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REVUE ROUMAINE DE BIOLOGIE

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SOMMAIRE

L'ANALYSE DES PHYTOCÉNOSES LIGNEUSES DE L'ALLIANCE *ALNO-ULMION* SUR LE TERRITOIRE DE LA ROUMANIE

V. SANDA, A. POPESCU

Le travail fait l'analyse des bocages appartenant à l'alliance *Alno-Ulmion* Br.-Bl. et Tx. ex Tschou 48 em. Müller et Görs 68 qui comprend jusqu'à présent 8 associations signalées sur le territoire de la Roumanie.

Les phytocénoses ligneuses de l'alliance *Alno-Ulmion* sont réparties surtout le long des rivières des régions des plaines, des collines et des montagnes ou sur les terrains dépressionnaires, périodiquement inondés le printemps. Les sols sont alluvionnaires, superficiels, mi-squellettiques ou squelettiques, gléisés et ici ce développe une flore qui se caractérise par de nombreuses espèces hygrophiles et sous-hydrophiles.

Alno-Ulmion Br.—Bl. et Tx. ex Tschou 48 em. Müller et Görs 68 (Syn. : *Alno-Padion* Knapp 42 ex Medwecka in Matuskiewicz et Borowik 57, *Alneto-Fraxinion* Scamoni et Pass. 59 p.p.).

Elle comprend les bocages d'aunes distribués le long des rivières ayant comme espèces caractéristiques : *Alnus glutinosa, A. incana, Prunus avium, Frangula alnus, Ulmus laevis, Carex brizoides, C. pendula, C. remota, Chaerophyllum hirsutum, Chrysosplenium alternifolium, Circaea luteiana, Equisetum hiemale, E. telmateia, E. sylvaticum, Festuca gigantea, Matteuccia struthiopteris, Thelypteris palustris, Myosoton aquaticum, Aegopodium podagraria.*

subal. Ulmion Oberd. 53

Fraxino-Ulmetum (Tx. 52) Oberd. 53

(Syn. : *Querco-Ulmetum* Issler 24, *Querco-Fraxinetum* Rudski 49, *Fraxino pannonicae-Ulmetum* Soó 60, *Ulmeto-Acereto-Fraxinetum* Păun (64) 66, *Carpino-Ulmetum carpinifoliae* (Meusel) Pass. 53).

C'est une association de plaine, la couche arborescente étant formée d'un mélange de frênes, aunes et chênes. I. Pop (1979) en analysant les phytocénoses de la forêt de Ciala (arrondissement d'Arad) constate que ces forêts de plaine, décrites sous différents noms, s'encadrent dans l'association *Fraxino-Ulmetum* (Tx. 52) Oberd. 53, qui ressemble très bien, de point de vue floristique, à celle de l'Europe centrale.

Les couches des arbres et des arbustes sont formée des espèces : *Fraxinus excelsior, Fr. angustifolia, Ulmus laevis, Quercus robur, Ulmus minor, Populus alba, P. nigra, Frangula alnus, Malus sylvestris, Acer campestre, Cornus sanguinea, Corylus avellana, Crateagus monogyna, Ligustrum vulgare, Prunus spinosa,* etc.

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Parmi les espèces herbacées, nous mentionnons : *Festuca gigantea*, *Carex brizoides*, *Galeopsis speciosa*, *Circaea lutetiana*, *Brachypodium sylvaticum*.

Dans l'association on a décrit les sous-associations suivantes : *quercetosum robori* I. Pop 79, *fraxinetosum angustifoliae* Oprea 76, I. Pop 79 (Syn. : *Alno-Fraxinetum angustifoliae muntenicum* Simon 60), *leucojetosum aestivi* Dobrescu et Vițalariu 79, *banaticum* Borza 62, *euonymetosum nani* Dobrescu 68.

En tant que faciès, nous signalons : *fraxinetosum (pojarkovianae)*, *aegopodietosum*, *caricetosum(pilosae)*, *poëtosum(silvicolae)*, *cardaminetosum(amarae)* Dobrescu 68, *rubietosum (caesii)* Dobrescu 68, Lupu 80, *sambucetosum nigrae*, *urticetosum dioicae* Lupu 80.

Fraxino(pallisae-angustifoliae)-Quercetum roboris Popescu, Sanda, Doltu 79

(Syn. : *Quercetum roboris romanicum* Sanda 70, *Ulmeto-Fraxinetum pallisae* (Borza 66) Sanda 70).

Elle est répandue dans le sud-est de la Roumanie et se développe sur les terrasses situées entre Călmățui et Buzău, sub un substrat bien approvisionné en eau, quelquefois même inondé, surtout le printemps.

La couche des arbres est formée de *Quercus robur*, *Ulmus minor*, *Fraxinus angustifolia* et *Fr. pallisae*, les deux dernières étant des espèces ponto-balkaniques plus thermophiles, adapteés sur les terrains en excès d'humidité (fig. 1).

Fig. 1. — Peuplement de *Fraxinus pallisae* et *Fr. angustifoliae* de la forêt de Spătaru (arrondissement de Buzău) inondé le printemps.

Les arbustes sont bien représentés, formant une couche assez compacte. Les espèces les plus représentatives sont : *Acer tataricum*, *Cornus sanguinea*, *Crataegus monogyna*, *Pyrus pyraster*, *Acer campestre*, *Frangula alnus*, *Ligustrum vulgare*, *Prunus spinosa*, *Euonymus europaeus*.

Fig. 2. — Aspect vernal avec *Leucojum aestivum* abondemment développé dans la forêt de Spătaru.

La couche herbacée est formée d'un grand nombre d'espèces mésophiles et sous hygrophiles, ces dernières favorisées par l'excès d'humidité du printemps. Parmi les espèces hygrophiles nous mentionnons : *Poa trivialis*, *Lythrum salicaria*, *Lysimachia nummularia*, *Leucojum aestivum* (fig. 2). Les plus nombreuses espèces mésophiles sont : *Asparagus officinalis*, *Polygonatum officinale*, *Melica uniflora*, *Brachypodium sylvaticum*, *Peucedanum latifolium*, *Lathyrus niger*, *Scutellaria altissima*, *Lamium purpureum*, *Serratula tinctoria*.

La sous-association *serratulo-peucedanosum* Sanda 70 se développe dans des microdépressions fangeuses pendant une bonne partie de l'année sur un sol de type bourbier. Les deux espèces qui caractérisent la sous-association, sont : *Serratula tinctoria* et *Peucedanum latifolium* qui se développent sur de sols mi-salés et humides.

La sous-association *fraxinetosum pallisae* (Krausch 65) comb. nova (Syn. *Fraxinetum pallisae* (Simon 60) Krausch 65, *Querco-Ulmetum* Issler 24 em. Soó 58 *leteense* Simon 60) est décrite dans le Delta du Danube, peuplant les parties plus basses des microdépressions de la forêt de Letea.

subal. **Alnion glutinosae-incanae** (Br.—Bl.15) Oberd. 53

La sous-alliance *Alnion glutinosae-incanae* groupe les associations d'aunes de l'étage montagnard et collinnaire où s'interpénètrent les deux espèces :

Alnus glutinosa et *Alnus incana* auprès desquelles on rencontre des espèces caractéristiques, comme : *Doronicum austriacum*, *Equisetum sylvaticum*, *Matteuccia struthiopteris*.

Stellario nemori-Alnetum glutinosae (Kästner 38) Lohm. 57

(Syn. : As. *Alnus glutinosa-Salix purpurea* Paucă 41, *Aegopodio-Alnetum praecarpaticum* Kárpati V., Kárpati I. et Jurko 63, *Alnetum glutinosae* Meijer-Drees 36).

Représente les bocages riverains édifiés par *Alnus glutinosa* qui se rencontrent le long des rivières des régions collinnaires et montagnardes. La composition floristique de l'association est riche et hétérogène. Cette hétérogénéite est le résultat de l'altitude à laquelle se trouve les phytocénoses ainsi que du facteur hydrique (le régime d'humidité).

Les espèces de reconnaissance de l'association sont : *Alnus glutinosa*, *A. incana*, *Fraxinus excelsior*, *Ulmus laevis*, *Frangula alnus*, *Humulus lupulus*, *Stellaria nemorum*, *Aegopodium podagraria*, *Matteuccia struthiopteris*, *Salvia glutinosa*, *Circaea lutetiana*, *Stachys sylvatica*, *Festuca gigantea*, *Carex remota*, *Equisetum telmateia*, *Geranium phaeum*, *Athyrium filix-femina*, etc. (tableau 1).

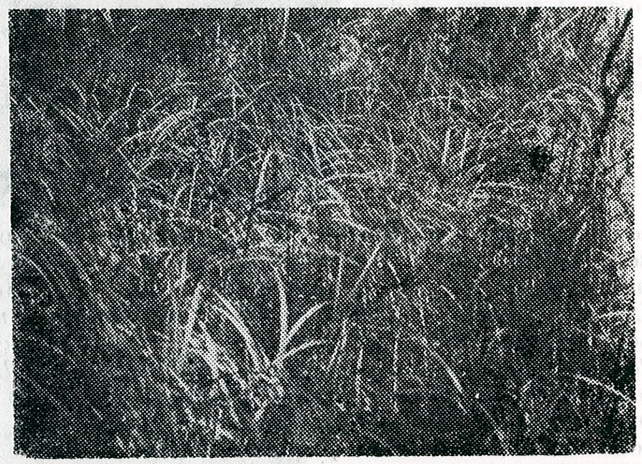

Fig. 3. — *Carex acutiformis* dominant dans la couche herbeuse des phytocénoses d'*Alnus glutinosa* dans la forêt de Chitila (NO de Bucarest).

Dans l'association sont décrites les sous-associations suivantes : *struthiopterietosum filicastri* Tx. 37, *festuco gigantei-salvietosum glutinosae* Turcu 70, *salicetosum purpureae* (Paucă 41) Popescu, Sanda, Doltu 79, *coryletosum avellanae* Coste 74, *piceetosum* Lungu 71, *ligulario-sphagnetosum (terris)* Lungu 71, *caricetosum brizoidis* Soó 62 (Syn. : *Alno-Caricetum brizoidis* Oberd. 53, *Carici (brizoidis)-Alnetum glutinosae* Horvat 38), *alnetosum incanae* Borza 59 (Syn. : *Alnetum incanae carpaticum* Klika 36, *Alnetum glutinosae-incanae* auct. roman. non Br.-Bl. 15, 30, *Telekio speciosae-Alnetum incanae* Coldea 90).

Tableau 1

Stellario nemori-Alnetum glutinosae (Kästner 38) Lohm. 57

N° de colonne	1	2	3	4	5	6	7	8	9	10	11
	5			10		18	10	17	10	16	10
Stellario-Alnetum et Alno-Ulmion											
Stellaria nemorum	II	V	V	III			III	III		I	I
Anus glutinosa	V	V	V	V	V	V	V	V	V	V	V
Alnus incana		II		III	III	I			II		
Fraxinus excelsior									I	I	II
Ulmus laevis	II		III		I					I	III
Frangula alnus	II				V	I			I	I	
Humulus lupulus	II	I		III			I	I	II	I	III
Aegopodium podagraria		I		V	II	III	III	IV	IV	V	
Matteuccia struthiopteris	III			II		V	III		III		
Salvia glutinosa	I			I	V	III	IV	I	III	I	II
Impatiens noli-tangere	III	III		I		III			I		III
Circaea lutetiana		II			II	II	III		III	I	III
Stachys sylvatica		IV		I				I		I	III
Chrysosplenium alternifolium				III			I		II	I	
Festuca gigantea		I			II	III	I		I	II	II
Caltha laeta		III									
Carex remota		III					III	III	III	I	I
Equisetum telmateia				III							I
Rumex sanguineus		II		I			I			I	
Galeopsis speciosa		III									
Geranium phaeum		III		II					I	II	
Athyrium filix-femina	III	III							III		II
Thalictrum aquilegiifelium	II										
Prunus padus				I						I	
Elscholtzia ciliata			I								
Ulmus glabra					I					I	
Vitis sylvestris											I
Carex pendula										I	I
Lysimachia nummularia		III	IV								
Equisetum hiemale											I
Dryopteris cristata											I
Equisetum sylvaticum		I									
Fagetalia											
Carpinus betulus	I			I		I				IV	III
Fagus sylvatica										I	I
Asarum europaeum	II	III		III			I	I		III	I
Cardamine impatiens	I	I		I			I		I	II	I
Scrophularia nodosa	I			II	I		I	V	II	I	
Geranium robertianum	I	V		II	II		IV		III	II	III
Euphorbia amygdaloides		II				I				II	I
Oxalis acetosella	I	III					I				I
Lamium galeobdolon	I			I							I
Lamium maculatum	II	I		I							
Dryopteris filix-mas	I	III		II			II			II	
Pulmonaria officinalis				II	II		I			II	I
Carex sylvatica					II						I
Physalis alkekengi					I		I	I		I	
Isopyrum thalictroides				II						I	

Tableau 1 (suite)

N° de colonne	1	2	3	4	5	6	7	8	9	10	11
Stellaria holostea				III							
Symphytum tuberosum				II			I				
Acer pseudoplatanus		III								I	I
Tilia cordata											I
Adoxa moschatellina				II			III				
Corydalis solida				I							
Sanicula europaea		III								I	
Symphytum cordatum								II			
Rubus hirtus		III		III			I				
Milium effusum				II						I	
Petasites albus		II					I		I		
Melica uniflora											I
Mercurialis perennis				II							
Allium ursinum										III	
Asperula taurina										I	
Helleborus odorus										I	
Epipactis helleborine							I			I	

I : *Stachys alpina* (1), *Daphne mezereum* (4), *Paris quadrifolia* (4), *Hepatica transilvanica* (4), *Epilobium montanum* (2), *Actaea spicata* (4), *Helleborus purpurascens* (4), *Cardamine glanduligera* (4), *Dentaria bulbifera* (10), *Galanthus nivalis* (10), *Ruscus aculeatus* (10), *Corydalis cava* (10), *Dryopteris robertiana* (7), *Myosotis sparsiflora* (6), *Viola mirabilis* (6) ;

Querco-Fagetea

Acer campestre	I	III		I						IV	III
Corylus avellana	I	III		I	III	III				IV	III
Cornus sanguinea	I			I	V					III	III
Sambucus nigra		IV		III		III		I	I	I	III
Crataegus monogyna	I	III	V		IV			II		I	III
Rosa canina	I						I				I
Viburnum opulus		IV	IV		I						II
Euonymus europaea		IV	V	I	II					I	I
Ligustrum vulgare					II			I		I	I
Clematis vitalba			V		IV	I	I		I	IV	III
Hedera helix	I										I
Brachypodium sylvaticum	I	IV			IV	III	I	IV		I	I
Fragaria vesca	I	IV		I		II	I				I
Poa nemoralis		III		III				III	I	I	
Lapsana communis		III		I	I			IV	I		
Viola reichenbachiana	I	I					I				
Moehringia trinervia					I	I					
Mycelis muralis				II		III			II		
Glechoma hirsuta						I			III		
Ficaria bulbifera		II	V	III		III			I		
Anemone ranunculoides				I						III	
Dactylis polygama				II	I						
Arum orientale					I	I				IV	
Veronica chamaedrys		I		I							
Pyrus pyraster	I									I	II
Populus tremula						I					II
Prunus spinosa	I		IV					I			
Anemone nemorosa	I		I	III	II		I				
Clinopodium vulgare		II						III			

I : *Acer tataricum* (5), *Rubus idaeus* (7) *Tamus communis* (5), *Crataegus oxyacantha* (11), *Scilla bifolia* (10).

Salicetea purpureae

Salix alba		I		I	III	I	IV	I	I		I
Salix fragilis			IV	I		I			II		

Tableau 1 (suite)

N° de colonne	1	2	3	4	5	6	7	8	9	10	11
Populus nigra					III			V	I	I	I
Populus alba					III	I	I				
Salix purpurea	I				I			I		I	III
Rubus caesius	IV	II			IV	I	I	V		IV	
Sapanaria officinalis										II	
Salix cinerea		I							III		III
Salix triandra					I				I	I	
Phragmitetea + Bidentetea											
Phragmites australis								II			
Lythrum salicaria	III							V			II
Myosolon aquaticum				II	I						
Lycopus europaeus	V	I		II	II		II		IV		
Mentha aquatica	IV				II		I		II		
Polygonum hydropiper	I				II			I	I		
Bidens tripartita	I				I			III			
Polygonum lapathifolium		I	III								
Mentha pulegium						I	I			I	

1 : *Lycopus exaltatus* (8), *Symphytum officinale* (6).

Artemisietea

Urtica dioica	III	IV	II	IV	I	I	III	IV	I		V
Eupatorium cannabinum	I			I	II	I			III	I	III
Calystegia sepium	III										I
Geum urbanum	I	III	IV	I	IV	III	II	II	I	III	I
Sambucus ebulus		II				III	I	IV	I		I
Glechoma hederacea	IV	III	IV	IV	III		III		III	III	I
Tussilago farfara	II	II		I	III		III			I	I
Galium aparine						IV			III		II
Alliaria petiolata	I			I				I			I
Stellaria media	I				II	IV	II	II			
Arctium lappa		II		II	I			III	III		I
Anthriscus sylvestris	I					I				II	
Erigeron annuus	I				I		I				
Telekia speciosa		III		III					I		
Cheliodonium majus									I	I	
Heracleum sphondylium			III	I		I				I	
Parietaria officinalis										III	
Valeriana officinalis					II				III		II
Equisetum arvense			II		II						
Rumex obtusifolius				I							I
Poa annua				IV			III			I	
Scutellaria galericulata									III		
Galeopsis speciosa					I					II	
Bilderdykia dumetorum										I	
Verbascum nigrum									II		
Artemisia absinthium		I							I		
Dipsacus sylvester											II
Conium maculatum									III		
Verbena officinalis									II		
Carpesium cernuum					III						

Molinio-Arrhenatheretea (incl. Filipendulo-Petasition)

Ranuculus repens	III	V		III	II	III	IV	V	IV		IV
Potentilla reptans	II	I		I	II		II	II		I	II
Prunella vulgaris	III	II			III	II	V				I
Agrostis stolonifera				III	I		III	I	IV		I
Petasites hybridus											V
Cirsium oleraceum		III				I			I		

Tableau 1 (suite)

N° de colonne	1	2	3	4	5	6	7	8	9	10	11
Galeopsis tetrahit				II		II					
Poa trivialis				I	I				III		I
Trifolium repens	V						III				
Caltha palustris				II							III
Mentha longifolia		II	IV	III				IV	III		III
Taraxacum officinale		II		V		II	I	II			
Poa pratensis		V			I	III	II	III	I		
Pteridium aquilinum	II										I
Rumex conglomeratus								V			
Hypericum tetrapterum											III
Epilobium hirsutum											V
Cirsium palustre					II						
Rumex crispus											II
Epilobium adnatum	II										
Geum rivale				II							
Cardamine pratensis				III			I				
Ajuga reptans	I			I		I					
Plantago major					II			IV			
Achillea millefolium		II		V	I			IV			II
Myosotis scorpioides	I					I	II	I			II
Equisetum palustre								IV			I
Stellaria graminea		II									
Scirpus sylvaticus				I							I
Bellis perennis					I					I	
Lolium perenne		I					II		I		
Galium palustre								III			III
Plantago lanceolata		I									I
Campanula rapunculoides						I	I				I
Cerastium holosteoides			III	I							
Angelica sylvestris	II				I						
Ranunculis acris					I						I
Campanula patula		III						I			
Trifolium pratense		I				I					
Carex vulpina							III				
Lychnis flos-cuculi								IV			I
Galium uliginosum				IV							
Daucus carota								III			

I : *Euphorbia stricta* (10), *Carum carvi* (2), *Juncus inflexus* (11), *Medicago lupulina* (2), *Rorippa, sylvestris* (7), *Deschampsia caespitosa* (1), *Agropyron repens* (2).

Compagnes											
Sonchus asper							III				
Chaerophyllum hirsutum	II										
Pulmonaria mollis	III										
Euphorbia cyparissias	II				II		II				
Abies alba	III										

I : *Sanguisorba minor* (2), *Potentilla anserina* (2), *Polygala vulgaris* (2), *Gentiana asclepiadea* (2), *Cicuta virosa* (2), *Cirsium arvense* (2), *Cardaminopsis arenosa* (2), *Origanum vulgare* (2), *Potentilla erecta* (2), *Juniperus communis* (2), *Trifolium medium* (2), *Cerinthe minor* (2), *Hypercum perforatum* (2), *Hieracium pilosella* (2).

II : *Plantago media* (2), *Cruciata glabra* (2), *Cynoglossum officinale* (2), *Senecio vernalis* (2), *Stachys germanica* (2), *Cynosurus cristatus* (2), *Lithospermum oficinale* (2).

L'endroit de réalisation des relevés : 1 — Raţiu O., Gergely, I., 1979, Ţara Oaşului ; 2 — Chifu Th. et collab., 1973, Valea Nemţişorului ; 3 — Mititelu D. et collab., 1971, Bazinul Trotuşului ; 4 — Kovács A., 1979, Monts Bodoc ; 5 — Dihoru Al., 1976, Département de Prahova ; 6 — Turcu Gh., 1970, La région entre Argeş—Rîul Doamnei ; 7 — Sanda V. et al., 1970, Valea Oltului—Călimăneşti ; 8 — Zaharia I., 1972 — Le bassin Gilort ; 9 — Boşcaiu N., 1970, Les Monts Ţarcu—Godeanu ; 10 — Coste I., 1975, Les Monts Locva ; 11— Paucă A., 1941, Les Monts Codru—Muma ;

En tant que faciès sont décrites celles de : *Cornus sanguinea* ssp. *australis*, *Equisetum telmateia*, *Anemone ranunculoides*, *Rubus caesius*, *Glecoma hederacea*, *Pteridium aquilinum*, *Urtica dioica*, *Sambucus ebulus*, *Poa annua*, *Lolium perenne* Coste 80, *euonymetosum nanae*, *salicosum*, *typhoidosum*, *violo-tozziosum*, *caricosum elongatae* Lungu, 71, *oreopteriodosum* Raţiu 70. On signale aussi dans l'association la variante régionale de *spiraeaetosum salicifoliae* Fl. Raţiu 68.

Alno incanae-Syringetum josikaeae (Borza 65 n.n). Raţiu et al. 84

C'est une association peu étudiée chez nous, l'espèce caractéristique *Syringa josikaea* étant connue seulement dans les Monts Apuseni. L'association se développe le long des vallées dans des conditions écologiques favorables pour le développement des espèces caractéristiques de l'alliance *Alno-Ulmion*. De la sorte, dans ces phytocénoses apparaissent : *Aconitum calybotryon*, *Carex brizoides*, *Angelica sylvestris*, *Stellaria nemorum*, *Dipsacus pilosus*.

Etant située à l'étage du hêtre, dans cette association apparaissent plusieurs éléments de *Fagetalia* et *Querco-Fagetea* parmi lesquelles, les plus représentatives sont : *Asarum, europaeum, Athyrium filix-femina. Mercurialis perrenis*, *Lamium maculatum Lamiastrum galeobdolon*, *Silene heuffelii*, *Milium effusum*, *Dryopteris cristata*, *Melica nutans*, *Geranium robertianum*, *Galium schultesii*.

La couche des arbustes, bien développée, est formée de : *Daphne mezezereum*, *Ribes uva-crispa*, *Corylus avellana*, *Sambucus nigra*, *Rosa spinosissima*, etc.

Carici remotae-Fraxinetum Koch ex Faber 36

(Syn. : *Alnetum glutinosae-incanae caricetosum remotae* Klika 41, *Alnetum glutinosae caricetosum remotae* Zólyomi 43).

L'association est peu connue chez nous. Dans la littérature elle est citée par R. Soó (1947, 1951), B. Zólyomi (1943) et N. Boşcaiu (1971).

Les phytocénoses de *Fraxinus excelsior* se développent auprès des rochers, formant des bocages où participent encore *Alnus incana* ainsi que *Tilia cordata*, *Ulmus glabra*, *Acer pseudoplatanus*. L'édificatrice de l'association est conditionnée par le substrat édaphique, prenant naissance dans les vallées montagnardes, sur les sols humides, argileux, riches en humus, là où se produisent les processus de glaisage.

La sous-association *alnetosum* Soó 63 est mentionnée dans les montagnes de Siriu (Gh. Dihoru, 1975) selon un relevé insignifiant, qui peut être compris dans l'association *Stellario nemori-Alnetum glutinosae*.

Carici acutiformi-Alnetum (Dostál 33) Soó 63

Se développe sur les terrains dépressionnaires, périodiquement inondés le printemps. Ce type de peuplement est caractéristique pour les cours lents des rivières des plaines, avec beaucoup de méandres, qui inondent le printemps les lacs dépressionnaires des tournants du lit. L'eau d'inondation et celle résultée de la nappe phréatique située à la surface, produit l'embourbement de ces terrains, favorisant le développement des

espèces *Alnus glutinosa, Salix fragilis, Ulmus laevis, Fraxinus angustifolia, Fraxinus pallisae* qui forment la couche arborescente bien définie.

La couche arbustive est dominée par *Viburnum opulus, Frangula alnus, Cornus mas, Crataegus monogyna, Corylus avellana, Cornus sanguinea, Sambucus nigra, Rubus caesius.*

L'humidité accrue du sol favorise l'installation de nombreuses espèces ligneuses, dans la plupart hygrophiles et sous-hygrophiles dont les plus caractéristiques sont : *Carex acutiformis* (fig. no 3), *C. riparia, Lycopus europaeus, Oenanthe equatica, Poa palustris, Caltha palustris, Polygonum hydropiper.*

L'association est connue en Munténia et Moldova.

Quercetum robori-pedunculiflorae Simon 60

Est décrite dans le Delta du Danube (Hasmacul-Omer, la forêt de Letea) où elle occupe les endroits les plus hauts du microrelief entre les dunes de sable, là où l'eau stangne pendant une courte période. Les endroits bas, excessivement humides longtemps, sont peuplés par *Fraxinus pallisae.*

Les espèces de reconnaissance de l'association sont : *Quercus pedunculiflora, Q. robur, Fraxinus pallisae,* et comme différentielles locales *Periploca graeca* et *Vitis sylvestris.* Dans les phytocénoses de *Quercus pendunculiflora* et *Q. rubor* on rencontre de nombreuses espèces caractéristiques à l'alliance *Alno-Ulmion* et à la classe *Querco-Fagetea*, par exemple : *Populus alba, Viburnum opulus, Rubus caesius, Symphytum officinale, Eupatorium cannabinum, Pyrus pyraster, Cornus sanguinea, Crategus monogyna, Epipactis helleborine, Lysimachia nummularia.*

La présence des microdépressions comprenant des accumulations d'humidité ou d'eau permanente ainsi que l'existence de la nappe phréatique peu profonde déterminent l'installation de nombreuses espèces hygrophiles et sous-hygrophiles dans ces phytocénoses.

Pruno-Fraxinetum Oberd. 53

Est mentionnee par Att. Szabó (1971) dans la région de Sărăţel—Chiraleş—Lechinţa. Le manque de données au sujet de cette association tur le territoire de notre pays nous fait douter de l'existence de ce cénotaxon dans la végétation de la Roumanie.

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COENOTAXONOMY OF DECIDUOUS FORESTS BELONGING TO THE *QUERCO-FAGETEA* Br.-Bl. ET VLIEGER 37 CLASS IN ROMANIA

A. POPESCU, V. SANDA

The paper analyzes a number of 14 forestry associations belonging to the *Querco-Fagetea* class; their taxonomic framing their characterization of the floristic structure of the arboretum as well as a synonymization of numerous coenotaxons is achieved succeeding thus a uniform dealing with deciduous forests in Romania.
The coenotaxonomy of the beech forests in Romania (1989) as well as the study of the groupings belonging to the *Alno-Ulmion* alliance (1992) made the object of two separate papers.

The *Querco-Fagetea* Br.-Bl. et Vlieger 37 (Syn.: *Carpino-Fagetea* Jakucs 60) class gathers the mesophyll deciduous forests on eutrophic and mesotrophic soils in the mountainous and sloping area and are well represented on both sides of the Carpathians. Towards the superior altitudes, the forests belonging to the *Querco-Fagetea* class are interpenetrating the spruce forest and towards the inferior altitude, with the thermophilous oak forests.

The characteristic species for the class are (apud R. Soó 1968 and N. Boşeaiu, 1971): *Acer campestre*, *A. platanoides*, *Arum maculatem*, *Astragalus glycyphyllos*, *Brachypodium sylvaticum*, *Bromus benekeni*, *Campanula rapunculoides*, *Cephalanthera longifolia*, *Tanacetum corymbosum*, *Clematis vitalba*, *Cornus mas*, *Corylus avellana*, *Crataegus monogyna*, *C. oxyacantha*, *Convallaria majalis*, *Cruciata glabra*, *Dactylis polygama*, *Digitalis grandiflora*, *Dryopteris filix-mas*, *Epipactis helleborine*, *Euonymus verrucosus*, *Galium schultesii*, *Geranium robertianum*, *Glechoma hirsuta*, *Geum urbanum*, *Hedera helix*, *Hypericum hirsutum*, *Melica nutans*, *M. uniflora*, *Moehringia trinervia*, *Neottia nidus-avis*, *Platanthera bifolia*, *Ligustrum vulgare*, *Poa nemoralis*, *Polygonatum latifolium*, *P. multiflorum*, *Pulmonaria officinalis*, *Pyrus pyraster*, *Quercus petraea*, *Q. robur*, *Sorbus torminalis*, *Staphylea pinnata*, *Scropularia nodosa*, *Solidago virgaurea*, *Symphytum tuberosum* ssp. *nodosum*, *Vicia dumetorum*, *V. sylvatica*, *Viola odorata*, *V. reichenbachiana*, etc.

FAGETALIA SYLVATICAE Pawl. 28

This order includes the deciduous forests on the moist soils, moderate wet or even wet, spread on the fieldgrowing layer up to the mountainous ones. The characteristic species of the order are most numerous, so we mention the most characteristic ones: *Adoxa moschatellina*, *Actaea spicata*, *Aegopodium podagraria*, *Allium ursinum*, *Anemone nemorosa*, *Asarum europaeum*, *Carex sylvatica*, *Chaerophyllum aromaticum*, *Circaea lutetiana*, *Corydalis solida*, *Cardamine bulbifera*, *Euphorbia amygdaloies*, *Fagus sylvatica*, *Festuca drymeia*, *Galium odoratum*, *Gymnocarpium dryopteris*, *Helleborus purpurascens*, *Hepatica nobilis*, *Lamiastrum galeobdolon*, *Lamium maculatum*, *Lathyrus vernus*, *Mercurialis perennis*, *Milium effusum*, *Myosotis sylvatica*, *Oxalis acetosella*, *Salvia glutinosa*, *Sanicula europaea*.

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Symphyto-Fagion Vida 59

(Syn. : *Fagion dacicum* Soó 64, *Fagion carpaticum* Moor 52 p.p.)

The beech trees in the Romanian Carpathians constitute a synchorologic complex well individualized within the European beech forests which enabled the outlining of some distinct associations. The alliance comprises a recognition species nucleus well individalized such as : *Symphytum cordatum*, *Cardamine glanduligera*, *Hepatica transsilvanica*, *Hieracium rotundatum*, *Silene heuffelii*, *Moehringia pendula*, *Primula leucophylla*, *Pulmonaria rubra*, *Ranunculus carpaticus*, *R. flabellifolius*, *Saxifraga heuffelii*, *Verbascum abietinum*, *Galium kitaibelianum*.

subal. **Moehringio muscosae-Acerenion** Boşcaiu (79) 82

(Syn. : Tilio-Acerion Klika 55, *Acerion dacicum* Soó 64, Moehringio-Acerion Boşcaiu 79, *Acerion pseudoplatani* Oberd. 57).

Differential species : *Aruncus sylvester*, *Lunaria rediviva*, *L. annua* ssp. *pachyrhiza*, *Moehringia muscosa*, *M. pendula*, *Phyllitis scolopendrium*, *Polystichum lobatum*.

1. **Spiraeo-Coryletum** Ujv. 44

(Syn. : As. *Spiraea ulmifolia-Corylus avellana* Ujv. 44, *Corylo-Tilietum* Vida 59, *Corylo-Euonymetum verrucosae* Dihoru 75, *Spireetum ulmifoliae* Zólyomi 39).

The *Corylus avellana* and *Spiraea chamaedryfolia* phytocoenosis on the rocky regions are little studied in our country. In their floristic composition we may meet species characteristic to the *Moehringio muscosae-Acerenion* Boşcaiu (79) 82 suballiance such as : *Moehringia muscosa*, *Tilia cordata*, *Actaea spicata*, *Fraxinus excelsior*, *Ulmus glabra*, *Polystichum braunii*, *Acer pseudoplatanus*, *Poa nemoralis*, etc., which justifies their inclusion in the above mentioned suballiance.

The characteristic species of the order and alliance met within these phytocoenoses are : *Dryopteris filix-mas*, *Pulmonaria rubra*, *Saxifraxa cuneifolia*, *Asarum europaeum*, *Fagus sylvatica*, *Mercurialis perennis*, *Athyrium filix-femina*, *Rubus hirtus*, *Scrophularia nodosa*, etc.

The *Spiraea* and *Corylus* shrubs occupy the rocky or stony sides which explains the presence of the saxicolous species. Sometimes, the *Euonymus verrucosus* species develops abundantly creating a facies.

2. **Acereto-Ulmetum** Beldie 51

(Syn. : *Aceretum pseudoplatani carpaticum* Sillinger 33, *Phyllitidi-Aceretum* Moor 52).

It is met along the quay with diffused light and accentuated atmospherical humidity, on calcareous detrius, on rendzino-type soils very rich in humus. The association comprises arboreti where *Acer pseudoplatanus*, *Ulmus glabra*, more rarely *Fagus sylvatica*, *Fraxinus excelsior*, *Abies alba*, *Picea abies*, etc.

The bushy layer is made of *Corylus avellana*, *Ligustrum vulgare*, *Euonymus europaeus*, *Rhamnus cathartica*.

The graminous species are those characteristic to the alliance and suballiance from which we mention : *Phyllitis scolopendrium*, *Lunaria rediviva*, *Polystichum setiferum*, *Moehringia muscosa*, *Cystopteris fragilis*, *Silene heuffelii*, *Scutellaria altissima*, *Helleborus purpurascens*, *Cardamine glanduligera*, *Senecio nemorensis* ssp. *fuchsii*, *Primula columnae*, *Aremonia agrimonioides*, *Potentilla micrantha*, *Gymnocarpium robertianum*, etc.

Within the associations three subassociations are described : *grossularietosum* Morariu et al. 67, *subcarpaticum* Soó 57 and *coryletosum colurnae* Borza 58.

subal. **Staphyleo-Tilienion platyphylli** Täuber 86

It groups the lime forests characterized by a series of differential Dacian-Balkan species such as : *Staphylea pinnata*, *Festuca drymeia*, *Glechoma hirsuta*, *Aremonia agrimonioides*, *Lathyrus venetus*.

3. **Carpino-Tilietum platyphylli** Täuber 86

The arboretum in the inferior course of the Mureş reaches the height of 10—18 m having a high consistency of 0.7—1.0.

The grasssy species with more significant abundance-dominance indexes met with this arboretum are *Festuca drymeia*, *Carex sylvatica*, *Galium odoratum*.

For the subassociation *aceretosum tatarici* Täuber 86 the differential species are : *Acer tataricum*, *Cornus mas*, *Quercus cerris*, *Primula acaulis*, which miss in the *typicum* subassociation.

4. **Staphyleo-Tilietum platyphylli** Täuber 86

For the *Tilia platyphyllos* and *Staphylea pinnata* arboretum the constant presence of the species *Ruscus aculeatus* is characteristic and forms a specific facies.

Among the grassy species with more significant abundance-dominance indexes we may mention : *Carex pilosa*, *Lathyrus vernus*, *Hedera helix*.

subal. **Epipactido-Fagenion** Boşcaiu et al. 82

It presents a coenotic cohesion and expressivity less evident than its Central-European vicarials *Cephalanthero-Fagion* Tx. 55 that comprises the thermophilous groups formed on calcareous substrate. Differential species : *Cephalanthera damassonium*, *C. longifolia*, *C. rubra*, *Tanacetum corymbosum*, *Campanula persicifolia*, *Vincetoxicum officinale*, *Epipactis atrorubens*, *E. helleborine*, *E. microphylla*.

5. **Polysticho (setifero)-Tilio-Fraxinetum** Soó 64

(Syn. : *Tilio-Fraxinetum* auct. roman. non Zólyomi 36, *Tilio-Fraxinetum transsilvanicum* Soó 50, Gergely 62).

The association develops on a rocky substrate on fixed detritus, on fast slopes with different expositions. The soil is thin, discontinuous and heaps mostly in cracks and on the shelves of the calcareous rocks. The tree layer is weakly developed with large glades, the individuals present contorsioned growing and the coronet has dried branches. The

most representative species are : *Tilia cordata, Fraxinus excelsior, Quercus petraea, Q. pubescens, Acer pseudoplatanus, Sorbus cretica.*

The bushes are present by : *Coloneaster integerrima, Spiraea chamaedryfolia, Rhamnus tinctoria, Euonymus verrucosus, Berberis vulgaris, Cornus mas, Crataegus monogyna.*

The grassy species make up a vegetation carpet well developed and the most characteristic are : *Poa nemoralis, Hierochloe australis, Sesleria heuffleriana, Helictotrichon decorum, Stellaria holostea, Galium album, Campanula trachelium, Phyllitis scolopendrium.*

It may be established that the structure and floristic composition of the phytocoenoses of this association is most heterogenous comprising characteristic species both for *Fagetalia, Querco-Fagetea* and for *Quercetalia pubescentis* (the *Aceri-Quercion* alliance).

subal. **Tilio-Fagenion (or.-taur.-silv.)** Dobrescu et Kovács 73

It represents a suballiance of maximum interference with phytocoenoses poor in Dacian elements ; there appear balkan and oriental (anatolic elements in the exchange, more thermophilous with a clarifying rôle.

6. **Querco petraeae-Tilio-Carpinetum** Dobrescu et Kovács 73

It develops on the sunny sides of the mountain, on grey-brown forest soil, weakly podzolized, light up to moderate acid. Edifying and recognition species : *Quercus petraea, Tilia tomentosa, Carpinus betulus, Cardamine quinquefolia, Melica nutans, Carex brevicollis, Asparagus tenuifolius.*

The phytocoenoses present the floristic structure made of (in its great majority) elements characteristic for the *Fagetalia* order and the *Querco-Fagetea* class but the existence of a species nucleus characteristic to the *Quercetea pubescenti-petraeae* class prints a moderate xerothermic-continental character to the association and an affinity with the other Est-Europran coenotaxons.

The tree layer is dominated by *Quercus petraea* among which *Quercus polycarpa, Q. dalechampii, Tilia tomentosa, Carpinus betulus, Acer campestre, Quercus robur, Sorbus torminalis, Fraxinus excelsior, Fr. coriariifolia, Ulmus minor*, may be met.

The grassy species that compose a well developed carpet are represented by : *Dactylis polygama, Cardamine quinquefolia, C. bulbifera, Carex brevicollis, Lathyrus venetus, Lithospermum purpureocaruleum, Melica nutans, Oryzopsis virescens, Laser trilobum*, etc.

The great number of thermophilous species of South origin differentiate the Moldovian phytocoenoses from those of the Central Europe.

The subassociations described : *poëtosum, melicetosum* and *caricetosum* Dobrescu et Kovács 73.

7. **Querco robori-Tilio-Carpinetum** Dobrescu et Kovács 73

(Syn. : *Querco robori-Carpinetum* auct. mold. non Soó et Pócs 57, As. *Quercus robur-Carpinus betulus-Tilia tomentosa* Dobrescu et Eftimie 66).

It is an association less spread in the Central Moldavian plateau than *Querco petraeae-Tilio-Carpinetum* but it appears well outlined. Its phytocenoses inhabit the sides of the mountains with reduced slopes sometimes on plateaus. It develops on rich soils, brown grey, humiferous with neutral reaction up to slightly acid.

The arborescent layer is made of *Quercus robur, Tilia argentea, T. cordata, Carpinus betulus, Fraxinus excelsior, Quercus pedunculiflora, Q. petraea, Ulmus minor.*

The bushes are well represented by the species : *Corylus avellana, Salix caprea, Viburnum lantana, Staphylea pinnata, Crataegus monogyna, Euonymus europaeus, Ligustrum vulgare, Cornus sanguinea, Euonymus verrucosus, Sambucus nigra.*

The grass-like forest, well developed, is represented by the following species : *Arum orientale, Corydalis bulbosa* ssp. *marschalliana, Fritillaria montana.*

Within the phytocoenoses considered relict, a significant fidelity is evidenced by *Euonymus nana* and *Primula vulgaris.* The following subassociations are evidenced within the association : *euonymetosum, primuletosum* and *melicetosum* Dobrescu et Kovács 73.

subal. **Lathyro hallersteinii-Carpinenion** (Boşcaiu 79) Boşcaiu et al. 82 (Syn. : *Carpinion betuli (Issler 31 p.p.)* Soó 62, *Carpinion* Oberd. 53, *Carpinion dacicum* Soó 62, *Lathyro halersteinii-Carpinion* Boşcaiu 79).

It comprises the *Carpinus betulus* associations and their mixture with *Quercus* or *Fagus* species. Recognition species : *Helleborus odorus, H. purpurascens, Lathyrus hallersteinii, Melampyrum bihariense* ssp. *römeri.*

8. **Querco robori-Carpinetum** Soó et Pócs (31) 57

(Syn. : *Querceto-Carpinetum* Tx. 30, 37 ; *Stellario-Carpinetum* Oberd. 57 ; *Carpino-Quercetum roboris* Borza 41, Csűrös et Kovács 62 ; *Quercetum roboris banaticum* Borza 62 ; *Evonymo nanae-Carpinetum* (Borza 31) Seghedin et al. (77) 78).

The association is spread in the field and hill area being characteristic to flat places, plateaus, on deep soils, typically mesophyll. The arborescent layer is dominated by *Quercus robur* and *Carpinus betulus* achieving a covering of 80—90% along which we may also find : *Fagus sylvatica, F. taurica, Prunus avium, Acer campestre, Malus sylvestris, Pyrus pyraster*, etc.

The bushes are present by the species : *Cornus sanguinea, Crataegus monogyna, Euonymus europaeus, Ligustrum vulgare, Clematis vitalba*, that achieve a cover of up to 30%. The grass-like cover is well represented by the species : *Asperula taurina, Anemone nemorosa, Aegopodium podagraria, Brachypodium sylvaticum, Carex pallescens, C. brizoides, Dactylis polygama, Helleborus odorus, Lamiastrum galeobdolon, Lamium maculatum, Lathyrus vernus, Lychnis flos-cuculi, Lysimachia nummularia, Melica uniflora, Polygonatum latifolium, Pulmonaria officinalis, Ranunculus auricomus, R. ficaria, Stellaria holostea, S. media, Scutellaria altissima, Scilla bifolia*, etc.

The following subordinated units are described : *moldavicum* Bârcă 73, *dacicum* Erika Schneider-Binder 73 (Syn. : *Querceto-carpinetum dacicum* Borza 31 apud Paşc. 43), *stachyetosum* Soó 58, facies with *Carex*

brizoides Harghitai 39, *corydaletosum* (Issler 36) Tx. 37 (Syn. : *Querco-Carpinetum corydaletosum* (Issler 36) Tx. 37, *austrotranssilvanicum* Borza 59 (Syn. : *Querco-Carpinetum* Tx. 37 *austrotranssilvanicum* Borza 59), *banaticum* Borza 62 (Syn. : *Querco-Carpinetum banaticum typicum*, *aceretosum* et *fraxinetosum* Paşc. 43), *coryletosum colurnae* Borza 58 (Syn. : *Querco-Carpinetum coryletosum colurnae* Borza 58), *ulmetosum* Borza 58 n.n. (Syn. : *Querco-Carpinetum ulmetosum* (Borza 58) Dobrescu et al. 69, Mihai 73), *betuletosum* (Paşc. 438) Popescu, Sanda, Doltu 79 (Syn. : *Querceto-Carpinetum betuletosum* Paşc. 43), *fagetosum* (Lupu 80) comb. nova (Syn. : *Querco robori-Fagetum sylvaticae* Lupu 80), facies *betulosum pendulae* Lupu 80, facies, *corylosum avellanae* Lupu 80.

9. **Querco cerris-Carpinetum** Boşcaiu et al. 66

The *Carpinus betulus* and *Quercus cerris* phytocoenoses present resemblances both as concerns the floristic composition and as concerns the stational conditions with *Querco petraeae-Carpinetum* Soó et Pócs 57 association. To both associations the codominant clarifiers are distinct but both associations have a phytocoenotic accentuated convergence by the structural similitude too, fact for which they have been considered ecological vicarials, conditioned by the lithological layer (N. Boşcaiu et al. 1966).

The two edifying species : *Carpinus betulus* and *Quercus cerris* find themselves in different codominant relations. The arborescent level the species : *Tilia platyphyllos*, *Acer campestre*, *Sorbus torminalis*, etc. may still be found.

The bushy layer is made of : *Ligustrum vulgare*, *Viburnum lantana*, *Crataegus monogyna*, *Cornus sanguinea*, *C. mas*, *Rosa canina*, etc.

The grass-like cover is well represented by the species : *Stellaria holostea*, *Melampyrum bihariense*, *Helleborus purpurascens*, *Euphorbia amygdaloides*, *Mercurialis perennis*, *Cardamine bulbifera*, *Primula acaulis*, *Melica uniflora* , *Galium schultesii*, *Geum urbanum*, *Trifolium medium*, *Vincetoxicum officinale*.

Owing to the interference of the recognition species groups this association establishes the relation between *Lathyro hallersteini-Carpinenion* and *Quercion pubescenti-petraeae*.

10. **Galio kitaibekiani-Carpinetum betuli** Coldea et Adriana Pop 88.

Vegetates in the Cozia massif on the sunny sides with medium slope (15—30°) and with podzolic-feriiluvialls soils, weakly acid.

In the arborescent layer of the coenoses, dominate the *Quercus petraea*, *Q. polycarpa*, and *Carpinus betulus* in the subdominant species.

In the grassy layer, the Carpathian-Balkan species *Galium kitaibelianum* is noticed in a high amount. Beside it, other geographical differentials appear such as *Lathyrus hallersteinii*, *Primula veris* ssp. *columnae*, *Potentilla micrantha* and *Agrimonia agrimonioides* that print an evident thermophilous character to the association. Among the dominant grassy species we may remind of : *Festuca heterophylla*, *Poa nemoralis*, *Dentaria bulbifera*, *Dactylis polygama* as well acidophilous species : *Luzula luzuloides*, *Pteridium aquilinum* and *Calamagrostis arundinacea*.

11. **Melico uniflorae-Tilietum tomentosae** (Sanda et Popescu 71) corr. hoc loco (Syn. : *Tilietum tomentosae* Sanda et Popescu 71).

The association has been mentioned as nomen nudum in Snagov and Radu Vodă forests. It develops on a brown-reddish wood-weakly podzolized soil, with 5 to 11 m depth phreatic water. It is very spread in the forests around Bucharest, but in those of Moldavia, too.

Recognition species : *Tilia tomentosa*, *Melica uniflora*, *Tilia cordata*, *Campanula trachelium*, *Hedera helix*.

In the tree layer, species as : *Quercus robur*, *Carpinus betulus*, *Fraxinus excelsior*, *Ulmus minor*, *Acer campestre*, etc., still appear.

The bushes are well represented by : *Crataegus monogyna*, *Ligustrum vulgare*, *Euonymus verrucosus*, *E. europaeus*, *Corylus avellana*, *Acer tataricum*.

The grassy carpet is made of a rather big number of species among which the most representative are : *Carex pilosa*, *Dactylis polygama*, *Polygonatum latifolium*, *Brachypodium sylvaticum*, *Carex divulsa*, *Lathyrus niger*, *Asparagus tenuifolius*, *Cruciata laevipes*, *Asarum europaeum*, *Scutellaria altissima*.

12. **Querco petraea-Carpinetum** Soó et Pócs 57

(Syn. : *Querco-Carpinetum typicum* Tx. (30) 37 p.p. ; *Carpino-Quercetum petraeae* auct. rom. pro Borza 41 ; *Carici pilosae-Carpinetum* R. et Z. Neuh. 64, 67, 68, 70 ; *Primulae veris-Carpinetum* R. et Z. Neuh. 64, 68, 70).

These phytocoenoses are rather sppead in Romania especially in the hilly area ; they develop on little steep slopes with different expositions on brown soils, sometimes podzolized with clay or sandy-clay texture. The arboretum of this kind is also found at the contact between field and hills at 200—600 m altitude.

Except for the edifying species *Quercus petraea* and *Carpinus betulus* we may also find *Quercus robur*, *Acer tataricum*, *Quercus farnetto*, in the arborescent layer and *Fagus sylvatica* at higher altitudes.

On the South and eroded slopes we may meet the species *Quercus cerris* and *Fraxinus ornus*. In these phytocoenoses we may also find the species : *Acer platanoides*, *A. campestre*, *Tilia tomentosa*, *Fraxinus excelsior*, *Populus tremula* and *Quercus pedunculiflora*, these last ones in the South-east and Easten parts of Romania.

The subarboretum presents a reduced covering and is made out of : *Crataegus monogyna*, *Ligustrum vulgare*, *Cornus sanguinea*, *Corylus avellana* and more rarely *Prunus spinosa*, *Cornus mas*, *Euonymus europaeus*, *E. verrucosus*, *Staphylea pinnata*.

The grassy layer is represented by a great number of species among which the most representative are : *Galium schultesii*, *Cardamine bulbifera*, *Lathyrus vernus*, *L. niger*, *Brachypodium sylvaticum*, *Polygonatum latifolium*, *Anemone ranunculoides*, *Veronica officinalis*, *Potentilla micrantha*. On valleys and sides with high humidity, there appear : *Pulmonaria officinalis*, *Festuca sylvatica*, *Carex sylvatica*, *Euphorbia amygdaloides*, *Galium odoratum*, *Asarum europaeum* (Table 1).

Table 1

Querco petraeae-Carpinetum Soó et Pócs 57

Number of row	1	2	3	4	5	6	7	8	9	10
Number of survey	15	14	19	11	5	30	6	29	10	5
Char. ass.										
Carpinus betulus	V	V	V	V	V	V	V	V	V	V
Quercus petraea	V	V	V	V	V	V	V	V	V	V
Carpinion										
Galium schultesii	V	III	II	I	IV	II	II	I	I	II
Carex pilosa	III	—	V	—	V	IV	III	—	II	IV
Acer campestre	III	II	III	I	V	IV	IV	II	III	III
Campanula trachelium	II	—	I	III	IV	II	—	—	—	—
Carex digitata	I	—	I	III	V	IV	—	I	—	—
Digitalis grandiflora	I	I	—	II	—	—	—	—	—	—
Hepatica nobilis	III	I	I	I	—	II	—	—	—	I
Lamium maculatum	I	I	—	—	—	—	—	—	—	—
Lathraea squamaria	—	II	—	—	—	II	—	—	—	—
Ligustrum vulgare	II	I	—	III	IV	II	I	II	II	—
Melamphyrum bihariense	—	II	—	V	V	II	—	—	—	—
Prunus avium	IV	II	III	V	V	IV	III	I	III	III
Ranunculus auricomus	IV	—	I	V	—	III	—	I	—	V
Ranunculus cassubicus	II	II	—	—	—	—	—	—	—	—
Ficaria bulbifera	I	I	—	I	—	III	—	—	—	—
Scilla bifolia	—	—	—	—	IV	II	—	I	—	—
Stellaria holostea	II	—	III	IV	V	V	II	—	II	II
Tilia cordata	III	—	I	V	IV	IV	—	I	III	II
Vicia pisiformis	I	—	—	III	—	—	—	—	I	—
inca minor	—	—	—	III	—	—	—	—	I	—
Viola mirabilis	I	—	—	IV	—	II	—	—	—	—
I: *Erythronium dens-canis* (8), *Ulmus minor* (3), *Vicia dumetorum* (1).										
IV: *Melampyrum nemorosum* (5), *Dactylis polygama* (9), *Carex umbrosa* (4),										
Symphyto-Fagion										
Aposeris foetida	II	II	—	—	—	—	—	—	—	—
Asarum europaeum	II	I	III	—	V	IV	—	I	—	—
Dentaria glandulosa	I	—	—	II	—	IV	—	—	—	—
Cephalanthera longifolia	III	I	—	I	—	II	—	—	—	—
Hedera helix	I	—	II	—	II	III	—	—	—	I
Lathyrus venetus	IV	III	—	—	—	—	—	—	I	—
Luzula luzuloides	II	III	—	I	—	I	—	III	—	III
Mercurialis perennis	I	—	—	I	IV	IV	—	—	—	—
Neottia nidus-avis	II	—	I	I	—	—	—	II	—	—
I: *Actaea spicata* (1), *Cephalanthera damassonium* (2), *Milium effusum* (1).										
Acerion										
Acer platanoides	IV	—	I	—	III	III	—	—	—	—
Acer pseudoplatanus	I	II	—	I	II	—	—	—	I	—
Ulmus glabra	—	I	—	V	III	III	—	I	I	—
Fagetalia										
Galium odoratum	II	IV	I	V	III	IV	—	I	II	II
Euphorbia amygdaloides	III	I	III	II	V	IV	IV	II	II	V
Lathyrus vernus	II	II	I	—	II	II	III	I	II	V
Carex sylvatica	I	I	I	—	—	I	—	II	II	II
Dentaria bulbifera	III	I	—	—	V	IV	III	II	—	IV
Galeobdolon luteum	I	—	—	IV	IV	—	II	—	—	II
Maianthemum bifolium	II	I	—	V	—	I	—	—	—	—
Dryopteris filix-mas	—	I	—	I	V	—	—	—	—	II
Fagus sylvatica	IV	III	I	III	III	IV	II	III	II	V

Table 1 (continued)

Number of row	1	2	3	4	5	6	7	8	9	10
Viola reichenbachiana	IV	III	IV	—	—	III	IV	I	III	V
Pulmonaria officinalis	I	I	II	I	II	III	II	—	II	II
Anemone nemorosa	I	I	—	I	—	I	V	—	—	—
Campanula rapunculoides	II	I	—	—	—	I	II	I	—	—
Mycelis muralis	IV	II	—	II	IV	IV	—	I	II	IV
Sanicula europaea	III	II	II	I	V	IV	—	—	—	—
Fraxinus excelsior	I	—	—	I	II	III	III	I	—	—
Aegopodium podagraria	I	I	II	—	—	IV	—	—	—	—
Geranium robertianum	—	II	I	V	—	—	—	—	I	II
Scrophularia nodosa	II	—	I	—	II	—	—	—	—	II
Ajuga reptans	II	IV	—	—	—	II	—	—	—	—
Allium ursinum	—	III	—	—	—	IV	—	—	—	—
Circaea lutetiana	—	I	—	—	—	II	—	—	—	—
Epilobium montanum	I	—	—	—	—	I	—	—	—	—
Galeopsis speciosa	—	I	—	III	—	—	—	—	—	—
Myosotis sylvatica	—	—	—	—	IV	II	—	—	—	—
Stachys sylvatica	I	I	—	I	IV	—	—	—	—	—
Isopyrum thalictroides	III	II	—	II	—	—	—	—	—	—
Viola sylvatica	I	—	—	—	—	I	—	—	—	—
Rubus hirtus	—	—	—	—	IV	IV	—	—	—	—
Cardamine impatiens	—	—	—	I	—	—	—	—	—	I
I: *Monotropa hypopitys* (2), *Daphne mezereum* (1), *Polygonatum verticillatum* (2).										
III: *Hepatica transsilvanica* (6).										
Querco-Fagetea										
Melica uniflora	II	V	I	II	II	II	III	I	IV	IV
Crataegus monogyna	IV	I	II	V	V	V	IV	II	IV	III
Poa nemoralis	III	II	II	V	—	IV	II	III	IV	III
Geum urbanum	I	I	I	V	—	I	II	I	II	III
Brachypodium sylvaticum	I	I	I	IV	—	I	—	I	III	—
Cornus sanguinea	III	I	II	I	V	III	—	I	III	—
Populus tremula	III	III	II	I	IV	IV	I	I	—	I
Fragaria vesca	II	I	—	V	—	II	II	II	IV	—
Corylus avellana	II	I	II	V	V	II	—	I	—	—
Euonymus europaeus	I	I	I	—	II	II	—	—	II	—
Lapsana communis	II	I	IV	III	III	—	I	—	—	—
Dactylis glomerata	IV	I	II	—	IV	I	—	—	—	—
Anemone ranunculoides	II	—	—	—	V	III	—	I	—	—
Lathyrus niger	V	I	III	—	—	I	III	—	—	—
Cruciata glabra	IV	III	—	I	II	II	—	III	—	IV
Malus sylvestris	I	—	I	—	—	III	—	—	—	—
Salvia glutinosa	—	II	—	IV	III	III	—	—	—	—
Melittis melissophyllum	III	I	—	I	—	—	—	I	—	—
Tilia tomentosa	—	—	—	—	V	III	IV	—	—	III
Quercus robur	I	II	II	—	—	—	—	—	—	—
Glechoma hirsuta	II	—	I	—	—	III	—	—	II	—
Cephalanthera longifolia	—	—	—	—	—	—	III	I	—	III
Pyrethrum corymbosum	II	—	—	II	—	—	—	I	I	—
Convallaria majalis	—	I	I	I	—	—	—	—	—	—
Polygonatum latifolium	—	I	II	II	—	—	—	—	—	—
Symphytum tuberosum	I	I	—	—	III	—	—	—	—	—
Cornus mas	II	—	—	—	—	—	III	I	—	—
Sedum maximum	—	I	III	—	—	—	—	—	I	—
Hieracium murorum	I	I	—	—	—	—	—	—	—	—
Polygonatum odoratum	I	—	I	—	—	—	—	—	—	—
Euonymus verrucosa	—	—	—	—	II	—	—	—	I	—
Festuca heterophylla	—	—	—	—	—	—	—	III	II	—
Epipactis helleborine	I	—	—	I	—	—	—	—	—	—

Table 1 (continued)

Number of row	1	2	3	4	5	6	7	8	9	10
Lonicera xylosteum	IV	I	—	—	—	—	—	—	—	—
Melica nutans	III	—	I	—	—	—	—	—	—	—
Moehringia trinervia	I	—	—	I	—	—	—	—	—	—
Quercus dalechampii	—	—	—	I	V	—	—	—	—	—
Staphylea pinnata	—	—	—	—	IV	—	—	—	I	—
I: *Viburnum lantana* (9), *Campanula persicifolia* (2), *Platanthera bifolia* (1).										
III: *Clematis vitalba* (6).										
V: *Galium sylvaticum* (4).										
Quercetea pubescenti-petraeae + Orno-Cotinetalia										
Sorbus torminalis	III	—	I	—	—	III	—	I	II	II
Buglossoides purpureocaeruleum	—	—	—	II	—	II	—	—	II	—
Tamnus communis	—	—	—	—	—	—	II	—	III	III
Trifolium medium	II	I	—	I	—	—	—	II	—	—
Viburnum opulus	I	I	—	—	IV	II	—	—	—	—
Astragalus glycyphyllos	II	I	—	III	—	—	—	—	—	—
Clinopodium vulgare	I	I	—	IV	—	I	—	—	—	—
Rosa canina	I	I	—	IV	—	—	—	—	—	—
Asperula taurina	—	—	—	—	—	II	—	—	II	—
Prunus spinosa	—	—	—	III	—	—	—	—	II	—
Fraxinus ornus	—	—	—	—	—	—	—	I	IV	—
Helleborus odorus	—	—	—	—	—	—	—	I	III	—
Aremonia agrimonioides	—	—	—	—	—	—	—	—	V	IV
Potentilla micrantha	—	—	—	—	—	—	—	II	II	—
Vincetoxicum hirundinaria	—	—	—	—	—	—	IV	—	II	—
Genista tinctoria	—	I	—	—	—	III	—	—	—	—
Lembotropis nigricans	—	II	—	—	—	III	—	—	—	—
I: *Carex montana* (1), *Viola hirta* (9), *V. alba* (1), *Acer tataricum* (3).										
II: *Ruscus hypoglosum* (9), *Silene viridiflora* (9), *Lychnis coronaria* (9), *Agrimonia eupatoria* (4).										
III: *Ruscus aculeatus* (9).										
Accompanying species										
Galium aparine	II	I	—	I	—	—	—	—	—	—
Hypericum maculatum	I	I	—	—	—	—	—	—	—	—
Pteiridium aquilinum	I	I	—	—	—	—	—	—	—	—
Prunella vulgaris	I	—	—	I	—	—	—	—	—	—
Abies alba	—	IV	—	I	—	—	—	—	—	—
Lysimachia nummularia	I	—	I	—	—	I	—	—	—	—
Nepeta pannonica	—	—	I	—	—	II	—	—	—	—
Sambucus nigra	—	—	—	I	—	II	—	—	—	—
Urtica dioica	—	I	—	I	—	—	—	—	—	—
Valeriana officinalis	—	I	—	I	—	—	—	—	—	—
Alliaria petiolata	I	—	—	I	—	II	—	—	—	—
I: *Hieracium sabaudum* (2), *Ajuga genevensis* (1), *Carex pallescens* (1), *Hypericum hirsutum* (1), *Betula pendula* (1).										
II: *Ophioglossum vulgatum* (8), *Hypericum perforatum* (4)										

Place of surveys: 1 — Burduja et al. 1973, Mărgineni Massif; 2 — Th. Chifu et al. 1973, Tg. Neamț—Neamț Monastery; 3 — Gh. Mihai, 1971, Hudești—Comănești (Botoșani); 4 — D. Mititelu et al. 1971, Trotuș Basin; 5 — D. Mititelu et al. 1986, Măgura Odobești; 6 — N. Ștefan, 1981, Rimnicul Sărat Basin; 7 — A. Popescu et V. Sanda, inedit, Cocorăștii Mislii et Cobia (Prahova and Dîmbovița Counties); 8 — Gh. Popescu, 1975, Bistrița Vîlcii Basin; 9 — Irina Hoborka, 1972—Dognecei Mountains; 10 — I. Pop et al. 1978, Zarand Mountains.

Within the association the following infracoenotaxons are known *transsilvanicum* Soó 57, facies with *Agrimonia agrimonioides* Schneider Erika 76, *frainettosum* Gh. Popescu, 74, Popescu et Păun 75; *moldavicum* Burduja et al. 73, Mititelu 73; *cytisetorsum leucotrichi* Turcu 70; *melicetosum uniflorae* Zólyomi 58, *caricetosum pilosae* Soó 62, *carpinetosum orientalis* Horeanu 81, *potentilletosum* Dumitru 80, facies *tiliosum tomentosae* et facies *fagosum* Drăgulescu 84, facies *caricosum brevicollis* Mihai et Sârbu 78.

13. **Melampyro (bihariensi)-Carpinetum** Soó 64

(Syn.: *Lathyro hallersteinii-Carpinetum* Coldea 75)

The association is known in Transsilvania and develops on titlted lands with varied exposition but less on the Northern sides of the mountains.

The floristic structure of phytoceoenoses is made of species characteristic to the *Symphyto-Fagion* alliance and to the suballiance *Lathyro hallerstenii-Carpinenion* which are also recognition species for the association. Among them we may cite: *Carpinus betulus*, *Melamphyrum bihariense*, *Helleborus purpurascens*, *Erythronium dens-canis*, *Silene dubia*, *Melica picta*, *Cardamine glanduligera*, etc.

In the arborescent layer in addition to the edifying and dominant species *(Carpinus betulus)*, *Quercus petraea* also participates with high abundance-dominance (1—3) as well as *Quercus robur*, *Tilia cordata*, *Prunus avium* (the last three species with less numerous specimens).

The subarboretum is made out of the *Crataegus monogyna*, *Viburnum lantana*, *Euonymus europaeus*, *Ligustrum vulgare*.

The floristic structure of the phytocoenoses of *Melampyro (bihariensi)-Carpinetum* is most resembling to those of *Querco petraeae-Carpinetum* and sometimes it presents the Constance = V and AD = 1—3. The following subassociations are described: *typicum* Soó 64, Codea 75; *praerossicum* Soó 64; *banaticum* Schrött 872; *tilietosum tomentosae* Coldea 75; *transsilvanicum* (Borza 41) Soó 69; *luzuletosum* Coldea 75; *quercetosum cerris* Coldea 75; *cephalantheretosum* Coldea 73, 75.

The facies *Allium ursinum* Coldea 72 also described whitin the association.

14. **Tilio (tomentosae)-Carpinetum** Doniță 68

(Syn.: *Tilio-Fraxinetum* sensu Borza 66 non Zólyomi (34) 36 et var. reg. *valachicum* Borza 66; *Tilio tomentosae-Carpinetum degradatum* Dobrescu et Kovács 73).

It is described in Dobrudja (the Babadag plateau) where it installs on sheltered valleys, on gentle slopes, on brown, humus wood soil, with high trophicity.

The arborescent layer is well represented as it is formed of the species: *Tilia tomentosa*, *Carpinus betulus*, *Quercus dalechampii*, *Tilia cordata*, *T. platyphyllos*, *Ulmus glabra*, *Acer platanoides*, *Fraxinus coriariifolia*. The great density of the arborescent layer hinders the bush development that makes a weaklier represented layer. The most frequent species are: *Cornus mas*, *Crataegus pentagyna*, *Euonymus verrucosus*, *Corylus avellana*.

Among the grassy species, the most frequent are: *Scilla bifolia*, *Corydalis bulbosa*, *Cardamine bulbifera*, *Galium odoratum*, *Mercurialis*

perennis, *Scutellaria altissima*, *Carex digitata*, *Lithospermum purpureo-caeruleum*, *Carpesium cernuum*.

The subassociations *vlasicum* Sanda et Popescu 71 (Syn. : *Ornithogalo-Tilio-Quercetum* Alexandrina Dihoru 76), *tilietosum cordatae* Doniţă 70, *typicum* Doniţă 70 and facies with *Quercus* Doniţă 70, are known.

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STRUCTURE ET DYNAMIQUE D'UNE POPULATION DE *CYTISUS SESSILIFOLIUS* L. DANS LES PÂTURAGES ABANDONNÉS DE L'APENNIN CENTRAL (ITALIE)*

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A population of *Cytisus sessilifolius* colonizing wide areas of the xeric pastures in the Nature Reserve of Torricchio (Italy) was examined. This species, probably, constitutes the first stage in the secondary succession leading to the beech forest in the central Appennine region. A permanent study area (10 × 10 m) was established in order to map the demographic units distinguishing them by diameter, height and age-state, and to study the age structure on 882 individuals sampled. Fruit and seed production following three classes of development were examined. The *C. sessilifolius* population is at least 22 years old, and established itself without any changes in the floristic combination of the local vegetation. Phases of colonization, stabilization, expansion and regression were identified. The mortality rate and the rarity of juvenile shoots in the areas covered by adults lead to the prevision of a future extinction of the local population. The developmental parameters were strictly related with the age-state and the seed production. A direct function between age and diameter was estimated by means of a regression analysis which allows future studies to be made without destructive sampling.

KEY WORDS: age states, size structure, mortality, age structure, dynamic phases, *Cytisus sessilifolius*.

INTRODUCTION ET BUT

Dans l'Apennin central l'abandon des pratiques culturales et du pâturage est très répandu et il donne lieu à des phénomènes propes aux successions secondaires qui, à travers des stades préforestiers, aboutissent à la reconstitution de la forêt.

L'étude du rôle de certaines populations d'espèces qui caractérisent les premiers stades de ces successions paraît important pour comprendre les temps et les mécanismes par lesquels elles s'installent, se développent et régressent, modifiant, dans l'ensemble, l'environnement et la végétation.

Les processus dynamiques du type que l'on a décrit ont été examinés à l'intérieur de la Réserve Naturelle de Torricchio qui s'étend sur 317 Ha, sur un territoire de montagne d'origine calcaire qui va de 800 à 1500 m, et dont la végétation est constituée, pour les trois quarts, par des pâturages d'origine secondaire et des prairies de fauche, tandis que les forêts d'*Ostrya carpinifolia* et *Fraxinus ornus* avec les forêts de *Fagus sylvatica* (surtout taillis ou taillis balivés) recouvrent 86 Ha environ.

L'institution de la Réserve (1970) et le régime de protection intégrale qui concerne la plupart de sa surface, faisant suite à un abandon déjà étendu des pratiques agricoles et pastorales, ont causé l'accéleration des processus dynamiques de la végetation, dont l'étude est très remarquable tant au niveau scientifique qui à un nivcau du gestionnaire (PEDROTTI, 1976).

* La recherche a été realisée avec l'aide financière de la Region Marche (Italie).

Les pâturages en particulier commencent à évoluer en donnant lieu à une succession secondaire qui aboutit à la hêtraie ; sur la base des connaissances actuelles, le premier stade de cette succession devrait être constitué par l'entrée de broussailles de *Juniperus communis* L. et *Cytisus sessilifolius* L. qui représentent les « pâturages buissonnés » décrites par Francalancia (1976) dans la Carte de la vegétation de la Réserve de Torricchio.

Cette recherche est la première d'une série qui a pour but de connaître le rôle du cytise à l'intérieur des successions sécondaires dans l'Apennin central, et notamment :

— l'influence des populations de *C. sessilifolius* dans la végetation des pâturages abandonnés ;

— le temps nécessaire au passage dans la phase ultérieure de la succession ;

— la structure des populations typiques de ces successions dans le but de comprendre les phénomènes principaux qui s'y déroulent, car l'évolution de la végétation dépend dernièrement de cela (Falinska, 1989 a).

Le cytise *(Cytisus sessilifolius* L.) est un arbuste fortement ramifié, qui a un portement prostré droit, c'est une espèce des pentes arides qui se trouve jusqu'à 1600 m dans l'Apennin central ; c'est un élément subatlantique avec une distribution méditerranéenne occidentale. Il s'agit en outre d'un arbuste dont les jets prostrés pourraient avoir la possibilité d'enraciner donnant origine à de nouveaux individus ou ensembles de jets (polycormones) difficilement identifiables.

Cytisus sessilifolius est connu en littérature comme espèce des lisières (manteaux) des forêts de colline et de montagne, qui entre dans les premiers stades évolutifs de la végétation des champs abandonnés dans la série de la hêtraie et du bois de charme noir et scutellaire (Ubaldi, 1976 ; Pedrotti, sous presse).

L'espèce (comme cela arrive dans la famille des *Leguminosae)* est objet de symbiose radicale avec des bactéries azoto-fixatrices, qui pourrait influencer de façon décisive l'environnement dans les stades de la succession.

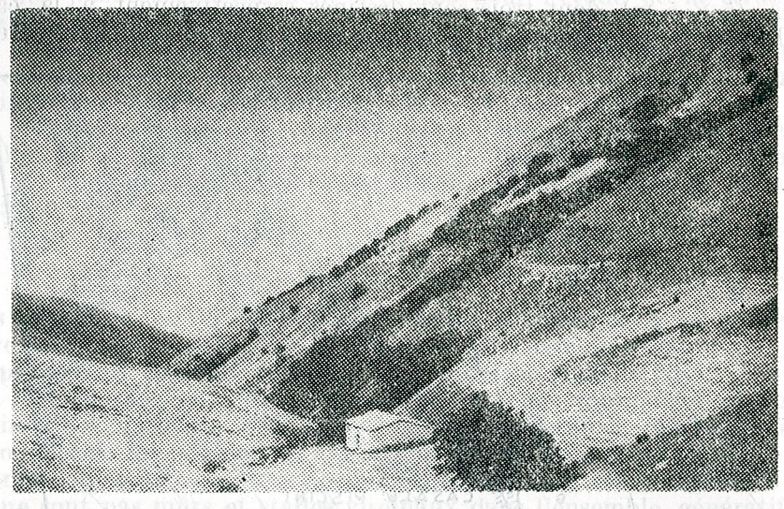
MATÉRIEL ET MÉTHODES

Cette recherche emploie une approche dynamique et populationniste du sujet, qui permet de décrire l'état d'une population en donnant des informations de type prédictif grâce aux approfondissements relatifs à la structure, à la forme et au cycle de développement des individus.

Pour l'application de telles méthodologies on a fixé (depuis 1986) une surface permanente d'étude (10 × 10 m) à l'intérieur d'une vaste et dense colonie de *C. sessilifolius* qui s'est installée depuis longtemps sur une pelouse xérique (Fig. 1 ; Planche 1 ; Canullo et Venanzoni, 1989).

La surface se trouve à 1150 m d'altitude sur un versant exposé au NO avec une inclinaison de 35° env. ; le substrat qui affleure, de type calcaire, marneux et siliceux (scaglia rosata) est riche en Fissures qui en

A



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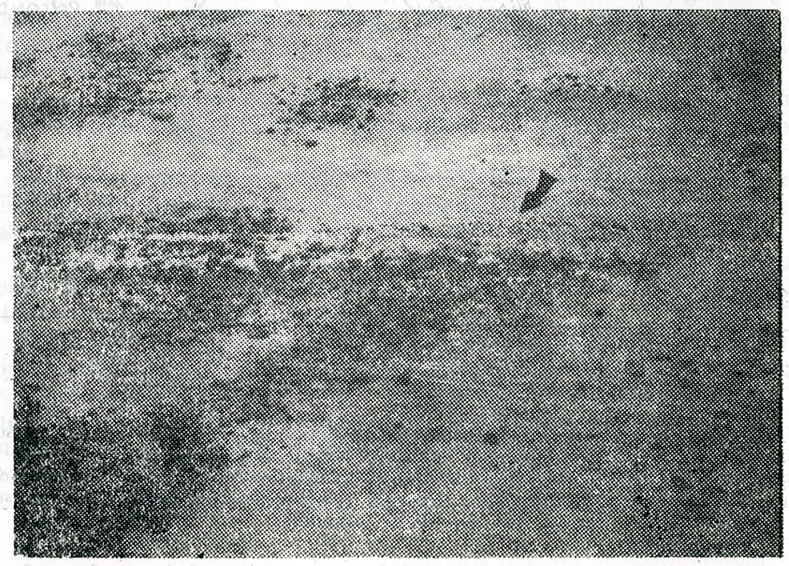

Planche 1. — A : *Cytisus sessilifolius* après la colonisation de la surface d'étude (photo : E. Orsomando août 1974) ; B : étage actuel de la même population (photo : R. Canullo, juillet 1986).

déterminent la perméabilité élevée et donc le caractère aride (Deiana et Pieruccini, 1976).

La surface permanente d'étude a été ultérieurement divisée en 4 carrés (5 × 5 m), dont trois sont destinés respectivement à la cartographie des unités démographiques, à la détermination de l'âge, au relevé

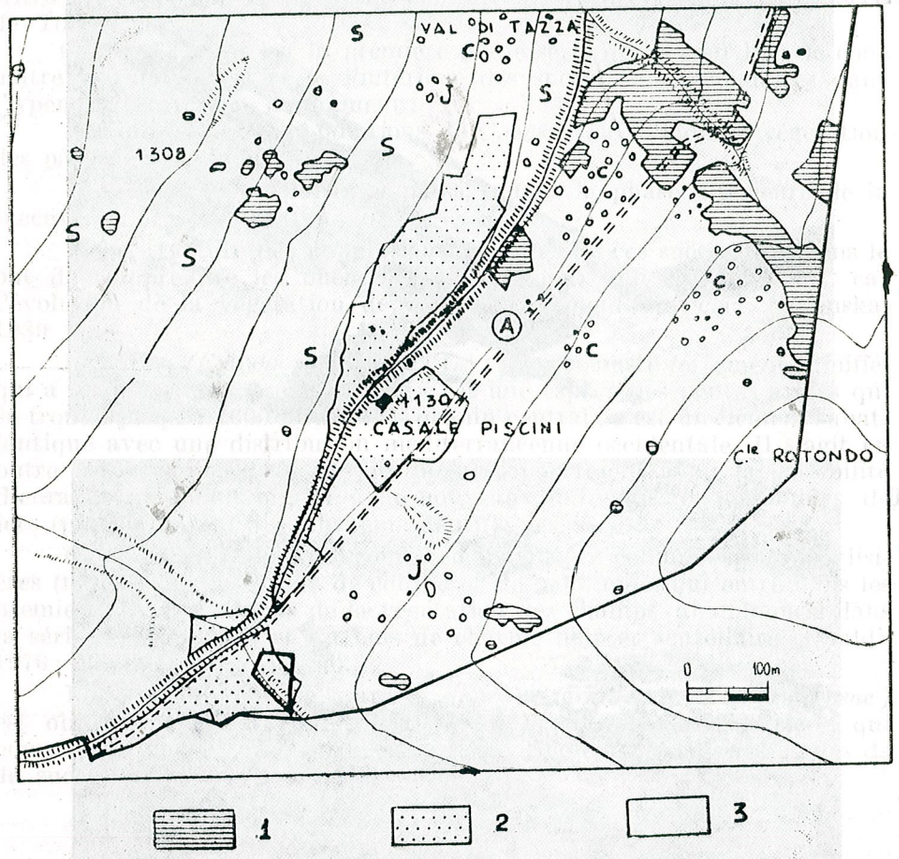

Fig. 1. — Localisation de la surface permanente d'étude (A) dans un pelouse xérique buissonnée à *Cytisus sessilifolius* de la Réserve naturelle de Torricchio.
1. Bois et individus de *Fagus sylvatica* ; 2. Prairie à *Cynosurus cristatus* ; 3. Pelouse à *Sesleria nitida* (s), *Festuca ovina* et *Brachypodium rupestre* ; —. limite de la Réserve ; C. *Cytisus sessilifolius* ; *J. Juniperus communis*.

de la végétation, tandis qu'un autre carré représente la surface de contrôle. La surface destinée à la cartographie a été fixée au réseau permanent avec des mailles de 1 × 1 m (pour un total de 25 sub-surfaces).

La vérification sur le terrain a permis d'assigner à son propre individu ces jets prostrés, non radicants et recouverts par une couche de sol et de litière, mais, à cause des difficultés liées à la nature du substrat, on

ne peut pas exclure que certains jets radicants aient été considérés sans en saisir l'appartenance à un individu polycormique. D'ailleurs l'observation des jets est généralement acceptée pour l'étude de la structure (Falinska, 1984).

L'unité démographique, pourtant, est un jet complexe qui, raisonnablement, ne doit pas se détacher beaucoup de la consistance réelle des individus dans la population prise en examen.

Pour chaque jet de *C. sessilifolius* à l'intérieur du réseau de cartographie, on a enregistré les coordonnées, le diamètre basal et la hauteur.

Le type de distribution a été élaboré par la formule de Kershaw (1973) :

$$\frac{\Sigma (x - \bar{x})^2}{x \cdot (n - 1)}$$

dans laquelle x est la densité pour chaque sub-surface, $\bar{x}$ est la densité moyenne et n le nombre des sub-surfaces ; il s'agit de distribution agrégée pour $d > 1$, casuelle pour $d = 1$ et uniforme pour $d < 1$.

Considérant en outre que l'âge n'aide pas toujours à prévoir la destinée des individus (surtout en ce qui concerne la mortalité et la reproduction) les jets ont été distingués, sur la base du stade d'âge, en : juvéniles (diamètre jusqu'à 1,5 mm, âge jusqu'à 2 ans), végétatifs (jets qui ne sont pas mûrs et stades virginaux dans l'ensemble, génératifs (en floraison), morts (Rabotnov, 1969 ; Krüsi, 1981 ; Falinska, 1985 ; Silvertown, 1987). Cette distinction concorde, en outre, avec les considérations de Brakham (1980) qui soutient l'emploi de catégories du même type.

Pour la mortalité on a exclu les individus morts depuis deux années (d'après la décomposition de l'épidermide et la fragmentation des rameaux).

La détermination de l'âge a été effectuée par l'échantillonnage destructif des 882 individus dans la surface préposée à ce but, et pour cela on a compté les anneaux de croissance sur les jets coupés à la base.

On a effectué en outre un relevé de la végétation dans la surface spéciale de 25 m² : l'attribution des valeurs, selon l'échelle Braun-Blanquet, a fait suite à la liste floristique.

Enfin, à l'époque de la maturation des fruits (août-septembre), on a distingué 3 classes de hauteur des jets génératifs ($I^{er} < 50$ cm ; II^{e} 50—80 cm ; $III^{e} > 80$ cm), et, à l'extérieur de la surface d'étude, on a recueilli par hasard un échantillon de 100 légumes pour chaque classe. Ensuite toutes les gousses, privées du pédoncule, ont été pesées (± 0,05 mg) et les graines contenues ont été comptées et pessées à leur tour, après une estimation du rôle du prasitisme.

RÉSULTATS

Dans le tableau 1 on a rapporté le relevé de la végétation à l'intérieur de la population de *Cytisus sessilifolius*. L'espèce se développe dans une pelouse xérique qui fait partie de l'alliance *Crepido lacerae-Phleion ambigui* (Biondi et Blasi, 1982) dans laquelle prévalent, comme couverture, les espèces herbacées vivaces et les petits arbustes.

Le spectre biologique (Fig. 2) voit les hémicriptophytes qui dominent (72%) suivies des caméphytes (18,5%), tandis que *C. sessilifolius* est la seule phanérophyte sur un total de 43 espèces ; la dominance des hémicriptophytes et la basse participation de thérophytes soulignent le

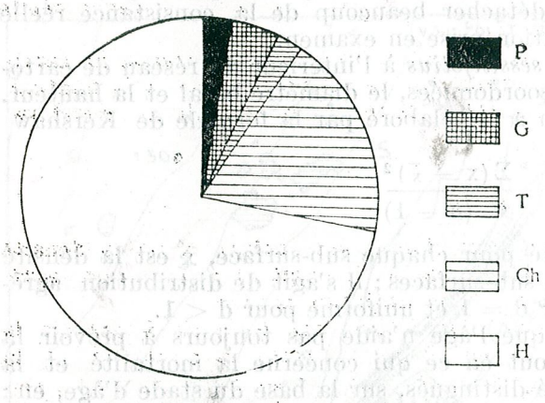

Fig. 2. — Spectre biologique de la végétation relevée à l'intérieur de la surface permanente d'étude.

caractère montagnard de la végétation, caractère d'ailleurs propre à toute la flore de la montagne de Torricchio (Ballelli et Francalancia, 1982). Les espèces carctéristiques et le rapport Ch/H (0,26) sont conformes à une association du type *Seslerio nitidae-Brometum erecti* (Biondi et Blasi, 1982 ; Biondi et Barelli, 1982).

Dans la surface d'étude (25 m²) on a relevé 848 individus (ou unité démographiques).

La structure horizontale et verticale peut être exprimée par la cartographie détaillée des unités démographiques selon les stades de développement, la hauteur et le diamètre : dans la figure 3 on a rapporté une des 25 sub-surfaces où telles caractéristiques sont conformes à la moyenne. On peut remarquer l'aspect à noyaux denses qui se diversifient quant au développement (hauteur maximale : 1,4 m).

Cette caractéristique est bien résumée par le coéfficient d dont la valeur (3,94) indique une distribution du type agrégé ; l'analyse faite seulement sur les jets juvéniles montre une tendance d'aggrégation inférieure ($d = 2{,}08$) tandis que les jets les plus anciens (âge > 13 ans) sont peu nombreux et distribués casuellement. La densité moyenne est de $33{,}9 \cdot m^{-2}$ ($\pm$ 11,6 ds).

Une élaboration ultérieure a consenti d'analyser et de dresser, par interpolation, la carte de la distribution des données recueillies (ou dérivées) dans l'entière surface d'étude.

La figure 4 montre particulièrement la distribution des jets juvéniles et génératifs, de la hauteur et du diamètre (moyenne dans chaque sub-surface). Les individus juvéniles sont plus concentrés dans le coin inférieur droit tandis que dans le côté gauche de la surface-échantillon ils sont peu nombreux ou absents ; ceux qui sont génératifs sont plus densément distribués dans le côté moyen gauche et dans le sommet supérieur droit, quoique présents dans toute la surface examinée ; pour le paramètres de développement il en est ressorti le même type de distribution

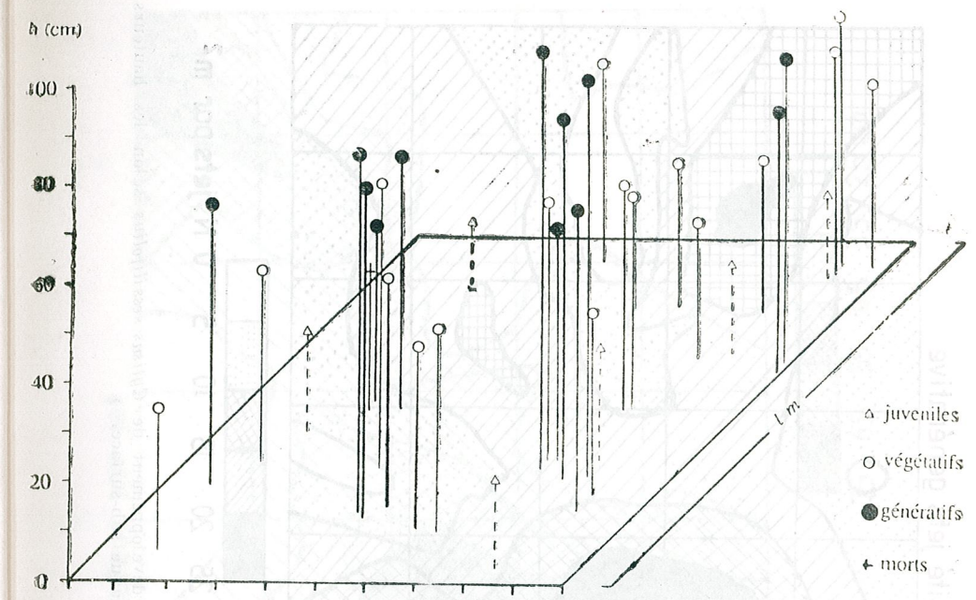

Fig. 3. — Représentation tridimensionnelle de la structure de *Cytisus sessilifolius* à l'intérieur d'une sub-surface : la composition et les valeurs sont conformes à la moyenne

Quantitativement, 45% des jets mappifiés se trouvent au stade végétatif, 35,5% en floraison, 10,7% sont représentés par les jets juvéniles tandis que 8,8% constituent l'incidence de la mortalité sur la population.

La distribution de ces rapports selon les classes de diamètre et les trois classes de hauteur déterminées pour la récolte des fruits, sont exprimées par les pyramides de la figure 5.

Les individus juvéniles sont compris dans la classe inférieure de diamètre ($x = 0{,}15$ mm) et de hauteur ($\bar{x} = 18$ cm). Les jets au stade végétatif sont concentrés surtout dans les classes inférieures et les individus génératifs ont des dimensions plus grandes même si une participation remarquable aux classes inférieures témoigne d'un certain niveau de précocité.

Le rapport entre les jets fleuris et ceux qui ne sont pas fleuris, pris comme indice de reproduction, correspond à 0,55 sur toute la population ; analytiquement, les classes de dimensions inférieures ont un indice de 0,17 et la deuxième classe de développement atteint un rapport pair à 2,5 qui augmente ultérieurement pour les classes supérieures (Fig. 5).

La production des graines (tableau 2) est différente pour les trois classes de hauteur (prises comme classesde développement) disposant toutes les valeurs dans un ordre qui grandit selon l'accroissement des dimensions des individus.

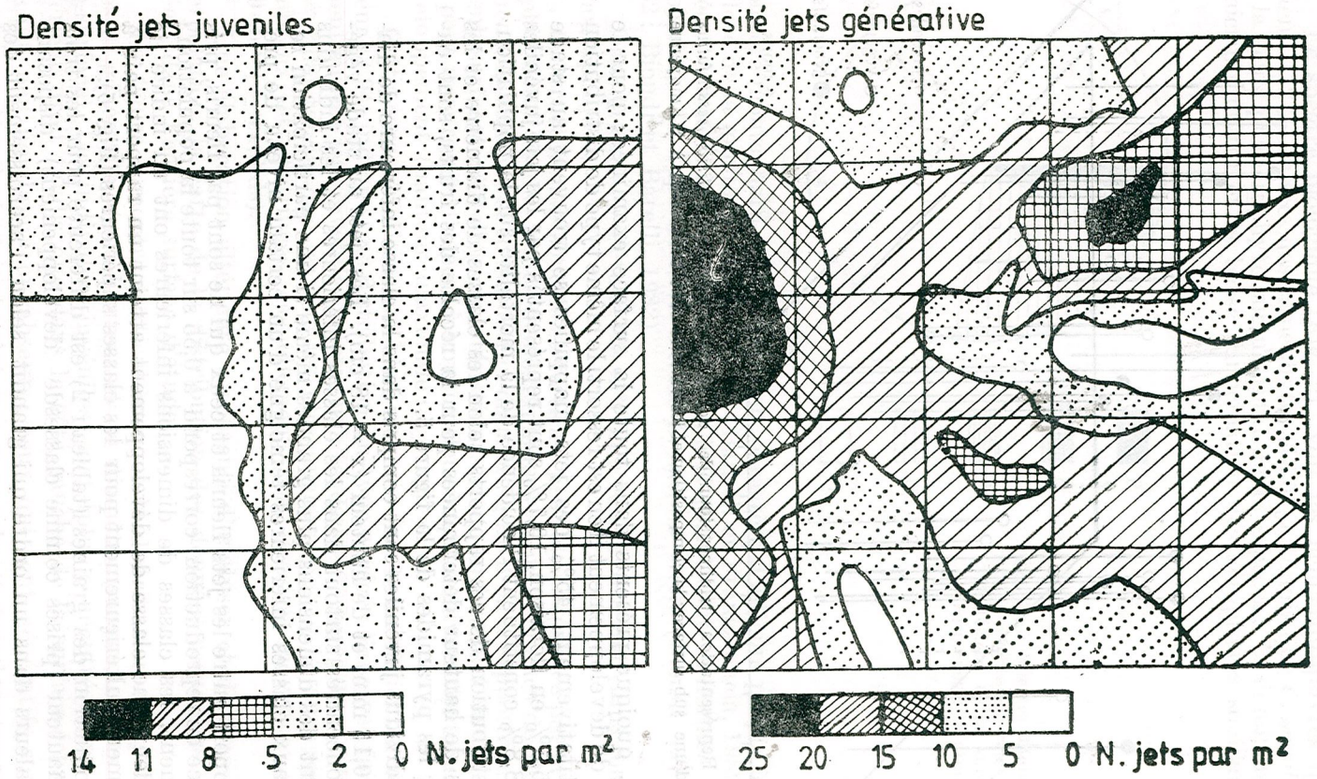

Fig. 4 — Distribution des jets juvéniles, génératifs es des paramètres de développement de *Cytisus sessilifolius* selon les hauteurs et les diamètres moyens pour chaque sub-surface.

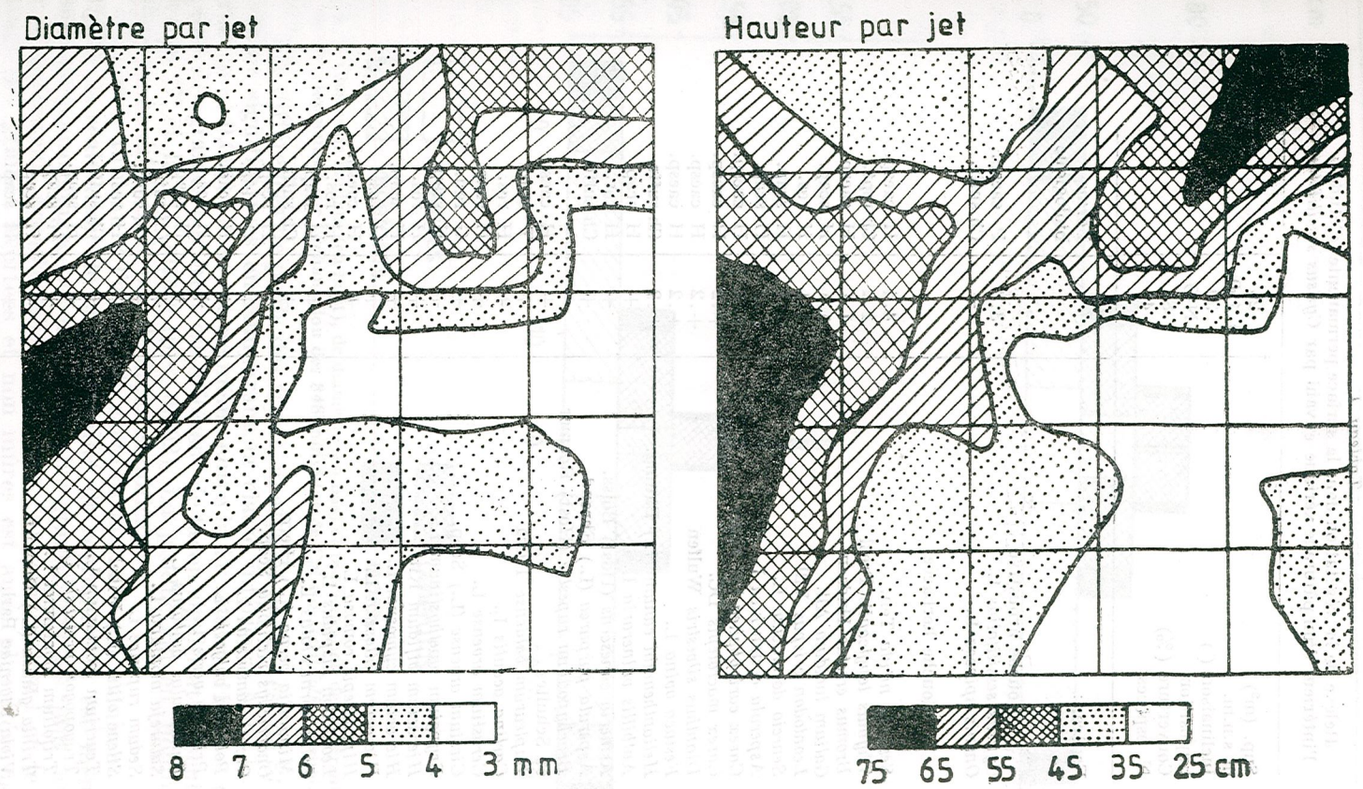

Fig. 4

Tableau 1

Relève de la végétation dans la surface permanente d'étude à l'intérieur d'un pâturage xérique envahi par *Cytisus sessilifolius*

Sup. (m²)	25	
m s.n.m.	1.150	
Inclinaison (°)	30	
Exposition	N-O	
Couverture (%)	85	
N. espèces	43	
Espèces :		Forme biologique
Couche arbustive		
Cytisus sessilifolius L.	3.3	P caesp.
Ononis spinosa L.	+	Ch suffr.
Couche herbacée		
Sesleria nitida Ten.	4.4	H caesp.
Thymus longicaulis Presl	1.2	Ch rept.
Bromus erectus Hudson	1.1	H caesp.
Galium lucidum All.	1.1	H scap.
Leontodon crispus Vill	1.1	H ros.
Senecio doronicum L.	1.1	H scap.
Asperula cynanchica L.	+.2	H scap.
Carex cariophyllea La Tourr	+.2	H caesp.
Carex macrolepis DC.	+.2	H caesp.
Dianthus silvestris Wulfen	+.2	H caesp.
Festuca ovina L.	+.2	H caesp.
Helianthemum canum (L.) Baumg.	+.2	Ch suffr.
Anthillis vulneraria L.	+	H scap.
Armeria canescens (Host) Boiss.	+	H ros.
Asperula purpurea (L.) Ehrend.	+	Ch suffr.
Brachypodium rupestre (Host) Roemer et Schultes	+	H caesp.
Bupleurum baldense Turra	+	T scap.
Carlina acaulis L.	+	H ros.
Cerastium arvense L.	+	H scap.
Cirsium arvense (L.) Scop.	+	G rad.
Eryngium amethystinum L.	+	H scap.
Hieracium bifidum Kit.	+	H ros.
Hieracium cymosum L.	+	H scap.
Hieracium pilosella L.	+	H ros.
Hippocrepis comosa L.	+	H caesp.
Leontodon hispidus L.	+	H ros.
Lotus corniculatus L.	+	H scap.
Minuartia verna (L.) Hiern	+	Ch suffr.
Onobrichys viciifolia Scop.	+	H scap.
Orchis sambucina L.	+	G bulb.
Polygala vulgaris L.	+	H scap.
Primula veris L.	+	H ros.
Ranunculus bulbosus L.	+	H scap.
Satureja montana L.	+	Ch suffr.
Sedum rupestre L.	+	Ch succ.
Silene otitis (L.) Wibel	+	H ros.
Teucrium montanum L.	+	Ch suffr.
Tragopogon pratensis L.	+	H scap.
Trifolium montanum L.	+	H scap.
Trinia glauca (L.) Dumort.	+	H scap.
Viola eugeniae Parl.	+	H scap.

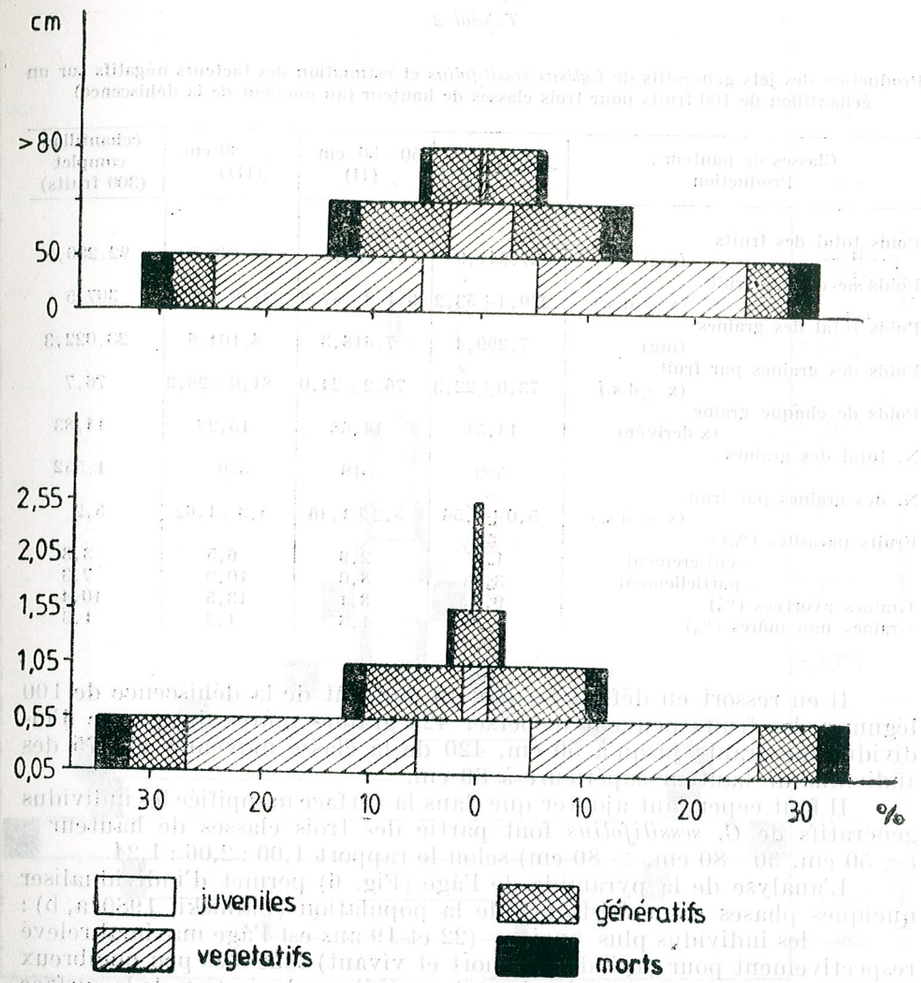

Fig. 5. — Classes de hauteur (en haut), de diamètre (en bas) et participation des différents stades de développement sur l'échantillon des 848 individus de *Cytisus sessilifolius* mappifiés.

Les différences de poids des fruits, analysées par le test de Student, s'avèrent significatives en comparant la classe inférieure avec la II^e^ et la III^e^ ($p < 0{,}001$) : il en est de même entre la II^e^ et la III^e^ ($p < 0{,}003$), le poids des graines par fruit est différent de façon significative seulement entre la I^er^ et la III^e^ classe ($p < 0{,}002$). La production en nombre des graines ne se différencie pas de façon significative.

Dans le tableau 2 l'action de certains facteurs qui réduisent déjà à l'origine la réalisation de la potentialité de reproduction est même exprimée en pourcentage. Les individus plus grands sont les plus atteints par le prédateur *Acanthoscelides obsoletus* Say *(Coleoptera)* et le pourcentage des graines avortées et non mûres est aussi plus élévé pour la classe de hauteur plus grande.

Tabelul 2

Production des jets génératifs de *Cytisus sessilifolius* et estimation des facteurs négatifs sur un échantillon de 100 fruits pour trois classes de hauteur (au moment de la déhiscence)

Classes de hauteur : Production	< 50 cm (I)	50—80 cm (II)	> 80 cm (III)	échantillon complet (300 fruits)
Poids total des fruits (mg)	27.941,9	31.125,5	33.183,5	92.250,9
Poids de chaque fruit (x ± d.s.)	279,4±53,2	311,3±57,9	331,8±72,7	307,5
Poids total des graines (mg)	7.299,4	7.618,3	8.104,6	23.022,3
Poids des graines par fruit (x ±d.s.)	73,0±22,3	76,2±24,0	81,0±26,3	76,7
Poids de chaque graine (x derivée)	14,51	14,68	15,29	14,83
N. total des graines	503	519	530	1.552
N. des graines par fruit (x ± d.s.)	5,0±1,56	5,2±1,46	5,3±1,62	5,2
Fruits parasités (%) :				
— entièrement	1,9	2,9	6,5	3,8
— partiellement	3,9	8,0	10,0	7,3
Graines avortees (%)	9,1	8,1	13,5	10,4
Graines non mûres (%)	0,8	1,3	1,7	1,3

Il en ressort en définitive que, au moment de la déhiscence de 100 légumes, les fruits peuvent disperser 425 graines mûres du groupe d'individus mesurants jusqu'à 50 cm, 420 de la classe moyenne et 375 des individus de hauteur supérieure à 80 cm.

Il faut cependant ajouter que dans la surface mappifiée les individus génératifs de *C. sessilifolius* font partie des trois classes de hauteur (< 50 cm, 50—80 cm, > 80 cm) selon le rapport 1,00 : 2,06 : 1,24.

L'analyse de la pyramide de l'âge (Fig. 6) permet d'individualiser quelques phases caractéristiques de la population (Falinski, 1980 a, b) :

— les individus plus anciens (22 et 19 ans est l'âge maximal relevé respectivement pour un individu mort et vivant) sont très peu nombreux et représentent les restes de la phase de première colonisation de la surface (qui pourtant remonte à l'époque 1965—1973) ;

— une phase ultérieure peut être identifiée, considérant les individus jusqu'à 13 ans qui constituent 1—7% de la population : cette phase (dans la période 1874—1980) correspond à la phase de stabilisation de la population ;

— dans la période 1981—1984 la population a été caractérisée par une phase d'expansion, comme en témoignent les pourcentages élevés relatifs à 3, 4, 5 et 6 and (19,8% 15,4%, 16,1% et 10,9%) ;

— la classe d'âge de 1—2 and comprend un nombre décroissant d'individus, caractérisant actuellement le début de la phase de régression.

L'âge moyen des individus dans la population est de 8,7 ans.

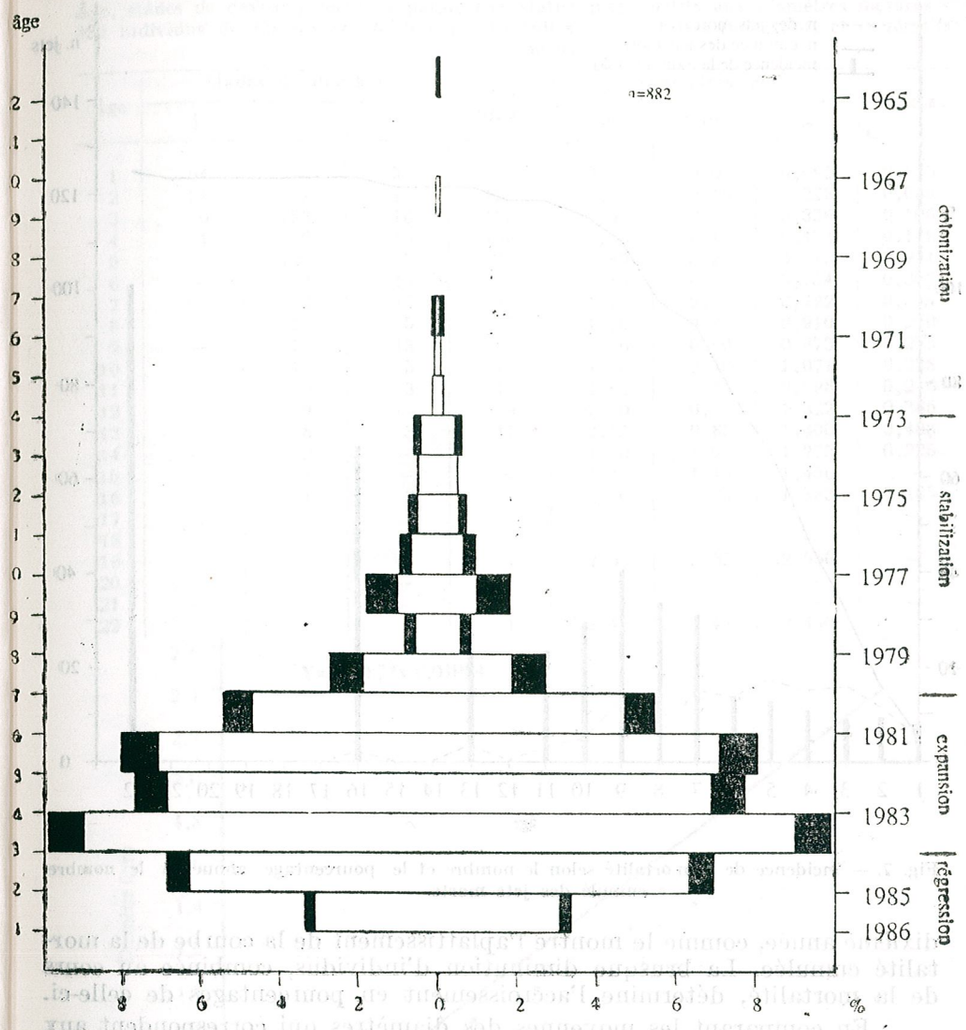

Fig. 6. — Pyramide des âges de *Cytisus sessilifolius ;* en noir les jets morts.

Dans la figure 7 on a exprimé l'incidence annuelle de la mortalité de *C. sessilifolius :* les stades juvéniles de cette espèce présentent une mortalité égale à 10—13% environ qui augmente à 23—40% dans l'intervalle 7—12 ans au-delà duquel les rapports sont discontinus à cause du petit nombre d'individus (tableau 3).

En termes absolus, on peut dire que le nombre des morts est presque constant dans les stades plus jeunes, et qu'il decroît au-delà de la

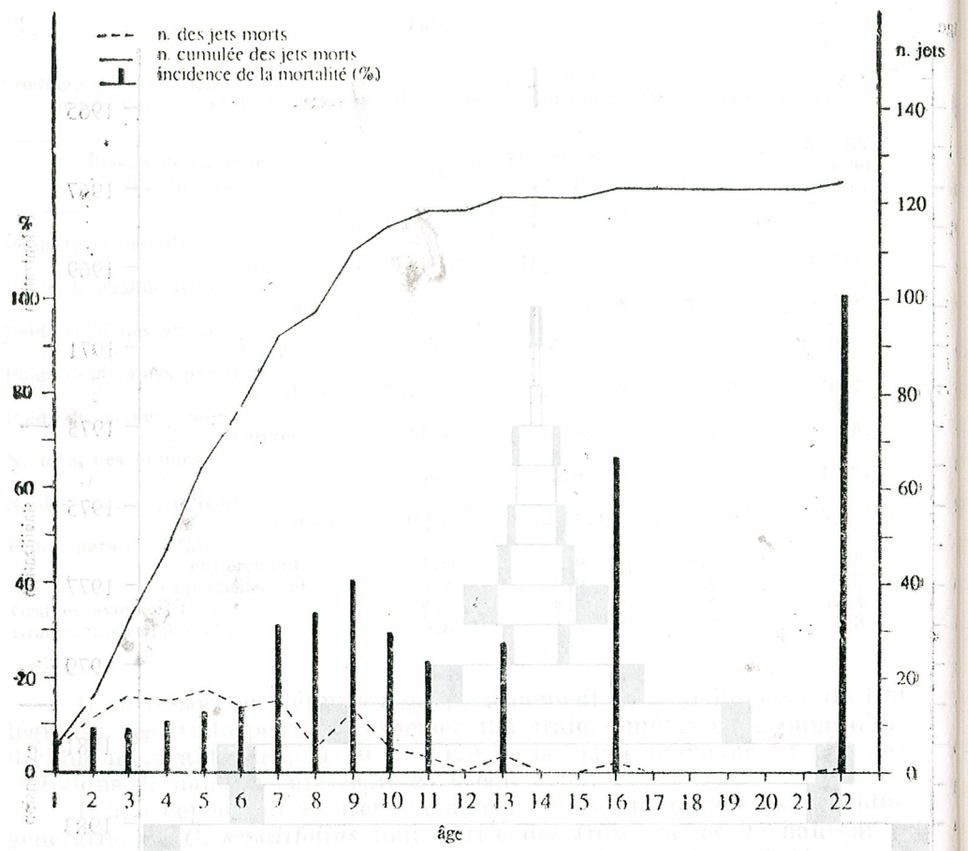

Fig. 7. — Incidence de la mortalité selon le nombre et le pourcentage annuel et le nombre cumulé des jets morts.

dixième année, comme le montre l'aplattissement de la courbe de la mortalité cumulée. La brusque diminution d'individus, combinée au cours de la mortalité, détermine l'accroissement en pourcentages de celle-ci.

En comparant les moyennes des diamètres qui correspondent aux âges (déterminées sur l'échantillon de 882 individus) on a recherché une courbe de régression (par la méthode des moindres carrés) qui est représentée par la droite de la figure 8 ; la fonction est significative à 99% et montre une relation en vertu de laquelle, à chaque année correspond un millimètre d'accroissement environ.

Les données qui se détachent davantage de la droite sont celles pour lesquelles les individus sont très peu nombreux, comme on peut le remarquer dans le tableau 3, où les échantillons mis en order par âge sont distingués selon les stades de développement, rapportant la moyenne et la déviation standard des diamètres relatifs, unies à l'intervalle des valeurs.

Tableau 3

Âge, stades de développement et paramètres statistiques relatifs aux diamètres mesurés sur 882 individus de *Cytisus sessilifolius* j = juvéniles ; a = adultes (végétatifs et génératifs) ; + = morts

Âge	Stades de develop.			n. échantillons	Diamètres (cm)			±d.s.
	j	a	+		max	min	x	
1	54	—	5	59	0,1	0,05	0,095	0,015
2	21	89	11	121	0,35	0,15	0,228	0,055
3	6	153	16	175	0,65	0,15	0,336	0,106
4	1	120	15	136	1,00	0,15	0,484	0,149
5	—	125	17	142	1,60	0,25	0,613	0,241
6	—	83	13	96	1,85	0,30	0,734	0,308
7	—	33	15	48	1,70	0,30	0,792	0,314
8	—	10	5	15	1,70	0,60	0,910	0,270
9	—	19	13	32	1,40	0,50	0,873	0,275
10	—	12	5	17	1,85	0,70	1,071	0,338
11	—	10	3	13	1,60	0,65	0,996	0,246
12	—	9	—	9	2,10	0,75	1,322	0,385
13	—	8	3	11	2,15	0,85	1,400	0,408
14	—	2	—	2	1,50	1,05	1,275	0,225
15	—	1	—	1	1,45	1,45	1,450	—
16	—	1	2	3	1,60	1,20	1,383	0,165
17	—	—	—	—	—	—	—	—
18	—	—	—	—	—	—	—	—
19	—	1	—	1	2,55	2,55	2,550	—
20	—	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—	—
22	—	—	1	1	2,45	2,45	2,450	—

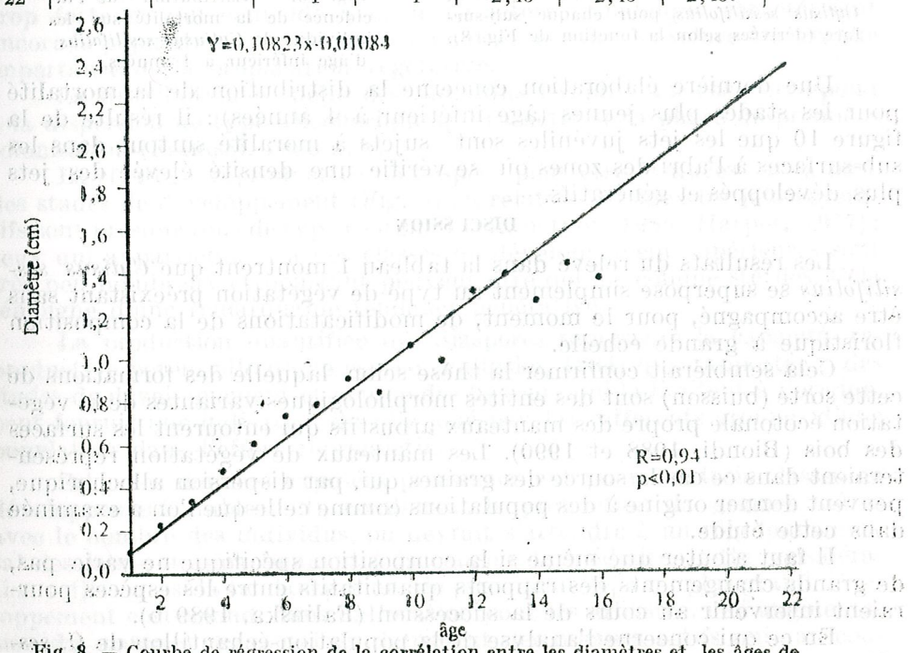

Fig. 8. — Courbe de régression de la corrélation entre les diamètres et les âges de *Cytisus sessilifolius*.
● = valeurs moyennes par année.

Selon la fonction de la courbe de régression on a attribué les classes d'âge à l'intérieur de la surface-échantillon, pour chaque sub-surface. Le résultat est la distribution rapportée èans la figure 9 qui met en relief le plus ancien âge moyen à l'extrême supérieur droit et dans la partie gauche du réseau, dans laquelle tombe la plus grande valeur moyenne.

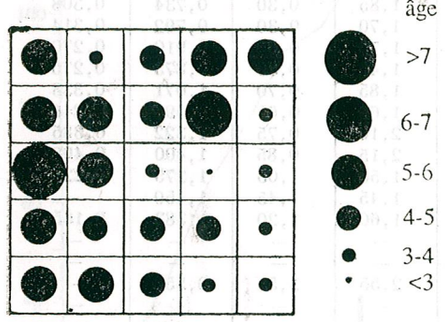

Fig. 9. — Distribution des âges moyens de *Cytisus sessilifolius* pour chaque sub-surface (dérivées selon la fonction de Fig. 8).

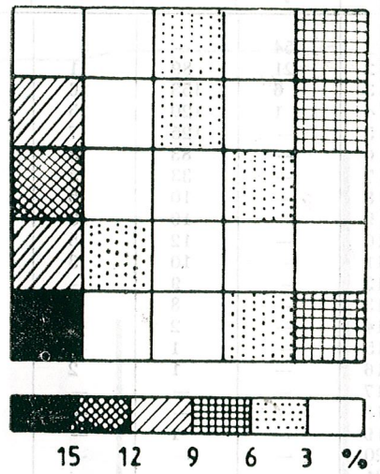

Fig. 10. — Distribution de l'incidence de la mortalité sur les individus de *Cytiusus sesilifolius* d'âge inférieur à 4 années.

Une dernière élaboration concerne la distribution de la mortalité pour les stades plus jeunes (âge inférieur à 4 années) : il résulte de la figure 10 que les jets juvéniles sont sujets à moralité surtout dans les sub-surfaces à l'abri des zones où se vérifie une densité élevée des jets plus développés et génératifs.

DISCUSSION

Les résultats du relevé dans la tableau 1 montrent que *Cytisus sessilifolius* se superpose simplement au type de végétation préexistant sans être accompagné, pour le moment, de modificatations de la composition floristique à grande échelle.

Cela semblerait confirmer la thèse selon laquelle des formations de cette sorte (buisson) sont des entités morphologiques variantes de la végétation écotonale propre des manteaux arbustifs qui entourent les surfaces des bois (Biondi, 1988 et 1990). Les manteaux de végétation représenteraient dans ce cas la source des graines qui, par dispersion allochorique, peuvent donner origine à des populations comme celle que l'on a examinée dans cette étude.

Il faut ajouter que même si la composition spécifique ne varie pas, de grands changements des rapports quantitatifs entre les espèces pourraient intervenir au cours de la succession (Falinska, 1989 b).

En ce qui concerne l'analyse de la population-échantillon de *C. sessilifolius*, les cartes de la distribution (Fig. 4 et 9) montrent une corrélation précise entre la distribution des jets génératifs et les paramètres de

développement en excluant tout à fait les jets juvéniles des surfaces à haute concentration d'individus adultes. Dans ces zones-là en effet, les individus les plus jeunes sont sujets à une mortalité élevée (Fig. 10). Ce phénomène pourrait être imputé à l'effet-ombre dû aux branches des adultes ou même à une sorte d'allélopathie (Andrezejewski et Falinska, 1986 ; Knapp, 1974).

En tout cas dans les sub-surfaces en question, la mortalité élevée des jets juvéniles laisse prévoir, dans une dizaine d'années, une régression rapide de la population.

Précédemment (Canullo et Venanzoni, 1989) on a montré comment la plus grande densité d'individus juvéniles coïncidait avec un degré élevé de mortalité à laquelle toutefois contribuent 90% des individus d'âge supérieur : à la lumière des élaborations actuelles (on n'a pas trouvé une relation significative) on pourrait exclure une mortalité qui dépend directement de la densité.

La comparaison avec la distribution de la densité totale illustrée par Canullo et Venanzoni (1989) montre en outre que dans la partie gauche de la surface d'étude la plus grande densité est déterminée par des individus plus mûrs, tandis que ce sont les jets juvéniles qui constituent un noyau à haute densité en bas à gauche. Ce type de structure horizontale fait penser que l'expansion de la population examinée se vérifie en direction ouest, en suivant la pente.

Le coefficient de distribution des jets ($d = 3{,}94$) montre une tendance à l'agrégation (définie par l'extension $\infty > d > 1$) qui n'est pas trop forte et qui coïncide avec le caractère dynamique caractérisant encore la population ; ce caractère, en outre, tendrait à exclure une grande importance de la propagation végétative.

La distribution casuelle des individus les plus anciens correspond à la dispersion de type allochorique qui caractérise la phase de première colonisation (Falinski, 1980 a, b).

La stratégie reproductive de l'espèce peut être évaluées sur la base des stades de développement (Fig. 5) en relation à l'âge : les jets végétatifs sont presque tous de type « virginal » (Rabotnov, 1985 ; Harper, 1977) ; ceux qui appartiennent à des classes de développement supérieures sont très peu nombreux et pourtant peuvent être définis comme stériles. Cela témoigne d'une remarquable tendance générative.

La production quantifiée des diaspores représente la capacité reproductive potentielle d'une espèce (Falinska, 1984) qui, rapportée à des stades de développement et épurée des facteurs qui la limitent à l'origine, peut fournir une indication du rôle joué par les différents groupes d'une population dans l'effort reproductif.

Selon les données exposées précédemment pour les trois classes de développement (tableau 2), combinant la production de graines mûres avec le nombre des individus, on devrait s'attendre à un rôle fondamental des jets qui appartiennent à la classe intermédiaire. Cette considération reflète aussi la tendance générale pour laquelle, à un plus grand développement correspond, dans l'allocation pour les arbustes, une augmentation du matériel structurel au détriment des organes actifs qui, au contraire, semblent prédominer dans les classes moyennes de développement (Ojea, Pereiras et Basanta, 1988).

La structure de l'âge de l'échantillon examiné est typique d'une population au début de la régression (Falinski, 1980 a, b ; Rabotnov, 1985 ; Stazi, 1986—1987) et la mortalité frappe particulièrement les individus qui ont un âge moyen élevé, différent de ce que l'examen préalable par classes d'âge suggérait (Canullo et Venanzoni, 1989 ; Fig. 7 ; tableau 3), et intéresse la phase de stabilisation.

La phase de colonisation intéresse une période très longue car, pendant que la surface était utilisée par le pâturage, l'espèce était contrôlée par les animaux étant ainsi en permanence, en conditions pionnières.

La corrélation directe (r = 0,94) et la régression entre âge et diamètre, rend ce dernier utile pour prédire le développement réel des individus et permet d'éviter des échantillonnages destructifs à travers l'emploi de la fonction de la Fig. 8. La distribution de l'âge dérivée dans le réseau permanent confirme les rapports étroits avec les paramètres de développement (hauteur et diamètre) et avec l'estimation de la fertilité (Fig. 4 et 9).

Dans l'ensemble, l'examen de la végétation, les observations relatives à la structure et à la distribution, la production de diaspores et la dissémination de type autochorique qui réussit seulement dans les surfaces libres, fait penser que l'expansion de la population est en train de se réaliser génératívement avec un faible poids de la multipilcation végétative.

La première colonisation de la surface par *C. sessilifolius* laisse une question en suspens sur la localisation de la source des graines et sa distance (Mc Clanahan, 1986) et sur le type de dispersion utilisé : la littérature suggère l'hypothèse de la myrmécochorie vérifiée en différentes situations et pour des espèces voisines (Ridley, 1930 ; Beattie, 1983) : la présence de l'élaïosome (riche en graisses) prédispose les grains à être pillées par les fourmis.

Debussche, Escarré et Lepart (1980) observent que les gousses sèches de *Cytisus purgans* (L.) Boiss. peuvent être dispersées aussi par les moutons pendant la pâture ; il faut dire toutefois que cette espèce a une gousse villeuse et à dimensions inférieures.

CONCLUSIONS

Les premières recherches sur la colonisation des pâturages xériques de l'Apennin central (Italie) par *Cytisus sessilifolius* concernent une population qui se développe sur la végétation des pâturages sans, pour le moment, la modifier dans sa propre composition spécifique.

Les données relatives à la structure de la population selon l'âge identifient précisément quatre phases dynamiques :

— colonisation (22—14 années) remontant à 1965—1973 ;
— stabilisation (13—7 années) dans la période 1974—1980 ;
— expansion (6—3 années) depuis 1981 jusqu'en 1984 ;
— régression (1—2 années), actuellement en cours.

Même si la phase de régression est déjà commencée, une douzaine d'années de dominance du cytise n'ont pas encore mené à un nouveau stade de la succession secondaire ; probablement de nouvelles espèces pourront s'installer lorsque la population aura laissé libres des espaces plus grands.

Parmi les caractéristiques examinées (développement, fertilité, production des graines) il y en a beaucoup qui confirment que la population peut se répandre surtout au moyen de la reproduction à distribution autochore, à laquelle participent en grande partie les individus mûrs qui appartiennent à la classe de développement intermédiaire.

La mortalité frappe surtout les classes aux âges moyens et élevés. La rareté des jets juvéniles dans les surfaces densément occupées par les adultes permet de prévoir l'extinction de la population de ces zones dans une dizaine d'années si les conditions et le dynamisme actuels se maintiennent.

La mortalité, en outre, est presque constante dans les sept dernières années ; pourtant le passage à la phase de régression doit être dépendant d'un plus faible succès reproductif.

On a relevé aussi une étroite relation entre paramètres et stades de développement et production de graines ; une proportion presque directe a été vérifiée entre âge et accroissement en diamètre : la fonction de la courbe de régression relative permettra ainsi de faire une évalution digne de confiance sans avoir recours à des échantillonnages destructifs.

Cette première approche du point de vue de la population à l'examen du rôle de *C. sessilifolius* dans les stades de la succession secondaire, a mis en évidence en outre la nécessité d'un examen des types de populations présentes dans la montagne de Torricchio pour en relever l'origine, le type et l'expansion depuis l'institution de la Réserve naturelle.

De cette façon il sera possible d'introduire le facteur temps dans les recherches en cours, tant par la répétition de quelques observation (diachronisme) qu'en analysant différents stades de la succession (synchronisme), avec référence en particulier aux rapports quantitatifs entre les espèces en relation avec des populations différentes de *C. sessilifolius*.

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ECOPHYSIOLOGICAL CHARACTERISTICS OF SOME VEGETAL POPULATIONS IN SPRUCE FORESTRY ECOSYSTEMS

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The paper comprises the results of the ecophysiological researches referring to the primary producers in two spruce fir forests situated in the upper basin of Sebeș, Sureanu mountainous massif in the Oașa hollow area and Prigoana Valley : the assimilatory pigments, the osmotic pressure, the concentration in carbohydrates, the pH of cellular sap and the water content of the assimilatory tissue. The registered values by the analyzed ecophysiological parameters are in a proportion corresponding to a normal productive level, the ratio of chlorophylls and the carotene have higher values than the theoretical ratio. The values of the osmotic pressure are higher for the ligneous species as compared to those of herbs, but in acknowledged limits for the nemoral species. The water content of the assimilatory tissue differs especially with the phenological moment and with the species analysed.

The forestry ecosystems are to be found on 25% of Romania's surface and represent the greatest part of the territory administered by man in the vertical structure.

The spruce forests represent the forestry vegetation placed even from the middle mountainous level; the spruce stand vegetates on the slopes moderately atilt, on all expositions especially near the forest limit or neighbouring the barren places. The geobotanical literature identifies the two kinds of forests analysed into the associations : *Oxalis-Piceetum abietis* Brezina et Hadac 62, *Piceetum subalpinum* Br. Bl. 39 Al. Borza describes even new facies, in the Sebeș Valley, i.e. facies *myrtillosum*.

Typologically, it belongs to the type of spruce with *Oxalis* (Pașcovschi and Leandru 1958) and spruce with *Vaccinium*.

MATERIAL AND METHODS

The ecophysiological research work performed by us concerns the two spruce forests situated in the upper basin of the Sebeș river, in the mountainous massif of Șureanu, in the Oașa valley at 1350—1500 m height in a 90 years old forest, on a brown acid, moder type humus soil, with poor trophicity, and a mean to high humidity regime, with a 4.5—5.5 pH medium and in the Prigoana valley found at 1500 m height, in an almost virgin forest, with 150 years old trees, on a podzol-type crude humus soil, with poor trophicity, strong acidity (pH 3.5—5.0), high humidity, but hardly accessible.

The collecting of samples was made from the dominant populations of herbs and shrubs.

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The concentration of assimilatory pigments is determined with "light" leaves, collected from the lower third of trees crown, while the leaves collected from herbs are of "shade"-type according to their level in the forest structure. The used methods are the spectrophotometrical ones : Comar and Zscheille (1941) method, improved by Bruinsma (1963) for chlorophyllian pigments and the Holm (1954) method, reconsidered by Fabian (1971), for carotenoid pigments.

The water content of the assimilatory tissue has been determined by gravimetrical methods, drying the material at 85°C.

The carbohydrate concentration has been determined by refractometric analysis (6).

The pH has been potentiometrically measured. The osmotic pressure of the cellular sap has been measured by the cryoscopic method.

RESULTS AND DISCUSSION

THE SPRUCE FOREST WITH OXALIS FROM OAȘA

The content in assimilatory pigments (chlorophyllian and carotenoid ones) has a character specific to each vegetal population (Table 1). By the edifying species, *Picea abies*, the total chlorophyll content varies from spring time to summer time, taking increasing values as compared to those registered for the species in similar ecosystems and for the coniferous species in general, they are smaller than the deciduous species (4, 5).

During the aestival season, an increase of the assimilatory pigment content is noticed for the herbaceous level which is correlated with the existence of a high photosynthesis. For example, *Luzula luzuloides* and *Senecio fuchsii* are to be mentioned (Table 1).

The carotenoid pigments, although in a lower quantity with coniferous trees as compared to deciduous trees, play an active part in light absorption both in vernal and aestival periods (2, 3).

The osmotic pressure registers higher values with the ligneous species than with the herbaceous ones; the values registered by *Picea abies* are comparable with those of the similar species in the ecosystem and integrate themselves within normal limits for these types of forests (1), (4), (8). The smaller values registered in the vernal season imply a more reduced osmotic effort of plants, mostly determined by a positive level of water supply of the station (Table 1).

Carbohydrate concentration of the cellular sap plays an important part in the osmotic adjustment ; the values are generally correlated directly with those of the osmotic pressure. The highest values are registered by *Picea abies* both in the vernal and aestival seasons (Table 1).

The pH of cellular sap, except for the *Oxalis acetosella* species registers values that render evident an acid-neuter character which implies a satisfactory metabolic activity under the existing site conditions.

The water content of the assimilatory tissue is high during the whole period of vegetation for the dominant herb species; lower values are characteristic for the leaves of the wood species (Table 1).

Table 1

Ecophysiological indexes characteristic for the primary producers in the spruce forests with *Oxalis* in Oașa (the Sebeș Valley)

Species	Cellular sap			Chlorophyll a + b × 10^{-4} g/g d.w.	Carotenoid pigments × 10^4 g/g d.w.	Ratio		Water content %
	Osmotic pressure (bars)	carbohidrates %	pH			Chlorophyll a/b	Chlorophyll/carotenoids	
Spring time								
Trees and shrubs								
Picea abies	27.39	18.00	3.9	31	6	1.77	5.17	58
Vaccinium vitis-idaca	—	—	—	29	5	1.79	5.80	55
Herbs								
Oxalis acetosella	10.60	5.50	1.9	90	18	1.97	5.0	80
Adoxa moschatellina	12.04	5.00	4.9	—	—	—	—	90
Luzula luzuloides	14.20	5.00	6.0	—	—	—	—	81
Summer time								
Trees								
Picea abies	22.60	13.00	4.0	35	8	3.92	4.37	58
Herbs								
Oxalis acetosella	14.68	5.50	2.9	78	20	2.01	3.90	86
Senecio fuchsii	18.76	5.00	6.0	91	36	6.15	2.61	87
Fragaria vesca	17.08	7.00	5.0	—	—	—	—	70
Luzula luzuloides	—	—	—	135	40	3.32	3.37	82
Calamagrostis arundinacea	15.88	7.00	8.0	—	—	—	—	84

Table 2

Ecophysiological indexes characteristic for the primary producers in the spruce forests with *Vaccinium* in Prigoana (the Sebeș Valley)

Species	Cellular sap osmotic pressure (bars)	Cellular sap Carbohydrates %	pH	Chlorophyll a+b (10^{-4} g/g d.w.)	Carotenoid pigments (10^{-4} g/gd.w.)	Rate		Water content %
						Chlorophyll a/b	Chlorophyll/ /carotenoids	
Spring time								
Trees and shrubs								
Picea abies	25.71	18.00	4.0	45	9	1.68	5.07	58
Vaccinium vitis idaea	27.63	17.00	4.8	—	—	—	—	53
Vacinium myrtillus	—	—	—	42	10	1.86	4.50	55
Herbs								
Oxalis acetosella	12.52	5.00	2.9	89	18	1.87	5.23	80
Soldanella montana	—	—	—	80	17	1.72	4.94	80
Summer time								
Trees and shrubs								
Picea abies	23.56	17.00	4.1	44	8	2.83	8.50	60
Vaccinium myrtillus	20.52	12.00	3.9	24	6	3.17	3.90	60
Vaccinium vitis idaea	20.44	14.00	4.5	17	6	3.52	3.73	60
Herbs								
Oxalis acetosella	12.76	7.00	2.3	98	25	2.92	3.92	80
Luzula luzuloides	—	—	—	103	28	3.12	3.75	76
Deschampsia flexuosa	13.24	5.00	6.0	58	16	3.14	3.62	82
Luzula sylvatica	13.96	5.00	5.2	115	28	2.67	4.38	74
Luzula pilosa	—	—	—	98	26	2.92	3.76	75

THE SPRUCE FOREST WITH *VACCINIUM* FROM PRIGOANA

The content in assimilatory pigments analysed with the wood species, *Picea abies* both for the two shrub species, *Vaccinium vitis-idaea* and *V. myrtillus* and the main herb populations may be influenced by the species and especially by the site conditions (Table 2).

The total chlorophyll content decreases with the shrub species in the aestival season, a condition made up for by the high density and the gravimetric weight registered at this vegetation level.

Although the content in assimilatory pigments increases with the most aestival species, however the lower photosynthetic level, mirrored also in the productive capacity of the ecosystem, is determined by the structure of phytocoenosis, the older age of the arboretum and especially by both the too high acidity of the substratum and an excessive humidity.

The osmotic pressure of the cellular sap, as an ecophysiological parameter of the hydric conditions, has higher values both for the dominant species *Picea abies* and *Vaccinium vitis-idaea*, considerably higher in the vernal season as compared to the aestival one (Table 2). The herbaceous species have an osmotic level that inscribes between the limits of variation known for nemoral species (1), (4), (5), (7), (8).

Carbohydrate concentration of the cellular sap varies in the same direction with the osmotic pressure, which is considerably higher for *Picea abies* as compared to herbs.

The pH values of the cellular sap vary between 1.9—2.9 with *Oxalis acetosella* and 8.0 with *Calamagrostis arundinacea*; the pH values inscribe in the acid field which shows a good metabolic activity concordant with a satisfactory productive level.

The water content present in the assimilatory tissue is high in this ecosystem too; it overpasses 80 percent and represents a consequence of the existence of a good water supply in the ground, on the one hand, and of a rich composition of species, on the other hand. Lower values are registered with *Picea abies*, *Vaccinium vitis-idaea* and *V. myrtillus* (Table 2), values that generally correspond both to the analysed phenaspect and to the type of species.

CONCLUSIONS

The assimilatory pigments are to be found in normal quantities in the two analyzed stations, for trees and herbs as well; depending on the species, there is a less amount in Prigoana and this fact is correlated with the lower productive level of this forest.

The relation between the chlorophyllian pigments and the carotenoid ones, although not theoretically corresponding, is characteristic for the species, especially in Oașa.

The values of the osmotic pressure are moderate with the studied species which implies the existence of a normal process of water absorption specific to the nemoral species from Oașa as compared with that from Prigoana, where the absorption process is more difficult.

The water content of the assimilatory tissue differentiates by the phenologic moment and by the species.

The pH of the cellular sap indicates a positive metabolic activity, especially for the Oaşa spruce forests.

The carbohydrate content is correlated with the values of the osmotic pressure, in both stations, with higher values for trees as compared to herbs.

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DEHYDROGENASE ACTIVITY OF CHLOROCOCCACEAN ALGAE GROWN IN DEFINITE NUTRIENT MEDIA

F. NÀGY-TÓTH

A dozen of Transylvanian unicellular green algae (*Scenedesmus acutus* Meyen strains Alba, Caz., Clj., Fortuna, Mono, Talab.; *S. intermedius* Chod., Caz.; *Choricystis guttula* Hind., Caz., Zah; *Chlorella luteoviridis* Chod., Arok; *C. minutissima* Fott et Novák., Rez.; *Chlorococcum* sp., Caz.) were grown in batch cultures in order to investigate their production potential in definite artificial nutrient media, their ability to expoit the given trophic pool and, as a reflection of the vital processes, to determine their dehydrogenase activity in correlation with their protein and chlorophyll contents.

Under standard living conditions a fairly good productivity was achieved with *Chlorella minutissima*, Rez., *Scenedesmus acutus*, Clj., and *S. acutus*, Caz., Talab and Alba. Molasses in concentration of 0.5 % enhanced the productivity of two other strains (Fortuna, Mono) of *S. acutus*, while CO_2 and N in a higher amount lowered it.

Dehydrogenase activity varied according to species and strains (0.021 — 0.138 and 0.365—0.834 mg triphenylformazan/mg dry weight), and growth conditions (0.17—0.310 and 0.040—0.757 mg triphenylformazan/mg dry weight). It was higher in algae grown under sub-optimal conditions; i.e. a negative correlation between dehydrogenase activity and growth intensity could be established, but the correlations were positive with the chlorophyll and protein contents of the cells.

Specificity and congruency of these algae appeared in different ways, e.g. in five of *S. acutus* strains (Caz., Alba, Talab., Fortuna, Mono) the productivity was nearly the same, while dehydrogenase activity differed significantly, especially, if it was related to different constituents. It is believed that specificity appears more evidently under harsh conditions.

The results registered during these experiments may contribute to a better taxonomy of *Chlorococcacae*, as well as to the selection of suitable algae for scientific and biotechnological purposes.

Many criteria are used to properly characterize algal species in order to establish their accurate taxonomic position, their natural places in the ecosystem. During the last four decades the physiological and biochemical markers have become more and more favourized not only because they can easily be determined with sophisticated experimental tools, but also because they help reveal biological potentials which reside in algal cells and can act if the environmental conditions allow. Moreover, the correlation of morphological, structural and physiological specificities of different algal species seems a useful and necessary task for phycology.

Dehydrogenase activity determined by the reduction of triphenyltetrazolium chloride (TTC) is regarded as an expression of the vitality of living cells (I. Szalai, V. Frenyó, 1962; P. L. Steponkus, F. O. Lanphear, 1967; H. Bergmann, 1974; R. Maier et al., 1981). Based on this reaction, methods have been developed to estimate plankton population density (A. A. Alleem, 1955) which were further standardized by electrochemical measurements (T. T. Packard, M. L. Healy, 1968) in order to reveal marine plankton metabolism (J. P. Christensen, T. T. Packard, 1977; F. D. King et al., 1978). Assayed in natural blue-green water bloom population and in its bacterium-free culture as well, it was found that there are substrateless and substrate-linked dehydrogenases; their acti-

vity was enhanced by sucrose and ethanol (M. I. Kuzmenko, 1980). Though, the global activity of dehydrogenases, i.e. the respiratory electron transport system (ETS) (T. T. Packard, 1971) can reveal many correlations with other physiological properties of algae (e.g. productivity, protein content, chlorophyll *a* amount /F. D. King et al., 1978; P. C. Garfield et al., 1979; F. W. Setchell, T. T. Packard, 1979/), it was rather seldom assayed in pure algal culture.

In order to know more about the algae living in different habitats of Transylvania we have dealt with for many years (F. Nagy-Tóth, A. Barna, 1987), a dozen of algae from the Collection of Pure Cultures (F. Nagy-Tóth, A. Barna, 1987) have been experimented in batch cultures. The aim was, primarily, to establish their production potential under different trophic conditions. It seems reasonable to determine dehydrogenase activity for sensing the balance between catabolic and anabolic processes during growth.

MATERIAL AND METHODS

In four experimental sets, six strains of *Scenedesmus acuius* Meyen, and one strain of *S. intermedius* Chod., two strains of *Choricystis guttula* Hind,. two *Chlorella* species (*C. luteoviridis* Chod., *C. minutissima* Fott et Novák.) and one species of *Chlorococcum* sp. were investigated. As it can be seen from the mark of the strains (Tables 1—4), all these algae have been collected from different habitats and presumably still possess though repressed, some of their ancestral specific properties which would emerge by improved growth conditions.

Table 1

Relationships between dehydrogenase activity and biosynthetic capacity in eight Chlorococcacean algae 12 days of culture, Knop-Pringsheim-Felföldy's nutrient solution, Vladimirova-Semenenko's type vessels, 13,500 lx)

Algae	Cells /µl	mg dry wght l/day	Protein %	Chloroph. %	Total pigm. %	Dehydrogenase activity, mg formazan			
						mg dry wght	10^6 cells	mg protein	mg chloroph-s
Chlorella luteoviridis, Arok	176,250	147	35.97	1.04	1.30	0.069	0.69	0.19	6.63
Choricystis guttula, Zah	12,190	38	64.62	0,63	0,91	0,138	5,20	0,21	21,90
Ch. guttula- Caz.	97,200	103	60,45	1,16	1,68	0,112	1,41	0,19	9,65
Chlorococcum sp., Caz.	65,200	197	39,57	1.11	1.36	0,094	3.39	0.24	8.47
Scenedesmus intermedius, Caz.	20,000	136	49.14	0.66	0.85	0.099	15.97	0.36	12.32
S. acutus, Caz.	134,375	382	17.67	0.60	0.88	0.031	1.05	0.18	5.17
S. acutus, Alba	142,800	316	24.69	0,69	0.88	0.053	1.40	0.21	6.78
S. acutus, Talab.	120,300	380	23.40	0.69	0.97	0.021	0.78	0.09	3.04

Table 2

Dehydrogenase activity and growth potential in four Chlorococcacean species (21 days of culture, Kuhl,Lorenzen's nutrient solution, vertical columns 5,500 lx)

Algae	Cells/µl	mg dry wght/ liter/day	Protein %	Dehydrogenase activity, mg formazan		
				mg dry wght	10^6 cells	mg protein
Chlocella luteoviridis, Arok	190,625	90	47.19	0.420	4.200	0.89
Chlorococcum sp., Caz.	38,500	100	40.38	0.485	26.40	1.20
Scenedesmus intermedius, Caz.	25,250	79	40.73	0.365	23.85	0.90
S. acutus, Alba	62,610	81	45.50	0.834	22.64	1.83

Table 3

Effects of CO_2 and N-concentration on the growth and dehydrogenase activity of two chlorococcacean algae (7 days of culture, Kuhl-Lorenzen's nutrient solution, Vladimirova-Semenenko's type vessels, 13,500 lx)

Algae	Growth conditions		Cells/µl	mg dry wght/ liter/day	Dehydrogenase activity mg formazan	
	CO_2 %	mg N/l			mg dry wght	10^6 cells
Chlorella minutissima, Rez	5	140	288,125	336	0.310	2.52
		280	309,375	359	0.210	1.70
	1.5	140	661,870	559	0,143	0.84
		280	575,940	427	0.202	1.04
Scenedesmus acutus, Clj	5	140	64,375	313	0.117	3.98
		280	58,450	297	0.190	7.73
	1,5	140	87,200	453	0.147	5.34
		280	75,000	339	0.295	10.42

In a set of experiments 8 algae were grown in Knop-Pringsheim-Felföldy's nutrient solution (L. J. M. Felföldy, 1961) (Table 1), while in the other three sets Kuhl-Lorenzen's solution (A. Kuhl, H. Lorenzen, 1964) was used (Tables 2—4). Experimental variants were set up by doubling the amount of nitrogen in Kuhl-Lorenzen's solution and by supplying it with molasses (in concentration of 0.5 and 1%) as well as by increasing the CO_2 content in the sterilized bubbling air from 1.5% to 5% (Tables 3—4). In all cases pH of the solutions was adjusted to the value of 6.7—7.0 and then the solutions were autoclaved (at 0 2 atm, 105°C for 1 hour). After cooling the samples were inoculated with an aliquot amount of algal suspension taken from subcultures in logarithmic growth phase. The resulting suspensions were poured into modified Vladimirova—Semenenko's vessels (F. Nagy—Tóth, 1987), or into vertical columns (S. Péterfi, F. Nagy—Tóth, 1967), each variant comprising 3—5 repetitions, and then placed in a setting up (S. Péterfi, F. Nagy—Tóth, 1967) where they were illuminated bilaterally at an intensity of

Table 4

Effects of C-sources and O_2 on the growth and dehydrogenase activity of two different strains of *S. acutus* (4 days of culture, Kuhl-Lorenzen's nutrient solution, Vladimirova-Semenenko' type vessels, 13,500 lx)

Algae	Experimental conditions	Cells/µl	mg dry w/l/day	NH_2-groups	Chlor. — s %	Total pigm. %	Dehydrogenase activity mg formazan			
							mg dry wght	10^6 cells	mg chlor — s	mg NH_2-group
Scenedesmus acutus, Fortuna	air + 1,5 % CO_2	23,200	140	44.65	3.88	4.54	0.096	2.84	4.55	0.215
	air + 1,5 % CO_2 + 0 5 % molasess	24,530	610	16.79	1.99	2.32	0.040	2.17	2.29	0.238
	air without O_2	5,250	40	17.62	1.75	2.13	0.36	14.45	52.56	2.043
	air without O_2 + 1 % molasses	250	11	37.05	0.131	1.69	0.47	55.92	103.54	12.680
		25,750	150	54.44	3.80	4.50	0.122	2.31	3.84	0.224
Scenedesmus acutus, Mono	air + 1 5 % CO_2	34,560	470	22.45	2.47	2.81	0.044	3.97	2.87	0.195
	air + 1 5 % CO_2 + 0 5 % molasses	4,190	20	19.54	1.175	2.71	0.757	10.90	3.18	3.874
	air without O_2	650	4	28.60	0.419	0.504	2.423	86.16	73.43	8.472
	air without O_2 + 1 % molasses									

6,500—7,000 + 6,500 — 7,000, or unilaterally at 5,500 lx and simultaneously bubbled (1 ml of mixture of sterilized air supplied with 1.5 %, CO_2 for 60—80 ml of algal culture) daily for 12 hours. Cultivation up to the end of the logarithmic phase of *S. acutus*, Fortuna (which is considered the reference strain in these experiments) lasted 5, 7 and 12 days in Vladimirova—Semenenko's vessels, and 21 days in vertical columns.

Dehydrogenase activity was determined based on a method used for a long time in soil microbiology * (S. Kiss, M. Boaru, 1965 ; M. Drăgan—Bulandra, 1978) and modified according to the nature of the higher plants or algal materials (P. L. Steponkus, F. O. Lanphear, 1967 ; H. Bergmann, 1974 ; R. Maier et al., 1981). The procedure applied was the following : samples of 50—100 ml (depending on cell densities) taken from the cultures were centrifuged (at 4°C, 4,500 rotations/min, for 15 min), washed thrice with McIlvaine phosphate buffer (pH 7.5) (L. Mázor, 1960) and finally resuspended with phosphate buffer to a volume of 10 ml. In graduated test-tubes 20—30 mg $CaCo_3$ sicc., 1.5—3 ml washed algal suspension, and 0.5 ml 3% TTC solution (2, 3, 5-triphenyltetrazolium chloride, Austranal, dissolved in McIlvaine's buffer) were successively added, and then stirred and incubated (28—30°C) for 24 hours. The triphenylformazan formed during incubation was extracted with 96% ethanol or acetone, used in an amount necessary to complete the mixtures in test-tubes to 10 ml, then the mixtures were stirred well and filtered repeatedly in graduated flasks (25 or 50 ml) and the formazan extracted was immediately measured photocolorimetrically (at 540 ± 10 nm with a FEK-56 M photocolorimeter). Dehydrogenase activity was expressed as mg triphenylformazan reported to dry weight of algae, cell number, and chlorophyll, or protein contents.

From the same experimental material, the optical and cell density (photocolorimeter FEK-56 M, at 597 ± 10 nm and Büreker's haemocytometer, respectively), the biomass production (mg/l of dry weight at 105°C of 50—100 ml algal suspension) and the productivity (dry weight/litre/day) of the cultures were also determined. The cell biomass was analysed for its protein and pigment contents. For the determination of the NH_2-groups the O. H. Lowry's method applied by G. E. Fogg (1966) and adapted by S. Péterfi et al. (1978) was used, whereas the total nitrogen was determined with a Kjeldahl procedure adapted by L. L. Shchetinina and V. A. Butenko (1957), and by K. E. Ginzburg et al. (1973). The pigments were determined according to A. Hager and T. Meyer-Bertenrath (1966).

RESULTS AND DISCUSSIONS

According to the purposes of the researches here dealt with, the algae were primarily considered on the basis of their productivity and their capacity to exploit definite nutrient pool in the given media. All other physiological properties actually determined, including dehydrogenase activity, were correlated with productivity. This biotechnological criterion varies within large limits depending on species, strains and growth conditions.

* Hearty thanks to Prof. Dr. I. Kiss for the multiple help granted during this work.

When grown simultaneously in identical standard nutrient solutions at higher light intensity (13,500 lux), two strains (Caz., Talab.) of *Scenedesmus acutus* gave the highest productivity (380 mg dry weight/litre suspension/day) (Table 1), but at lower light intensity (5,500 lux) *Chlorococcum* sp., Caz. was the most productive (Table 2). Nevertheless, weaker light diminished the productivity of all species, mostly that of *Scenedesmus intermedius*, Caz. and *S. acutus*, Alba (Table 2). Perhaps these algae are more sensitive to light than *Chlorococcum* sp., Caz. and *Chlorella luteoviridis*, Arok.

Of all species examined the highest productivities were recorded in *S. acutus*, Fortuna grown in Kuhl-Lorenzen's nutrient solution supplemented with 0.5% molasses (610 mg dry weight/litre/day), *Chlorella minutissima*, Rez. grown in standard solution (559 mg dry weight/litre/day) (Table 3), and *S. acutus*, Mono (470 mg dry weight/litre/day) grown in nutrient solution enriched with molasses in an amount of 0.5% (Tables 3, 4). It is perhaps interesting to mention that strain Fortuna reached its stationary growth phase earlier than strain Mono, which could be considered a physiological specificity. Nevertheless, these kinds of differences arose more evidently under unfavourable conditions, e.g. lack of O_2 in the environment, excess of molasses (Table 4). The slowest growth was recorded in the cultures of *Choricystis guttula*, Zah (the productivity was ten times lower than in *S. acutus*, Caz. and Talab). (Table 1), while the most inhibited cultures were those deprived of oxygen and those supplied with molasses in a proportion of 1% (Table 4). Of course, cell numbers cannot be compared at all, or they can be compared only very cautiously, even in the same strain if grown under different conditions, because of the variability of the size of its cells. The cultivation conditions, as can be seen from these data and as it has been repeatedly revealed, have a profound influence on the growth, the productivity and chemical composition of algae. Thus, increasing the CO_2 content in the bubbling air (from 1.5 to 5%) without or with doubling the nitrogen amount in Kuhl-Lorenzen's nutrient solution (from 140 to 280 mg/l) decreased, nearly at the same rate, the growth and multiplication of both *Chlorella minutissima*, Rez. and *Scenedesmus acutus*, Clj. It is relevant to note the difference in sensitivity, i.e. the specificity of these two algae; *Chlorella* showed higher dehydrogenase activity when CO_2 concentration was higher and N-content lower, while *Scenedesmus* was more dehydrogenase-active when CO_2 was lower and N higher. The higher concentrations of CO_2 and N strengthened mutually their effects, acted synergically on *S. acutus*, Clj. (Table 3). But molasses, as organic carbon source, in an amount of 0.5% in the nutrient solution enhanced the growth of both strains (Fortuna, Mono) of *S. acutus*. Nevertheless, in double amounts and under anoxic conditions its effect was strongly inhibitory.

Dehydrogenase activity varied between 0.021 and 0.834 mg formazan/mg dry weight of algae, namely between 0.021—0.138 and 0.365—0.834 mg, depending on algal species and strains, respectively, as well as between 0.117—0.310 mg and 0.040—0.757 mg, depending on growth conditions (Tables 1—4). Thus, as it occurs fairly frequently in the physiological processes of algae, variations induced by external factors can be as strong as the specific factors, or even stronger than the specific

ones. Otherwise, these values are comparable to those published by M. I. Kuzmenko (1980) in mixed population of "water bloom" and axenic algal cultures, respectively, namely 18.75—51.97 mg formazan/g dry weight and 35.33—84.59 mg when the substrate was enriched with sugars. Furthermore, the limits of variations recorded can also be considered quite usual on the basis of productivities (in average 200—600 mg dry algae/litre/day) of these algae, which lie between the limits quoted as good achievements (e.g. 0.117—0.189 and 0.654 g dry weight/litre/day by *Chlorella pyrenoidosa* and 0.71 g/litre/day by *Scenedesmus quadricauda* [H. Witsch, R. Harder, 1961; V. A. Chesnokov, 1962; J. Komárek, J. Ruzicka, 1969]).

Relating the dehydrogenase activity to cells, or protein and chlorophyll contents the feature of the physiological status of these algae appears, obviously, different. It is, of course, determined first of all by the size of cells, which varies also within rather broad limits. Considering, for instance, the three strains of *S. acutus* when grown in stronger light, dehydrogenase activity gave fairly close values, i.e. 0.79, 1.05 and 1.40 μg formazan/10^3 cells (Table 1), but in weaker light it became nearly 20 times higher (Table 2). However, the *S. intermedius*, Caz cells in stronger light, having lower productivity, seemingly being inhibited, manifested more intense, nearly 10 times higher dehydrogenase activity than under weaker light conditions in which case this activity was nearly equal with that of *S. acutus* Alba. The activity varies on the same manner with *Chlorella luteoviridis*, Caz. and *Chlorococcum* sp., Caz., too.

Differences in growth conditions (light intensities, nutrient solutions, vessels) of the same four species of algae cultivated successively (Tables 1, 2) can, certainly, explain their differences in dehydrogenase activity, too. But, there is another reason, namely, their differences in age; the first experiments lasted 12 days, while the second ones 21 days. And according to H. Bergmann (1974), dehydrogenase activity increases with the age of cells.

Total protein content varied within wide limits (17.67—64.62 %) and, generally, correlated (positively with dehydrogenase activity (Tables 1, 2), which agrees with the conclusions of P. C. Garfield et al. (1979). Similarly, the positive correlation seems to be valid when dehydrogenase activity is related to the amount of proteic NH -groups (Table 4).

The highest dehydrogenase activity was recorded when it was reported to the chlorophyll content of the cells (73.43 and 103.54 mg formazan/mg chlorophylls), namely, in the two strains (Fortuna, Mono) of *S. acutus* grown in nutrient solution supplemented with 1% molasses (Table 4). It appears again that the growth conditions have stronger effects on dehydrogenase activity than it would be determined by endogenous properties, i.e. specificity of algae. Since dehydrogenase activity varies in a higher degree than the chlorophyll and total pigment contents, it was difficult to ascertain any doubtless relations between these two physiological characteristics. Thus, while the total pigment contents in three strains of *S. acutus* were nearly the same, dehydrogenase activity differed significantly (Table 1). Nevertheless, positive correlations between chlorophyll content and dehydrogenase activity seem to be valid in these experiments too, as it was found by F. W. Setchell and T. T. Packard (1979). Otherwise, the chlorophyll content of the cells was

comprised between the limits of 0.131—3.88%, while that of total pigments between 0.88—4.54%, which correspond to the mean values given for green algae (P. Böger, 1964; F. Nagy—Tóth et al., 1980). In cells grown in media containing molasses the pigment contents were lower (Table 4), probably because of heterotrophic nutrition, according to L. Bergmann (1955), though C. Eyster et al. (1958) found a little higher chlorophyll concentration in heterotrophically grown *Chlorella pyrenoidosa*. Such differences could be due to the fact that different algae have different heterotrophic potentials (F. Martinez et al., 1987). The pigment contents were, generally, negatively correlated with the density of the normal cultures (Table 1). No significant differences among *Scenedesmus* strains could be noticed under such conditions. However, in slowly grown and in inhibited cultures, chlorophyll and total pigment contents were lower (e.g. *Choricystis guttula*, Zah, as well as *S. acutus*, Fortuna and Mono grown in solutions containing molasses or under anoxic conditions).

Noteworthy is the negative correlation of dehydrogenase activity with the cell density of the cultures, which, otherwise, is in good agreement with the productivity, as well. When cell densities were about the same in the two strains (Fortuna, Mono) of *S. acutus*, dehydrogenase activities were close too (Table 4), which follows, probably, from the close mean sizes of the cells. This congruency can be argued by the opposite finding which results from the significant difference in cell sizes and dehydrogenase activities of *Chlorella minutissima*, Rez. and *S. acutus*, Clj. (Table 3).

CONCLUSIONS

Considering all relations of dehydrogenase activity with other physiological characteristics (both positive and negative correlations), found in these experiments, two main conclusions appear evident: (1), the extreme variability of dehydrogenase activity determined either by species and strains or growth conditions, and (2) the harder the life conditions the higher the dehydrogenase activities are. These statements seem obvious if the tolerance or sensitivity of algae as well as the respiratory electron transport system are considered. Different species/strains started to grow under identical life conditions gave, after a while, mostly different results according to their intrinsic ecological, physiological and genetic properties (Tables 1, 2). However, convergence could appear, but not in all characteristics. Thus, three strains (Caz. Alba, Talab.) of *Scenedesmus acutus* had almost the same productivities, but they differed in respect of their protein content and dehydrogenase activity. Similarly, other two strains (Fortuna, Mono) of *S. acutus* in normal nutrient solution had close cell densities, productivities, chlorophyll and total pigment contents, but differed in proteic,NH_2 content and dehydrogenase activity. The specific differences were amplified by unfavourable trophic conditions (Table 4). Consequently, comparison of the data will contribute to a better taxonomic identification of the species and, even to a higher extent, to the selection of suitable algae for scientific and biotechnological purposes.

Dehydrogenases which are located in mitochondria, microsomes and cytosol (A. L. Lehninger, 1976) "consist of a complex chain . . . that transport electrons from catabolized foodstuff of oxygen" (T. T. Packard, 1971) and their activity is an estimate of the metabolic activity of phytoplankton (F. D. King et al., 1978) and soil (S. Kiss, M. Boaru, 1965; M. Drăgan- Bularda et al., 1984), a measure of the respiration rate (T. T. Packard et al., 1971; H. Bergmann, 1974; R. Vintilă et al., 1989). Consequently, it seems obvious that dehydrogenase activity is more intense under sub-optimal living conditions which bring about more intense metabolic activities, especially catabolic processes and, finally, this activity results in negative correlations with productivities, and in positive ones with the protein and chlorophyll contents, as well as with the age of cultures.

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

I. PROGESTERONE EFFECT ON "IN VITRO" CULTURES OF *NICOTIANA TABACUM* cv. *XANTHI* SOMATIC TISSUES

AURELIA BREZEANU, GINA COGĂLNICEANU, DOINA CÂRCIUMĂRESCU

The progesterone influence on *Nicotina tabacum* cv. *Xanthi* callus proliferation and on some morphogenetic processes induced in "in vitro" culture of somatic tissues was investigated. The concentrations of 0.25; 1.25; 2.5; 5; 10; 25 mg progesterone L^{-1} were used. The action of progesterone is complex, the concentration dependent and selective with respect to the type of explant and morphophysiological peculiarities. The highest concentration used (25 mg$\cdot$ L^{-1}) proved to have toxic effects, causing the suppression of indirect organogenesis, progressive etiolation and finally explant extinction.

It has been proved that steroids are present in plants. Simons and Grinwich (9) detected the presence of androsterone (5α-androst-16-en-3--one), androgene (4 androsten-17 β-01-3-one, plus 5 α-androstan-17 β-01-3-one), estrogene (1, 3, 5 (10)-estratrien-3-01-17 one, plus 17 β-estradiol) and progesterone (4-pregnen-3, 20-dione) by radioimmunoassay in plant tissues from 128 species belonging to 50 families. No connection between the widespread occurrence of steroids in plants and species, tissue, organ, sex, age, or any metabolic specialized pathway has been found. The function of these steroids in plants is not yet known.

Plant tissue cultures represent a useful experimental system for the investigation of the biosynthesis and of the metabolism of steroids (10), (11), and at the same time, for the biotechnological exploration of their biosynthetic capabilities (6). The experimental data in this field are scarce so far. We mention the research of Graves and Smith (5) and Furuya et al. (4) who have emphasized that suspension culture of *Nicotiana tabacum* and *N. rustica* are capable to metabolise progesterone.

In this context, the possible involvement of progesterone in the modulation of some morphogenetic processes on somatic tissue culture of *Nicotiana tabacum cv. Xanthi* has been investigated and is presented in the paper.

MATERIALS AND METHODS

The *Nicotiana tabacum cv. Xanthi* plants were used in our experiment. The explants were represented by pieces of somatic tissue (petiole, mesophyll, nodato area, internodato area) of about 5—10 mm, excised from the aseptic developed plants with 8—12 fully expanded leaves. The explants were aseptically inoculated on agarised culture medium represented by the basal Murashige and Skoog (8) containing B_5 medium's vitamins and supplemented with NAA (1.0 mg $\cdot$ L^{-1}) or 2,4 D (0.5 mg $\cdot$ L^{-1}) as phytohormones sources. Incubation was performed at 26—28°C $\pm$ 2°C temperature under an illumination regime of approximatively 3000 lux

and a photoperiod of 16 h light daily or in continuous light. The progesterone pulvis influence an the tobacco tissue cultures was analysed by exogenous application in the culture media in concentrations of : 0.25 ; 1.25 ; 2.5 ; 5 ; 10 ; 25 mg · L ^{2}. Experiments were done on 15—30 specimens/explants type/concentration order. All variants were repeated three times.

RESULT AND EISCUSSIONS

a) THE BEHAVIOUR OF THE *N. TABACUM* SOMATIC EXPLANTS ACCORDING TO THE "IN VITRO" CONDITIONS

The reactivity of the explants belonging to different categories of organs and tissues according to the "in vitro" conditions varies significantly being dependent on their morphophysiological peculiarities and on the nature of the exogenous auxin (Table 1). Both callus initiation

Table 1

Various explants reactivity "in vitro" conditions

Nature of the explant	Exogenous auxin	Callus induction	Indirect organogenesis		Direct organogenesis	Abnormal indirect organogenesis
			Rhizogenesis	Caulogenesis		
Foliar mesophyll	NAA	++	++	–	–	–
	2,4 D	++	++	–	–	+
Petiole	NAA	+++	++	+	–	–
	2,4 D	+++	++	+	–	+
Nodato area	NAA	+	+	–	++++	–
	2,4 D	+	+	–	++++	–
Internodato area	NAA	++++	++	+	–	–
	2,4 D	++++	++	+	–	+

– absence of reactivity
+ weak reactivity
++ satisfactory reactivity
+++ good reactivity
++++ strong reactivity

and induction of some morphogenetically processes appeared more intensively at internodata and petiole level. We also observed these phenomena in previous experiments using other cultivars and species of the genus (1), (2), (3), (7). As regards the effect of exogenous auxins, we mention that the presence in the culture medium of NAA (1.0 mg · L^{-1}) determined in most categories of explants an intensive calogenesis (Plate

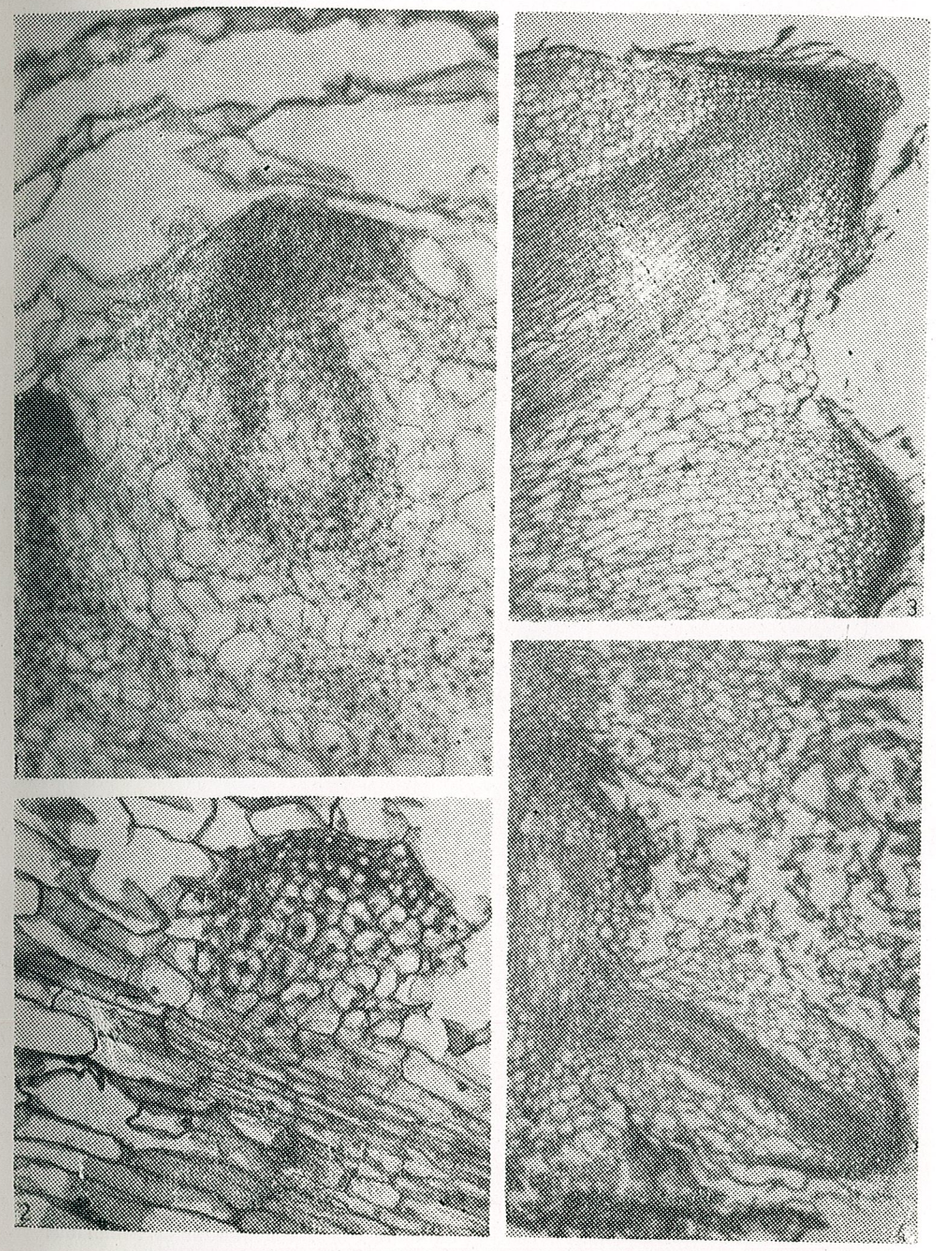

Plate I. — The meristemoidal organogenetic clumps in the callus culture of *N. tabacum* cv. *Xanthi* from superficial layers (Fig. 1) and deep layers (Fig. 2) sometimes originated from vascular strands. Root cap of the differentiated roots primordia from superficial meristematic areas (Fig. 3) and from the deep meristematic areas of the callus (Fig. 4).

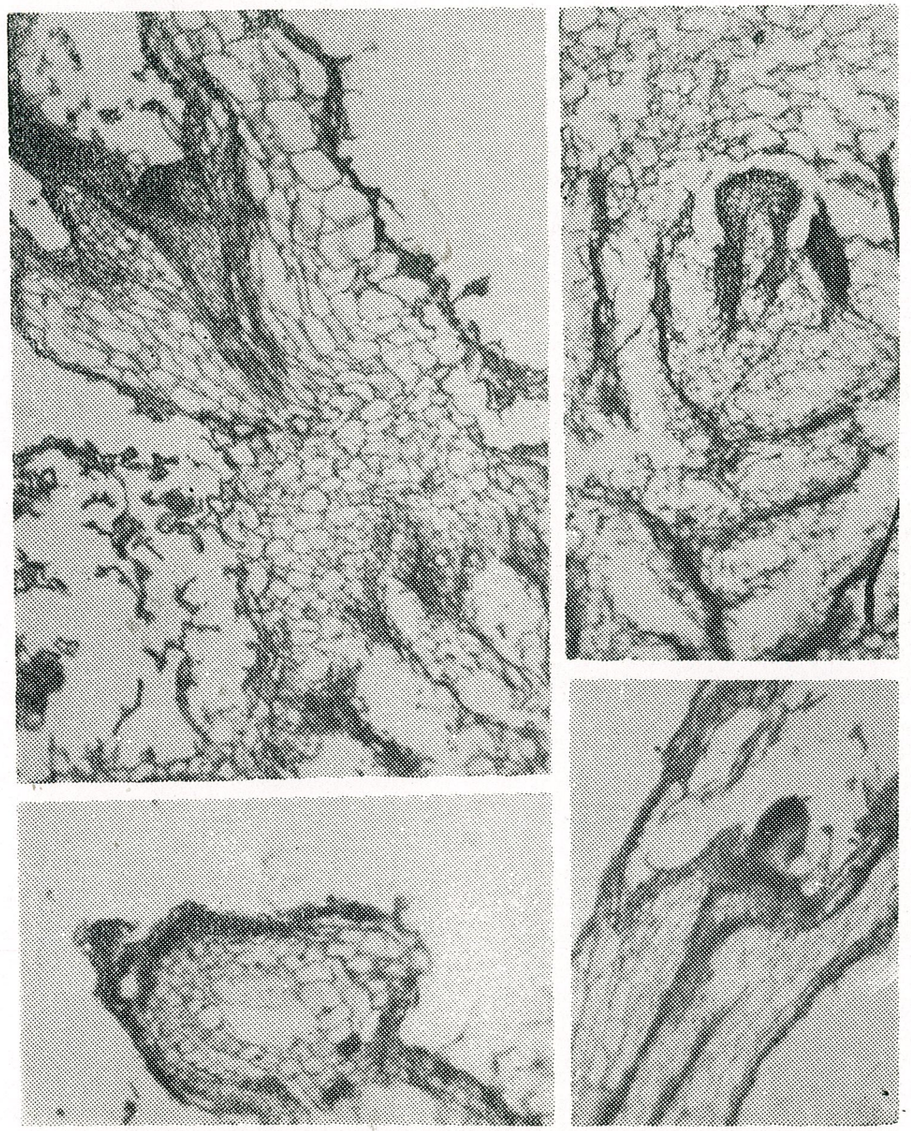

Plate VI. — The particular histological aspects regarding the differentiation of anomalous shoot primordia in the callus under treatment with 2,4 D and progesterone.

II Fig. 1) followed mainly by the formation of roots and sporadically of shoots. The development patterns of roots and shoots were similar to those described in the literature by us and others (1), (2), (3), (7) (Plate I and Plate III, Fig. 1). The new organs originated from the cells located in superficial areas of the callus (Plate I, Fig. 1, 3) as well as from the deep areas of the vascular clumps or strands (Plate I, Fig. 2, 4). The addition in nutritive medium of 2, 4 D (0.5 mg · L^{-1}) inhibited callus differentiation and altered the normal developmental program of roots and shoots in all cultured explants except for the nodato area. The anomalous formations which did not evolve either in typical roots or shoots developed in this case (Plate IV). The inoculum from the nodato area presented a particular evolution, a multiple axillary shoot developed vigorously (100%) in the presence of NAA as well as of 2, 4 D.

b) EFFECT OF PROGESTERONE IN SOMATIC TISSUE CULTURES

In our experimental conditions, the exogenous administration of progesterone had an increased inhibitory effect depending on the concentration, on callus initiation and growth as well as on the induction of some morphogenetical processes. The phenomenon materialised by a progressive decrease of callus quantity as well as by loosing its capacity of morphogenessis (Plate II and III). In the presence of 25 mg · L^{-1} progesteron the callus did not differentiate at all and at a short time after inoculation the degradation and necrosis of explants appeared. This may suggest a possible interference between the action of progesterone either with dedifferentiation processes (loss of some metabolic abilities) or with the reactivation of mitotic divisions. The mechanism of these effects can be speculated at present only. Apparently the administration of progesterone in moderate concentrations (1—5 mg · L^{-1}) did not affect the pigmentation, the texture and friability of the callus.

The sensibility of the inoculum to progesterone decreased in the order : leaf > petiole > internodato area > nodato area. This may be explained by the large excision area of the leaf explant as compared with petiole or internodato area.

The administration of progesterone in a concentration of 25 mg · L^{-1} proved to be toxic, independent of the type of explant used. It causes a progressive etiolation and a complete loss of the callus induction capacity and of the organogenetic differentiation ability (Plate III, Figs. 3, 4). The modification is irreversible and leads to extinction. Lower concentrations of progesterone (0.25—5 mg · L^{-1}) caused a less intensive etiolation that did not affect the developmental potency of explants (Plate III, Fig. 2). Usually, etiolation starts from the excision edge of the inoculum and is propagated along the veins (Plate V, Figs. 1—2).

The nodato area explants had a low production of callus, calogenesis being preceded by the development of multiple axillary buds. It is interesting that development started very soon, 3—4 days after inoculation, with 100% success, independent of the concentration of administered progesterone. This may be correlated with the presence of meristematic centers of shoot primordia, which preexist in nodal area. It could be pos-

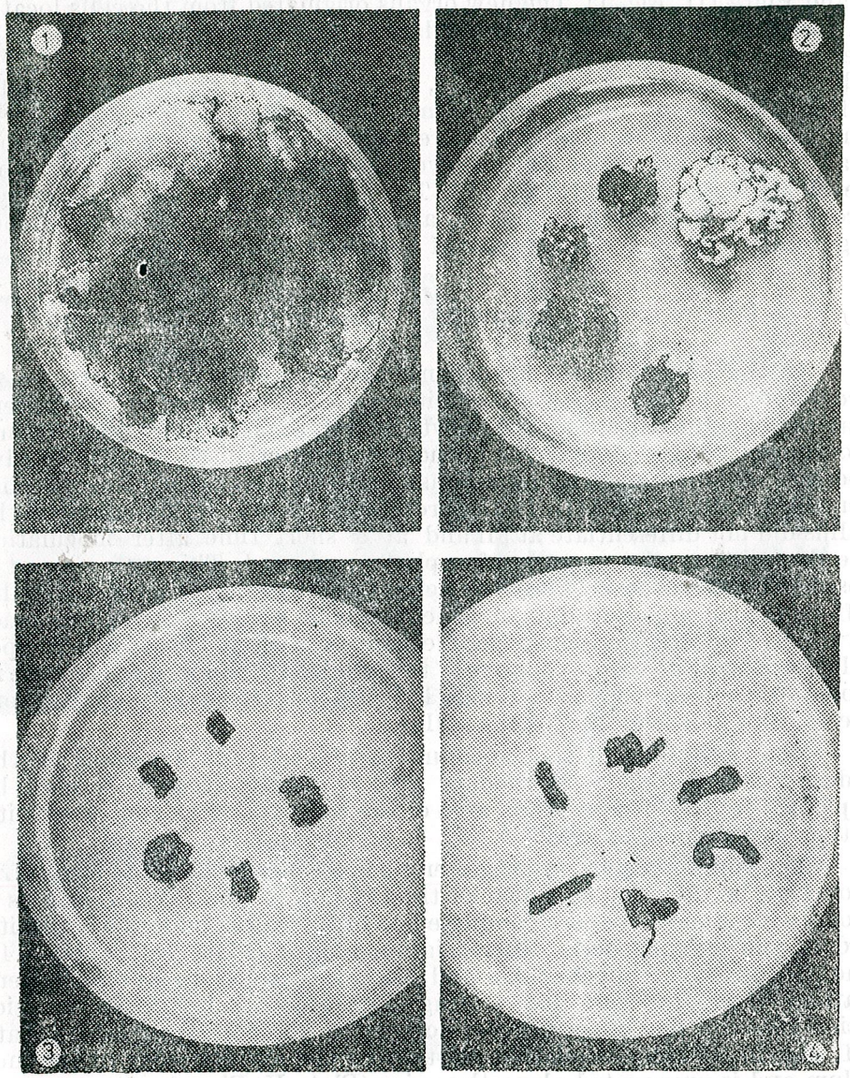

Plate II — Morphological peculiarities of the *N. tabacum* callus tissue (Fig. 1) and the influence of progesterone in concentrations of 1.25 mg. L^{-1} (Fig. 2), 10 mg. L^{-1} (Fig. 3) and 25 mg. L^{2} (Fig. 4). The strong inhibitory effects function of the concentration on callus initiation and proliferation were observed.

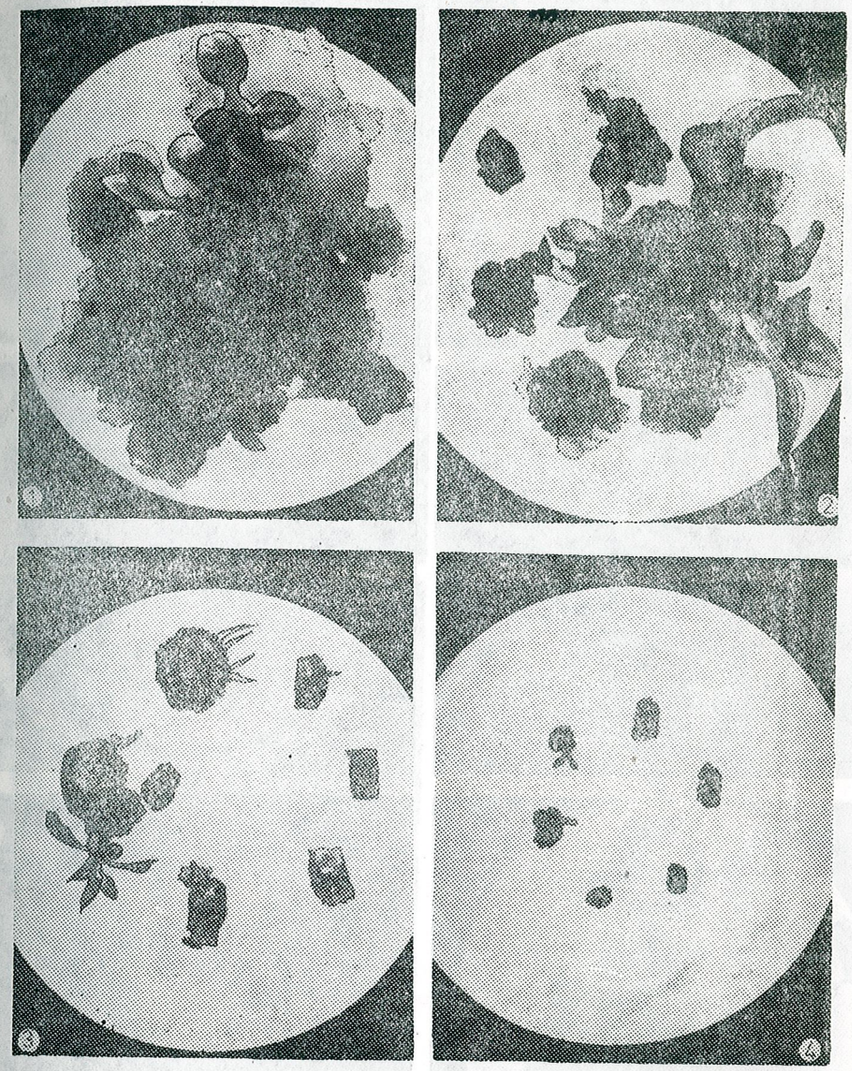

Plate III. — The inhibition of the shoots differentiation in the callus culture as a result of the progesterone treatment (Fig. 1) — control, (Fig. 2) 0.25 mgç L^{-1}, (Fig. 3) 5mg. L^{-1}, (Fig. 4) 25 mg. L^{-1}.

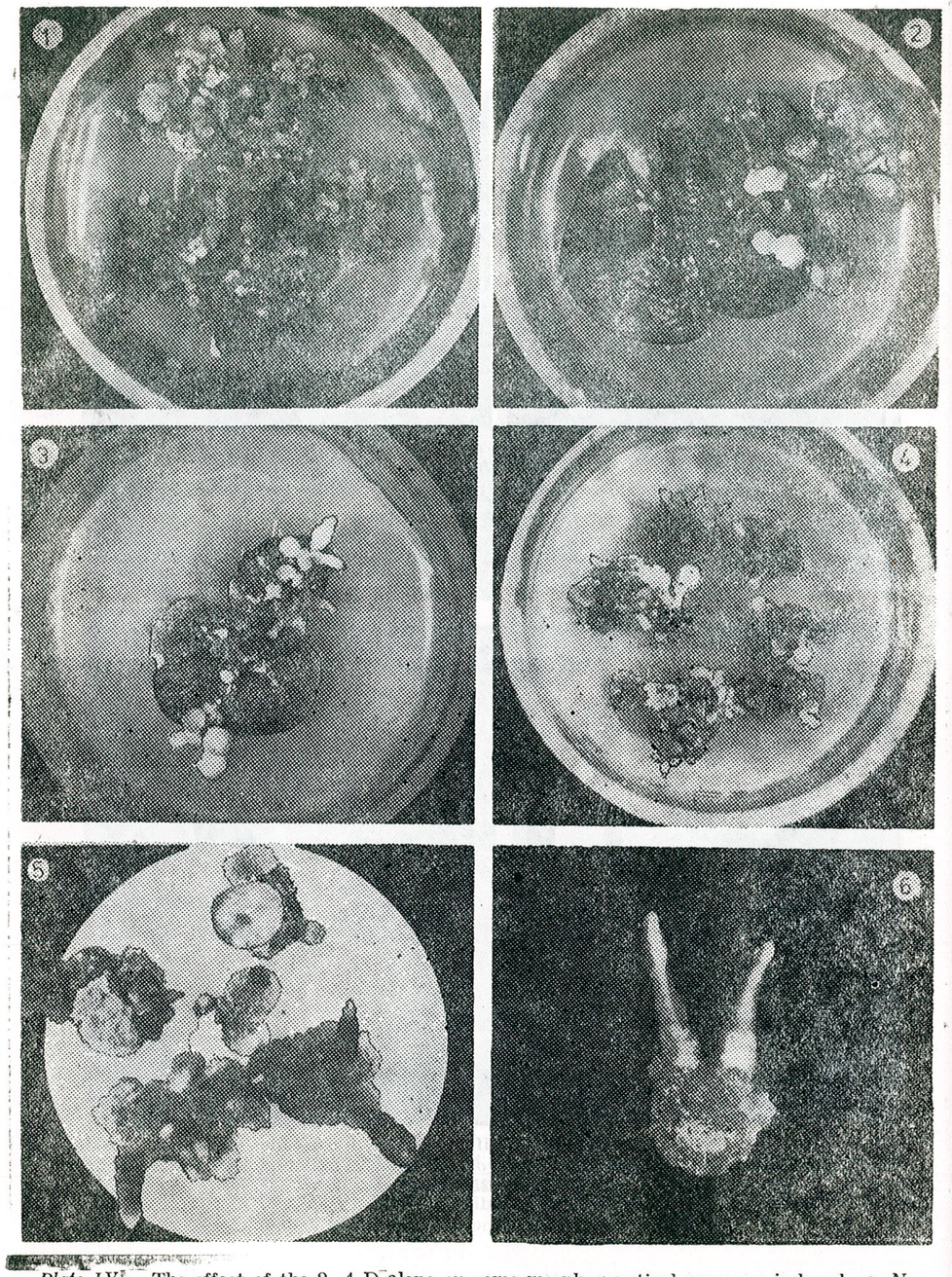

Plate IV — The effect of the 2, 4 D alone on some merphogonetical processes induced on *N. tabacum* callus culture (Fig. 1, 2, 5, 6) and in combination with progesterone in different concentrations (Fig. 3, 4).

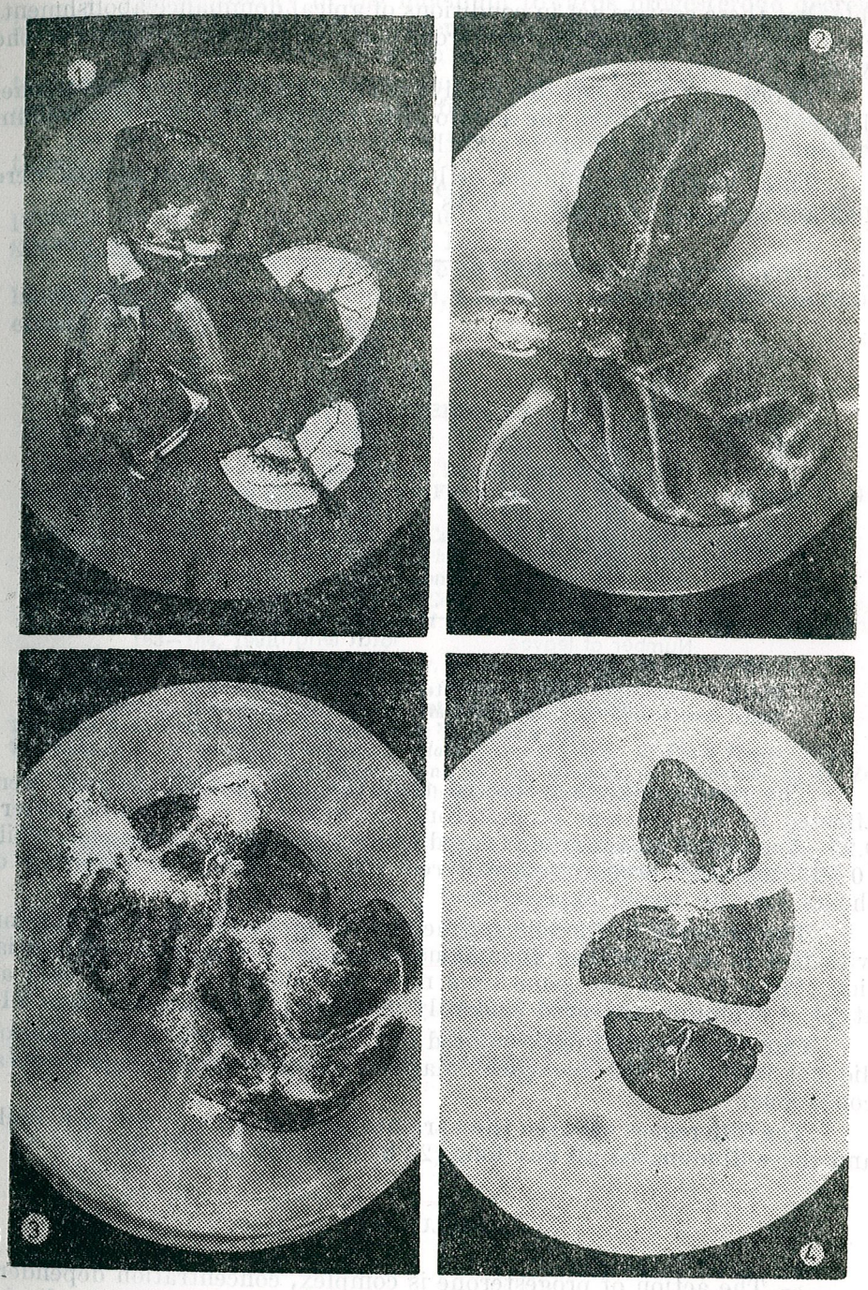

Plate V — The inhibitory effects of progesterone on the callus initiation and morphogenesis induced in explants of leaves. The characteristic progressive etiolation process of the inoculum was observed.

sible that these centers act in conditions of apical dominance abolishment, when exogenous auxin and progesterone had no significant influence on the axillary bud development.

In order to test a possible tardive or remanent effect of progesterone, differentiated shoots from the nodato area were passed on the MS medium without auxin or progesterone and their development was analysed.

Total length, the number of leaves and the average growth were monitored (Fig. 1) for the 18 days period.

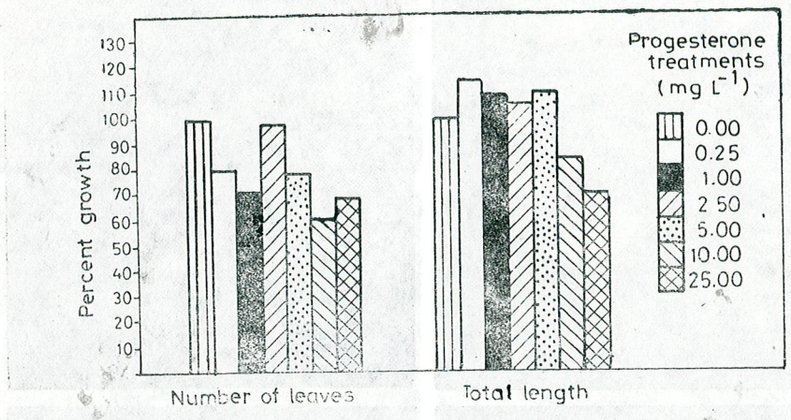

Fig. 1. — Remanent effect of progesterone on the development of differentiated shoots from the nodato area after subculture on rooting medium in the absence of progesterone.

The results obtained showed that the parameters analysed were affected by the previous progesterone treatment in a different manner : 0.25—5 mg · L^{-1} progesterone caused control like shoots behaviour, while 10—25 mg · L^{-1} progesterone altered the developmental potential of shoots for the whole experimental period.

The presence in the culture medium of progesterone in association with 2, 4 D increased the anomalous organogenic primordia differentiation (Plate IV). At the same time numerous shoots primordia with an atypical evolution appeared evidently in histological analysis (Plate VI).

Our experimental data pointed out that the length of illumination did not influence the biological material responses under experimental conditions.

The differences between the variants illuminated only for 16 h daily and those illuminated all day long (24 h) were not significant.

CONCLUSIONS

— The action of progesterone is complex, concentration dependent and selective with respect to the type of explant and the morphophysiological peculiarities.

— The sensibility of the inoculum towards progesterone decreases in the order : leaf > petiole > internodato area > nodato area.

— The highest concentration used (25 mg · L^{-1}) proved to have toxic effects, causing the suppression of the organogenetic potential via callus, progressive etiolation and finally the explant extinction.

— Explants from the nodal area were less influenced by progesterone with regard to the proper developmental programme.

— The remanent inhibitory effect of progesterone was obvious especially for 10—25 mg progesterone concentrations, affecting the length growth and the number of shoot leaves.

— Further investigations are needed in order to understand the biochemical and structural changes, caused by the presumptive hormonal effects of progesterone in plants.

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STUDIES ON THE REACTIVITY OF DIFFERENT SOYBEAN EXPLANT TYPES (*GLYCINE MAX* L. MERR.) TO "IN VITRO" CULTURE CONDITIONS

VICTORIA CRISTEA, DORINA CACHIȚĂ-COSMA

This study has been carried out due to the great economic value of *Glycine max* (L) Merr. Micropropagation and "in vitro" callusogenesis have been investigated according to the hormonal balance and to the types of explants: cotyledonary nodes, epicotyl, embryonary axis and primary leaves. The best multiplication capacity was initially recorded in cotyledonary nodes, while callusogenesis occurred in explants of hypocotyl, cotyledons, cotyledonary nodes and roots.

Soybean (*Glycine max* (L) Merr.) is one of the most important culture vegetables from all over the world, owing to its oil and protein content. Therefore specialists in the field of traditional culture techniques, as well as in the field of "in vitro" vegetal culture and genetic engineering, pay a special attention to this plant.

Although *Glycine max* (L) Merr has been initially refractory to "in vitro" cultures, some wild *Glycine* species gave very satisfactory results: *Glycine canescens*, *Glycine tomentella* Mayata (Kameya and Widholm 1981) and *Glycine clandestina* Wendl (Lazzeri et al. 1985).

The "in vitro" multiplication research which has been carried on over the *Glycine max* species and which started in the 60' s, has become successful between 1977 and 1980 (Oswald et al. 1977 and Cheng et al. 1980). These authors present a multiplication method starting from the cotyledonary nodes cellular suspensions, but with a low efficiency. It was but Christianson et al. (1983), Lippmann and Lippmann (1984), Wright et al. (1986) who gave an account of an efficient method for soybean "in vitro" multiplication.

There are two major ways for soybean aseptical multiplication as resulting from the subsequent literature published: shoot organogenesis (Cheng et al. 1980, Saka et al. 1980, Kartha et al. 1981, Tilton and Russel 1984, Barwale et al. 1986, Wright et al. 1987, etc.) and somatic embryogenesis (Christianson et al. 1983, Lippmann 1984, Lazzeri et al. 1987 a, b, etc.).

Within the framework of the researches which have been carried on to obtain "in vitro" photoautotrophic cell suspensions, a leading part was played by those on soybean (Klerk-Kiebert et al. 1982, Horn et al. 1983, Martin et al. 1984, Nelson et al. 1984, Barwale et al. 1986, Cannon et al. 1986).

The success in applying standard genetic engineering methods on soybean is limited by the absence of an efficient transformation system as well as of a system to regenerate transformed tissues (Christou et al. 1987). However, protoplast generation studies, genetics and genetic engineering studies have been notified lately (Niizeki and Kita 1981, Pederson et al. 1983, Hood et al. 1986, Zakri 1986, Christou et al. 1987, Esnault et al. 1987). Thus several researches have been accomplished for inducing

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somaclonal variations, for gene transfer with a view to improving oil and soybean protein quality, to inducing resistance to insects, viruses or herbicids, to inducing "crown gall" tumours by *Agrobacterium tumefaciens*.

As resulting from the above-mentioned authors' research, the "in vitro" proliferation through neoformed shoots has been realized starting from cotyledonary nodes, epicotyl, embryonary axis, primary leaves. In this paper, because of the great reaction variability which depends upon the subgenus and the variety of the studied plant, we aimed at presenting the "in vitro" behaviour of different explant types of a certain soybean variety.

MATERIAL AND METHODS

Glycine max Acmée variety was used. The seeds were disinfected with Ca hypochlorite 7%, with Tween 20, for 40 minutes. The seed inoculation was realised depending on the experimental variants, on culture media presented in Table 1.

Table 1

Culture media for *Glycine max*

— Macroelements, microelements and FeEDTA-after Murashige-Skoog	
— Pyridoxine	— 1 mg/l
— Thyamine	— 1 mg/l
— Nicotinic acid	— 1 mg/l
— myo-Inositol	—100 mg/l
— Succrose	— 30 g/l
— Agar	— 7 g/l
pH — 5.7	

Variants mg/l

1	BAP	— 0.025	8.	NAA	— 1(
	2,4 D	— 2			
2	BAP	— 2.5	9	BAP	— 10
	2,4 D	— 1		2,4 D	— 2
3	BAP	— 2.5	10	BAP	— 2.5
	2,4 D	— 2		2,4 D	— 0.1
4	BAP	— 2.5		IAA	— 0.1
	2,4 D	— 5	11	2,4 D	— 0.1
5	BAP	— 2.5		IAA	— 1
	2,4 D	— 10	12	BAP	— 5
6.	2,4 D	— 10		2,4 D	— 0.1
				IAA	— 1
7	BAP	— 2.5	13	BAP	— 10
	NAA	— 10		2,4 D	— 0.1
				IAA	— 1
			14	2,4 D	— 1
				IAA	— 1

The experimental variants were as follows :

I — initial sterile seeds, inoculated on medium 3, were preserve in the dark and after 36 days the generated plantlets were broken up an reinoculated on the same type of medium. The following explant types wer

inoculated : root and hypocotyl fragments, cotyledons, epicotyls and apex with the primary leaf primordia. The inoculi were further on kept in the dark at about 20—24°C. Observations were made 70 days after inoculation.

II — initial inoculation of seeds on the media 2—8, their composition representing a synthesis of the hormonal balance which is to be found in the literature. Incubation was accomplished at normal temperature and photoperiod — 16 h light/8 h dark.

Observations were periodically made, 14 and 26 days after inoculation.

After 30 days from the inoculation, the generated callus or the plant fragments were transferred on culture media 1, 3, 7 and 9. Observations were made after 60 and 88 days. After 47 days from the last transfer, the callus was repicated on media 3 and 5—8. Observations were made after 23 days from the transfer. The final observations were made after 115 days from the last transfer.

RESULTS AND DISCUSSIONS

I. As it has been made evident by the literature in the field, the callus generating capacity differs, depending on the explant origin. Thus, in an initial phase the hypocotyl axis fragments have the best callusogene capacity. The epicotyl and cotyledons also generate the callus. The hypocotyl and cotyledon callus, generated in the dark, has a beige-whitish colour and a greater volume than the one generated by apex or root fragments. The callus which is generated in the dark by the latter two explant-types is white-greenish.

II. Following the observations which were made after 14 days from the seed inoculation, it is to be noticed that the plantlets on NAA-10 mg/l medium as unique phytohormone-present the best growth and rhizogenesis. At the same time, white friable callus is generated at the basis of the hypocotyl, favourable to cell suspension inducing.

Phenomena of multiplication and shoot generation from the cotyledonary node appear on a BAP-2,5 mg/l medium, beside NAA-10 mg/l. This phenomenon was made evident in 1980 by Cheng, too, in the presence of an auxin and of BAP.

On the media 5 and 6 with 10 mg/l 2,4 D(BAP-2,5 mg/l at medium 5) plant evolution and callusogenesis is weaker than on medium 3 (2,4D-2 mg/l, BAP-2, 5 mg/l).

The greater amount of callus is generated on medium 3, which we use as a medium generally favourable to the majority of species, in what callusogenesis inducing is concerned.

After 26 days from the inoculation (Table 2), on the medium 8 (NAA 10 mg/l), 73% from the plants show multiplication, and 2—6 shoots with leaves generate from the cotyledonary node (Fig. 1 a). The radicular system is well developed and the callus is reduced. Multiplication is obtained on medium 3 and 7 too, and at 50% from the plants on media 2 and 4. On medium 3,12% of the plants show 2—4 roots on the superior side of cotyledons, with or without white callus.

Fig. 1. a, b. — Soybean seed evolution on medium 8, (a) and 6 (b) after 26 days from inoculation.

Table 2

Soybean seed evolution on culture media with various hormonal balance, after 26 days from inoculation

Medium variants (mg/l)	Multiplication — % from total/ variants	Rhizogenesis	Callus generation from :	Cotyledon colour	Epicotyl
2 BAP = 2.5 2,4 D = 1	52	yes	— roots — cotyledons	— green	grown
3 BAP = 2,5 2,4D = 2	35	yes and at 12 % from the plants roots on the cotyledons too	— roots — hypocotyl — cotyledons — *great*	— dark green	grown
4 BAP =2.5 2,4 D = 5	54	yes	— roots — cotyledons	— green	grown
5 BAP = 2.5 2,4 D = 10	—	yes	— roots — hypocotyl — cotyledons	— green	ungrown
6 2,4 D = 10	—	weak	— hypocotyl — cotyledons — *a little*	— yellowish, some necrosed	ungrown
7 BAP =2.5 NAA	35	yes	— roots — hypocotyl — cotyledons — *a little*	— dark green	ungrown
8 NAA =10	73	very good	— roots — hypocotyl — cotyledons — *a little*	— green	grown

60 days after transferring the generated callus on the above-mentioned media, the following remarks are to be made :

— on medium 3, the callused cotyledonary nodes, or the callus generated on the inferior side of the cotyledon, generates 3—5 shoots. On this medium the callus is green-yellowish or bright-green ;

— on medium 7, the callus is dark green ;

The callus generated on top of the root, from the cotyledonary node or from the cotyledons, generates 3—4 shoots (Fig. 2 a,b).

On medium variants 5 and 6 there are no multiplication phenomena to be noticed (Fig. 1 b).

After 88 days from inoculation, the shoots appear on the cotyledonary node callus, on media 1, 7, 9, without any possibility to make a

correspondence between the hormonal balance of the medium and the shoot generation from the callus. However, all the cotyledonary nodes which had shown multiplication phenomena on media 1, 7 and 9, proceeded from medium 7, with NAA-10 mg/l and BAP-2, 5 mg/l. As it can be noticed both in the first inoculation stage and after the transfer a cytokinin-auxin hormonal balance (with NAA in excess) is favourable to multiplication and rhizogenesis.

Fig. 2. — Shoot generation from the callus obtained from the cotyledonary node (a) or from the cotyledon (b).

Without transferring the explants, after 107 days from inoculation, shoot and root appearance is to be observed, out of the callus which was generated not only by the cotyledonary nodes but by the apex and cotyledon as well; the rhysogene origin callus can also generate roots (Table 3).

The percentage of plants which multiplied or the generated roots have been calculated related to transfer medium variants.

Thus, a higher rhizogenesis percentage (40%) compared to shoot generation (20%) has been noticed, when the auxin level (2, 4 D) is 80 times greater than the cytokimin level (BAP) (Table 3).

When the BAP level is 5 times greater than the 2, 4 D, the 35% multiplication predominates, as compared to 5% rhizogenesis. When the hormonal balance is in equilibrium (medium 3), this is shown for none of the two phenomena, although at the initial inoculation on this medium roots are generated on cotyledons at the undivided plants (Table 2). The presence of NAA as an auxin, together with BAP, favourises root generation and rhizogenesis to the same extent.

The callus generating shoots proceed from the superior fragments of the plant-cotyledonary node, cotyledons and apex. The regenerating capacity of the cotyledonary nodes and of apexes could be explained by the existence of the meristematic tissues, a very interesting problem being the multiplication through the cotyledon-generated callus. This phenomenon could be possibly associated to the presence of reserve substances from cotyledons.

Rhizogenesis appears at calluses which derive from all the plant levels-roots, hypocotyl, cotyledonary node, cotyledons. At this stage the majority of calluses grow, with their surface covered by small white concrescences that can be associated to some secretory processes. The aspect of this callus is that of a callus in its early stages of somatic embryogenesis release.

Results concerning the generation and culture of soybean cell suspensions will be published in another paper.

Table 3

Shoot and root generation from soybean callus, initiated from different plantlet parts (root, hypocotyl, cotyledonary node, cotyledon, epicotyl, apex) after 107 days from the first transfer of initiation media

Medium variant (mg/l)		Initial media from which explants have been transferred:									
		1 BAP = 2.5, 2,4D = 1		3 BAP = 2.5, 2,4D = 2		4 BAP = 2.5, 2,4 D = 5		5 BAP = 2.5, 2,4D = 10		7 BAP = 2.5, NAA = 10	
		a. shoot generation	b. rhizogenesis	a.	b.	a.	b.	a.	b.	a.	b.
BAP = 0.025	% from total/var.	—	100	—	—	—	—	—	100	100	—
1 2,4 D = 2	callus origin	—	cotyledon	—	—	—	—	—	cotyledon roots	cotyledonary node apex	—
BAP = 2.5	% from total/var.	—	—	—	—	—	—	—	—	—	—
3 2,4 D = 1	callus origin	—	—	—	—	—	—	—	—	—	—
BAP = 2.5	% from total/var.	48	48	—	—	—	—	—	—	30	30
7 NAA = 10	callus origin	cotyledon	cotyledon	—	—	—	—	—	—	cotyledonary node	cotyledonary node sup, hypocotyl
BAP = 10	% from total/var.	—	—	50	25	100	—	—	—	25	—
9 2,4 D = 2	callus origin	—	—	cotyledonary node	cotyledonary node	apex	—	—	—	cotyledonary node	—

Table 3 (continued)

Medium variants mg/l		Shoots generation	Rhizogenesis
1 BAP = 0.025	% from total variants	20	40
2,4D = 2	callus origin	— cotyledonary node — apex	— cotyledonary node — roots
3. BAP = 2.5	% from total variants	—	—
2,4 D = 1	callus origin	—	—
7. BAP = 2.5	% from total variants	16	16
NAA = 10	callus origin	— cotyledon — cotyledonary node	— cotyledon — cotyledonary node — hypocotyl
9. BAP = 10	% from total variants	35	5
2,4 D = 2	callus origin	— cotyledonary node — apex	— cotyledonary node

CONCLUSIONS

Previously presented data have revealed the possibility of micropropagation in *Glycine max* (L.) Merr. by means of shoots generated from seeds inoculated on culture media accurately balanced hormonally or from the callus generated from cotyledons or cotyledonary nodes. Caulogenesis or rhizogenesis are presented according to the hormonal balance of culture media.

The generated callus sometimes reveals stages of somatic embryogenesis. As a result of repeated transfers, a friable callus inducing cell suspensions is obtained.

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CANDIDA BOIDINII ICCF26 IMPROVEMENT BY PROTOPLAST FUSION

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The proper conditions for protoplast isolation from an industrial *Candida boidinii* strain were established. The dynamics of protoplast obtaining from cells grown on glucose or methanol, as carbon and energy source, was analysed on microelectronographs. *C. boidinii* protoplasts were fused with *Candida utilis* protoplasts in order to obtain fusion products improved in thermotolerancy and with efficient growth on methanol. Our study proved that *C. boidinii* ICCF26 could be improved by protoplast fusion.

In recent years much attention has been focused on yeasts called methylotrophs, which facultatively use methanol as carbon and energy source. Investigations regarding the industrial applications of methylotrophs for the biomass and metabolites production, such as vitamins and aminoacids, have been performed (Trotsenko and Bystryk, 1990). At the same time, methylotrophic yeasts represent an ideal host for heterologous gene cloning at industrial level, due to the presence of a very well regulated promotor (the promotor of *AOX* gene (CREGG et al. 1987)). About 50 strains were reported as methanol utilisers (TANI, 1984). Among these, *C. boidinii* was especially used for biomass and metabolites production.

Protoplasts represent very important tools for both yeast improvement by parasexual hybridization and basic studies on membranes, cell wall and enzymes. Generally, protoplasts could be easily obtained in many yeast species, but it is necessary to establish the proper conditions for each one.

The aim of this study was to establish the proper conditions for protoplast isolation from an industrial strain of *C. boidinii*, isolated from the environment (Olarascu, 1989), in order to use them for fusion with *C. utilis* protoplasts. The purpose of protoplast fusion between *C. boidinii* and *C. utilis* was to obtain fusion products which had to be thermotolerant and could efficiently use methanol as carbon and energy source. As to our knowledge, *C. boidinii* has not been yet improved by protoplast fusion.

At the same time, the dynamics of protoplast isolation was analysed on microelectronographs. Some considerations regarding the ultrastructure of *C. boidinii* cells grown on methanol and glucose were made, too.

MATERIAL AND METHODS

The following yeast strains were used :

— *Candida boidinii* IICCF26, isolated from the environment, was kindly supplied by Dr. N. Olarascu from The Chemical and Pharmaceutical Research Institute — Bucharest.

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— *Candida utilis* 14, was kindly supplied by Dr. V. Herlea from the Department of Microbiology, University of Bucharest.

Media and cultivation. The minimal medium used contained: 0.25% KH_2PO_4, 0.25% $(NH_4)_2SO_4$, 0.08% $MgSO_4 \times 7H_2O$, 0.006% $FeSO_4 \times 7H_2O$, 0.04% maize extract, trace elements and 1.5% methanol or 2% glucose as carbon source.

Cultivation was carried out in batch culture in minimal medium for 48 hours and the growth was appreciated as optical density at 530 nm.

Protoplast isolation: Cells were grown until middle exponential growth phase was achieved. The cells were treated by 14.3 mM beta-mercaptoethanol in 0.05 M Tris-HCl Buffer, pH-7.5, at a cell density of 1×10^7 cells/ml for 1 hour at 30°C. Then the cells were treated by lyophilized snail gut juice at a concentration of 1 mg/ml in 0.1 M phosphate buffer, pH-6, osmotically stabilised by 0.6 M KCl, at a cell density of 1×10^7 cells/ml for 1, 2 or 3 hours. *C. utilis* protoplasts were thermally inactivated after a method described by Saddekni et al. (1988), but higher temperatures were used. The viability of heat-inactivated protoplasts was determined by their regeneration ability reported to a control protoplast sample kept at 30°C. Finally the inactivation at 55°C was chosen.

Protoplast fusion was carried out by 40% polyethylene glycol (PEG), molecular weight 4000, in 0.05 M Tris-HCl Buffer, pH-7.5, containing 0.01 M $CaCl_2$.

The electronmicroscopic study was carried out by fixing the cells and protoplasts in 3% glutadaldehyde solution. followed by postfixing with 2% OsO_4 and embedding in Epon 812 resin. The ultrathin sections obtained with a Tesla ultramicrotom were stained by uranyl acetate and lead citrate according to HAYAT (1972) and examined under a Philips microscope.

To detect the localization of catalase activity, the cells fixed with glutaraldehyde were treated by 3, 3'-diaminobenzidine (DAB) after the method of FUKUI et al. (1975).

RESULTS AND DISCUSSION

PROTOPLAST ISOLATION IN *CANDIDA BOIDINII* ICCF26

In order to establish the proper conditions for protoplast isolation in *C. boidinii*, we benefitted by our experience on *S. cerevisiae* and *S. carlsbergensis* (PETCU et al., 1990). In this respect, we used the same small concentration of beta-mercaptoethanol (14.3 mM) like in *Saccharomyces*, considering that high concentration could damage the cell membrane. In our preliminary experiments we tried to use the same concentration of snail gut juice (0.4 mg/ml) like in *Saccharomyces*, incubating the cells for a long time (21 hours), but this did not give a good yield of protoplasts. For this reason, we increased the snail gut juice concentration to 1 mg/ml and reduced the time of incubation. In these conditions we obtained a good yield (95%) of protoplasts after 3 hours of incubation in snail gut juice solution, from cells grown on medium with methanol or glucose.

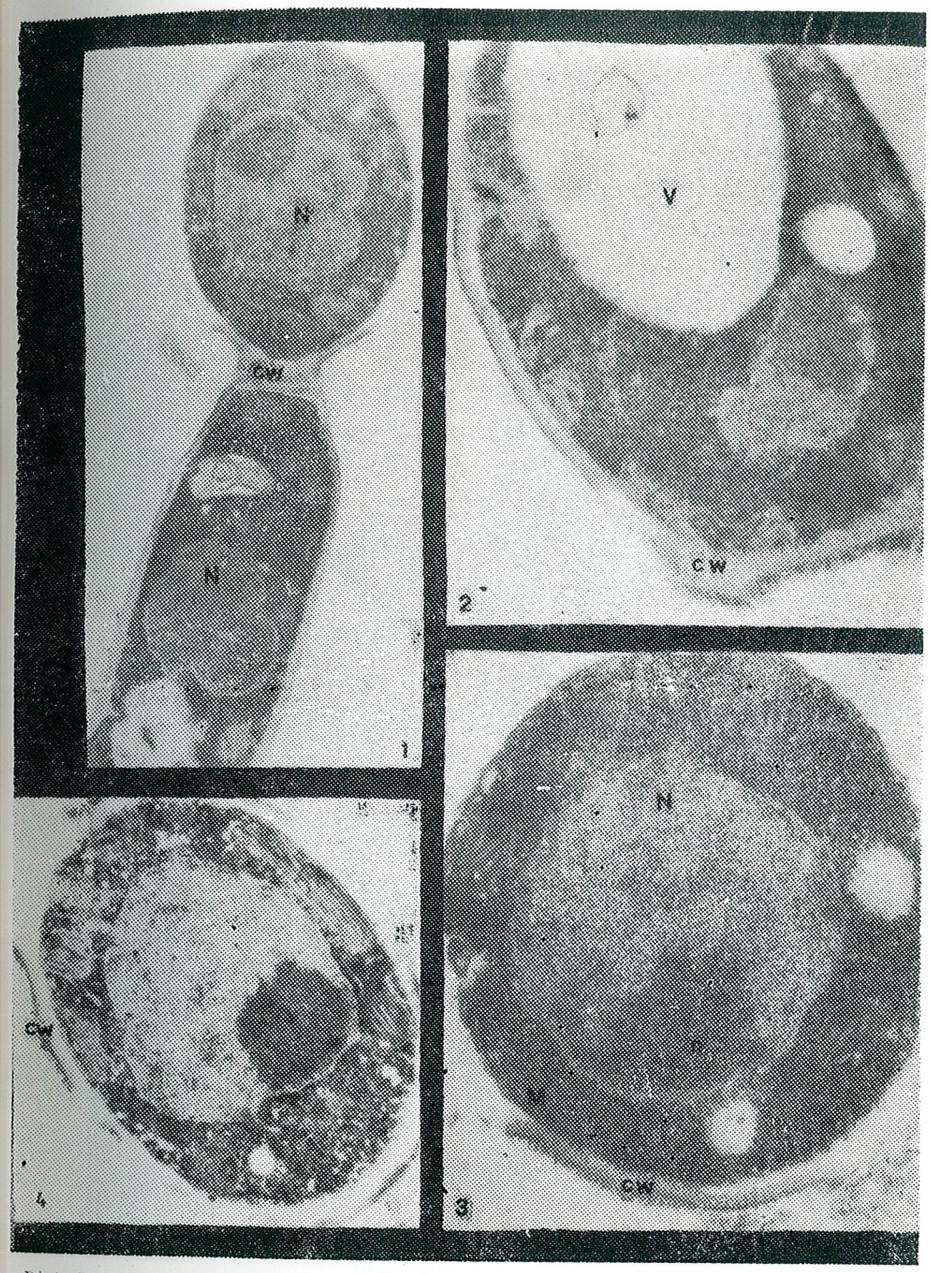

Plate I. — The ultrastructure of *C. boidinii* ICCF26 cells grown on glucose and of their protoplasts,
Fig. 1. — Two attached cells of *C. boidinii*. One of them is developing a protoplast.
Fig. 2. — The ultrastructure of a *C. boidinii* cell. A thick cell wall could be observed.
Fig. 3. — and Fig. 4. — *C. boidinii* cells after 2 hours of lytic treatment.

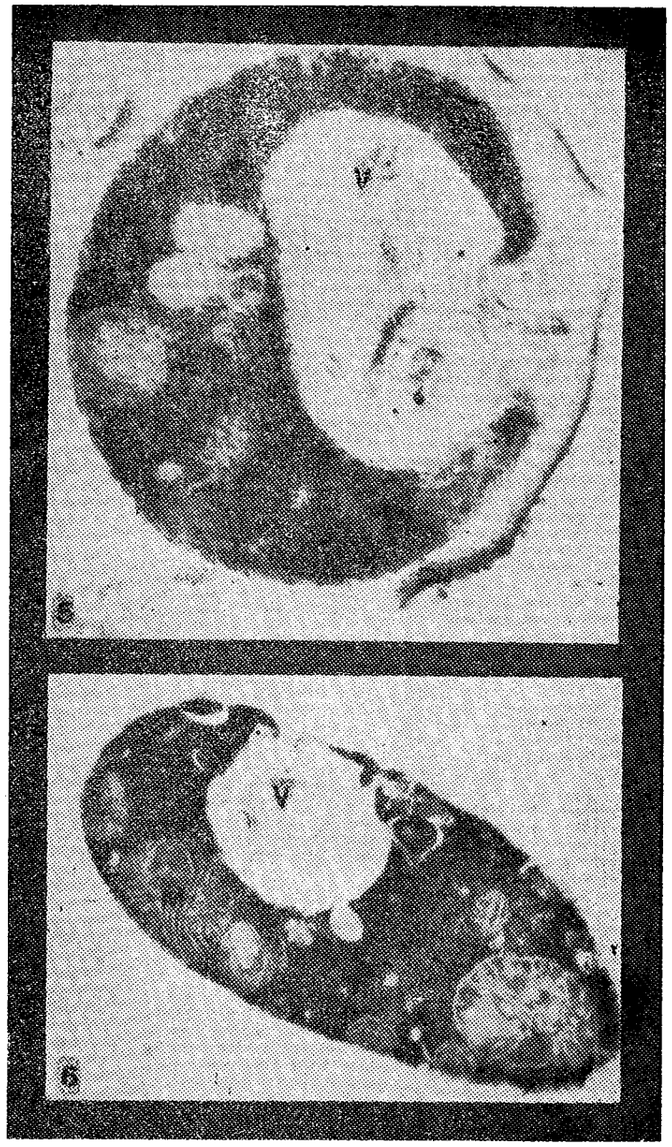

Fig. 5. — and Fig. 6. — Proper protoplasts obtained after 3 hours of lytic treatment.

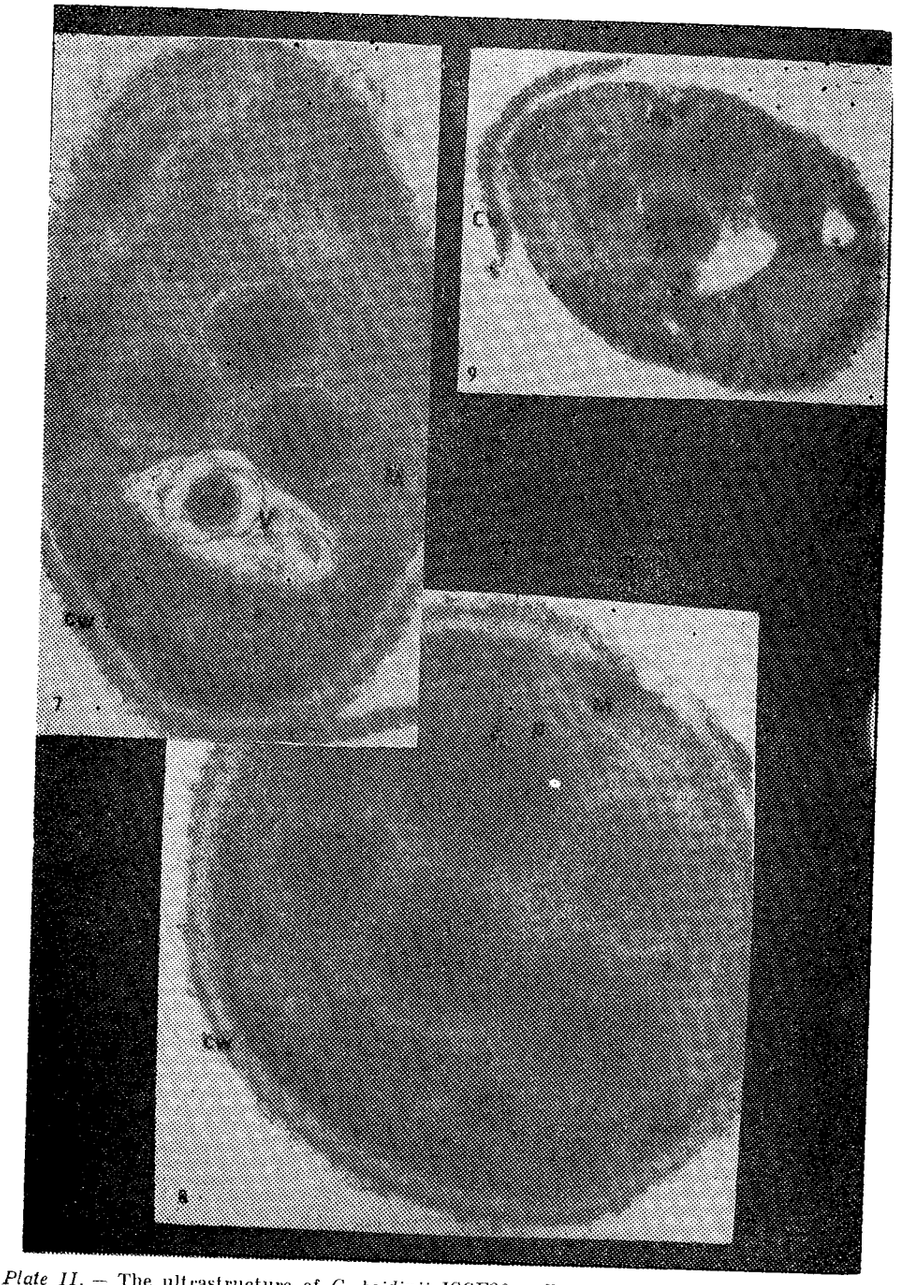

Plate II. — The ultrastructure of *C. boidinii* ICCF26 cells grown on methanol and of their protoplasts.

Fig. 7. — The ultrastructure of a *C. boidinii* cell grown on methanol.
Fig. 8. — *C. boidinii* cell with a thick cell wall.
Fig. 9. — *C. boidinii* cells after 2 hours of lytic treatment.

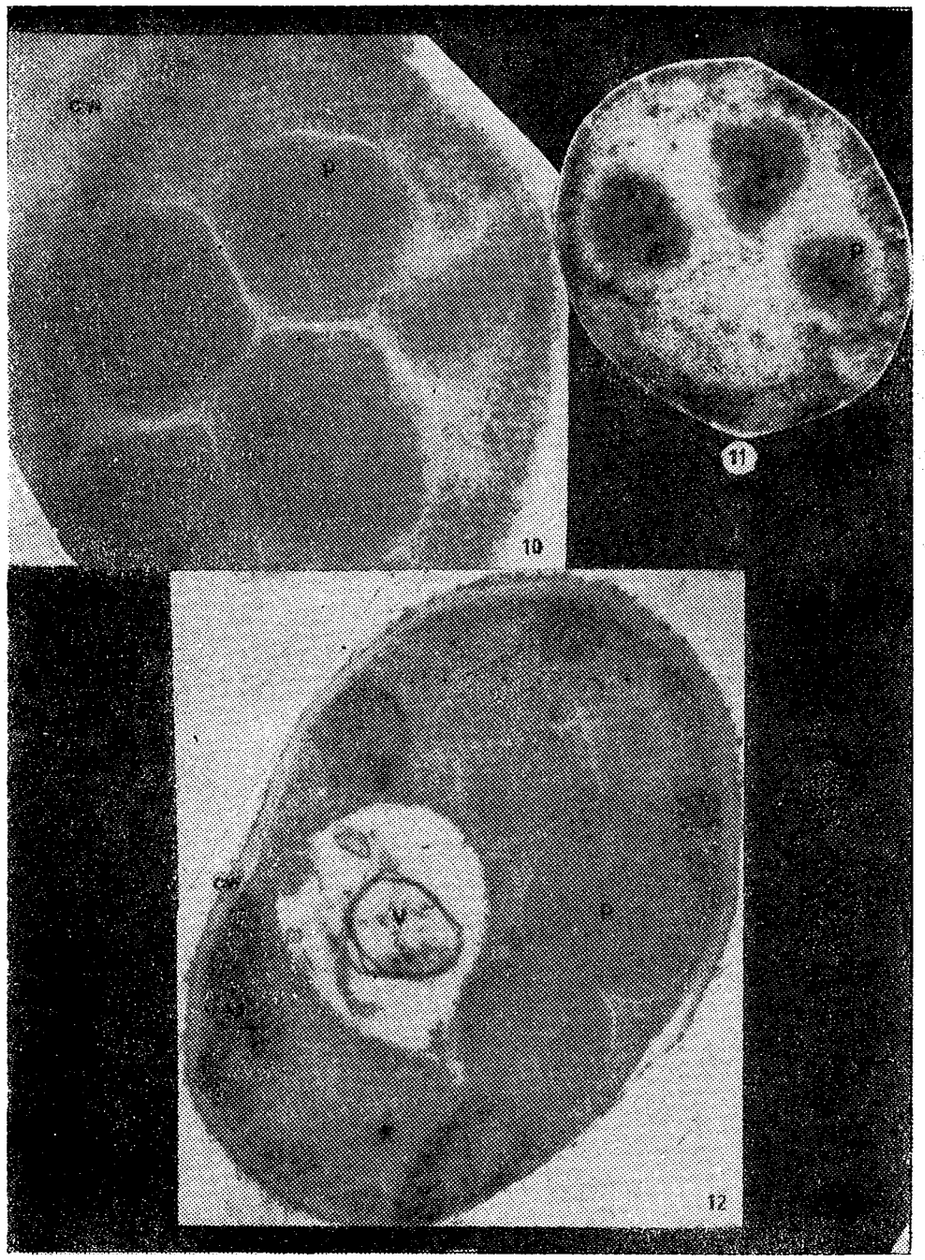

Fig. 10. — *C. boidinii* cells after 2 hours of lytic treatment.
Fig. 11. — Proper protoplast obtained after 3 hours of lytic treatment.
Fig. 12. — DAB reaction in peroxisomes of *C. boidinii* cell.

The conditions for *C. utilis* protoplast isolation were the same as in *C. boidinii* resulting a good yield of protoplasts (94%) after 3 hours of incubation with lytic enzymes.

The dynamics of protoplast obtaining was analysed on microelectronographs, too (Plate I and Plate II). During the successive stages of protoplast isolation, it could be noticed that the lytic action could progress on the whole surface of the cell wall uniformly (Fig. 4 and Fig. 7) or especially on restricted regions (Fig. 1, Fig. 2). It is possible that this could depend on the fact that the cell was free (Fig. 4 and Fig. 7) or was attached to another one (Fig. 1 and Fig. 2). We noticed that in the case of free cells the lytic action could progress uniformly in different points of the cell wall. In the case of attached cells the lytic action was primarily expressed in the free part of the cell wall and then in the region by which the cell was attached with another one (Fig. 1, and Fig. 2). This could be explained by the fact that the region of attachment between two cells of the cell wall is thicker and maybe more protected. These differences in lytic action could be noticed both in cells grown on glucose or methanol after 1 hour of digestion by snail gut juice.

After 2 hours of lytic treatment both in cells grown on glucose and methanol the digestion was extended to larger regions of the cell wall, but small fragments of cell wall still remained (Fig. 3, Fig. 4, Fig. 9, Fig. 10).

After 3 hours of incubation, proper protoplasts were obtained (Fig. 5, Fig. 6 and Fig. 11). Despite Dziengel et al. 1977) conclusions, that in methanol grown cells protoplasts are more hardly obtained than in glucose grown cells, our method permitted us to obtain a good yield of protoplasts in both situations.

It has been generally appreciated that the protoplasts have a spherical form. But we could seldom notice protoplasts without any cell wall material having an ellipsoidal form (Fig. 6). This could raise some problems concerning the forces which act for keeping the shape of the protoplasts in the absence of the cell wall.

Both in glucose grown cells and in protoplasts obtained from them we could not detect any microbodies, as could be expected (Fig. 1 — Fig. 6).

In the methanol grown cells and in their protoplasts we always could observe microbodies (Fig. 7 — Fig. 11). Microbodies are organelles generally present in yeasts grown on methanol. (Veenhuis, 1976). They are delimited by a single unit membrane and contain the enzymes employed in the first steps of methanol pathway : alcohol-oxidase, catalase and dihydroxyacetone-synthase. Probably microbodies exist to protect the cell against the toxic effect of H_2O_2 which results from alcohol-oxidase activity. H_2O_2 is rapidly metabolized by catalase present in microbodies too, so its efect on cell metabolism is not exerted.

In cells grown 18 hours on methanol we could observe 2 to 6 peroxisomes. They could represent 70—80% of the cell volume. It is significant that some of peroxisomes have intimate relationships with vacuole (Fig. 7, Fig. 9, Fig. 12), suggesting an exchange of cell material between these structures. It could be expected that the peroxisomal enzymes suffer posttranslational processing in reticulum endoplasmic, Golgi Apparatus and Vacuole. At the same time, relationships with mitochondria could be noticed, too (Fig. 7).

In some peroxisomes we could observe crystalloid structures (Fig. 7, Fig. 10), occupying the whole peroxizome (Fig. 7 and Fig. 10). OSUMI et al. (1982) supposed that the crystalloid structure was determined by particles of alcohol-oxidase and catalase whereas VEENHUIS (1983) suggested that only alcohol-oxidase was present in the crystalloid matrix.

DAB reaction indicated us a catalase activity dispersed in the whole peroxisome, while in mitochondria this reaction activity was located only at the level of membranes (Fig. 12).

CANDIDA BOIDINII IMPROVEMENT IN THERMOTOLERANCY BY PROTOPLAST FUSION WITH *CANDIDA UTILIS*

The experimental model for *C. boidinii* improvement is presented in Fig. 13 and it was based on protoplast fusion with *C. utilis*. Yeast strains which belong to *C. utilis* are thermotolerant (that means that they could grow at higher temperatures than 30°C), even if they have an associative temperature profile (MADEIRA LOPEZ, 1985). The aim of the fusion was to transfer thermotolerancy genetic determinants from *C. utilis* to *C. boidinii*. To be sure that the fusion products would belong to *C. boidinii*, *C. utilis* protoplasts were heat-inactivated. At 55°C *C. utilis* protoplasts were killed because we could not obtain any revertants after incubating them in regenerating medium. Taking into account that at this temperature protoplasts are killed and DNA is not denatured, we could consider that *C. boidinii* protoplast fusion with heat-inactivated *C. utilis* protoplasts is in fact a genetic transformation. In spite of this fact, we still name the transformed cells of *C. boidinii*, fusion products. The selection of *C. boidinii* transformed cells was made on the basis of copper resistance acquired from *C. utilis* (Fig. 13).

The fusion products obtained were tested for growth on methanol both at 28°C and 37°C. As could be seen in Table 1, some of the fusion products had a better growth at 37°C, exceeding both *C. boidinii* and *C. utilis*. At 28°C the same fusion products had a better growth than *C. utilis*. But at this temperature none of them achieved the growth capacity of *C. boidinii*. The fusion products were however of interest for their growth capacity at 37°C.

Our conclusion is that protoplast fusion could be used for methylotrophic yeast *C. boidinii* improvement, but protoplast fusion does not mean a simple addition of the best qualities from two strains. Sometimes a favourable quality is accompanied by some less favourable ones.

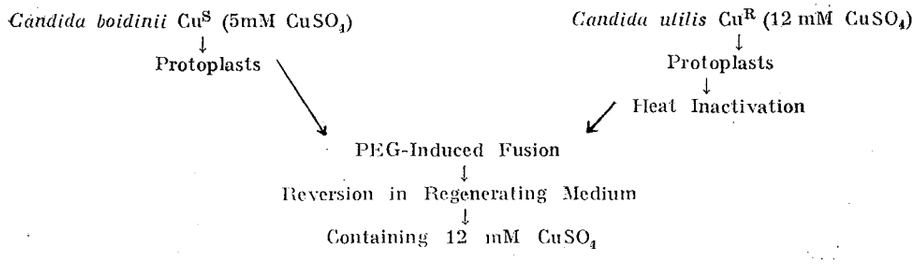

Fig. 13. – Protoplast fusion between *C. boidinii* and *C. utilis*.

Table 1

Growth capacity of some fusion products and of *C. boidinii* and *C. utilis*

	28°C		37°C	
Strain	Growth OD_{530nm}	Residual Methanol g%	Growth OD_{530nm}	Residual Methanol g%
C. boidinii	4.9	0.0936	0.816	0.300
C. utilis	1.08	0.225	1.3	0.202
FP-1	1.320	0.284	0.960	0.293
FP-2	1.183	0.2066	1.130	0.336
FP-3	1.833	0.102	1.310	0.283
FP-4	1.783	0.140	1.716	0.290
FP-5	2.550	0.100	1.880	0.204
FP-6	2.06	0.110	2.03	0.115

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