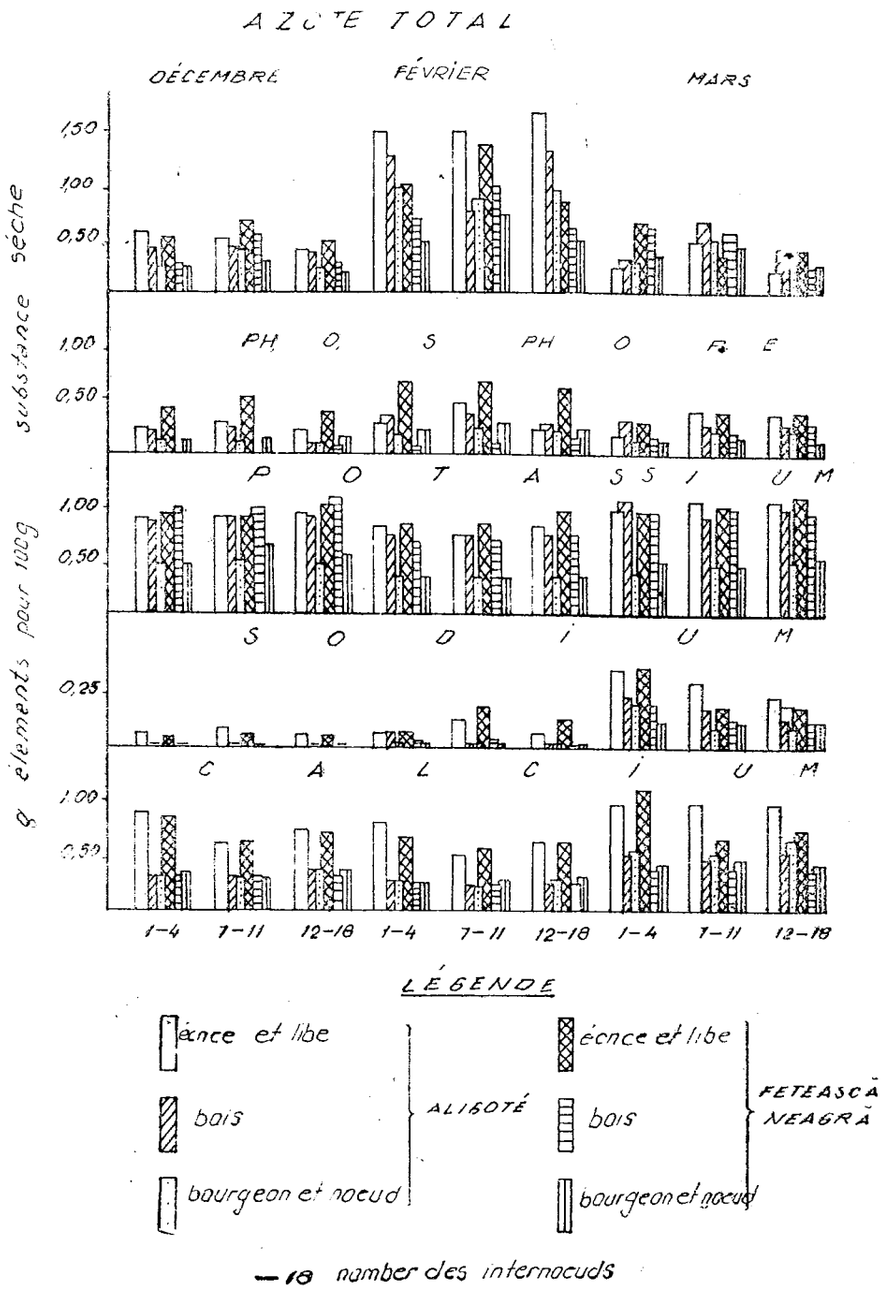

Fig. 1. — La quantité des éléments minéraux pendant la période de repos chez les espèces Aligoté et Feteasca neagră.

On a apprécié le contenu des éléments minéraux par la méthode de la minéralisation par voie humide avec de l'acide sulfurique at on a déterminé l'azote total par la méthode Kejeldahl, le phosphore colorimétrique, le potassium, le sodium et le calcium au photomètre à flamme.

RÉSULTATS ET DISCUSSIONS

Des données obtenues (fig. 1) il résulte que l'azote souffre des modifications quantitatives pendant le repos forcé. On constate une augmentation quantitative tout aussi au sarment qu'au bourgeon avec le nœud d'insertion. Au mois de mars, lorsque le processus d'accroissement des bourgeons commence, la quantité d'azote diminue.

La variation quantitative du phosphore présente la même dynamique mais la valeur des différences pour chaque détermination est inférieure.

La variation quantitative les cations est marquante dans les tissus conducteurs. Le potassium manifeste un accroissement quantitatif au mois de mars, tout aussi dans le liber que dans l'écorce, et de même dans le bois.

Au niveau des bourgeons avec le nœud d'insertion, la variation est moins évidente.

Le cation Na^+ manifeste un développement quantitatif évident au mois de mars.

Cette croissance se rapporte tout aussi à l'axe du sarment qu'au bourgeon avec le nœud d'insertion. Il est possible que ce cation par sa présence ait une contribution à une hydration plus active des tissus du sarment, au moment de la croissance.

La variation du cation Ca^{++} démontre une augmentation quantitative surtout dans les tissus qui réalisent leur différence au printemps c'est-à-dire l'écorce et phloeme (Kramer, p. J., 1963). Cet élément est nécessaire pour les membranes des cellules en constituant une partie composante des pectines.

Il y a des différences entre les bourgeons du même sarment, par rapport à la position de ceux-ci au long du sarment. Les bourgeons situés vers le milieu du sarment présentent des quantités plus grandes d'azote de phosphore et de potassium, par rapport à ceux de la base et du sommet.

En ce qui concerne le sodium et le calcium, leur quantité est plus grande vers la base du sarment, fait qui démontre le processus de différentation et de maturation des tissus qui se trouvent dans cette partie du sarment dans un rythme plus rapide.

CONCLUSIONS

1) Pendant le repos forcé, dans l'axe du sarment et dans les bourgeons avec le nœud d'insertion, la quantité d'azote augmente.

2) Au printemps, lorsque les bourgeons commencent à croître la quantité d'azote diminue, celui-ce étant utilisé dans les processus de néogenèse.

3) La variation quantitative des cations a lieu évidemment dans le tissu conducteur.

— Le potassium augmente du point de vue quantitatif au mois de mars dans l'écorce et le liber toute comme dans le bois.

— Le sodium augmente au point de vue quantitatif dans l'axe du sarment, spécialement dans l'écorce et le liber, vers le printemps.

— Le calcium augmente dans l'écorce et le liber.

4) Les bourgeons situés vers le milieu du sarment de réproduction ont des quantités plus grandes d'azote, de phosphore, de potassium par rapport aux bourgeons situés à la base ou au sommet du sarment de réproduction (sarment productif).

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Center of Biological Researches
Iași
Blvd. Copou 20 A, Iași 6600 Romania

PLANT REGENERATION FROM CROWN GALLS OF POTATO *(SOLANUM TUBEROSUM* L.*)*

ALEXANDRINA PĂTRAȘCU-GĂLIN, PETRUȚA CORNEA, DOINA CÂRCIUMĂRESCU

Tumors with regenerative capacity were induced in potato Desirée cultivar by nopaline C_{58} and octopine A_6 strain of *Agrobacterium tumefaciens*. Some differences regarding the tumoral characteristics of the two bacterial strains were noted. The tumors induced by octopine A_6 strain differentiated especially rooting shoots on free hormone MS medium and that induced by nopaline C_{58} strain differentiated roots on the same medium.
Opine assay was positive for tumors and for the shoots and the roots differentiated from them. The potato transformants we obtained were susceptible to superinfection with both the octopine A_6 and nopaline C_{58} strain of *A. tumefaciens*. They have an abnormal morphology (short internodes, small leaves) and a poor capacity of rooting and of tuberization as compared with untransformed plants. Nevertheless, after a few subcultures on the same media these characteristics modify and the transformed plants resemble more and more with the control plants.

Potato *(Solanum tuberosum* L.) is a major crop plant which is able to be infected naturally by Agrobacterium strains (3) inducing tumors (galls). The causal agent of crown gall tumorigenesis is the Ti plasmid and the molecular basis of this process is the transfer and integration of a definite segment located on Ti plasmid (T-DNA) in the plant genome (19).

Potato can be transformed by *Agrobacterium tumefaciens* and regenerated into plants by using wild-type strains (12) or binary vector harboring disarmed. *A. tumefaciens* strains (1), (13), (16), (17), (18). The gene transfer techniques developed for potato need an efficient regeneration procedure of the transgenic cells combined with minimal somadonal variation. Diploid potato clones have been transformed by electro poration of protoplasts with different selectable markers and resulting diploid regenerated plants have been used in somatic hybridization; it has been shown that hybrid cell selection on the basis of antibiotic or herbicide resistances brought by the parents of fusion is an efficient method for the recovery of tetraploid somatic hybrids (7).

At the International Potato Center (C.I.P.)in Peru genetic engineering research projects are concentrated on the incorporation and expression of synthetic and natural genes regarding nutritional value or the resistance to pests and diseases in potato, using Agrobacterium plasmid vectors. This has been successful in inserting the synthetic gene coding for synthetic protein containing a high content of essential aminoacids (particularly lysine and methionine) in potato plants and at obtaining organ specific (tuber) expression (4). Recently MacKenzie et al (6) introduced by genetic transformation into potato cultivar Russet Burbank the coat protein gene from potato virus S(PVS) which confers the resistance to the subsequent infection with this virus and also to PVS RNA.

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MATERIALS AND METHODS

Two strains of *Agrobacterium tumefaciens* namely one nopaline strain C_{58} with plasmid $pTiC_{58}(nos^+ tm^+)$ and one octopine strain A_6 with plasmid $pTiA_6(ocs^+tm^+)$ were used in our experiments. Nopaline strain C_{58} is a gift from dr. Mlinarova-Czechoslovakia and octopine strain A_6 belongs to the collection of our laboratory.

The bacterial strains were grown on ML medium with 2% agar and bacterial suspension for infection on ML broth for 48 hours at 28°C.

Potato Desirée cultivar was used as recipient of T—DNA both plants grown in the greenhouse and in vitro obtained plants by the meristem cultures method used by us earlier (14) and multiplied by the layering technique on nutritive basal hormone free MS medium (9).

For in vivo infection leaf fragments were dipped in bacterial suspension for 10′ after a previous sterilization of leaves for 3′ in a 0.1% $HgCl_2$ solution containing a few drops of Tween 20. After sterilization the leaf fragments were washed three times in distilled water. For in vitro material stem segments were wounded and then inoculated with bacterial suspension. The infected material was placed on MS medium without hormones and after 48 hours was transferred on the same medium but supplemented with carbenicillin (500 mg/l) or ampicillin (500 mg/l) for elimination of bacteria. After 5 subcultures on this medium, the tumors were excised and transferred on MS hormone free medium without antibiotic.

Stem segments of uninfected plants and of transformants obtained directly from tumors were placed on two variants of MS basal medium for inducing of tuberization. These media noted T_{10} and T_{11} contained 4 mg BAP/l and 8% sucrose and 4 mg BAP/l and 6% sucrose respectively. The cultures were exposed daily to 5000 lx for 16 hours and for tuber formation to 1000 lx for 8 hours and to dark for 16 hours.

The presence of opines was evidenced by the TLC method on silicagel plates and by ninhydrine 0.1% straining (15) as compared with control plants. Histological analyses were performed according to standard techniques, namely a fixation in Navashin-Brown's solution and Erlich hematoxyline staining.

RESULTS ET DISCUSSION

Transformation of stem internodes of potato Desirée cultivar with *A. tumefaciens* strain A_6 and C_{58} resulted in tumors with regenerative capacity. Small tumors developed after 2 weeks on the infected plants at the all wounding sites (Fig. 1).

Histological analysis of the tumors revealed a mosaic of cellular types (Fig. 2) which evolved to shoots, roots or presented different stages of cell senescence. We noted some differences in tumoral characteristics of the two bacterial strains used regarding the tumoral habitus and the induction of endogenous hormonal activities. This aspect was revealed by other authors too (5). The tumors induced with octopine strain A_6 are green or lighthrown and have a great regenerative capacity for both roots and shoots (Figs 3 and 4); the tumors induced with the nopaline

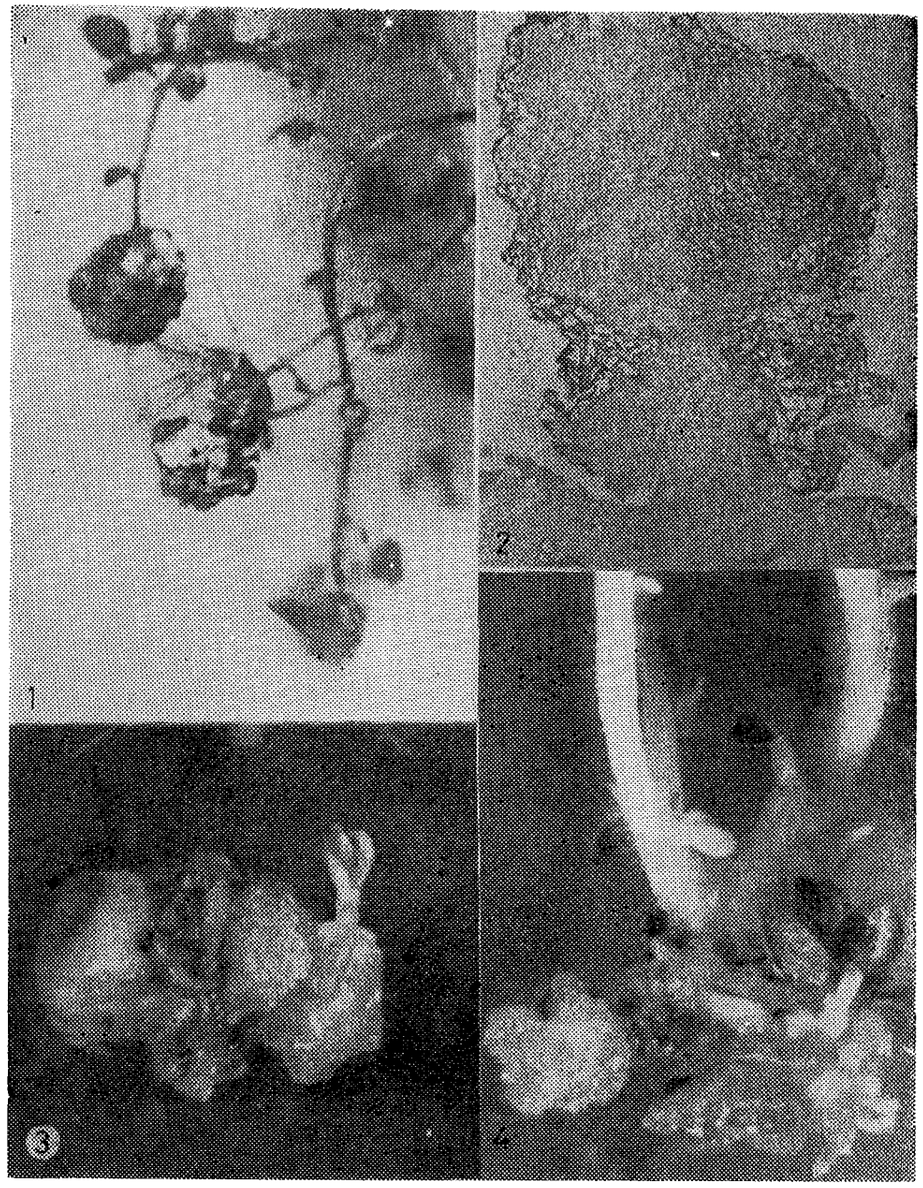

Fig. 1. — In vitro tumors induced by nopaline C_{58} strain of *Agrobacterium tumefaciens* on shoots of potato Desirée cultivar.
Fig. 2. — Histological aspect of the tumorous tissue induced by octopine A_6 strain of *A. tumefaciens;* a mosaic of cellular types is revealed.
Figs. 3. and 4 — Tumors with shoots differentiated from them on free hormone MS medium; the octopine A_6 strain was used for tumor inducing.

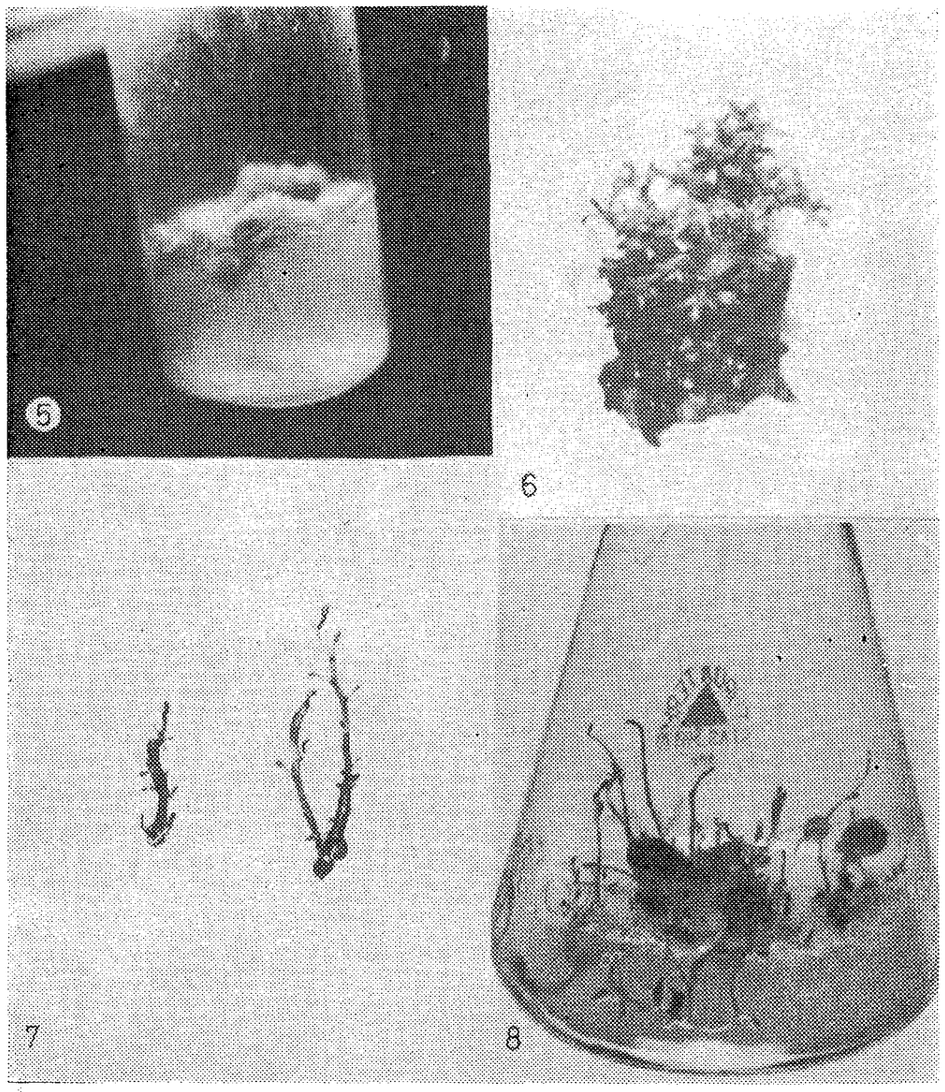

Fig. 5. — Tumors with roots differentiated on free hormone MS medium ; the nopaline C_{58} was used for tumor inducing.
Figs. 6 and 7. — Shoots with abnormal morphology differentiated from tumors.
Fig. 8. — In vitro tuberization on shoots differentiated from tumors induced by *A. tumefaciens*.

strain C_{58} are green with a glomerular habitus, grow more rapidly and develop roots especially (Fig. 5). The excised tumors continued to grow when they were transferred on the basal hormone free MS medium. Opine assay was positive for tumors and for the shoots and the roots developed from them. These characteristics prove the transformed state of the analysed tissues.

The shoots differentiated from tumors were abnormal morphologically (short internodes, small leaflets) and had a very poor rooting capacity on the MS medium without hormones (Figs 6 and 7) comparatively with the untransformed shoots. Nevertheless, after a few subcultures on this medium, their morphology changed resembling with that of the normal shoots ; their internodes became longer, the leaves were larger, rooted easier and could be multiplied by the usual layering technique.

The transformed potato plants we obtained were susceptible to superinfection with both the octopine A_6 and nopaline C_{58} strain of *A. tumefaciens*. After the superinfection was performed, new tumors developed and these regenerated shoots too. The opine assay was positive for them. The cuttings of these shoots and of control plants were placed on the T_{10} and T_{11} variants for tuberization. For control material round minitubers were induced within 4 weeks on stoloniferous shoot with stolons emerging from nodes above the agar. No difference was noticed between the two variants of culture medium used. In the case of shoots regenerated from tumors, tubering was induced only within 10 weeks, the minitubers were smaller, elongated (Fig. 8) and the t_{10} variant was more adequate.

In conclusion, both tested strains of *Agrobacterium tumefaciens* are virulent for potato Desirée cultivar. The nopaline C_{58} strain and the octopine A_6 strain were able to induce tumors which differentiated shoots and roots. In this regard our observations were not similar with those of other authors (20) who considered that only nopaline strains of *A. tumefaciens* were able to induce teratoma on very few host species, but the octopine strains induced unorganized tumors on all host species. In our experiment, the nopaline and octopine strains of *A. tumefaciens* having unchanged T—DNA were able to induce tumors which regenerated roots and rooting shoots. These results are quite different from the situation for the other species (8), (21) but are similar with the others (10), (11). We used also the nopaline strain C_{58} for tumor inducing to three grape vine cultivars and obtained tumors which differentiated roots (2).

Transformants of potato Desirée cultivar are susceptible to superinfection. Their phenotype, the rooting and the tuberization capacity on adequate culture media are different from that of control plants but these characteristics modify after a few subcultures on these media and they resemble more and more with the control.

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Romanian Academy, Institute of Biology
Splaiul Independenței 296, sector 6,
79651 — Bucharest

Cultivation and Processing of Medicinal Plants, L. Hornok Ed., Akadémiai Kiadó, Budapest, 1992, 338 pp., 114 figs, 20 tabs, ISBN 963 05 60218.

Taking into account the actual state-of-the-art in phytotherapy, an increase of interest for vegetal products was evidenced all over the world. Medicinal plants may constitute raw materials for pharmaceutical products, and this fact led to reconsidering the existing fund of such plants.

Principal aspects considered by the book deal with the techno-logical conditions for growing, storing and extraction of active principles from medicinal plants, stressing on the biological aspects of plants; fighting against vegetal/animal pests, as well as identification of novel clones, richer in valuable compounds.

The book is the fruit of a cooperation between 11 specialists from the Research Institute for Medicinal Plants (Budapest), the University of Horticultural Sciences and Food Industry (Budapest), as well as some other departments and companies from Hungary.

The presented material is structured on three parts.

The first one contains the following chapters:

a) general aspects on medicinal plants, where notions related to the definition of medicinal plants, their role and importance, as well as their production in Hungary, are presented;

b) biological considerations regarding: heredity and variability of medicinal plants; active compounds contained; taxonomy for the main cultivated species; environmental factors; breeding; methods for controlling and identification of different clones of such plants;

c) technical — and technological aspects related to medicinal plants and the particular conditions of mechanization and use of chemical fertilizers are presented;

d) processing of medicinal plants, with emphasis on operations like: drying, storing as well as extraction of essential oils and other active principles.

In the second part the cultivation of some 37 European and Mediteranean species is presented. For each species, data regarding historical aspects, introduction in Pharmacopeia; morphological and biological features; content of active compounds; the way of sowing and culturing; preparation of soil; minerals needed; use of pesticides; cropping; storing, etc, are mentioned.

In the third part, a number of 24 species of medicinal plants, cultivated in the temperate regions but sometimes in tropical and subtropical climates as well (but which are important for European consumers), are described. This part also includes some tables with data for cultivation and collecting of medicinal plants.

This is a useful handbook for the cultivation and application of medicinal plants, both for specialists in this field, and also for those with an interest for the utilization of such plants, a field with ancient traditions, which actually shows an ever-increasing interest.

Dr. V. Sanda

Biological Indicators in Environmental Protection, Margit Kovacs. Ed., Académiai Kiadó, Budapest, 1992, 207 pp. 62 figs, 55 tabs, ISBN 963 05 60275.

It is well-known that plants are valuable biological indicators for the degree of pollution in the environment. Recently, a scale of sensitivity was reported, taking into account the macroscopical symptomatology (such as cellular plasmolysis, decrease of the cellular content or deformation of chloroplasts) as well as ecophysiological and biochemical changes which are a consequence of accumulation of different pollutants in green plants.

The MAB (Man and Biosphere) program of UNESCO for monitoring the environment using biological indicators, as well as the impact produced by different pollutants upon terrestrial and aquatic ecosystems, had as a result a world-wide cooperation aimed to diminish the hazardous effects of pollutants.

Inferior plants, such as fungi, lichens, bryophytes, are sensitive bioindicators for the pollution degree of the environment. By studying the distribution pattern of diverse species (especially of lichens) in the surroundings of intensely polluted urban areas, the following types of species were detected : strongly sensitive, sensitive, moderately tolerant, and tolerant species, respectively.

Herbaceous plants are to a great deal sensitive to pollution with sulphur dioxide (which produces necrosis of the foliar limb) as well as heavy metal ions, evidenced in different organs such as the roots, leaves, stems, inflorescences and fruit.

Trees show different degrees of sensitivity to pollutants such as SO_2, HF, H_2SO_4 or heavy metal ions. For instance, species such as : *Abies alba, Picea abies, Pseudotsuga menziesii, Pinus sylvestris, P. strobus, Biota orientalis, Larix decidua,* are very sensitive to sulphur dioxide. The pH value of cellular juice in some trees *(Tilia platyphyllos, Acer platanoides, Fraxinus excelsior)* in urban areas (Frankfurt am Main, Germany) is in the 2.7—3.5 range, whereas in unpolluted zones it is in the 3.4—4.4 range.

Aquatic plants (algae, mosses, phanerogamae of coastal zone) are indicator species for the degree of acidity of the environment. A large diversity of species was determined, from oligotrophic (found in environments poor in nutrients) to eutrophic (which indicate habitats rich in mineral salts), such as : *Butomus umbellatus, Ceratophyllum demersum, Epilobium hirsutum, Potamogeton pectinatus, Rorripa amphibia, Rumex hydrolapathum, Sagittaria sagittifolia, Scirpus lacustris.*

Excepting for soil acidification, accumulation of heavy metals leads to modifications in vegetal tissues, especially in roots, but also modifications of the foliar mesophile, in the distribution of annual rings of trees (the sensitivities of trees vary depending on the maturity of the tree, the young ones being more exposed to atmospheric pollutants).

Physiological processes, such as photosynthesis, respiration, transpiration, etc. undergo dramatic changes under the influence of pollutant agents such as hydrogene sulphide, ozone, heavy metals. Such pollutants also affect the reproductive processes of plants : anomalies in germination of pollen grains (by monstruosities of pollinic tubules), reduction in the number of cones, etc. were evidenced.

By studying the accumulation rate of heavy metals in plants, clones of cultivated species were produced in which such accumulations are diminished.

The complexity of structural modifications produced in the presence of pollutants, determinations of sensitivity gradients for large groups of plants (inferior and superior) as well as changes in the metabolism, are used for measuring the effects of pollution upon the environment.

The book is an excellent synthesis of all researches in the field of biological (vegetal) indicators used for the protection of the environment. By its wide addressing biologists, agronomists, sylvicultors, environmentalists, as well as hobbists of the vegetal kingdom, this book represents a compendium of information in the field, especially for the pollution phenomenon and its impact on the living matter.

Dr. *V. Sanda*
Dr. *A. Popescu*

M. ŞERBAN, NATALIA ROŞOIU, Biological Active Substances in Sea Organisms, Editura Academiei Române, Bucureşti, 1992, 344 p., 94 fig., 124 tab.

The volume "Biological Active Substances in Sea Organisms", written by Prof. dr. Mihai Şerban, correspondent member of the Romanian Academy and Natalia Roşoiu emphasize the increased interest in sea resources revaluation which led to the initiation of numerous research programmes as Pharmacean and SMOM in France, National Sea Grand Program in USA, in order to discover new sea active substances. In our country, especially in the Romanian Institute for Sea Research, has been initiated a program for sea biological resources revaluation with pharmaceutical purposes. In this context, there have already been ratified two new drugs : *MACRONIL*, a defated and deproteinated extract of the fish, with properties in reducing the

density of total microflora and of *Staphylococcus aureus* in respiratory tract and the second *ALFLUTOP R*, realized as an injectable solution and as a spray, obtained from small sea fish, with antirheumatic, antiinflammatory, trophic and biostimulatory properties.

The authors stress the importance of sea organisms like uni and pluri cellular algae, some invertebrates (spongiae, jelly fish, corals, worms, star fishes, crustaceous) as well as some fishes, which biosynthesize a variety of biological active substances grouped into two classes : antibiotics and sistemic drugs (vitamins, hormons, enzymes, antitumoral substances, halucinogens, neuroactive and cardioactive substances).

The volume is structured on eight chapters dealing with : antibiotics, toxic substances and biogenic amines, biological active natural extracts (from animals and algae), enzymes and natural enzyme effectors, heterolipids, vitamins and hormones.

The monograph's authors, well-known researchers in our country, approach the most recent and significant aspects on the obtaining, biochemical characterization, pharmacodynamic properties and clinical and industrial applications of different bioactive substances isolated from sea organisms.

Many of the presented researches belong to the authors, are of the most recent in the field and have already proved their applicability in pharmaceutical industry.

Each chapter includes a large bibliography overviewing a great amount of up-to-date information on the investigated substances.

Some of the biological active substances obtained from sea organisms presented in the volume are : *Cephalosporin C,* a fungal antibiotic ; *Ectionine,* an antibiotic from *Microciona* : mucopolyglucides extracted from *Mya* and *Mitilus,* protective against hyperlipemia and hypercolesterolemia ; *Laminarin,* isolated from brown algae and *Laminaria,* useful in the therapy of Basedow disease, mixoedema and goitre ; *vitamin E* obtained from green algae, with direct action on animal fertility ; *insulin* purified from sea invertebrates, etc.

The present volume proves to be a valuable synthesis of the researches on biological active substances from sea organisms, both in Romania and in the world, as well as a top research realized by two important personalities of the romanian school of biochemistry.

The book is of interest for both specialists : biochemists, pharmacologists, physicians, biologists and all those concerned on knowing and revaluating the natural resources of the sea.

Dr. V. Sanda
Dr. A. Popescu

AVIS AUX AUTEURS

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

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

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

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