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VALERIANELLA LASIOCARPA — TRIPLE TRAP FOR THE BOTANIST

BY

G. DIHORU

The variability of the fructiferous calyx of *Valerianella lasiocarpa* has determined its confusion with *V. eriocarpa* and *V. coronata* in Romania, and probably with *V. kotschyi* in other countries, too.

The wide variation in the shape of the fructiferous calyx in *Valerianella lasiocarpa* (Steven) Betcke (Coode, 1967) determined its confusion either with *V. eriocarpa* Desv. or *V. coronata* (L.) DC. in the Romanian botanical literature.

1. In flora of Romania *V. eriocarpa* has been mentioned for a long time by both former botanists and present ones (Grecescu 1898, 1901; Prodan 1939; Borza 1949; Morariu 1965; Sanda, Tutunaru 1965) in the south-east of the country. In fact it was mistaken with the specimens of *V. lasiocarpa*, where the fructiferous calyx is less developed, that is, it appears as disciformis and obtuse 3-toothed (with another 3 intermediary slight teeth) up to short cyatiformis, 6-toothed, with teeth acute or acuminate but not uncinate (var. *lasiocarpa*) (Dihoru 1975).

Many botanists fell in this trap on which we come back and underline that the two species are different by the following diagnemas (Dihoru 1969, 1970):

Valerianella lasiocarpa	*Valerianella eriocarpa*
— Stem in the lower part puberulent all around;	— Stem in the lower part puberulent only on the ribs;
— Bracts ovate, ciliate on the margins;	— Bracts lanceolate, sparsely on the margins;
— Fructiferous calyx disciformis 3-toothed — up to cyatiformis 6-toothed to the middle, teeth not-or with uncinate arista;	— Fructiferous calyx tubulous 6-toothed in the superior 1/4, teeth acute;
— Sterile loculus subequal with the half of the fertile one (outside it appears as a high rib);	— Sterile loculus very narrow or absent (outside it appears as a narrow and low rib);
— Fruit dorsal smooth, hairness not on rows, ventral groove not gibbose.	— Fruit dorsal carinate, hairness ± on rows, ventral groove gibbose.

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2. It is difficult to estimate that the specimens of *Valerianella lasiocarpa* could be mistaken with *V. coronata*, because in identifying the latter species, the indicative diagnema is too striking, and the fructiferous calyx in *V. lasiocarpa* was not known in its all variation, that is, it was not already known that it has perfect convergent forms with the calyx of *V. coronata*. The convergence of the fructiferous calyx in *V. lasiocarpa* with the *V. coronata* one is surprising, so, in the absence of the genuine samples of *V. coronata* for comparison, you cannot be convinced that what is meant is *V. lasiocarpa*. That is the way we explain the fact that var. *stribrnyi* Velen. was allotted to species *V. coronata*. Many previous and present botanists (Brandza 1898; Prodan 1939; Borza 1949; Ciocîrlan, Chirilă 1960; Sanda, Tutunaru 1965; Dihoru 1975; Sârbu 1978) fell in this trap. I myself — although I had investigated the species *V. lasiocarpa* — allotted the specimens a calyx cytiformis and an uncinate mucro at the apex of teeth (var. *stribrnyi* (Velen.) Dihoru comb. nova) to *V. coronata*, with the remark that the fruits are smaller (2.8—3.3 mm incl. calyx), different from *V. coronata* which has larger fruits (4 — 5 mm, incl. calyx) (Dihoru 1975, Pl. 2, 1—9).

If between *V. eriocarpa* and *V. lasiocarpa* there are clear differences between *V. coronata* and *V. lasiocarpa* var. *stribrnyi*, they are nonsignificant:

V. lasiocarpa var. *stribrnyi*	*V. coronata*
— Fruits brown, under 2 mm (1.8—2.0 mm, ventrally measured excl. calyx) or *ca.* 3.5 mm (incl. calyx); fertile carpela ovate, *ca.* 2.35 mm (Fig. 1, 1 a).	— Fruits blackish, over 2 mm (2.0—2.5 mm, ventrally measured excl. calyx) or 4—5 mm (incl. calyx); fertile carpela ovate lanceolate, *ca.* 3 mm (Fig. 1, 2a).

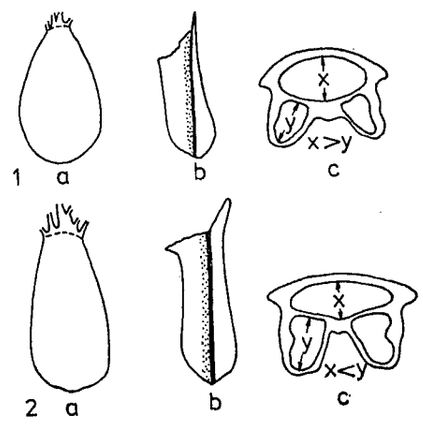

Fig. 1. — Fruits of *Valerianella lasiocarpa* (1) and *V. coronata* (2) *a*, dorsal fruits; *b*, lateral fruits; *c*, cross sections in fruits (*x* — height of fertile loculi, *y* — height of sterile loculi).

— Fructiferous calyx (thin) with a few hairs on the margin, the distance (span) between two opposed teeth is (2.5) 3.0—3.7 mm.	— Fructiferous calyx (thick) without hairs on the margin, the span between two opposed teeth being 4—5 mm.
— The nerves of the fructiferous calyx (brownish in water) are not prominent.	— The nerves of the fructiferous calyx (pale in water) are prominent.
— Ventral groove ovate, slightly carinate on the bottom.	— Ventral groove oblong unobviously carinated on the bottom.
— Ratio length : breadth of the fertile loculus = 2, and the fertile loculus is higher than the sterile one (Fig. 1, 1c).	— Ratio length : breadth of the fertile loculus = 3.3, and the fertile loculus is less high than the sterile one (Fig. 1, 2c).

3. According to some authors (Lincevski 1958; Katina 1965 etc.), what we name *V. lasiocarpa* var. *stribrnyi* corresponds to the species *V. kotschyi* Boiss., which I could not examine but I consider it doubtful in the region where it has been indicated with small fruits (*ca.* 2 mm) because the true species has much larger fruits (3.5 — 5 mm, incl. calyx) (Coode 1967).

DISCUSSIONS

Some authors (Coode 1967) treat realistically these closely related species *(V. coronata, V. lasiocarpa, V. eriocarpa* and even *V. kotschyi)* while others (Ernet, Richardson 1975) characterize in the same manner ("sterile loculi reduced to slender ribs") both *V. lasiocarpa* and *V. eriocarpa*. It is possible that Lincevski, 1958 and Katina, 1965, might confuse *V. lasiocarpa* var *stribrnyi* with *V. kotschyi*. The sections in fruits of *V. coronata* and *V. lasiocarpa* show about the same relationship between the fertile loculus and the sterile ones and an entirely different one for *V. eriocarpa* (Coode 1967; Dihoru 1975).

Ventrally seen, the sterile loculi of *V. lasiocarpa* appear really thin but in their section they are about 1/2 from the fertile one. The form of the ventral groove of *V. lasiocarpa* and *V. coronata* is differently characterized by some authors (Ernet, Richardson 1975; Boissier 1875; Post 1932; Velenovsky 1898).

In the West of Romania (Banat), the true *V. coronata* grows, and in the South-East, *V. lasiocarpa*, with a calyx variable in shape and size, being mistaken with either *V. eriocarpa* (unconfirmed in the flora of our country) or *V. coronata*. As specimens with an apparently 3-toothed calyx after cutting off give new shoots whose fruits have a cyatiformis calyx, as we have met in many other populations of the species, we consider that there are not two subspecies but two varieties, i.e. var. *lasiocarpa* and var. *stribrnyi* (Velen.) Dihoru h.l. (Dihoru 1975).

REFERENCES

1. Boissier E., *Flora Orientalis* 3, Genevae et Basiliae, 1875.
2. Borza Al., *Conspectus Florae Romaniae* 2, Cluj, 1949.
3. Brandza D,. *Flora Dobrogei*, Bucureşti, 1898.

4. Ciocîrlan V., Chirilă C., Lucr. St. Instit. Agron. Bucureşti, 1960.
5. Coode M.J.E., Notes Roy. Bot. Gard. Edinburgh, 1967, **27**, *3*.
6. Dihoru G., Considerations on some species of *Valerianella* Mill. in D. Jordanov (Edit). *Problems of Balkan Flora and Vegetation*. Sofia, 1975.
7. Ernet D., Richardson I.B.K., *Valerianella* in Tutin T. G. *et al.* (Edit.), *Flora Europaea* **4**, Cambridge, 1975.
8. Grecescu D., *Conspectul Florei României*, Bucureşti, 1898 and *Supplement*, 1909.
9. Katina Z. F., *Valerianaceae* in *Viznacinik roslin Ukraini*, Kiev, 1965.
10. Lincevski I. A., *Valerianella* in V. Komarov (Edit.), *Flora S.S.S.R.* **23**, Moscova-Leningrad, 1958.
11. Morariu I., *Valerianaceae* in Săvulescu T. (Edit.), *Flora R.P.R.* **8**. Bucureşti, 1961.
12. Post G., *Flora of Syria, Palestine and Sinai* **1**. Beirut, 1932.
13. Prodan I., Bul. Fac. Agronomie Cluj, 1939, **8**.
14. Sanda V., Tutunaru V., Com. Bot. S.S.N.G. Bucureşti, 1965, **3**.
15. Sîrbu I., *Flora şi vegetaţia din bazinul Chinejii şi al Prutului între Rogojeni-Mastacani*, Rezumatul tezei de doctorat Iaşi, 1978.
16. Velenovski J., *Flora Bulgarica, Supplementum*. Pragae, 1898.

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RECHERCHES CARYOLOGIQUES SUR L'*ACHILLEA SCHURII* SCHULTZ-BIP.

PAR

AURICA TĂCINĂ

The paper presents the observations made on some populations of *Achillea schurii* Schultz-Bip. in the Bucegi massive (South Carpathians of Romania). The investigated species has a diploid set of chromosomes $2n = 18$, among which 5 metacentrical pairs and 4 submetacentrical pairs. Chorological and morphological conditions confirm the existence of taxa *Achillea schurii* Schultz-Bip. and *Achillea oxyloba* (DC.) Schultz-Bip. as species.

Le genre *Achillea* L. est représenté, dans la flore de la Roumanie, par 28 espèces, appartenant aux sections : *Ptarmica* (Neck.) Kock et *Millefolium* (Adans.) Kock [4]. Dans nos recherches, les préoccupations ont été dirigées surtout vers l'espèce *Achillea schurii* Schultz-Bip. de la section *Ptarmica* (Neck.) Koch. Cette espèce représente un élément alpin des Carpates de S et E, ayant en tant qu'espèce vicariante, l'*Achillea oxyloba* (DC.) Schultz-Bip., répandue dans les Alpes de S et les Apennins [7].

Quoique bien apparentée à *Achillea oxyloba*, l'*Achillea schurii* en diffère par toute une série de caractères morphologiques qualitatifs. L'*Achillea schurii* est un taxon endémique ayant une aire limitée aux massifs des Carpates de S et E : Ciucaş, Bîrsei, Bucegi, Iezer, Făgăraş, Ţibleş, Rodnei, Rarău, Călimani, Ceahlău, Hăghimaş, Buzăului.

MATÉRIEL ET MÉTHODE

Les recherches ont été effectuées sur les populations du massif des Bucegi-Vîrful cu Dor (fig. 1). Le matériel de travail avait compris les méristèmes radiculaires obtenus des semences germées en vaisseaux

Fig. 1. — *Achillea schurii* Schultz-Bip. — Vf. cu Dor (versant nord-vest).

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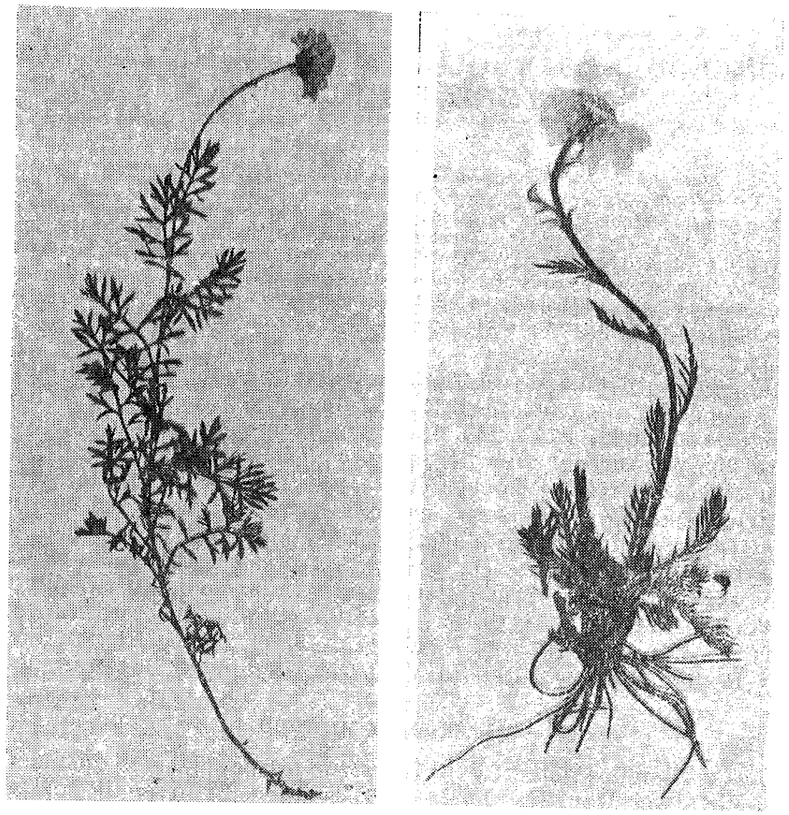


Fig. 2. — *a. Achillea schurii* Schultz-Bip. ; *b. Achillea oxyloba* (DC.) Schultz-Bip.

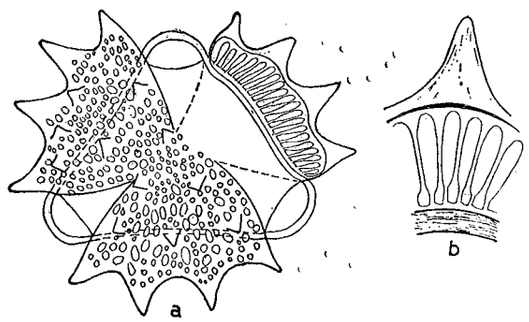
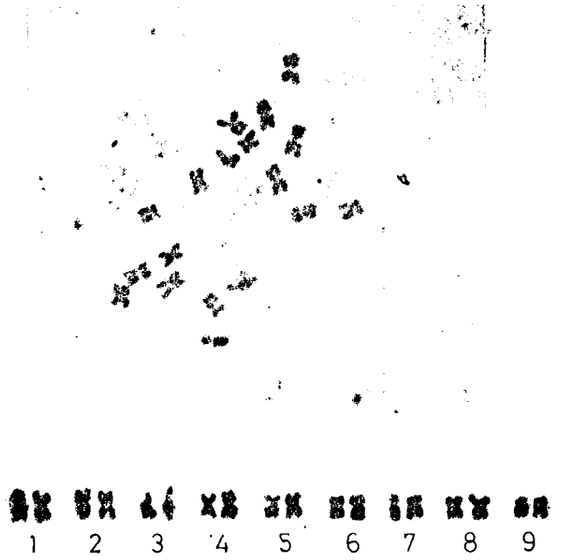


Fig. 3. — *a.* Pollen-vue apicale (*Achillea schurii*) ; *b.* Portion de la sporoderme, bien augmentée, vue en section optique (selon I. Tarnavschi, N. Mitroiu).



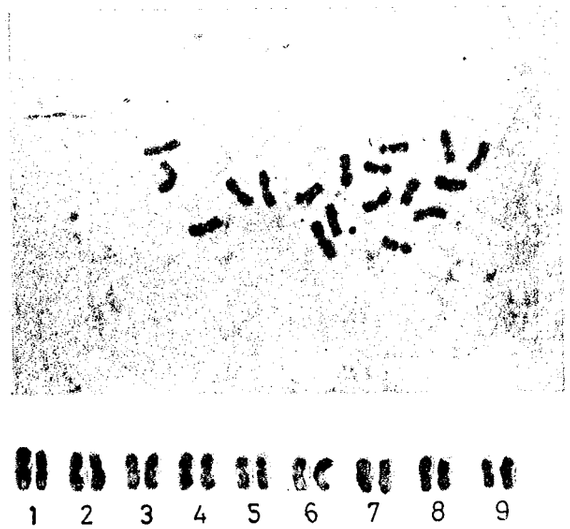
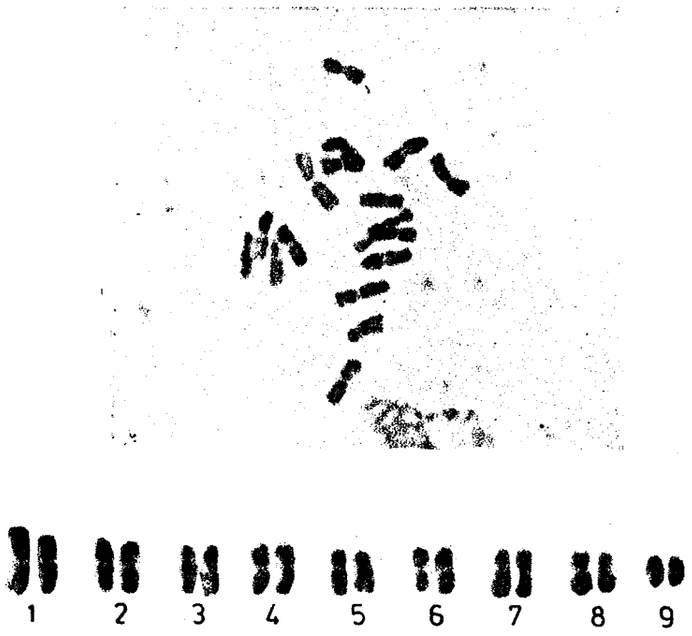


Fig. 4, 5. — Métaphase de la mitose à l'*Achillea schurii* Schultz-Bip. ($2n = 18$) et chromosomes homologues dans le set diploïde.



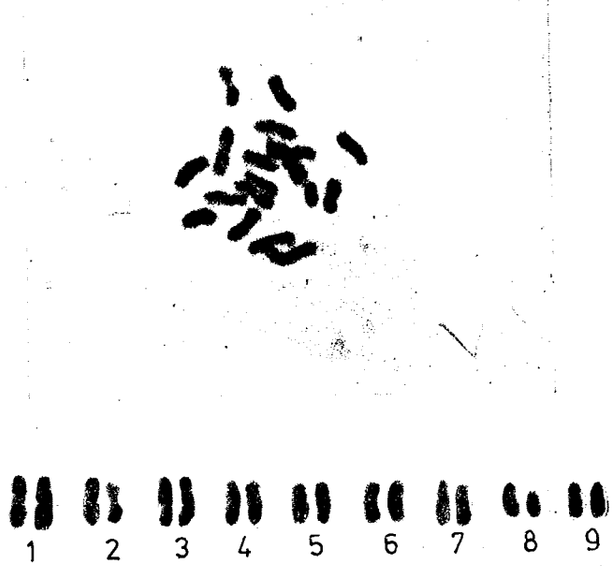


Fig. 6, 7. — Métaphase de la mitose à l'*Achillea schurii* Schultz-Bip. ($2n = 18$) et chromosomes homologues dans le set diploïde.

Petri. Le prétraitement des méristèmes radiculaires — avec colchicine 0,1% pendant 2,5 heures ; le fixage — dans un mélange d'alcool—acétique glacial 3 : 1. L'hydrolyse a été réalisée en 15 minutes en acide chlorhydrique (HCl) 1 N, suivie par la coloration avec réactifs Schiff.

L'examen des préparations squash a été fait au microscope MC_1 et les microphotos ont été obtenues à une augmentation directe de 400x.

RÉSULTATS ET DISCUSSIONS

L'*Achillea schurii* Schultz-Bip. est traitée en tant qu'espèce dans la Flore de la Roumanie [3], [4] et dans la Flore Européenne [5] en tant que taxon, étant considérée sous-espèce de l'espèce *Achillea oxyloba* (DC.) Schultz-Bip. Les deux taxons présentent une série de caractères morphologiques de similitude qui conduisent à l'idée d'un étroit voisinage (fig. 2*a*, *b*). Dans ce sens, on peut parler aussi d'une morphologie ressemblante du pollen des deux espèces (fig. 3*a*, *b*) — selon I. Tarnavschi, N. Mitroiu [7]. Il y a aussi certains caractères morphologiques de différentiation nette entre les deux taxons, à savoir : les feuilles bipinées, les lacinies involucrales obtuses, le papus présenté sous forme d'une courte couronne [2] ce qui justifie notre opinion de considérer l'*Achillea schurii* en tant qu'espèce, et non pas sous-espèce de l'*Achillea oxyloba*.

Sous l'aspect caryologique, pour le genre *Achillea*, le nombre de base, $n = 9$ [1,6].

A la suite des observations faites du matériel analysé il résulte que pour l'*Achillea schurii*, $n = 9$, $2n = 18$ (fig. 4—7). Le nombre des chromosomes déterminé par nous confirme les données de la littérature [1] De l'analyse du caryotype il résulte que des 9 paires de chromosomes, 5 paires sont métacentriques (I, II, IV, VI, VIII) et 4 sous-métacentriques (III, V, VII, IX) (fig. 4—7). La présence des chromosomes métacentriques et sous-métacentriques confère au caryotype un caractère symétrique, primitif, ce qui reflète en même temps l'ancienneté du taxon en discussion.

Conformément aux données caryologiques, nous pouvons dire que l'*Achillea schurii* est une espèce diploïde ($2n = 18$), un paléoendémisme* ayant en tant qu'espèce vicariante l'*Achillea oxyloba* avec le set diploïde $2n = 18$ [5] avec lequel elle avait occupé peut-être, dans le préglaciaire, un territoire commun. Dans la période post-glaciaire, les deux espèces étant isolées, leur évolution a été dissemblable, en devenant des espèces indépendantes.

Dans ce cas, les données caryologiques confirment les arguments morphologiques et corollogiques en faisant preuve de l'âge du taxon, ainsi que du rang systématique.

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* *Schëzoendemism dans la classification établie par Favarger, Contandriopoulos (1961), Favarger (1972).*

BIBLIOGRAPHIE

1. Ciopik V. I., *Visokogornaia flora ucrainschih Carpat*, Naukova dumka, Kiev, 1976, 116.
2. Hegi G., *Illustrierte Flora von Mitteleuropa*, München, 1928, **6**, *2*, 552.
3. Prodan I., *Achilleele României*, Cluj, 1931, 9—10.
4. Prodan I., Nyarady E., *Flora R.P.R.*, Ed. Academiei, București, 1955, **9**, 366.
5. Richardson I.B.K., Genul Achillea L., în: *Flora: Europaea*, Cambridge, 1976, **4**, 159—165.
6. Tarnavschi I.T., Bull. Grăd. Bot., Cluj, 1947, **27**, *1*.
7. Tarnavschi I.T., Mitroiu Natalia , St. și cerc. Biol., Seria biol. veget., 1959, **11**, *3*, 241.

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CONSIDÉRATIONS CONCERNANT LA FLORE MÉDICINALE SPONTANÉE DU DÉPARTEMENT DE TULCEA (ROUMANIE)

PAR

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In the medicinal spontaneous flora of the Tulcea county, more than 150 species have been identified. They belong to 44 families, most of them supplying a quantity of raw material up to hundreds of tones.

Dans le cadre du lever de la flore médicinale spontanée sur le territoire de la Roumanie, exécutés par l'Institut pour le contrôle d'État des Médicaments et Recherches Pharmaceutiques, en collaboration avec la Faculté de Biologie de Bucarest, pendant l'année 1976 on a effectué de recherches dans le département de Tulcea.

Situé à l'extrémité est du pays, le dép. de Tulcea présente un relief, un sol, une hydrographie et un climat particuliers, ce qui détermine une flore et une végétation spécifiques.

Au point de vue géomorphologique le dép. de Tulcea comprend deux grandes unités de relief : une partie ancienne — le haut relief d'érosion ou *Masivul Dobrogean* — et une partie plus récente — la basse terre, inondable.

Le haut relief d'érosion comprend cinq unités géomorphologiques :

1. Les Monts Măcin, le résultat de l'orogénèse hercynien de la fin du paléozoïque, se présentent aujourd'hui comme des collines ayant 100 — 400 m d'altitude, à quelques sommets plus hauts.

2. Les collines de Niculițel, qui représentent la continuation vers l'est des Monts Măcin, ont des hauteurs plus petites.

3. Les collines de Tulcea s'étendent plus loin vers l'est et ont des hauteurs encore plus petites.

4. Le plateau de Babadag, situé entre les Monts Măcin et les collines de Tulcea au nord, et le Plateau Casimcea vers le sud, à hauteurs comprises généralement entre 200 et 350 m.

5. La partie nord du Plateau Casimcea.

La partie basse du département comprend trois unités morphologiques :

1. la plaine alluviale prédeltaïque, représentée par la prairie du rivage droit du Danube ;

2. la plaine fluvio-maritime du Delta du Danube ;

3. la plaine littorale-lagunaire de Razelm-Golovița.

L'hydrographie du haut relief du district est très pauvre et constituée de quelques petits ou très petits ruisseaux, qui se jettent dans les

lacs ou dans le Danube. En ce qui concerne l'hydrographie de la partie basse, le rapport entre la terre ferme et l'eau est toujours en faveur de l'eau, toute la région étant parsemée d'un réseau de bras du Danube, canaux, marais, lacs.

Le climat des hautes régions est continental excessif, avec de grands contrastes thermiques de l'été à l'hiver et de pluies rares à caractère d'averses. Le climat des basses régions, de la mer Noire, est littoral-maritime, avec des contrastes thermiques plus petits.

En ce qui suit, nous présentons les plantes médicinales qui se trouvent dans des quantités plus grandes dans les principales zones biogéographiques, en exprimant en tonnes les quantités de matière première séchée qui peut être obtenue de chaque espèce de tout le département.

En établissant les quantités que nous recommandons à être récoltées, nous avons également en vue la protection de la nature, surtout dans le cas des plantes pérennantes (dont on récolte les racines, en détruisant ainsi la plante même).

Nous allons discuter aussi la teneur en principes actifs des plantes médicinales récoltées des localités différentes.

RÉSULTATS OBTENUS

1. ÉVALUATION QUANTITATIVE DES ESPÈCES DE PLANTES MÉDICINALES

A. Zone de forêts

Les forêts occupent des superficies assez petites à l'échelle du dép., mais couvrent une bonne partie des Monts Măcin, des collines de Niculiţel et du Plateau de Babadag.

Le nombre d'espèces de plantes médicinales qui poussent dans ces forêts n'est pas grand, mais quelques-unes se trouvent en très grande quantité.

Parmi les arbres nous mentionnons tout d'abord les espèces de *Quercus*, qui constituent des forêts pures ou mélangées avec d'autres feuillus : *Q. petraea* (Matt) Liebl., *Q. pedunculiflora* C. Koch., *Q. robur* L., *Q. polycarpa* Schur., *Q. dalechampii* Ten., *Q. frainetto* Ten., *Q. virgiliana* Ten., Cortex = 138,8.

Robinia pseudacacia L., arbre cultivé, souvent sauvage, est trouvé fréquemment, mais surtout au bord des forêts de la zone des Monts Măcin et des collines de Niculiţel, Flores = 43,2.

Un arbre très nombreux dans les forêts du dép. et surtout dans les Monts Măcin et les collines de Niculiţel est *Tilia*. Parmi ces espèces, *T. tomentosa* Mnch. est beaucoup plus fréquent, étant dominant localement ou codominant en associations, donnant aux forêts de Nifon, Niculiţel et au Monastère Cocoş, pendant la floraison, un charme à part, Flores = 155,2.

Les autres espèces sont plus rares: *T. cordata* Mill., Flores = 7,3 et *T. platyphyllos* Scop., Flores = 5,3.

Fraxinus excelsior L. est nombreux dans certaines forêts des Monts Măcin, des collines de Niculiţel et du Plateau de Babadag. Folium 34,6.

Fraxinus ornus L., arbre ou arbuste, constant jusqu'au codominant en association, est rencontré dans toutes les trois zones de forêts du haut relief, Folium = 40.

Outre les arbustes, dans la zone de forêts poussent encore des arbustes d'importance médicinale. On les rencontrent surtout au bord des forêts, en défrichements, coupures, brousses, clairières. De ces arbustes on peut obtenir de grandes quantités de matière première.

Mentionnons : *Coryllus avellana* L., Folium = 44,9 ; *Elaeagnus angustifolia* L., Folium = 3,4 ; *Crataegus monogyna* Jacq., Fructus = 16,7 ; *Rosa* sp. Fructus = 1,7 ; *Cotinus coggygria* Scop., Folium = 46,3 ; *Cornus mas* L., Fructus = 19,5 ; *Ligustrum vulgare* L., Flores = 7 ; *Sambucus nigra* L., Flores = 7,6.

Mentionnons encore la plante voluble *Clematis vitalba* L., Herba = 33,2.

Lieu	Espèces	Herba	Flores	Folium	Rhiz.	Rhiz. Radix
Plateau de Babadag, Casimcea, aux bords des forêts	*Urtica dioica* L.	—	—	29,8	—	—
	Polygonum aviculare L.	4,2	—	—	—	—
	Adonis vernalis L.	6,8	—	—	—	—
	Hypericum perforatum L.	4,6	—	—	—	—
	Rubus sp.	—	—	7,9	—	—
	Fragaria viridis Durch	—	—	5,2	—	—
	Geum urbanum L.	—	—	—	6,5	—
	Filipendula hexapetala Gilib.	—	2,9	—	—	—
	Agrimonia eupatoria L.	4,2	—	—	—	—
	Astragalus glycyphyllos L.	8,3	—	—	—	—
	Aegopodium podagraria L.	11,1	—	—	—	—
Plateau de Babadag Casimcea	*Lithospermum arvense.* L.	1,5	—	—	—	—
	Scrophularia nodosa L.	1,8	—	—	—	—
	Theucrium chamaedris L.	10,1	—	—	—	—
Aux bords des chemins des plateaux de Babadag et Casimcea	*Leonurus cardiaca* L.	1,7	—	—	—	—
	Calamintha officinalis Mnch.	3,0	—	—	—	—
	Origanum vulgare L.	6,9	—	—	—	—
	Cynanchum vincetoxicum (L) Pers.		—	—	—	2,5
	Galium verum L.	1,2	—	—	—	—
	Galium aparine L.	19,5	—	—	—	—
	Sambucus ebulus L.			fructus		4,3
	Artemisia vulgaris L.	15,9	—	—	—	—
Aux bords des chemins et endroits cultivés	*Artemisia absinthium* L.	26,5	—	—	—	—
	Arctium lappa L.	—	—	—	—	4,9
	Onopordon acanthium L.	2,5	—	—	—	—
	Cichorium intybus L.	—	—	—	—	2,0

De nombreuses plantes herbacées ou sous-arbustes d'importance médicinale poussent dans des forêts ou dans les zones de forêts. Nous mentionnons celles qui se trouvent en plus grande quantité.

À l'intérieur des forêts, dans des endroits hummides et ombrageux, surtout sur les collines de Niculiţel, pousse *Asarum europaeum* L., Rhizoma = 1,1.

Dans les mêmes stations, sur les collines de Niculițel et dans le Plateau de Babadag, on trouve *Asperula odorata.*, Herba = 2.

On mentionne encore les plantes semi-parasites *Loranthus europaeus* Jacq., Folium cum stipites = 9,1, surtout dans le Plateau de Babadag et le Plateau de Casimcea et *Viscum album* L., Folium cum stipites = 2,9, surtout dans le Plateau de Babadag.

Un nombre plus grand d'espèces se trouve au bord des forêts, des clairières, etc., comme on peut voir dans le tableau de la page 13.

B. La zone de steppe et la région rocheuse

Dans cette zone nous avons encadré les prairies non cultivées et utilisées comme pâturages, les terrains cultivés ainsi que les côtes et sommets rocheux des collines, dépourvus de forêts. On y trouve peu de plantes médicinales, dont nous mentionnons les principales dans le tableau qui suit:

Espèces	Flores	Herba
Capsella bursa pastoris (L) Medik	—	1,2
Verbascum sp.	—	0,09
Teucrium polium L.	—	5,5
Thymus sp.	—	23,9
Eryngium campestre L.	—	11,4
Xanthium spinosum L.	—	3,7
Achillea sp.	19,5	—

On cite encore *Artemisia austriaca* Jack. (ainsi que d'autres espèces semblables), plante non médicinale, mais qui, avec les espèces de *Thymus*, donne la couleur et surtout l'odeur spécifique à peu de terres non cultivées de la steppe de Dobrogea.

2. LA TERRE BASSE, INONDABLE

A. Végétation terrestre

La végétation terrestre se trouve sur la rive droite du Danube et sur les schorres du Delta, ainsi que vers le lac Razelm.

Excepté les saules de la rive droite du Danube, nous avons fait des recherches surtout sur le Delta du Danube et sur la région vers le lac Razelm.

Parmi les arbres, citons les espèces de *Salix*, nombreuses surtout dans les clairières du Danube, le Delta du Danube, les zones à proximité du lac Razelm, souvent dans les endroits marécageux Cortex = 346,2.

Parmi les plantes herbacées on trouvent: *Eupatorium cannabinum* L. souvent aux bords des canaux, Herba = 3; *Glycyrrhiza echinata* L., Radix = 12,2; *Althaea officinalis* L., Radix = 14,8; *Calystegia saepium* (L) R. Br. toujours au bords des canaux.

De petites quantités de *Convolvulus arvensis* L. se trouvent dans la zone de haut relief, Herba = 2,7; on cite encore *Solanum dulcamara* L., stipites = 39,9; *Solanum nigrum* L., Folium = 1,5; *Lycopus europaeus* L., Herba = 2,1; *Plantago major* L., Folium = 6,1; *Plantago lanceolata* L., Folium = 3,8; *Gnaphalium uliginosum* L., Herba = 1,5; *Matricaria chamomilla* L., Flores = 20,4; *Chrysanthemum vulgare* (L) Bernh., Flores = 44,6; *Artemisia maritima* L., Herba = 5,3.

B. Végétation de marécage et aquatique

Dans cette catégorie nous avons considéré les plantes qui poussent dans les endroits marécageux, aux bords des lacs, des flaques d'eau, des canaux, ainsi que celles qui poussent dans l'eau, à savoir: *Rumex hydrolopathum* Huds., Radix = 24; *Polygonum hydropiper* L., Herba = 22,9; *Nuphar luteum* (L) Sm., Rhizoma = 51; *Nymphaea alba* L., Rhizoma = 57; *Lythrum salicaria* L., Herba = 14; *Trapa natans* L., Fructus = 55; *Symphytum officinale* L., Radix = 1,9; *Mentha pulegium* L., Herba = 14,8; *Mentha aquatica* L., Herba = 22,5; *Mentha longifolia* (L.) Nath., Herba = 10,6; *Alisma plantago aquatica* L., Rhizoma = 2,1.

Outre les espèces citées, il y a d'autres tenant tant des zones du haut relief d'érosion, que de la basse terre inondable, qui se trouvent en quantité réduite, certaines même dans un nombre restreint de localités. Parmi celles-ci nottons: *Equisetum maximum* Lam., Herba = 0,8; *Juglans regia* L., Folium = 3,4; *Morus* sp., Folium = 1,7; *Hippophaë rhamnoides* L., Fructus = 0,7; *Prunus spinosa* L. (d'après Dihoru et Doniță — *P. moldavica* Kotov), Fructus = 2,8.; *Gleditschia triacanthos* L., Folium = 0,6; *Melilothus officinalis* (L) Medik., Flores = 0,8; *Cicuta virosa* L., Herba = 0,8; *Heracleum sphondylium* L., Radix = 0,9; *Teucrium scordium* L., Herba = 0,7; *Marrubium vulgare* L., Herba = 0,6; *Prunella vulgaris* L., Herba = 0,5; *Stachys sylvatica* L., Herba = 0,6; *Plantago media* L., Folium = 0,6; *Artemisia annua* L., Herba = 0,8; *Senecio jacobaea* L., Herba = 0,5; *Centaurea calcitropa* L., Herba = 0,6; *Convallaria majalis* L., Folium = 0,6.

II. DÉTERMINATION DE LA TENEUR EN PRINCIPES ACTIFS

Pour déterminer la qualité des plantes médicinales qui poussent dans le dép. de Tulcea, nous avons effectué des analyses quantitatives des principes actifs de certaines plantes récoltées dans diverses localités du dép. Les résultats obtenus nous permettent de faire certaines observations intéressantes.

Ainsi les espèces de l'*Achillea* (autres que *A. millefolium*) et *Matricaria chamomilla* ne constituent pas une matière première médicinale de bonne qualité, étant totalement ou presque dépourvues d'azulen, l'un des principes actifs de base de ces plantes qui poussent dans d'autres régions du pays.

D'autres espèces médicinales sont, au contraire, de qualité supérieure. Ainsi, les espèces de *Thymus* sont particulièrement riches en huile volatile, dépassant parfois de 400% la teneur minimale prevue dans la Pharmacopée Roumaine VII.

CONCLUSIONS

1. Situé à l'extrémité est du pays, le dép. de Tulcea présente un relief, un sol, une hydrographie et un climat particuliers, ce qui détermine une flore et une végétation spécifiques.

2. La flore médicinale spontanée est assez riche et variée. Nous avons identifié plus de 150 espèces, appartenant aux 44 familles. La plupart de ces espèces permet d'obtenir de la matière première séchée en quantités qui varient de quelques centaines de kg. à quelques centaines de tonnes.

3. Les analyses de laboratoire montrent que quelques-unes de ces espèces sont particulièrement riches en principes actifs, ce qui a une grande importance pour l'industrie pharmaceutique.

4. La valorisation supérieure des plantes médicinales du dép. de Tulcea peut apporter d'importants bénéfices à l'économie nationale.

BIBLIOGRAPHIE

1. Dihoru Gh., Doniță N., *Flora și vegetația podișului Babadag*, Ed. Academiei, București, 1970.
2. *** *Flora R.S.R.*, Ed. Academiei, București, Vol. I—XII, 1952—1972.
3. Zitti R., Retezeanu M., *Flora medicinală spontană din raionul Caransebeș*, Lucrările prezentate la Conferința națională de farmacie, București, 1958.

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BEITRÄGE ZUM STUDIUM DER *MOLINIO-ARRHENATHERETEA* TX. (1937) 1970 IM LOCVA-GEBIRGE (SÜDWESTEN RUMÄNIENS)

VON

I. COSTE

The paper analyses in a coenotaxonomic unitary view the mesophyllic grassland associations in the Locva Mountains belonging to *Molinio-Arrhenatheretea* class (Tx·37) 70.

Das Locva-Gebirge, im Südwesten Rumäniens zwischen der Donau und dem Unterlauf der Nera gelegen, stellt ein gut geprägtes Massiv dar, das in die Richtung Ost-West verläuft. Nach Osten hin besteht es aus weiten Hochebenen von 500—600 m Höhe, während im Westen die bis 350 m hohen Hügel immer niedriger werden. Entlang der Donau und Nera dehnen sich verschieden große Auen aus, die mit der Hügelzone durch ein 200—250 m hohes Terrassengelände verbunden sind.

Vom geomorphologischen Standpunkt aus ist dieses Gebiet durch proterozoische und paläozoische kristalline Schiefer gekennzeichnet. Die Randzone besteht aus quartären Ablagerungen von Kieselstein, Ton und Löß.

Das gemäßigte Festlandklima entspricht der Köppen Cfbx-Formel. In Moldova Veche werden Jahresdurchschnittstemperaturen von 11,2°C verzeichnet, die Niederschlagsmenge beträgt 560,1 mm, mit einem Maximalwert im Mai und einem Minimum im Dezember. Die Durchschnittstemperatur in den zentraler gelegenen Teilen des Massivs ist um 1—2°C kleiner und die Niederschlagsmenge erreicht 700 mm.

Die Böden dieses Gebietes stellen spezifische Gruppen der geomorphologischen Einheiten dar : Schwemmlandböden in den Tälern, Tschernoziom auf den niederen Geländeterrassen, brauner und gelbbrauner Waldboden im Hügelland, brauner und saurer Gebirgsboden auf den Höhen des Massivs ; auf den Lößablagerungen sind nichthumifizierte, lössoide Böden.

Die natürliche, diesem Gebiet charakteristische Vegetation besteht auf den Bergspitzen und Südhängen aus *Quercus petraea*, *Q. cerris* und *Q. farnetto* und aus *Fagus silvatica* — Wäldern mit *Carpinus betulus* und *Tilia tomentosa* in den Tälern. Die seit über 2000 Jahren andauernde intensive und stete Anwesenheit von menschlichen Siedlungen in den Randgebieten des Massivs, bestimmte die große Ausbreitung mezophiler und xerophiler Wiesen, die verschieden stark durch die menschliche Tätigkeit beeinflußt wurden.

Die durchgeführten Studien stützen sich auf die Methodologie der phytosoziologisch-sygmatischen Schule [2,1] ; in der synthetischen

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2—c.1090

Tafel wird die mittlere Abundanz-Dominanz und die Konstante der Arten in den analysierten Gesellschaften dargestellt.

Die Überprüfung der systematischen Position der Gesellschaften die zu *Plantaginetalia majoris*, Tx. et Prsg. 1950 gehören, veranlaßte die einheitliche Betrachtung aller mezohygrophiler und mezophiler Wiesen in Europa durch die Erweiterung der Klasse *Molinio-Arrhenatheretea* Tx. 1937, so daß auch die charakteristischen Wiesen-Tritt-Gesellschaften darin eingeschlossen werden [8]. Im Sinne dieser Auffassung, die wir als berechtigt ansehen, haben die in diesem Gebiet identifizierten Gesellschaften folgende zönotaxonomische Anordnung.

Molinio-Arrhenatheretea Tx. (1937) 1970

Trifolio fragiferi—Agrostietalia stoloniferae (Oberd. 1967) Tx. 1970
Agropyro — Rumicion crispi Nordh. 1940

1. *Rorippo silvestris — Agrostietum stoloniferae* (Moor 1958) Oberd. et Th. Müll. 1960

2. *Junco inflexi — Menthetum longioliae* Lohm. 1953 W. Koch 1926
Molinietalia Calthion Tx. 1939

3. *Epilobio palustre — Juncetum effusi* (Paucă 1941) Oberd. 1957

4. *Scirpetum sylvatici* Schw. 1944.
Plantaginetalia majoris (Tx. et Prsg. 1950) Tx. 1970

5. *Juncetum tenuis* (Diem. Siss. et Westh. 1940) Schw. 1944

6. *Plantago majori — Lolietum perennis* (Linkola 1921) Beger. 1930
Arrhenatheretalia Pawl. 1928
Arrhenatherion (Br. — Bl. 1925) W. Koch 1926

7. *Arrhenatheretum elatioris* Br. — Bl. 1919

8. *Poaeto trivialis — Festucetum pratensis* (Borza 1959) I. Moldovan 1970.

9. *Festuco rubrae — Agrostietum tenuis* (Horv. 1951)

1. *Rorippo silvestris—Agrostietum stoloniferae* (Moor 1958) Oberd. et Th. Müll. 1960

Diese Gesellschaft ist in den Donau- und Nera-Auen zu finden, in Senken mit eutrophen, beweideten Böden.

Die reiche floristische Zusammensetzung (Tab.1.1) umfaßt neben mezohygrophilen und hygrophilen Arten der *Molinio-Arrhenatheretea* auch zahlreiche Transgressionsarten, die einen unterschiedlichen Ruderalisierungsgrad anzeigen.

Der Struktur nach besteht die Gesellschaft aus zwei augenscheinlichen Schichten : einer niederen mit trittfesten Arten (*Agrostis stolonifera*, *Trifolium repens*, *Ranunculus repens*, *Potentilla reptans* usw.) und einer höheren, weniger individualisierten.

In Rumänien als *Agrostetum albae* Ujv. 1941 angeführt, wurden die Phytozönosen von *Agrostis stolonifera* zuerst zu *Molinio-Arrhenatheretea* gehörig angesehen, u.zw. als Auenwiesen mit unterschiedlichem Ruderalisierungsgrad. Die vorliegende Arbeit will in diesem Sinne die Bedeutung des menschlichen Einflusses unterstreichen.

Tabelle 1

Th. Müll 1961.

1. *Rorippo silvestris — Agrostietum stoloniferae* (Moor 1958) Oberd et Th. Müll 1961.
2. *Junco inflexi — Menthetum longifoliae* Lohm. 1953.
3. *Epilobio palustre — Juncetum effusi* (Paucă 1941) Oberd. 1957.
4. *Scirpetum sylvatici* Schw. 1944.
5. *Juncetum tenuis* (Diem., Siss. et Westh. 1940) Schw. 1944.
6. *Plantago majori — Lolietum perennis* (Linkola 1921) Beger 1930.
7. *Arrhenatheretum elatioris* Br.—Bl. 1919.
8. *Poaeto trivialis — Festucetum pratensis* (Borza 1959) I. Moldovan 1970.

Gesellschaft	1		2		3		4		5		6		7		8		Festuca arundinacea + III (3) ; Galega officinalis + I(3).
Zahl der Aufnahmen	12		10		5		15		8		14		9		11		
	AD	K	AD	K	AD	K	AD	K	AD	K	AD	K	AD	K	AD	K	
Agropyro – Rumicion et Trifolio – Agrostietalia																	
Agrostis stolonifera	5	V	+—2	IV	+—2	V											
Carex hirta	+	II	+	I													
Mentha logifolia	+	II	+	V	+	IV											
Ranunculus repens	+—1	III	—1	V	—3	III											
Juncus inflexus	+	II	+—2	IV													
Juncus compressus		I	2—5	V													
Rorippa sylvestris	+	I	+	II													
Trifolium hybridum	+	III	—		—												
Trifolium fragiferum	+	II															
Lycopus exaltatus	+	I	+	II	+	III											
Inula britanica	+	I	+	III	—												
Rumex conglomeratus	+	—	+	I	+	I											
Rumex crispus	+	I	+	I	+	+											
Symphytum officinale	+	I	+	—	+	I											
Verbena officinalis	—	—	—	I	—	—											
Ranunculus sardous	+—1	III	+	—	—	—											
Gratiola officinalis	+	I	+	I	—	—											
Calthion et Molinietalia																	
Juncus effusus	—	—	—	—	3—5	V											
Epilobium palustre	—	—	+	I	+—2	II											
Scirpus silvaticus	—	—	—	—	—	—											

Tabelle 1 (Fortsetzung)

Gesellschaft	1		2		3		4		5		6		7		8	
Zahl der Aufnahmen	12		10		5		15		8		14		9		11	
	AD	K	AD	K	AD	K	AD	K	AD	K	AD	K	AD	K	AD	K
Hypericum tetrapterum	–	–	–	–	+	I	+	I	–	–	–	–	–	–	–	–
Juncus articulatus	–	–	–	–	+	I	+	I	–	–	–	–	–	–	–	–
Lythrum salicaria	+	II	+	I	+–3	V	+	II	–	–	–	–	–	–	–	–
Equisetum palustre	+	II	+	II	+	I	+–1	IV	–	–	–	–	–	–	–	–
Lysimachia nummularia	+	I	+	II	+	IV	+–1	III	–	–	–	–	–	–	–	–
Lysimachia vulgaris	+	I	–	–	+	I	+	I	–	–	–	–	+	II	–	–
Mentha aquatica	–	–	+	III	+–3	III	+	V	–	–	–	–	–	–	–	–
Galium palustre	+	I	+	I	+	II	+	II	–	–	–	–	–	–	–	–
Poa palustris	–	–	+	I	+	II	–	–	–	–	–	–	–	–	–	–

Lychnis flos-cuculi + II(3); *Stellaria nemorum* + III(3); *Calystegia sepium* + II; *Carex remota* + II(3); *Carex leporina* + I(3); *Aegopodium podagraria* + I(4)

Lolio — Plantaginion et Plantaginetalia

Lolium perenne	+	I	+	I	–	–	–	–	+	I	2–5	V	+	I	+	I
Plantago major	+	I	+	I	–	–	–	–	+–1	V	+–2	IV	–	–	–	–
Plantago lanceolata	+	II	–	–	–	–	–	–	+	V	+–2	IV	+	I	–	–
Trifolium repens	+–2	III	–	–	–	–	–	–	+–1	IV	+–2	III	–	–	–	–
Juncus tenuis	–	–	–	–	+	II	–	–	+–5	V	–	–	–	–	–	–
Cynodon dactylon	+	I	–	–	–	–	–	–	–	–	+–1	II	+	II	–	–

Arrhenatherion, Cynosurion et Arhenatheretalia

Arrhenatherum elatius	–	–	–	–	–	–	–	–	–	–	–	–	3–5	V	+	I
Festuca pratensis	+	II	–	–	–	–	–	–	–	–	+	I	+–1	V	5	V
Dactylis glomerata	+	II	–	–	–	–	–	–	–	–	+–3	V	–	–	+–1	IV
Bromus mollis	+	II	–	–	–	–	–	–	–	–	+	I	+	IV	+	II
Chrysanthemum leucanthemum	–	–	–	+	+	I	–	–	–	–	–	–	+	III	+	III
Rhinanthus rumelicus	+	II	–	–	–	–	–	–	–	–	–	–	+	IV	+	IV
Trifolium campestre	+	II	–	–	–	–	–	–	–	–	+	II	+	IV	+	IV
Euphorbia virgata	–	–	–	–	–	–	–	–	–	–	–	–	+	II	+	I
Galium mollugo	–	–	–	–	–	–	–	–	–	–	–	–	+	II	+	IV
Salvia pratensis	–	–	–	–	–	–	–	–	–	–	–	–	+	I	+	I

Gesellschaft	1		2		3		4		5		6		7		8	
Zahl der Aufnahmen	12		10		5		15		8		14		19		11	
	AD	K	AD	K	AD	K	AD	K	AD	K	AD	K	AD	K	AD	K
Tragopogon orientale	–	–	–	–	–	–	–	–	–	–	–	–	+	III	+	IV
Equisetum ramosissimum	–	–	–	–	–	–	–	–	–	–	–	–	+	II	+	II
Ononis arvensis	+	I	–	–	–	–	–	–	–	–	–	I	+–1	II	+	I
Rorippa pyrenaica	–	–	–	–	–	–	–	–	+	III	–	–	–	–	+	I
Daucus carota	+	I	–	–	–	–	–	–	–	–	–	–	+	II	–	–
Pastinaca sativa	–	–	–	–	–	–	–	–	–	–	–	–	+–1	IV	+	I
Lotus corniculatus	–	–	–	–	–	–	–	–	–	–	+	I	+	I	–	–
Vicia grandiflora	+	I	–	–	–	–	–	–	–	–	–	–	+	III	+–1	IV

Silene vulgaris + IV(7); *Lathyrus latifolius* + I(7); *Campanula patula* + I(7); *Anthoxanthum odoratum* + I(8); *Centaurea banatica* + I(8). *Knautia arvensis* + I(8); *Linum hirsutum* + I(8); *Trifolium incarnatum* + I(8);

Molinio — Arrhenatheretea

Poa trivialis	+	III	+	II	+	I	+	III	+	I	–	–	–	–	+	I
Poa pratensis	+–1	IV	–	–	–	–	–	–	–	–	–	–	+	IV	+–1	V
Alopecurus pratensis	+	I	+	I	–	–	–	–	–	–	–	–	–	–	+	I
Achillea milefolium	+	II	–	–	–	–	–	–	+	III	+	II	+	V	+	II
Taraxacum officinale	+	I	+	I	–	–	–	–	+	I	+	I	+	I	–	–
Ranunculus acris	+	I	–	–	+	II	–	–	+	II	–	–	+	I	–	–
Cichorium intybus	+	III	–	–	–	–	–	–	–	–	+	III	+	II	+	V
Cerastium holosteoides	+	I	–	–	+	I	+	I	–	–	–	–	–	–	+	I
Prunella vulgaris	+	III	+	I	+	II	–	–	+	III	–	–	–	–	–	–
Rumex acetosa	+	I	–	–	–	–	–	–	–	–	–	–	+–1	V	+	II
Trifolium pratense	+	I	–	–	–	–	–	–	+	II	+	I	–	–	+	III
Medicago lupulina	+	I	–	–	–	–	–	–	+	I	+	III	+	I	+	II
Lathyrus nissolia	+	I	–	–	–	–	–	–	+	I	–	–	+	I	+	I
Lathyrus pratensis	–	–	–	–	–	–	–	–	–	–	–	–	+	II	+	I
Medicago sativa	+	I	–	–	–	–	–	–	–	–	–	–	–	I	–	–
Glechoma hederacea	+	I	–	–	–	–	–	–	+	I	–	–	+	II	–	–
Vicia cracca	–	–	–	–	–	–	–	–	–	–	–	–	+	I	+	I

Tabelle 1 (Fortsetzung)

Gesellschaft	1		2		3		4		5		6		7		8	
Zahl der Aufnahmen	12		10		5		15		8		14		9		11	
	AD	K	AD	K	AD	K	AD	K	AD	K	AD	K	AD	K	AD	K
Festuca rubra + III (5); *Agrostis tenuis* + III (5); *Veronica serpyllifolia* + I(7);																
Phragmitetea (incl. Magnocaricion)																
Typha angustifolia	+	I	+	I	+	I	--	–	–	–	–	–	–	–	–	–
Carex vulpina	+	I	–	–	+	I	–	–	–	–	+	I	–	–	+	I
Carex melanostachya	–	–	–	–	+	I	+	I	–	–	–	–	–	–	–	–
Carex riparia	–	–	+	I	–	–	+	II	–	–	–	–	–	–	–	–
Heleocharis palustris	+	I	–	–	–	–	+	I	–	–	–	–	–	–	–	–
Veronica anagallis–aquatica	+	I	+	I	–	–	–	–	–	–	–	–	–	–	–	–
Phragmites australis + I(1); *Iris pseudacorus* + I(1); *Bolboschoenus maritimus* + I(1); *Stachys palustris* + I(4).																
Andere Arten																
Stachys annua	+	II	–	–	–	–	–	–	–	–	–	–	+	II	+	III
Vicia sativa	+	II	–	–	–	–	–	–	–	–	–	–	+	I	+	I
Vicia hirsuta	+	II	–	–	–	–	–	–	–	–	–	–	+	II	+	II
Convolvulus arvensis	+	II	–	–	–	–	–	–	+	I	–	–	+	I	–	–
Lathyrus tuberosus	+	I	–	–	–	–	–	–	–	–	–	–	+	III	–	–
Salvia nemorosa	–	–	–	–	–	–	–	–	–	–	+	I	+	IV	+	III
Rubus caesius	+	I	–	–	+	I	–	–	–	–	–	–	–	–	–	–
Calamagrostis arundinacea	+	I	–	–	–	–	+	I	–	–	–	–	–	–	+	I
Juncus buffonius	–	–	–	–	–	–	–	–	+–5	V	–	–	–	–	–	–
Mentha pulegium	+	I	+	II	+	I	–	–	–	–	–	–	–	–	+	I
Aegilops cylindrica	–	–	–	–	–	–	+	I	–	–	+	I	–	–	–	–
Bromus arvensis	–	–	–	–	–	–	–	–	–	–	+	I	+	I	+	I
Polygonum aviculare	–	–	–	–	–	–	–	–	+	V	+–1	III	–	–	–	–
Poa annua	–	–	–	–	–	–	–	–	+	I	+	II	–	–	–	–
Capsella bursa-pastoris	–	–	–	–	–	–	–	–	–	–	+	II	+	I	–	–
Senecio vernalis	+	I	–	–	–	–	–	–	–	–	+	I	–	–	–	–
Malva neglecta	–	–	–	–	–	–	–	–	–	–	+	II	–	–	–	–
Hordeum murinum	–	–	–	–	–	–	–	–	–	–	+	II	–	–	–	–
Eryngium campestre	–	–	–	–	–	–	–	–	–	–	–	–	+	II	+	I
Asparagus officinalis	–	–	–	–	–	–	–	–	–	–	–	–	+	II	–	–
Falcaria vulgaris	–	–	–	–	–	–	–	–	–	–	–	–	+	II	–	–
Festuca valesiaca	–	–	–	–	–	–	–	–	–	–	–	–	+	II	–	–
Dorycnium herbaceum	–	–	–	–	–	–	–	–	–	–	–	–	+	II	–	–

Note: Aus Platzmangel fehlen aus der Tabelle 52 Arten die zufälliger Weise vorkommen (in 1–2 Aufnahmen) die auch von zönologischem und syndynamischem Standpunkt aus unbedeutend sind.

Die Gesellschaft ist auf Plätzen mit feuchtem, verdichtetem Boden zu finden und kann im Falle einer starken Zerstörung des Bodens allmählich in *Junco-Menthetum*, Lohm. 1953 übergehen, oder nach Trittverminderung in *Alopecuretum pratensis* Now. 1928.

2. *Junco inflexi-Menthetum longifoliae* Lohm. 1953

Diese Gesellschaft ist in dem Gebiet gut vertreten u.zw. in Form von Streifen den Bächen entlang oder auf Flächen mit Wasserüberschuß, die dem Tritt von Tieren ausgesetzt sind.

Die floristische Zusammensetzung (Tab. 1.2) ermöglicht unschwierig die Einordnung zum *Agropyro-Rumicion*-Verband sowie die Unterscheidung einer vertikal zweischichtigen Struktur. Wir sahen die Eingliederung einiger Zönosen aus der Donau-Au in diese Gesellschaft als notwendig an, u.zw. jener, die als zu *Lythro-Juncetum effusi* Todor et al. 1970 gehörig beschrieben wurden. Für diese relativ häufig auftretende Gesellschaft sind Bekämpfungsmaßnahmen notwendig, da sie keinen wirtschaftlichen Wert aufweist.

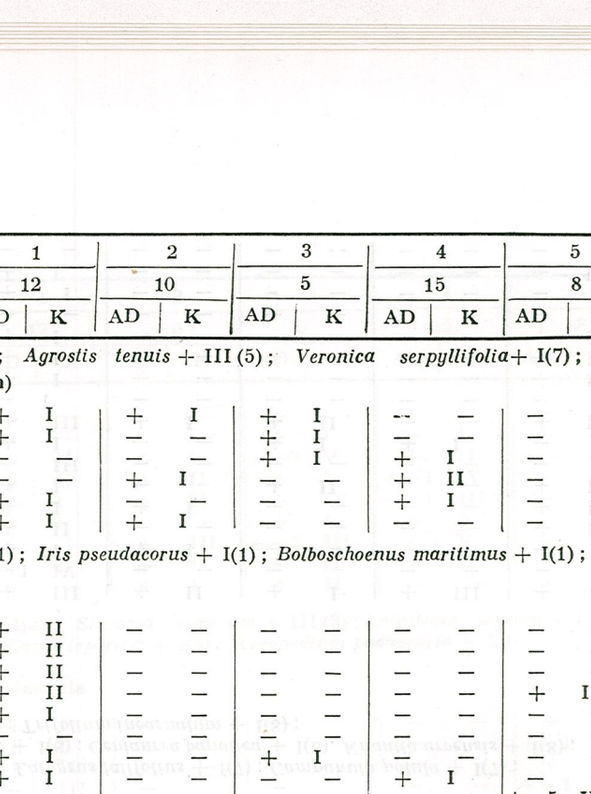

Abb. 1–*Epilobio palustre – Juncetum effusi* (Paucă 1941) Oberd. 1957.

3. *Epilobio palustre – Juncetum effusi* (Paucă 1941) Oberd. 1957

Diese Gesellschaft ist im Hügelland den Bächen entlang und in jenen Niederungen verbreitet, in denen sich das Wasser längere Zeit staut, u. zw. vorwiegend in den Tälern Radimna, Micoş, Ogliara, Zlatiţa, Pîrîul Cerbului und Craeul Văcăreţ.

Die floristische Zusammensetzung (Tab. 1.3) ist durch einen Kern hygrophiler und hygromezophiler Arten gekennzeichnet, die für *Calthion* Tx. 1939 charakteristisch sind, zu denen noch zahlreiche Transgressionsarten hinzukommen, die aber weniger konstant sind und die sich neben der dominierenden Art entwickeln können. Nach unserer Meinung ist es

berechtigt in diese Gesellschaft auch die von anderen Autoren genannten Zönosen einzufügen (7,5) u.zw. jene die sich in der Interferenzzone zwischen den Verbänden *Calthion* und *Agropyro — Rumicion* befinden.

4. *Scirpetum sylvatici*, Schw. 1944

Die aus *Scirpus sylvaticus* gebildeten Phytozönosen findet man auf Gleieböden entlang der Bäche, die Böden dauernd feucht halten. Die Phytozönosen bilden etwa 9 — 200 m² große Flecken im Radimna-,Nera-, Zlatița-, Ogliara- und Țesna-Tal.

Zur floristischen Zusammensetzung (Tab. 1.4) zählen 44 hygrophile und mezohygrophile Arten, die den ständigen Wasserüberschuß bestätigen und ohne weiteres deren Einbeziehung zum *Calthion* erlaubt.

Vom physionomischen Gesichtspunkt aus ist die Gesellschaft durch die fast ausschließliche Vorherrschaft von *Scirpus sylvaticus* gut individualisiert.

5. *Juncetum tenuis* (Diem. Siss. et Westh. 1940) Schw. 1944

Diese Gesellschaft wird in Gebieten angetroffen, die von forstwirtschaftlich ausgebeutet werden, u. zw. in den Lichtungen entlang der Wege, am Waldrand auf verdichtetem, feuchten Boden z.B. im Radimna- und Dobrița-Tal.

Die floristische Zusammensetzung (Tab. 1.5) ist durch verstärkte Transgression einiger Arten aus den benachbarten Wiesen gekennzeichnet (*Agrostis tenuis*, Festuca rubra), was auf die Entwicklungsrichtung der Phytozönosen dieses Gebiets im Falle der Beendigung der Trittbelastung hinweist, u. zw. auf die Vereinigung mit der Wiesenvegetation.

6. *Plantago majori—Lolietum perennis*. (Linkola 1921, Beger 1930)

In dem untersuchten Gebiet ist diese Gesellschaft häufig als Flekken am Wegrand zu finden, auf mäßig verdichteten, stickstoffhaltigen Böden.

Die floristische Zusammensetzung der Gesellschaft (Tab. 1.6) umfaßt zahlreiche für *Molinio-Arrhenatheretea* charakteristische mezophile Arten, die zusammen mit einer Reihe von Transgressionsarten aus *Chenopodietea* und *Artemisietea* den Zönosen einen heterogenen Charakter verleihen. Im allgemeinen herrscht in der Gesellschaft *Lolium perenne* vor, kann aber auch Flecken haben in denen *Plantago major*, *P. lanceolata* oder *Trifolium repens* vorherrschen. Insbesondere letzterer Typ zeigt viele Gemeinsamkeiten mit *Trifolio-Lolietum perennis* Resmeriță et al. 1967, Krippelowa 1967.

Die floristische Zusammensetzung, Physionomie, Struktur und Dynamik der Gesellschaft berechtigt deren Betrachtung zusammen mit den natürlichen Dauerwiesen.

7. *Arrhenatheretum elatioris*, Br. — Bl. 1919.

Die Gesellschaft ist der Nera entlang ziemlich begrenzt verbreitet, auf leichten, feuchten Böden mit guter Nährstoffversorgung.

Trotz der reichen, aus vielen Arten bestehenden floristischen Zusammensetzung (Tab. 1.7) weist die Gesellschaft einen hohen Grad von Homogenität auf und eine deutlich termophile Nuance. Die komplexe Struktur wird aus drei vertikalen Schichten gebildet, die ineinander überlaufen.

Da es gegenwärtig in Rumänien keine eingehende Analyse der Gesellschaft gibt, werden die aufgefundenen Flecken zu der im weitesten Sinne aufgefaßten Gesellschaft gezählt, mit der Einschränkung, daß sie wahrscheinlich auch zu *Dauco-Arrhenatheretum* (Br. —Bl. 1919) Görs 1966 hinzugezählt werden kann, als deren mitteleuropäische Variante.

8. *Poaetotrivialis—Festucetum pratensis* (Borza 1959) I. Moldovan 1970.

Die zu dieser Gesellschaft gehörenden Wiesen wurden in der Nera-Au auf nährstoffreichen Schwemmlandböden und auf entwässerten Böden gefunden, aber auch in mäßig feuchten Obstgärten in Belobreșca und Radimna.

In der floristischen Zusammensetzung (Tab. 1.8) sind zahlreiche Kennarten für *Arrhenatheretalia*, was deren Einordnung zu diesen berechtigt, zum Unterschied von einigen beschriebenen mezohygrophilen Zönosen, wie z. B. *Festucetum pratensis*, Soó (1938) 1955, die zu *Alopecurion pratensis* Pass. 1964 gezählt werden.

Die in der Donau-Au von I. Todor und Mitarb. (1970) und P. Raclaru und M. Alexan (1970) beschriebenen Phytozönosen entsprechen der von uns angegebenen Gesellschaft. Innerhalb der Gesellschaft sind einige

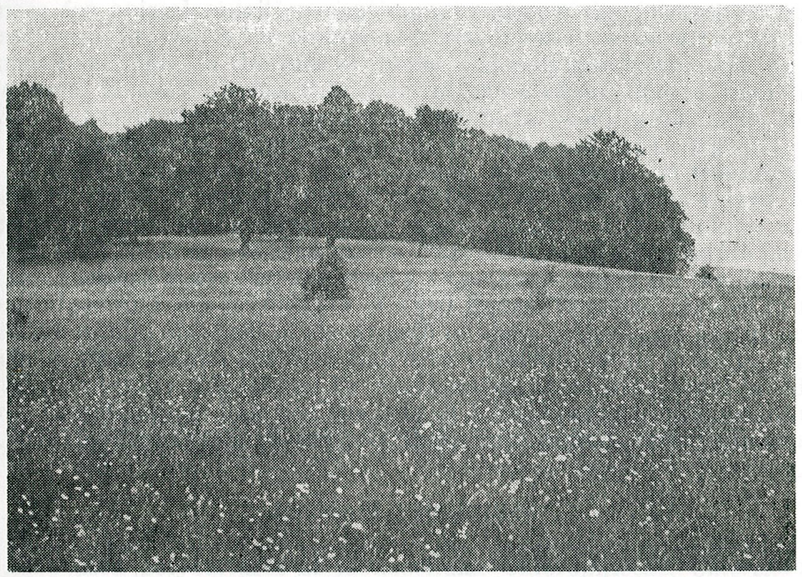

Abb. 2 — *Festuca rubrae—Agrostietum tenui* Horv. 1951.

Aspekte unterscheidbar, die der Untergesellschaft „*poetosum pratensis*" *Siroki 1956* eigen sind. Durch Beweidung wird die Gesellschaft von Wiesen abgelöst in denen *Lolium perenne* vorherscht; im Falle einer übertriebenen Entwässerung und Trockenlegung erscheinen Wiesen mit *Festuca valesiaca*.

9. *Festuco rubrae — Agrostietum tenuis* Horv. 1951

Die Gesellschaft bedeckt beträchtliche Flächen im Locva-Gebirge. Sie tauchte als sekündäre Wiese dort auf, wo *Carpino-Fagetum* und *Quer-*

cetum gerodet wurden. In : „Studium der *Festuco rubrae—Agrostietum tenuis* Jorv. 1951-Gesellschaft in dem Locva-Gebirge (Banat) — Lucr. ştiinţ. Inst. Agron. Timişoara, vol. XIV — Timişoara, 1977, S. 37—45“ wurde diese eingehend behandelt.

LITERATURVERZEICHNIS

1. Borza Al., Boşcaiu N., *Introducere în studiul covorului vegetal*, Bucureşti, 1969.
2. Braun—Blanquet J., *Pflanzensoziologie*, Wien—New York, 1964.
3. Coste, I., Lucr. şt. Inst. Agr. Timişoara — seria agron. **XIV**, Timişoara, 1977, 37—45.
4. Passarge H., Pflanzengesellschaften des Nordostdeutschen Flachlandes I, Jena, 1964.
5. Raclaru P., Alexan M., Stud. cerc. biol. — seria bot., **25**, 3. Bucureşti, 1973, 205—214.
6. Sissingh G., Mitt. flor.,-soz. Arbeitsgem. *14* Todenmann ü. Rinteln, 1969, 179—192.
7. Todor I., Gergely I., Bîrcă C., Contrib. bot, Cluj, 1971. 203—256.
8. Tüxen R., Ber. Naturhist. Ges., *114* Hannover, 1970, 77—85.

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GROWTH OF UNICELLULAR GREEN ALGAE IN NUTRITIVE SUBSTRATE WITH ADDITION OF WASTES FROM ANIMAL-BREEDING COMPLEXES

BY

ALEXANDRINA DIHORU, D. CADAR and M. VINTILĂ

A suspension of Chlorella and Scenedesmus is grown in a Knop-Pringsheim nutritive and drinking water substrate with addition of waste water from a bull-rearing complex. Concentrations of 25 per cent washing waters stimulate culture growth and development, a strong depollution being concomitantly noted. Higher washing water concentrations partially inhibit growth, the culture multiplication index varying from 2 to 6.

The question of profitable alga growth and production of an algal biomass for animal nutrition continues to arouse the interest of many researchers as proved by the numerous works devoted to the administration of unicellular algae in the diet of mono- and polygastric animals. At the same time, authors discuss the value of these algae, due to their high nutrient content, in improving animal products (wool, milk, meat, eggs) and in increasing the weight of animals [1], [2], [4], [5], [6], [8], [9].

Recent researches have been focused, in the main, on the administration of algae to animal diet in the form of suspensions mixed with fodders, to enhance digestibility, or concomitantly with drinking water through the system of watering places. This mode of administration proved effective because it avoided the energy expenses required by the dehydration of algae and the production of algal flours. We shall discuss in this paper the results obtained in the growth of algae suspensions by the cultivation of some microalgae in a nutritive substrate or in drinking water partially or totally substituted by waste waters from the animal breeding sector. Investigations followed the rate of biomass growth and accumulation and the depolluting capacity of these autotrophic microorganisms.

MATERIAL AND METHOD

We used polycultures of monocellular Chlorophiceae, *Chlorella* and *Scenedesmus*, grown in a Knop-Pringsheim nutritive substrate with the following composition : (g/l)

KNO_3 — 1
$Ca(NO_3)_2$ — 0.1
K_2HPO_4 — 0.2
$MgSO_4$ — 0.1
Fe citrate—0.025
microel. sol. Arnon — 0.5 ml

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distilled water — 1000 ml or in drinking water, without addition of salts, mixed with waste water from a bull-rearing complex.

The algae were grown in flasks with a flat bottom (500 and 2000 ml) and in anatomic vessels (2 000 ml) in the following variants :

Variant	Nutritive substrate, %	Waste water, %	Drinking water, %	Waste water, %
I	100	—	100	—
II	75	25	75	25
III	50	50	50	50
IV	25	75	25	75
V	—	100	—	100

Cultures were continually illuminated (day and night) from either side with fluorescent lamps (some 6000 lx) and bubbled for some 8 hrs/day. The temperature varied from 25 to 30°C. Addition of waste waters with an initial pH at 9.5 required modification and uniformization of pH (6.5) the optimum value for the growth of the species studied in all variants.

Every five days the following assayings were performed :

— pH with the help of the marker paper ;
— biomass accumulation, gravimetrically ;
— dynamic growth — in the haematocyte ;
— total N and raw protein by the Kjeldahl method ;
— oxidability indicator — by oxidation of organic substances with $KMnO_4$ in acid substrate.

RESULTS AND DISCUSSIONS

Washing waters carry a significant content of dejections (some 20 g s.u/l) in a wide range of exhaust and decomposition products, fodder remains, detergents, a.o., which reduce the capacity of light to enter the substrate.

Yet, in spite of this drawback, *Chlorella* and *Scenedesmus*, having a great adaptive ability, developed not only under normal conditions of growth (Knop-Pringsheim + drinking water substrate), but also under the apparently adverse conditions of waste water addition.

1. DEVELOPMENT OF MICROALGAE POLYCULTURE IN A KNOP-PRINGSHEIM SUBSTRATE WITH AND WITHOUT ADDITION OF WASTE WATERS

Starting from an initial suspension of $32 \cdot 10^6$ cell/ml, the culture was found to be some five times denser after a 15-day interval. Growth and development processes did not significantly alter pH values.

Growth processes were stimulated by an addition of 25 per cent washing water. In other variants, the development of algae was inversely proportional to the quantity of waste water added. We wish, nevertheless, to point out that after a period of accomodation the algae developed also in the variant with a waste water substrate alone, although cellular agglomeration and lysis processes often occurred (Fig. 1).

In terms of numerical growth, the waste water was found to have a stimulating effect on the accumulation of the biomass in variant II (some 1 g s. u/l). In the other variants, lower yields were registered. The reduced quantities of vegetal material /l of the culture substrate were somewhat

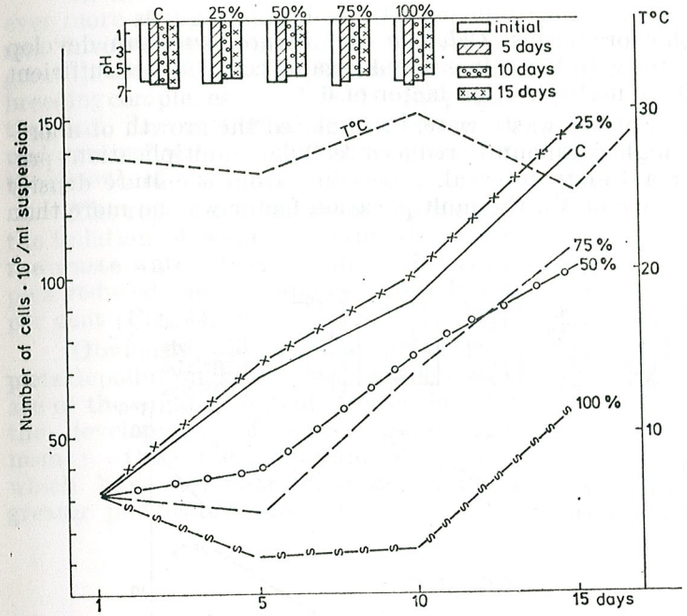

Fig. 1. — Numerical growth rate of microalgae in a Knop-Pringsheim nutritive substrate with addition of waste waters from an animal-breeding complex.

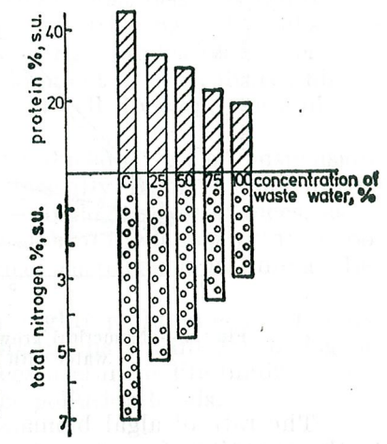

Fig. 2. — The raw protein content in a suspension of algae developed in the Knop-Pringsheim nutritive substrate with addition of waste waters.

compensated for by the chemical composition of the algae in which proteins amounted to 45 per cent in the Knop-Pringsheim substrate, and to over 25 per cent in the variants with waste water addition (Fig. 2).

In this way, algal protein yield might compete with best-quality hays (e. g. lucerne hay which comprises 18 — 20 per cent raw protein).

2. DEVELOPMENT OF UNICELLULAR GREEN ALGAE IN DRINKING WATER WITH OR WITHOUT WASTE WATER ADDITION

As autotrophic organisms, *Chlorella* and *Scenedesmus* can develop also in drinking water with low mineral and organic content, yet sufficient for ensuring a culture multiplication factor of 6.

Moderate additions of waste waters stimulated the growth of microorganisms while higher amounts reduced cellular multiplication processes. Thus, after a 15-day interval, proceeding from a culture density of $36 \cdot 10^6$ cell/ml (variant V), the multiplication factor was no more than 2 (Fig. 3).

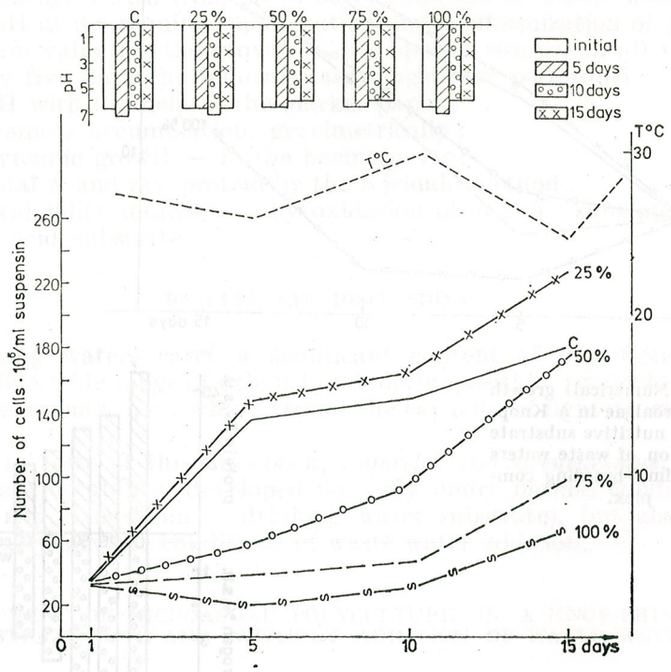

Fig. 3. — Numerical growth rate of microalgae grown in drinking water with addition of waste waters.

The rate of algal biomass accumulation was inversely proportional to the quantity of waste water added, which always ranged below unity.

Our results agree well with some literature data on the use of waste waters from animal-breeding complexes for the intensive growth of green microalgae. Thus, Boersma [3] scores positive results in alga growth by

using a diluted swine wastes substrate, which is by no means inferior, in point of chemical composition, to the standard one. It contains significant amounts of N, P, Mg, S, Ca, Na, Zn, B, a.o. At the same time, Kuznetsov and Pozdeeva [7] obtained green algae cultures in a nutritive substrate with waste waters from the swine-rearing complexes.

In the circumstances in which environmental pollution becomes an ever more stringent problem, the depolluting role of algae, by its effects and involvements, becomes a matter of great interest. Animal dejections from the breeding complexes, which lead to the pollution and degradation of the soil, subsoil, underground waters, could also be used for the growth of algae.

The repeated cultivation of algae and the isolation of some resistant strains in the waste waters from a bull-rearing complex reduced the oxidability index by 50 per cent (Fig. 4).

Obviously, we cannot speak of complete depollution, but a considerable decrease of the organic content is propitious for the development of other vegetal organisms: Caricetae, Phragmitetae, a.o., which have a higher organization and a greater purification capacity.

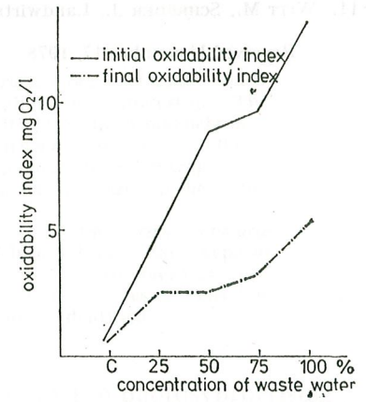

Fig. 4. — Reduction of waste waters pollution rate by populating them with autotrophic organisms

CONCLUSIONS

1. A polyculture of *Chlorella* and *Scenedesmus* becomes optionally heterotrophic if grown in a Knop-Pringsheim and drinking water substrate with addition of waste water from a bull-rearing complex. A 25 per cent drinking water amount stimulates the development of the culture; higher concentrations partially inhibit the process of cell division, the multiplication factor varying from 2 to 6.

2. Biomass accumulation is somewhat reduced : 1 g s.u/l suspension, therefore, algae growth is worthwhile especially from a quantitative viewpoint (higher protein content, active biological substances, a.o.) and much less from a quantitative one although in the latter instance, too, the possibility of using them, in the form of suspension, in animal diet should not be overlooked.

3. Due to their capacity of populating highly polluted environments, algae prove to be the only autotrophic organisms within the biological chain, able to utilize and produce substances useful for life (amino acids, vitamins, a.o.) and, at the same time, reduce pollution levels.

REFERENCES

1. Avudji M. A., Tr. VNII Karakulevodstvo, 1971, **17**, 240—244.
2. Beremski C., Tomova D., Mitkov S., Nicolova T., Bakalivanov S., Cerkezov N., Belorecikov D., Jivot. nauki, 1973, **10**, *5*, 99—105.

3. Boersma L., Feedstuffs, 1975, **47**, *22*, 20—21.
4. Ghetia N., Svinovodstvo, 1973, *2*, 17.
5. Gonciarenko A., Svinovodstvo, 1972, *10*, 17—18.
6. Hintz H. F., Heitman H. Jr., Weir W. C., Torell D. T., Meyer J. H., J. An. Sci., 1966, **25**, *3*, 675—681.
7. Kuznetsov A. K., Pozdeeva E.S., Nauci Tr. Ukr. s-h. Acad., 1976, 191, 33—34.
8. Melzer E., Cz. Chem.-Techn., 1973, **2**, *11*, 431—432.
9. Pomirovski N.G., Nurnazarov A. V., Ref. J. Jivot., 1973, *3*.
10. Schröder J., in *Symposium on new developments in the provision of amino acids in the diets of pigs and poultry*, New York, 1973, tome II, p. 251.
11. Witt M., Schröder J., Landwirtsch. Forsch., 1967, **20**, *2—3*, 148—156.

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DATA CONCERNING THE ABSORPTION SPECTRUM OF *SCENEDESMUS ACUTIFORMIS* INTACT CELLS

BY

FR. NAGY-TÓTH, V. SORAN

The absorption spectrum of the *Scenedesmus acutiformis* strain "Hársas" intact cells was measured with a Leitz-Ortholux MPE type photometer microscope. The monochromatic light was achieved intercalating a Leitz-type linear monochromator with mirror (Geradsicht-Spiegelmonochromator) with a dial of 370 — 1,100 nm, between the source (a 12 V/100 W incandescent lamp) and the microscope. The transmission of the monochromatic light, cell by cell, was measured in the region comprised between 400 and 700 nm.
On the basis of morphological data and the absorption spectrum, *Scenedesmus acutiformis* cells (nonsynchronized culture) were divided into 6 types. Each type of searched cell presents a characteristic absorption spectrum with significant differences concerning the relative quantity of pigments. In the 6 types of cells the peaks of chlorophylls *a* and *b* and of carotenoids could be identified.

The knowledge of the absorption spectrum of the photosynthetising cells *in vivo*, or of the isolated chloroplasts, is particularly important for the explanation of the photosynthesis processes.

The absorption spectrum of intact cells, of isolated chloroplasts, or of chloroplast fragments has been studied by spectrophotometry of some suspensions [2], [3], [5], [6], [8], [17], [18], [24], [25], [31]. These investigations ascertained that the spectrophotometry of suspensions, especially those of algae, presents some disadvantages, namely :

a) The pigments were present only in a very small part of the suspension volume. Their nonhomogeneous distribution leads to the appearance of the "sieve effect" phenomenon which causes a denaturation of the absorption spectra.

b) The dispersion of light modifies the real absorption spectrum of pigments. However, the errors may partially be corrected by various technical procedures [11].

These disadvantages can be avoided by microspectrophotometry. This method also allows to investigate different morphological and physiological types of cells in a culture, especially in a nonsynchronized population. The microspectrophotometry [1], [14], [30], a specific method of quantitative cytochemistry, applied in these experiments, allows also to record the absorption spectrum of the intact cell pigments of the algae. It was successfully used by Thomas *et al.* [28], [29] for studying the absorption spectrum of the intact cells of *Chlorella pyrenoidosa* and *Anabaena*. It seems to us particularly lucrative since it offers the possibility to rediscover *in vivo*, cell by cell, different forms of chlorophyll, as well as their absorption spectra.

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MATERIAL AND METHOD

The material used was a pure, monodesmoide nonsynchronized population of *Scenedesmus acutiformis* strain "Hársas". The static (nonbubbled) culture, but agitated daily, has been grown in a Knop-Pringsheim nutrient solution. It was illuminated by a 60 W incandescent bulb 12 hrs daily.

The measurements of the light transmission, cell by cell, were performed on fresh microscopical preparations, took out of a droplet from the suspension. A MPE-type Leitz-Ortholux photometer microscope was used. The monochromatic light was achieved in the microscope field by introducing a Leitz-type linear monochromator with mirror (Geradsicht-Spiegelmonochromator), with a dial of 370 to 1,100 nm, between the light source and the microscope. The light source was a 12 V and 100 W "Carl Zeiss (Jena)" incandescent microscope lamp.

The transmission of the monochromatic light was measured only between 400 and 700 nm. This limitation follows from subjective and objective causes. In the case of wavelengths shorter than 400 nm the microscopic objects cannot be observed any more, while in the case of wavelengths longer than 700 nm the measurement is not possible because of VFS 9 A type photocell with secondary device of electron amplification (Photozelle mit Secundär-Elektronen-Vervielfacher) which, being part of the apparatus, has its sensibility threshold at 700 nm.

In the wavelengths region investigated the different pigments have their maximum of absorption [4], [5], [10], [23], therefore the measurements were made in every 5 nm, separately for each morphological or physiological type of cell, excepting the yellow and green domain, in which they were made in every 20 nm only.

For each investigated wavelength the medium absorbance (optical density) as well as the standard deviation were calculated in respect of each cell type.

RESULTS AND DISCUSSION

There were determined the absorption spectra of 105 cells of *Scenedesmus acutiformis*. According to their morphological aspects, as well as to the different stages of development, and to the absorption spectra recorded, the investigated cells can be divided into the following categories (Figs. 1, 2):

1. Vigorous vegetative cells with a high chlorophyll content (40 cells);
2. Vegetative cells with a lower chlorophyll content (25 cells);
3. Dividing cells having a fragmented protoplast and containing less chlorophyll (14 cells);
4. Autospore mother cells with a lower chlorophyll content (12 cells);
5. Autospore mother cells rich in chlorophylls (6 cells);
6. Hyaline cells with extremely little chlorophylls (8 cells) (Figs. 1,2).

It is ascertained that in the absorption spectra of the *Scenedesmus* cells obtained by the method of microspectrophotometry the peaks characteristic of the main photosynthetic pigments can be identified : chloro-

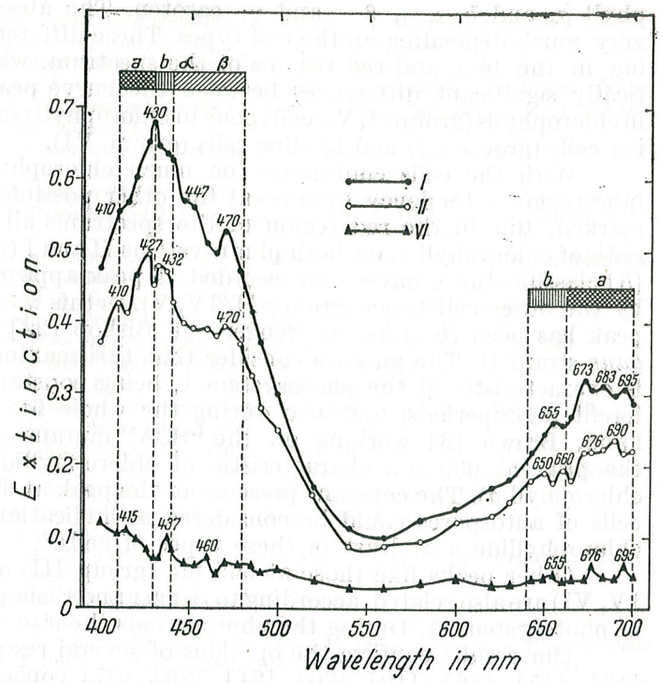

Fig. 1. — Absorption spectrum of *Scenedesmus* intact cells *in vivo*. I — vigorous vegetative cells with higher chlorophyll content; II — vegetative cells with lower chlorophyll content; VI — hyaline cells with the lowest chlorophyll content; a — chlorophyll-*a* zones; b — chlorophyll-*b* zones, α–, β–, γ — carotenoids.

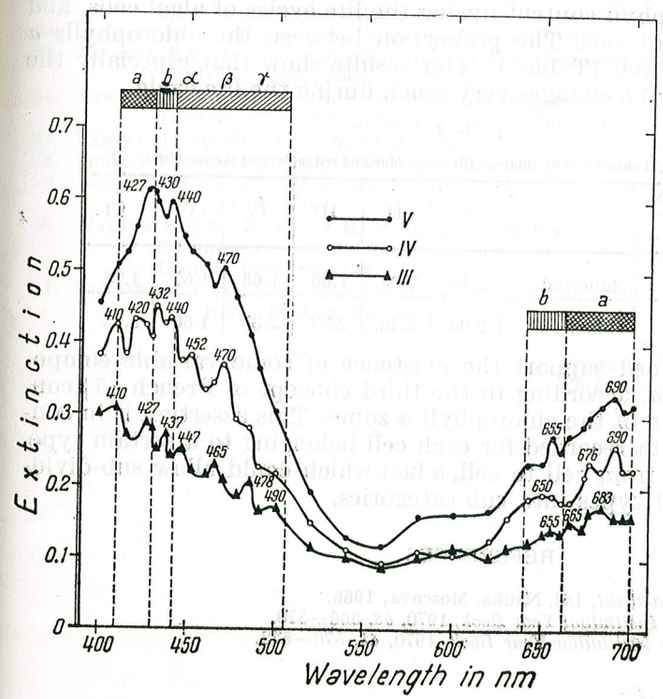

Fig. 2. — Absorption spectrum of *Scenedesmus* intact cells *in vivo*. V — autospore mother cells with higher chlorophyll content; V— autospore mother cells with lower chlorophyll content; III — dividing cells with fragmented protoplasmand; lower chlorophyll content; aI — chlorophyll-*a* zones, b | αchlorophyll - *b* zones 9 —, β — γ — ecarotenoids.

phyll *a*, and *b*, α—, β— and γ—caroten. The absorption spectra diffe very much depending on the cell types. These differences are more strik ing in the blue and red regions of the spectrum, where there are statis tically significant differences between the curve peaks of the cells ric in chlorophylls (groups I, V), cells poor in chlorophylls (groups II, IV), divid ing cells (group III) and hyaline cells (group VI).

With the cells containing too much chlorophylls (group I) in th blue region a tendency to conceal the other possible peaks has been re marked. But in the red region of the spectrums all the peaks characte ristic of chlorophyll *a*, for both photosystems II and I (according to French' [5] classification), have been recorded. A peak appears evident at 690 nm by the other cell types (groups II, IV, V). In this wavelength, similarly, a peak has been recorded by Senger and Bishop [22] in *Scenedesmus obli quus* strain D. The authors consider that this maximum of chlorophyll *a* is characteristic of the photosystem I, being constant in cultures of dif ferent photoperiods, and also during the whole life cycle. On the othe hand, Brown [3] working on the "SCA" mutant of *Chlorella* considers the peak at 690 nm characteristic of chlorophyllide *a* (a precursor o chlorophyll *a*). The constant presence of the peak at 690 nm in the mothe cells of autospores could be considered an indication of the existence o chlorophyllide *a* at least in these types of cells.

Other peaks like those at 665 nm (group III) and at 676 nm (group IV, VI) are also related, according to Senger and Bishop [22], to the activity of photosystem II. During the photoperiods the size of these peaks varies.

Our results confirm the opinions of several researchers [7], [9], [12], [13], [15], [16], [19], [20], [21], [26], [27] concerning the variations of the relative chlorophyll content during the life cycles of algal cells, and according to life conditions. The proportion between the chlorophylls *a* and *b*, also varies as well (Table 1). Our results show that especially the quantity of chlorophyll *b* changes very much during the life cycle.

Table 1

Maximum of the light absorption by chlorophylls in the blue and red regions of the spectrum

Cell type / Chlorophyll		I	II	III	IV	V	VI
Chlorophyll-*a*	blue/red	2.18	2.06	1.66	1.68	1.67	1.78
Chlorophyll-*b*	blue/red	2.06	2.46	2.22	2.35	1.60	2.12

The data obtained support the existence of some variable components of chlorophyll *a*, according to the third concept of French [5] concerning the estimation of the chlorophyll *a* zones. This assertion is in concordance with the data recorded for each cell belonging to a certain type. We found variations from cell to cell, a fact which could allow sub-dividing of the mentioned types into sub-categories.

REFERENCES

1. Brodskii V. I., *Trofika kletki*, Izd. Nauka, Moscova, 1966.
2. Brown J. S., *Carnegie Institution Year Book*, 1970, *68*, 566—570.
3. Brown J. S., *Carnegie Institution Year Book*, 1970, *68*, 570—572.

4. Davies B. H., *Analysis of carotenoid pigments*, In Goodwin T. W. (ed.), *Chemistry and Biochemistry of Plant Pigments*, Academic Press, London, New York, 1965, 489—532.
5. French C. S., *Carnegie Institution Year Book*, 1970, *68*, 578—587.
6. French C. S., Prager L., *Absorption spectra for different forms of chlorophyll*, In Metzner H. (ed.), *Progress in Photosynthesis Research, Vol. 2*, Publ. Internat. Union Biol. Sci., Tübingen, 1969, 554—564.
7. Gense M.T., Guérin-Dumartrait E., Leclerc J. C., Mihara S., Phycologia, 1969, **8**, *2*, 135—141.
8. Goedheer J.C., *Carotenoids in blue-green and red algae*. In Metzner H. (ed.), *Progress in Photosynthesis Research*, Vol.2, Publ. Internat. Union Biol. Sci., Tübingen, 1969, 811—817.
9. Hase E., *Cell division*. In Lewin R.A. (ed.), *Physiology and Biochemistry of Algae*, Academic Press, New York, Londra, 1964, 617—624.
10. Holden M., *Chlorophylls*. In Goodwin T. W. (ed.), *Chemistry and Biochemistry of Plant Pigments*, Academic Press, Londra, New York, 1965, 461—468.
11. Kok B., *Atti del 2° Congresso Internazionale di Fotobiologia*, Torino, 1957, 369—383.
12. Komárek J., Simmer J., Biol. Plant. (Praha), 1968, **7**, 409—424.
13. Lafèber A., Steenbergen C.L.M., Nature (London), 1967, **213**, 527—28.
14. Lison L., *Histochemie et cytochemie animales, Vol. 1*, Ed. Gauthier-Villars, Paris, 1960.
15. Lorenzen H., Ruppel H.G., Planta (Berl.), 1960, **54**, 394—403.
16. Markarova E. N., Baslavskaya S.S., Bot. Zhurn., 1965, **50**, 1, 68—70.
17. Metzner H., *Optical properties of algal suspensions. Productivity of Photosynthetic Systems (Models and Methods)*, Trebon (Czechoslovakia), 1969, 210—213.
18. Murchio J. C., Allen M. B., Photochem. & Photobiol., 1962, **1**, 259—266.
19. Paasche E., Physiol. Plant., 1968, **21**, 66—77.
20. Pirson A., Lorenzen H., *Synchronized Dividing Algae*, Ann. Rev. Plant. Physiol., 1966, **17**, 439—458.
21. Ruppel H. G., Flora (Jena), 1962, **152**, 113—138.
22. Senger H., Bishop N.I., *Changes in the photosynthetic apparatus during the synchronous life cycle of Scenedesmus obliquus*. In Metzner H. (ed.), *Progress in Photosynthetic Research, Vol. 1*, Publ. Internat. Union Biol. Sci., Tübingen, 1969, 425—434.
23. Shapozhnikov D. I., *Pigmenty plastidy zelenykh rastenii i metodika ikh issledovanya*, Izd. Nauka, Moscova, Leningrad, 1964.
24. Tageeva S.V., Brandt A.B., Korshunova V.S., Biofizika, 1962, **7**, 240—243.
25. Tageeva S.V., Brandt A.B., Korshunova V.S., Generosova I. P., Izvestia Akad. Nauk. SSSR, Ser. Biol., 1963, **3**, 391—404.
26. Tamiya H., *Growth and cell division of Chlorella*, In Zeuthen E. (ed.), *Synchrony in Cell Division and Growth*, John Wiley & Sons Inc., New York, 1964, 247—305.
27. Tamiya H., *Synchronous Cultures of Algae*, Ann. Rev. Plant Physiol., 1966, **17**, 1—26.
28. Thomas J., Nature (London), 1970, **228**, 181—183.
29. Thomas J., Phondke G. P., Tatake G.V., Gopal-Ayengar A. R., Photochem. & Photobiol., 1970, **11**, 85—101.
30. Walker P.M.B., Richards B. M., *Quantitative microscopical techniques for single cells*, In Brachet J., Mirsky A.E. (ed.), *The Cell, Vol. 1*, Academic Press, New York, Londra, 1959, 91—138.
31. Zakharova V. A., Eroshin N. S., Sidko F. I., Izvestia Sibirsk. Otd. Akad. Nauk SSSR, Ser. Biol., 1969, **3**, 36—40.

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L'ACTION DU Na-DODÉCYLSULFURICUM DES EAUX POLLUÉES SUR QUELQUES PROCESSUS PHYSIOLOGIQUES CHEZ L'ALGUE *CHLORELLA VULGARIS*

PAR

V. PETREA

Using as a test object grenn alga *Chlorella* the toxicity degree of Na dodecylsulfuricum, used as housekeeping detergent in industry, has been determined. It has been determined that in relative small concentrations ranging between $5 \cdot 10^{-6}$ and $1 \cdot 10^{-2}$ g/100 ml solution, the substance stimulates the algal growth and photosynthesis, but over these concentrations it has an inhibitory effect. On the other hand, the algal respiratory process is less influenced.
The culture medium exhibits a pH variation, being somewhat acidic at the beginning and becoming alkaline after 1 — 2 days.

La substance Na-dodécylsulfuricum, détergent largement utilisé dans l'industrie, autant que dans le ménage, peut devenir un agent polluant pour les eaux, lorsque les mesures nécessaires sont ignorées. Ce fait nous a déterminé d'établir sa toxicité, en étudiant son action sur la croissance, la photosynthèse, la respiration et le pH, dans le milieu de culture d'algue *Chlorella vulgaris*.

L'action du Na-dodécylsulfuricum sur la croissance a été établie, en déterminant le poids sec des algues crûes dans un milieu nutritif, contenant diverses quantités de la substance étudiée.

Dans ce but, ont été utilisés des vases en verre, avec 200 ml de solution nutritive Knopp-Pringsheim, à laquelle on a ajouté diverses quantités de substance, pour réaliser des concentrations entre $5 \cdot 10^{-6}$ et 1.10^{-2}g pour 100 ml de solution nutritive. Chaque milieu de culture a été inoculé d'un ml de suspension d'algues, contenant un mg d'algues sèches et tenu à la lumière fluorescente de 6 600 lx.

La croissance a été appréciée en déterminant le poids sec, après 6 et 14 jours. Des résultats obtenus (fig. 1), on constate que la croissance est stimulée jusqu'à la concentration de 5.10^{-3} g pour 100 ml de solution ; au-dessus de cette concentration la croissance est inhibée.

La photosynthèse a été déterminée par la méthode Warburg après 6, 24, 48 et 72 heures. On constate (fig. 2) qu'au commencement, la substance a une action de stimulation de la photosynthèse, mais, peu à peu, cette action diminue.

La respiration a été déterminée aussi par la méthode Warburg, mais ce phénomène est moins influencé par le Na-dodécylsulfuricum (fig. 3).

On a aussi déterminé le pH du milieu de culture pendant 8 jours, avec l'électrophotocolorimètre (FEK 56). On constate (tabl. 1) qu'au commencement, la réaction du milieu est acide, mais, 24 heures après,

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elle devient alcaline et l'alcalinité augmente à mesure que la concentration du Na-dodécil sulfuricum, dans le milieu, augmente aussi. On observe que le Na dodécil sulfuricum en concentrations entre 5.10^{-6} et 1.10^{-}

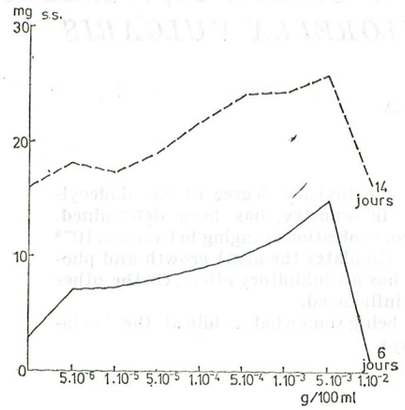

Fig. 1. — Le poids sec des algues cultivées en milieux contenant quantités diverses de Na-dodécylsulfuricum.

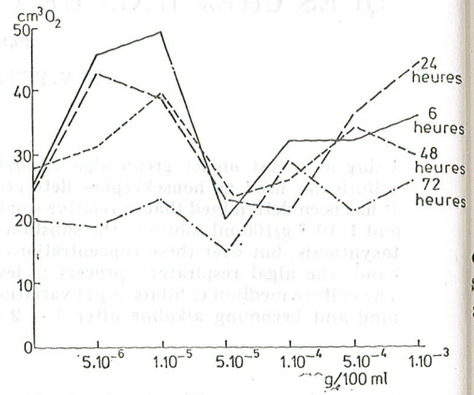

Fig. 2. — Influence du Na-dodécylsulfuricum sur la photosynthèse.

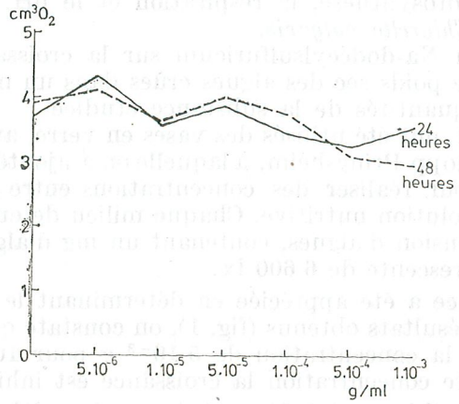

Fig. 3. — Influence du Na-dodécylsulfuricum sur la respiration.

exerce une action stimulatrice sur la croissance et la photosynthèse des algues. Au-dessus de cette concentration est enregistrée une action inhibitrice. Ces résultats viennent confirmer ceux obtenus par Naishtein S. A. et Yurovskaia E. M. [3] avec les pesticides Heptaclor et Aldrin, qui aux concentrations de 0,1 à 10 mg % ont une action stimulatrice sur la croissance des microorganismes.

Nos résultats sont en concordance avec ceux obtenus par Krulikova E. [1], Lujnova M. I. [2] et Spirescu I. [6], qui révèlent, que quelques pesti-

Tableau 1

Variation du pH dans le milieu de culture après n jours

Concentration de la substance	0	1	2	3	4	6	8
Contrôle	6,10	7,20	7,30	7,30	7,20	7,70	7,00
$5 \cdot 10^{-6}$	6,50	7,30	7,50	7,50	7,45	8,10	7,30
$1 \cdot 10^{-5}$	6,80	7,35	7,65	7,65	7,55	8,40	7,30
$5 \cdot 10^{-5}$	6,85	7,35	7,70	7,70	7,70	8,55	7,30
$1 \cdot 10^{-4}$	6,90	7,40	7,70	7,75	7,75	8,70	7,40
$5 \cdot 10^{-4}$	6,95	7,50	7,80	7,90	7,80	8,80	7,35
$1 \cdot 10^{-3}$	6,97	7,60	8,00	8,30	8,00	9,10	7,50
$5 \cdot 10^{-3}$	6,95	7,10	8,50	8,90	8,40	9,40	7,60
$1 \cdot 10^{-2}$	7,00	6,50	7,40	8,20	8,20	8,90	7,80

cides, en concentrations modérées, n'inhibent pas la croissance et la photosynthèse, tandis que, en concentrations plus grandes, manifestent une action inhibitrice.

BIBLIOGRAPHIE

1. Krulikovska E., *Experimentalnaia vodnaia toksikologhia*, Riga, 1976, *6*.
2. Lujnova, M.I., Schtman, L.M., Jarkov, V.I., *Symposium zur VIII Wirkungsmechanismen von Herbiziden und Synthetischen wachstumregulatoren*, Halle—Wittenberg, 1973.
3. Naishtein, S.A., Yurovskaia, E. M., *Experimentalnaia vodnaia toksikologhia*, Riga, 1972, *3*.
4. Petrea, V., Rev. Roum. Biol. — Biol. vég., 1974, **19**, *1*.
5. — Rev. Roum. Biol. — Biol . vég., 1978, **23**, *1*.
6. Spirescu, Ioana, Rev. Roum. Biol. — Biol. vég., 1978, **23**, *1*.

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GROWTH, DRY MATTER PRODUCTION AND MINERAL METABOLISM OF *FESTUCA PRATENSIS* L AND *FESTUCA RUBRA* L GROWN IN MIXTURE ON A LIMED PODZOLIC SOIL

BY

LUCIA STOICOVICI and ŞT. GALLÓ

The communication deals with the study of two grasses, *Festuca pratensis* L and *Festuca rubra* L, grown in pots on pseudogleyed podzolic soil for two years. The soil has been treated with two liming levels on a mineral fertilizer base. At four harvest dates the dry matter production is at both species brought about in a different way in relation to their ecological requirements. A favourable effect on a mixture composed of 75 % *Festuca pratensis* and 25 % *Festuca rubra* has been stated at 10 t/ha $CaCO_3$ treatment of the soil. In the first year of vegetation and at last harvests, the relative replacement rate favourably shifts towards *Festuca pratensis*. The 20 t/ha $CaCO_3$ level significantly influences nitrogen, calcium, potassium accumulation in the aboveground components of *Festuca pratensis*.

This paper includes information relevant to growth characteristics of two grasses, one of which is widely distributed on acid soils. Consequently, there is investigated the relation between the growth rate, dry matter production, mineral metabolism of species and their soil environment (acid soil), as well as the plants response to various components of soil chemical environment. In this relation the competition effect between species is taken into account.

MATERIAL AND METHODS

The plant species *Festuca pratensis* L and *Festuca rubra* L (Braşov local varieties, 1971 harvest) were grown in plastic containers (vessel surface = 176 cm^2) filled with 2.300 kg air-dried soil. The soil used was a pseudogleyed podzolic soil (from Baia Mare) and had a pH of 3.7 (in ClK) with hydrolytic acidity of 10.06 me% [3]. On a mineral fertilizer uniform base composed of : superphosphate 20 % P_2O_5 (P_{80} kg/ha), ammonium nitrate 33.5 % (N_{120} kg/ha), potassic salt 40 % K_2O (K_{120} kg/ha), two levels of calcium carbonate were added to the soil : 10t/ha (Ca_{10}) and 20 t/ha (Ca_{20}) nearby the control (Ca_0). Three replicates for each variant were given. Soil humidity was maintained at 70 % from water holding capacity.

The cultural scheme of the two species grown in various ratios is given in Table 1. Seeds were put to germinate in April 19,1972. After thinning out, the number of seedlings remained 12 per pot (replicate). The first harvest took place 47 days from seeding. Subsequently, plants

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were harvested at 10 days intervals and at the same time measurement were made. The green plant material was then placed in an oven at 80°C for 24 h and the dry weights determined. The material brought into powde

Table 1

Cultural scheme for species *Festuca pratensis* and *Festuca rubra*

Variant	t/ha $CaCO_3$ treatment	Seed percent representation		
		Festuca pratensis	*Festuca rubra*	
1	0 10 20	100(12 seeds)	0(0 seeds)	
2	0 10 20	75(9 seeds)	25(3 seeds)	
3	0 10 20	50(6 seeds)	50(6 seeds)	For each variant four harvest dates
4	0 10 20	25(3 seeds)	75(9 seeds)	
5	0 10 20	0(0 seeds)	100(12 seeds)	

was analysed for total nitrogen (Kjeldhal method), phosphorus (by means of photocolorimetry), calcium and potassium (by means of flame photometry.) Chemical analyses related to each treatment variant have been performed on mean samples.

RESULTS

Dry matter production of the aboveground parts is highly influenced by different levels of calcium carbonate found in soil, but there are also emphasized the species ecological features (Fig. 1). Whereas dry weight of *Festuca pratensis* in control does not exceed 0.25 g, in Ca_{10} variant the production reaches up to 3.61 g; however, the maximum level of $CaCO_3$ determines at this species but scarcely a larger dry matter production. *Festuca rubra* tolerates well the acidic conditions in the control variant but its production increases progressively from one harvest to another (Fig. 1) and exceeds that of *F. pratensis*. In mixtures *F. rubra* has a positive response to limed levels. Especially at 20 t/ha $CaCO_3$ in monoculture of *F. rubra*, an increase in the dry weight is noticed. In treatment variants, however, the yield of *F. rubra* is permanently lower with respect to *F. pratensis*.

The value ρ[4] known as the relative replacement rate of species a, in our case *F. pratensis*, with respect to species b, that is *Festuca rubra*, at the *n*th harvest with respect to the *m*th

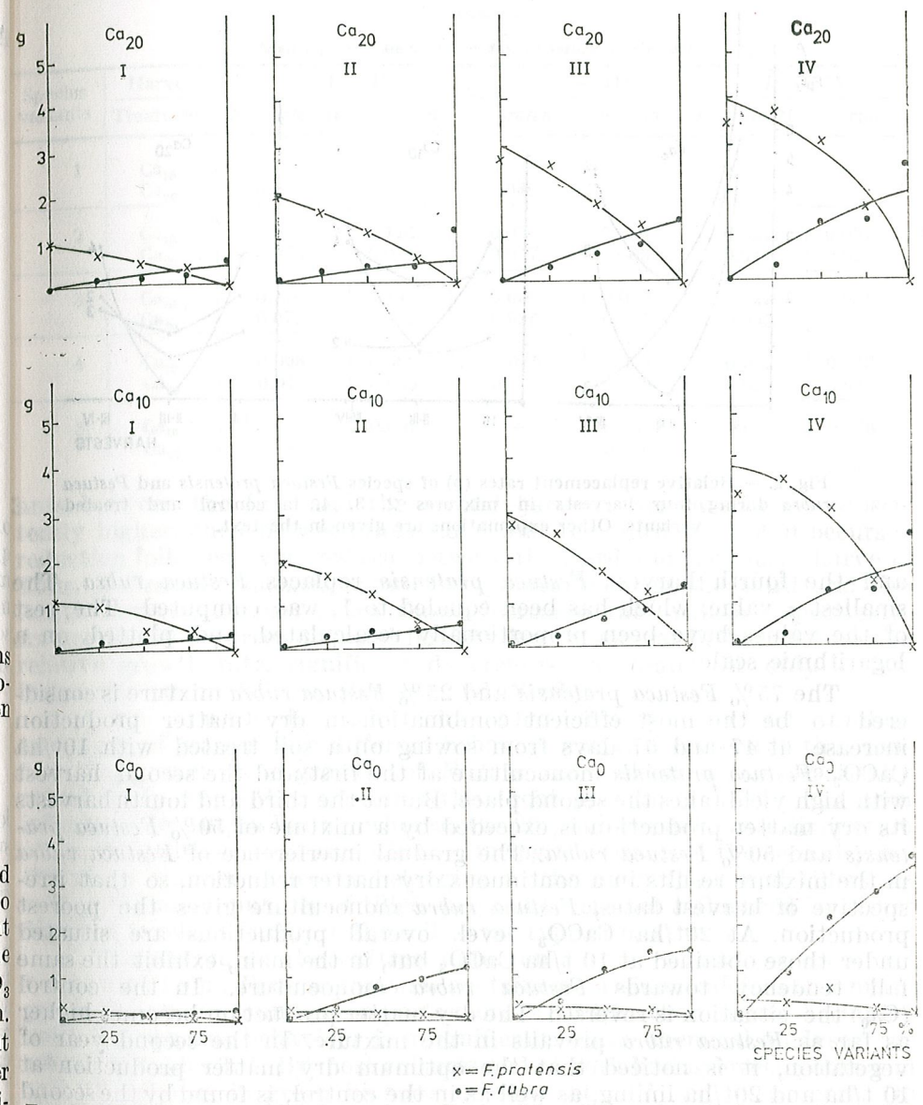

Fig. 1. — Relative dry weight change (on ordinate. g) in mixtures (on abscissa, species participation percentage) at harvest dates I, II, III, IV, and in treated variants.

harvest was calculated. In figure 2, if the curve inclination is to the right, we consider that species a replaces species b, and if the inclination

s to the left, species b replaces species a. Furthermore, from th general trend of the curves it follows that between the first and the thir harvest *Festuca rubra* replaces *Festuca pratensis* and between the thir

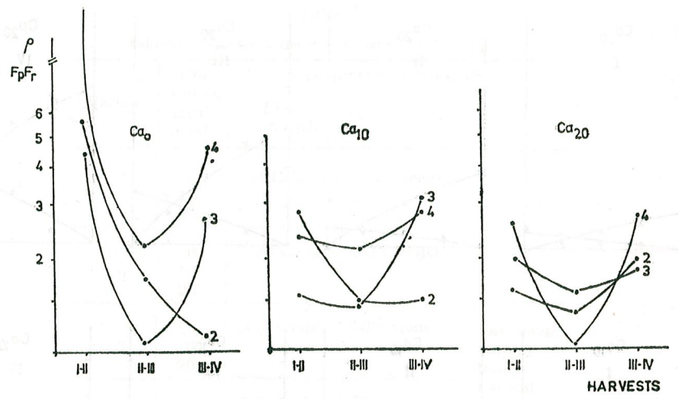

Fig. 2. — Relative replacement rates (ρ) of species *Festuca pratensis* and *Festuca rubra* during four harvests in mixtures 2, 3, 4, in control and treated variants. Other explanations are given in the text.

and the fourth harvest *Festuca pratensis* replaces *Festuca rubra*. Th smallest ρ value, which has been equaled to 1, was computed. The res of the values have been proportionally recalculated and plotted on a logarithmic scale.

The 75% *Festuca pratensis* and 25% *Festuca rubra* mixture is consid ered to be the most efficient combination in dry matter productio increase, at 47 and 57 days from sowing on a soil treated with 10t/h $CaCO_3$. *Festuca pratensis* monoculture at the first and the second harves with high yield takes the second place. But at the third and fourth harvest its dry matter production is exceeded by a mixture of 50% *Festuca pra tensis* and 50% *Festuca rubra*. The gradual interference of *Festuca rubr* in the mixture results in a continuous dry matter reduction, so that irre spective of harvest dates, *Festuca rubra* monoculture gives the poores production. At 20t/ha $CaCO_3$ level, overall productions are situated under those obtained at 10 t/ha $CaCO_3$ but, in the main, exhibit the same fall tendency towards *Festuca rubra* monoculture. In the control (Ca_0) the situation is reversed. The dry matter production becomes higher as far as *Festuca rubra* prevails in the mixture. In the second year of vegetation, it is noticed that the optimum dry matter production at 10 t/ha and 20t/ha liming, as well as in the control, is found by the second and the third mixtures in agreement with plants response in the first growing year. In the case of *Festuca pratensis* growing from the first to the fifth variants, with 10 t/ha and 20t/ha $CaCO_3$ added to the soil, the relative growth rate between harvests (Table 2) (considering the dry matter accumulation) [2], presents a steady decline in unit time.

Particularly so, between the third and the fourth harvests, the rate chiefly falls. The plant ageing is supposed to interfere. With *Festuca rubra* the relative growth rate is different. Initially, in mixtures and treated vari-

Table 2

Relative growth rate mg/mg/10 days at various species ages

Species variants	Harvests	I — II		II — III		III — IV	
	Treatment	*F. pratensis*	*F. rubra*	*F. pratensis*	*F. rubra*	*F. pratensis*	*F. rubra*
1	Ca_{10}	0.058	—	0.033	—	0.019	—
	Ca_{20}	0.065	—	0.043	—	0.020	—
2	Ca_{10}	0.064	0.114	0.036	0.004	0.027	0.057
	Ca_{20}	0.075	0.074	0.047	0.008	0.041	0.028
3	Ca_{10}	0.110	0.147	0.065	0.029	0.022	0.051
	Ca_{20}	0.078	0.092	0.036	0.065	0.056	0.059
4	Ca_{10}	0.098	0.089	0.025	0.078	0.032	0.043
	Ca_{20}	0.048	0.045	0.065	0.065	0.020	0.041
5	Ca_{10}	—	0.091	—	0.037	—	0.076
	Ca_{20}	—	0.085	—	0.020	—	0.062

ants between the first and the second harvest there are values consistently higher, then between the second and the third harvest it occurs a reduction followed by a new rise between the third and the fourth harvest. Thus, if *Festuca pratensis* shows low values between the third and the fourth harvest, *Festuca rubra* shows increased rate values respectively. As previously reported [1], soil fertility has in general little effect on the relative growth rate, significant differences are found in only a few instances, e.g. in *Lolium*, *Agrostis* and *Nardus*.

The mean height of the main shoot exhibits two types of grasses with different behaviours as can be seen in the control (Ca_0), where the growth of *Festuca rubra* shoot is not drastically retarded. In any other mixtures the shoot growth in height has been influenced by 10t/ha liming, whereas the 20t/ha level induces a diminution of growth (with certain exceptions). During the first and the second year, the mean number of leaves belonging to the main shoot and tillers, as well as the mean number of tillers per plant were not influenced by either different levels of $CaCO_3$ in the soil or the type of mixture.

Irrespective of the cutting date, in the first year of vegetation, *Festuca pratensis* contains potassium, calcium and nitrogen in the aboveground green parts more at 20 t/ha than at 10 t/ha liming level. Phosphorus behaviour is insignificant. Some chemical analyses are set out in tables 3, 4. By most cultures and at both liming levels, potassium and phosphorus in green biomass decrease at half and even more. On the contrary, calcium in the green parts accumulates nearly twice especially in plants grown on a soil with 10t/ha $CaCO_3$. In nitrogen dynamics in time there is recorded a curve reaching a maximum value at the second harvest, then a decline occurs to the third harvest. With *Festuca rubra* there are rather indefinite differences in the accumulation of potassium,

calcium and phosphorus (partly total nitrogen). The dynamics of nutri tive elements in time is relatively little significant with respect to *Festuc pratensis*. From the initial to the final harvest, potassium, phosphorus

Table 3

Nutritive elements content in *Festuca pratensis* green biomass. The first variant with regard to the seed mixture

Harvests	t/ha $CaCO_3$ treatment	%K	%Ca	%P	% Total nitrogen
I	Ca_0	2.05	0.21	0.04	3.50
	Ca_{10}	3.17	0.29	0.17	3.24
	Ca_{20}	3.44	0.38	0.21	4.48
II	Ca_0	2.57	0.10	0.03	7.70
	Ca_{10}	3.07	0.33	0.16	6.53
	Ca_{20}	3.30	0.49	0.15	8.03
III	Ca_0	2.41	0.11	0.05	3.60
	Ca_{10}	1.87	0.55	0.15	5.30
	Ca_{20}	1.99	0.57	0.15	5.90
IV	Ca_{10}	2.67	0.09	0.04	2.00
	Ca_{10}	1.71	0.59	0.14	3.64
	Ca_{20}	1.88	0.54	0.11	4.65

Table 4

Nutritive elements content in the green biomass of species *Festuca pratensis* and *Festuca rubra*. The third variant with regard to the seed mixture

Harvests	t/ha $CaCO_3$ treatment	*Festuca pratensis* %K	%Ca	%P	%total N	*Festuca rubra* %K	%Ca	%P	%total N
I	Ca_0	1.68	0.89	0.04	3.14	1.96	0.27	0.01	—
	Ca_{10}	2.43	0.19	0.03	1.88	2.68	0.20	0.24	5.20
	Ca_{20}	2.76	1.04	0.11	4.75	2.87	0.30	0.04	4.25
II	Ca_0	2.58	0.66	0.03	2.25	2.07	0.05	0.04	3.83
	Ca_{10}	2.74	0.33	0.16	7.90	2.90	0.15	0.25	10.40
	Ca_{20}	3.29	0.45	0.20	7.20	2.83	0.28	0.04	3.35
III	Ca_0	2.87	0.51	0.04	4.93	2.74	0.06	0.17	8.50
	Ca_{10}	1.99	0.47	0.15	5.67	2.10	0.18	0.22	7.20
	Ca_{20}	2.31	0.44	0.15	6.90	2.16	0.20	0.13	7.50
IV	Ca_0	1.44	0.16	0.03	1.22	3.57	0.06	0.21	5.95
	Ca_{10}	2.06	0.48	0.13	0.82	2.28	0.16	0.14	0.47
	Ca_{20}	2.04	0.56	0.14	2.20	2.36	0.15	0.14	2.30

nitrogen decrease over time. Irrespective of mixtures, in plant material harvested before flowering (*Festuca pratensis* and *Festuca rubra*) there is an accumulation of potassium in the control, of calcium in Ca_{10} and Ca_{20} variants and of phosphorus in Ca_{20} variant.

CONCLUSIONS

The experience with *Festuca pratensis* L. and *Festuca rubra* L. species, grown in monoculture and mixtures in different ratios, was carried out in greenhouse on a pseudogleyed podzolic soil. In this experiment through various levels of liming introduced on a uniform mineral fertilizer base (N, P, K) and at different harvest dates, the following conclusions are to be drawn :

1. The dry matter production increment in the aboveground parts of *Festuca pratensis* is recorded at 10/ha $CaCO_3$ treatment. Comparatively, *Festuca rubra* yield is permanently lower in all treated variants but in control. In this way, the ecological behaviour and particular adaptative value of grasses in monoculture and mixtures are evidenced.

2. The relative replacement rate of species (the ρ value), with small differences between treatment variants, shows that between the first and the third harvest *Festuca rubra* replaces *Festuca pratensis* and between the third and the fourth harvest *Festuca pratensis* replaces *Festuca rubra*.

3. The most efficient mixture, namely 75% *Festuca pratensis* with 25% *Festuca rubra* on a liming base of 10 t/ha $CaCO_3$ and at 47 and 57 days from seeding, has been considered. It is regarded the species overall yield in the first and second year of vegetation.

4. The relative growth rate in *Festuca pratensis* decreases in unit time especially between the last harvests. With *Festuca rubra* the relative growth rate appears in a different way.

5. A significant increase of K, Ca, N contents in the aboveground green parts of *Festuca pratensis* was determined by the maximum level of $CaCO_3$ in the soil. With plant ageing, a decrease of nitrogen, phosphorus and potassium and an increase of calcium respectively take place in both grasses.

REFERENCES

1. Higgs D.E.B., James D. B., J. of Ecol., 1969, **57**, *2*, 553—563.
2. Mac Coll D., Cooper J. P., J. Appl Ecol., 1967, *4*, 113—127.
3. Stoicovici E., Bilaos I., Stoicovici Lucia, St. Solului, 1969, **7**, *2*, 55—68.
4. Van Den Bergh J. P,. *Distribution of pasture plants in relation to chemical properties of the soil*, in : Rorison I. H., *Ecological Aspects of the Mineral Nutrition of Plants*, Blackw Sci. Publ. Ltd., Oxford-Edinburgh, 1969, 12—17.

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THE EFFECT OF X RAYS ON ROOT MERISTEM OF BROAD BEAN *(VICIA FABA)*

II. VARIATION OF MICRONUCLEI NUMBER AFTER IRRADIATION

BY

CONSTANŢA SPÂRCHEZ, V. SORAN and Z. URAY

The number of micronuclei within the root meristem cells of broad bean (*Vicia faba* var. *minor)* was counted at 24 and 48 hours after irradiation. The plant material, seedlings of 5 days, was irradiated with X rays in the following doses : 50, 100, 150, 200, 300 and 500 R. The results show a good correlation between the applied doses and the increased number of micronuclei. Concerning the relationship between the DNA content and the number of micronuclei there is a good correlation only in a particular case, covering the range of 50 — 200 R doses.

Evans, Neary and Williamson [3] observed for the first time the relationship between the chromosomal damage and the production of micronuclei on broad bean roots after neutrons and gamma rays action. Quite recently Heddle [4], Heddle and Harris [5], Schmid [6], [7] and other scientists have elaborated a new and rapid method, the so-called "micronucleus test", for the detection of chromosomal damage after different treatments (irradiation and chemical action).

Our purpose was : 1) to find if there is a linear correlation between the applied doses and the number of micronuclei and 2) if a similar correlation can be established between the DNA content and the number of micronuclei.

MATERIAL AND METHODS

Many of the methods used in this paper were previously published by us [8]. Here we refer to the micronuclei number method. They were counted on interphase cells and for each dose of irradiation about 2 000 cells were computed. The number of micronuclei was expressed in per cents as compared to the total number of cells.

RESULTS AND DISCUSSIONS

Fig. 1 shows the relationship between applied doses of X rays and the number of micronuclei in percents for 2 000 cells. The relation is quite linear especially 24 hours after irradiation. At 48 hours after irradiation there is a similar relationship, differing at 300 and 500 R when the percent of micronuclei suddenly increased. We have also computed the coefficient of correlation ("r") and found a very strong correlation

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between the number of micronuclei formed and the doses applied. The "r" was 0.99 at 24 hours and 0.97 at 48 hours after irradiation. This linear relation proves that in spite of the renewing action of "quiescent zone" of the root [1], [2] after irradiation, the chromosomal damage took place irrespective of conspicuous repair and increase of the DNA amount at 300 and 500 R.

In this connection we also computed the coefficient of correlation between the DNA content per nucleus and the number of micronuclei after different doses of irradiation. The general realationship is a very weak one, "r" being 0.10 at 24 hours and 0.24 at 48 hours after irradiation. The

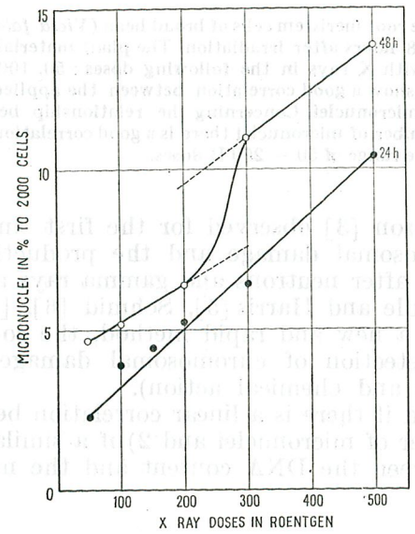

Fig. 1. — The relation between the applied doses of X rays and the number of micronuclei within meristemic cells of broad bean (*Vicia faba*).

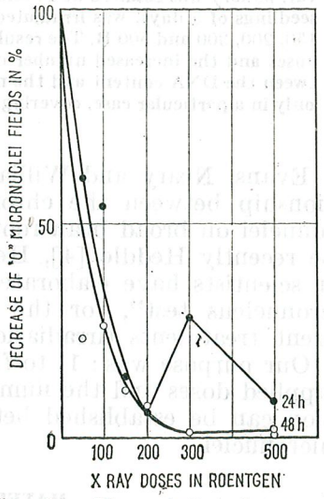

Fig. 2. — The relation between the applied doses of X rays and the decrease of "O micronuclei" fields in per cents, within meristemic cells of broad bean (*Vicia faba*).

lack of correlation results from the increase of the DNA content per nucleus at 300 and 500 R. If the coefficient of correlation was computed for a limited section of the curve, i.e. between 50 and 200 R, a good correlation was obtained, but it was negative; "r" = —0.97 at 24 hours and — 0.93 at 48 hours after irradiation. This means that the number of micronuclei increases when the DNA content per nucleus decreases. In fact, the number of micronuclei depends on the X rays doses applied and not on the DNA content per nucleus.

Fig. 2 shows the relation between the doses of X rays applied and the decrease of "O micronuclei" fields in per cents. The relation is expressed by a decreasing exponential curve. Fig. 3 shows, on histograms, the distribution of micronuclei per cells, expressed in per cents to 2 000 cells, at different doses of irradiation and at 24 hours and respectively 48 hours

after irradiation. The histograms show that the frequency of more micronuclei per cells increased with X rays doses. In spite of the fact that the DNA content per nucleus has increased again at 300 and 500 R, the histo-

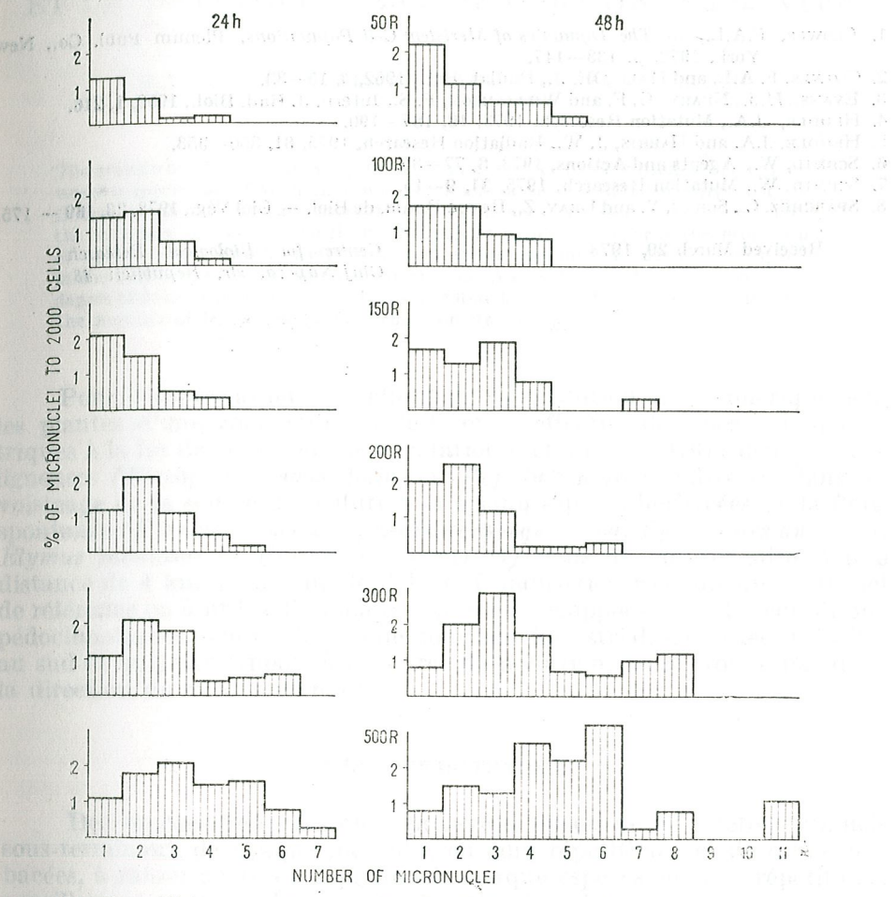

Fig. 3. — Histograms of micronuclei distribution per cell at different doses of X rays at 24 hours and 48 hours after irradiation.

grams show the appearance of more micronuclei, i.e. 6 — 11 per cell, as compared to 4 — 5 at 50 R. This is a clear proof that more chromosomal damages took place at higher doses of X rays.

CONCLUSION

The "micronucleus test" proved to be a better method for the estimation of chromosomal damage than the variation of DNA content

per nucleus in the case of broad bean (*Vicia faba* var. *minor*) meristemic roots irradiated in living conditions.

REFERENCES

1. Clowes, F.A.L., In *The Dynamics of Meristem Cell Populations*, Plenum Publ. Co., New York, 1972, p. 133—147.
2. Clowes, F. A.L. and Hall DE. J., Radiat. Bot., 1962, *3*, 15—33.
3. Evans, H.J., Neary, G. F. and Williamson, F. S., Intern. J. Rad. Biol., 1959, **1**, 216.
4. Heddle, J.A., Mutation Research, 1973, **18**, 187—190.
5. Heddle, J.A. and Harris, J. W., Radiation Research, 1975, **61**, 350—353.
6. Schmid, W., Agents and Actions, 1973, **3**, 77—85.
7. Schmid, W., Mutation Research, 1975, **31**, 9—15.
8. Spârchez, C., Soran, V. and Uray, Z., Revue Roum. de Biol. — Biol Vég., 1978, **23**, 169 — 175.

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L'INFLUENCE DE LA POLLUTION SUR LA CROISSANCE ET LE DÉVELOPPEMENT DE CERTAINES PLANTES

PAR

I. M. PEICEA, GABRIELA FIȘTEAG et ILEANA BUICULESCU

The results of biometrical analyses made at the end of vegetation period in two wooden species and five herbaceous species in a zone polluted mainly with SO_2, fluorides and powders are presented in the paper. One can notice that under the influence of noxae, significant differences appear as regards the production of wooden mass, length of offsprings and stems, and only in case of some species in the number of leaves and their dimensions. A new index of calculation of the degree of damage in wooden plants is suggested, based on the relationship between the number of leaves on an offspring and its weight.

Pour l'appréciation des effets de la pollution atmosphérique sur les plantes d'une zone industrielle *, on a effectué des mesures biométriques à la fin de la période de végétation (octobre — 1976) à deux espèces ligneuses *(Elaeagnus angustifolia* et *Populus nigra)* cultivées dans le voisinage de la source de pollution et a cinq espèces herbacées de la flore spontanée *(Plantago lanceolata, Euphorbia sequeriana, Phragmites australis, Elymus sabulosus* et *Pulicaria dysenterica)*, dans un endroit situé à une distance de 4 km de la zone de l'objectif industriel. En tant que matériel de référence on a utilisé les mêmes espèces développées dans des conditions pédoclimatiques semblables d'une zone non industrialisée située à 35 km au sud et qui, par rapport à la source de pollution, ne se trouve pas dans la direction du vent dominant.

MÉTHODE DE TRAVAIL

Des espèces ligneuses on a prélevé à raison de 50 rejetons annuels sous-terminaux de chaque individu, en cinq répétitions, et de celles herbacées, à raison de 10 exemplaires de chaque espèces en trois répétitions, pareillement pour les deux zones (témoin et polluée).

La signification de la différence des paramètres étudiés — présentés dans les tableaux nº 1 (les espèces ligneuses) et nº 2 (les espèces herbacées) — a été analysée du point de vue statistique à l'aide du teste « t » [3]. Pour l'illustration plus suggestive des modifications de certains paramètres, dans le cas des espèces *Elaeagnus angustifolia* et *Populus nigra* on a établi leurs courbes de variation par classes de fréquence.

* Les concentrations maximales des noxes dégagées par le Combinat des engrais chimiques dans la zone observée ont été (en mg/m³) : SO_2=15—18, fluorures=3,45 — 15,8, CO= 52 — 53, poudre de phosphates 22 — 38,7.

RÉSULTATS ET DISCUSSIONS

Les données présentées dans les tableaux nos 1 et 2 montrent que les plantes de la zone industrielle subissent d'importantes perturbations au niveau des processus de croissance et développement, exprimées par la diminution de certains organes, de la taille et de la biomasse des individus. On remarque ainsi des diminutions très significatives de la longueur et de la grosseur de la tige d'*Elaeagnus angustifolia* (fig. 1 et 2), de la lon-

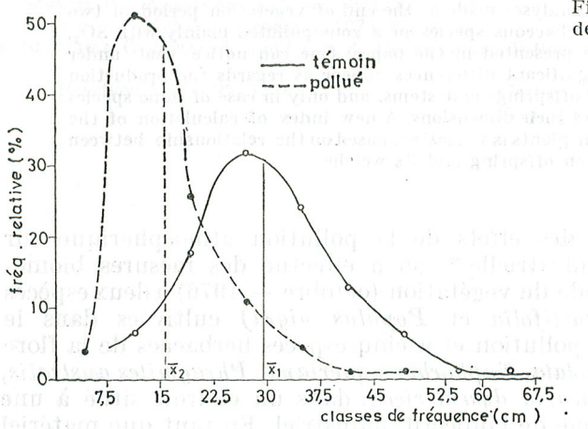

Fig. 1. — Variation de la longueur des tiges annuelles de l'*Elaeagnus angustifolia*.

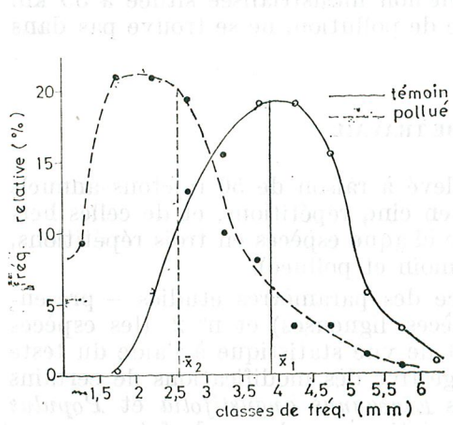

Fig. 2. — Variation de la grosseur des tiges annuelles de l'*Elaeagnus angustifolia*.

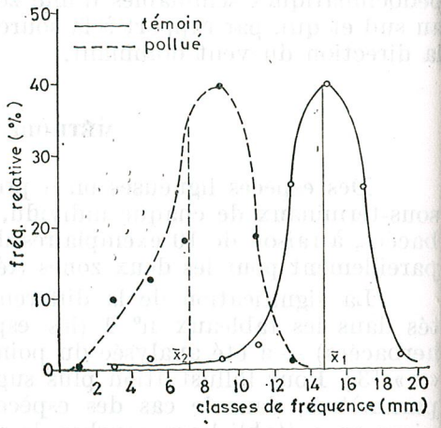

Fig. 3. — Variation de la longueur des boutons terminaux du *Populus nigra*.

gueur du bouton terminal de *Populus nigra* (fig. 3) et distinctement significatives en ce qui concerne la longueur des tiges des espèces herbacées *Euphorbia sequeriana*, *Plantago lanceolata* et *Phragmites australis*.

A la différence de l'autre espèce ligneuse étudiée, la longueur (fig. 4) et la grosseur (fig. 5) des tiges de *Populus nigra* de la zone polluée, présentent des valeurs dont les différences (en plus ou en moins) par rapport aux épreuves de référence sont comprises en limites insignifiantes. Le même phénomène d'inhibition de la croissance des rejetons de certaines espèces et de stimulation d'autres, a été évidencié aussi par A. S. Sitnikova [5] dans une zone avec pollution industrielle.

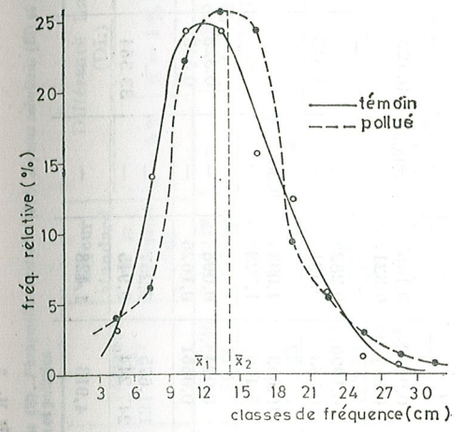

Fig. 4. — Variation du longueur des tiges annuelles du *Populus nigra*.

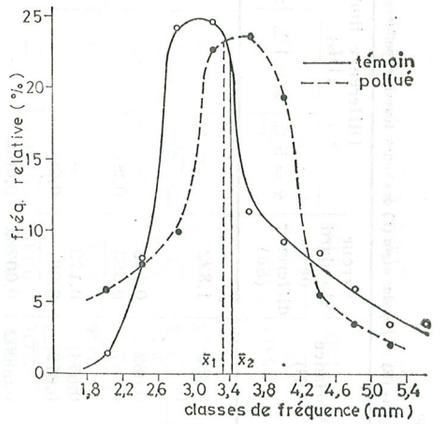

Fig. 5. — Variation de la grosseur des tiges annuelles du *Populus nigra*.

De nos recherches, il résulte que sans tenir compte du sens de variation de croissance en longueur des rejetons sous l'influence des noxes, le processus d'accumulation de substances dans les tiges ligneuses est incomodé, en constatant une diminution du poids sec de 71,3% pour *Elaeagnus angustifolia* (différence très significative) et de 45,3% pour *Populus nigra* (différence significative), (tableau no 1).

Le nombre des feuilles développées par un individu (les espèces herbacées) ou sur une tige annuelle (les espèces ligneuses) s'est avéré un paramètre variable ayant de tendances différentes (de croissance ou de diminution). De la sorte, à la plupart des espèces de la zone polluée a lieu une réduction remarquable du nombre des feuilles, les valeurs étant significatives (tableaux nos 1 et 2) ou non significatives pour *Elaeagnus angustifolia* (fig. 6). Par contre, l'espèce *Pulicaria dysenterica* présente un nombre de feuilles de 8,57% plus grand dans la zone polluée (valeur insignifiante) et *Populus nigra* développe sur un rejeton un nombre de feuilles de 42,29% plus grand par rapport au témoin (différence très significative), phénomène exprimé d'une manière suggestive par les courbes de variation (fig. 7).

Dans le cas des espèces ligneuses, en rapportant le nombre des feuilles à un centimètre de rejeton ou mieux à un gramme de substance sèche de tige, on constate une augmentation et un épaississement distinctement significatif du nombre des feuilles à l'unité respective, dans la zone polluée,

Tableau n° 1

L'analyse biométrique des espèces ligneuses *Elaeagnus angustifolia* (E) et *Populus nigra* (P) des zones témoin et polluées

Les paramètres	Espèce	Témoin ($\overline{X}_1$)	Pollué ($\overline{X}_2$)	Différence ($\overline{X}_1-\overline{X}_2$) (d)	Différence %	Variance (S^2)	Erreur standard de la différence (Sd)	DL α = 5 %	DL α = 1 %	DL α = 0,1 %	Signification de la différence (d ≷ DL 0,1 ; 1 ; 5 %)
Longueur de la tige* (cm)	E	29,60	16,00	13,60	45,95	8,853	1,822	—	—	9,50	d > DL 0,1 % TS**
	P	13,80	14,40	— 0,60	4,17	0	—	—	—	—	N
Grosseur de la tige (mm)	E	3,90	2,70	1,30	33,33	0,062	0,158	—	—	0,30	d > DL 0,1 % TS
	P	3,43	3,34	0,09	2,62	0,387	0,393	0,90	—	—	d < DL 5 % N
Poids sec tige ligneuse (g)	E	1,22	0,35	0,87	71,31	0,074	0,172	—	—	0,86	d > DL 0,1 % TS
	P	0,64	0,35	0,29	45,31	0,019	0,087	0,29	—	—	d = DL 5 % S
Poids sec 1 feuille (g)	E	0,044	0,036	0,008	18,18	0,00002	0,00283	0,0065	—	—	d > DL 5 % S
	P	0,194	0,185	0,009	4,64	0	—	—	—	—	— N
Poids sec feuilles/rejeton (g)	E	1,562	0,972	0,590	37,77	0,0612	0,1565	—	0,525	—	d > DL 1 % DS
	P	2,832	4,687	— 1,855	39,58	0,257	0,321	—	—	1,616	d > DL 0,1 % TS
Poids sec rejeton (tige + feuilles) (g)	E	2,782	1,326	1,456	52,54	0,1995	0,2825	—	—	1,424	d > DL 0,1 % TS
	P	3,472	5,037	— 1,565	31,07	0,383	0,391	—	1,313	—	d > DL 1 % DS
Nombre de feuilles/ rejeton	E	35,50	27,00	8,50	23,94	40,653	4,033	9,30	—	—	d < DL 5 % N
	P	14,60	25,30	—10,70	42,29	7,466	1,728	—	—	8,71	d > DL 0,1 % TS
Nombre de feuilles/1cm rejeton	E	1,202	1,687	— 0,485	28,75	0,024	0,098	—	0,328	—	d > DL 1 % DS
	P	1,058	1,757	— 0,699	39,78	0,0661	0,1626	—	0,545	—	d > DL 1 % DS
Nombre de feuilles/1g substance sèche tige	E	29,098	77,143	—48,045	62,41	131,675	7,257	—	—	36,582	d > DL 0,1 % TS
	P	22,813	72,285	—49,472	68,44	247,244	9,945	—	33,364	—	d > DL 1 % DS
Longueur bouton terminal (mm)	P	14,80	7,30	7,50	50,68	4,913	1,428	—	—	7,19	d > DL 0,1 % TS

• Tige = rejeton annuel sans feuilles;

Tableau n° 2

L'analyse biométrique des espèces herbacées *Plantago lanceolata* (P), *Euphorbia sequeriana* (E), *Phragmites australis* (Ph), *Elymus sabulosus* (El) et *Pulicaria dysenterica* (Pu) des zones témoin et polluées

Les paramètres	Espèce	Témoin ($\overline{X}_1$)	Pollué ($\overline{X}_2$)	Différence ($\overline{X}_1-\overline{X}_2$) (d)	Différence %	Variance (S^2)	Erreur standard de la différence (Sd)	DL α = 5 %	DL α = 1 %	DL α = 0,1 %	Signification de la différence (d ≷ DL 0,1 ; 1 ; 5 %)
Longueur de la tige (cm)	P	16,36	11,89	4,47	27,32	17,5526	1,3248	—	3,5955	—	d > DL 1 % DS*
	E	45,44	40,60	4,84	10,65	29,05	1,7488	—	4,7462	—	d > DL 1 % DS
	Ph	119,20	99,30	19,90	16,69	522,36	7,2274	—	19,5950	—	d > DL 1 % DS
	El	75,75	70,20	5,55	7,33	235,8842	4,8567	9,8372	—	—	d < DL 5 % N
	Pu	49,39	46,96	2,43	4,92	109,23	3,3015	6,6871	—	—	d < DL 5 % N
Nombre des feuilles	P	10,05	6,00	4,05	40,29	6,8684	0,8287	—	—	2,9592	d > DL 0,1 % TS
	E	105,00	39,55	65,45	62,33	408,38	6,5564	—	—	23,4129	d > DL 0,1 % TS
	Ph	11,15	7,47	3,68	33,00	3,7368	0,6112	—	—	2,1825	d > DL 0,1 % TS
	El	5,95	5,10	0,85	14,29	1,5986	0,3998	0,8097	—	—	d > DL 5 % S
	Pu	12,25	13,30	— 1,05	8,57	20,7894	1,4418	2,9203	—	—	d < DL 5 % N
Longueur des feuilles (cm)	P	19,90	6,39	13,51	67,89	4,7063	0,6860	—	—	2,4497	d > DL 0,1 % TS
	E	1,80	1,90	— 0,10	5,56	0,2239	0,1496	0,3030	—	—	d < DL 5 % N
	Ph	22,02	22,14	— 0,12	0,54	16,5471	1,2863	2,6054	—	—	d < DL 5 % N
	El	40,96	38,15	2,81	6,86	222,33	4,7151	9,5504	—	—	d < DL 5 % N
	Pu	3,85	3,34	0,51	13,24	0,2526	0,1589	—	0,4312	—	d > DL 1 % DS
Largeur des feuilles (cm)	P	1,00	0,90	0,10	10,00	0,0615	0,0784	0,1587	—	—	d < DL 5 % N
	E	0,26	0,28	— 0,02	7,69	0,0047	0,0217	0,0439	—	—	d < DL 5 % N
	Ph	1,56	1,50	0,06	3,84	0,1718	0,1310	0,2653	—	—	d < DL 5 % N
	El	0,88	0,77	0,11	12,50	0,0228	0,0478	0,0968	—	—	d > DL 5 % S
	Pu	1,15	1,08	0,07	6,09	0,02578	0,0507	0,1026	—	—	d < DL 5 % N
Nombre des inflorescences	P	2,40	1,30	1,10	45,83	1,3611	0,5217	1,0567	—	—	d > DL 5 % S
	Pu	10,25	8,15	2,10	20,49	45,0605	2,1227	4,2995	—	—	d < DL 5 % N
Longueur des inflorescences (cm)	P	1,22	1,12	0,10	8,20	0,1050	0,1452	0,2941	—	—	d < DL 5 % N
Nombre des branches latérales	Pu	5,70	4,20	1,50	26,31	13,6118	1,1666	2,3629	—	—	d < DL 5 % N

• TS = très significatif ; DS = distinctement significatif ; S = significatif ; N = non significatif

les différences étant plus évidentes pour *Populus nigra* (tableau nº 1). Les valeurs rapprochées et relativement constantes de ce rapport, pour les deux espèces ligneuses—dont la variation du nombre des feuilles est

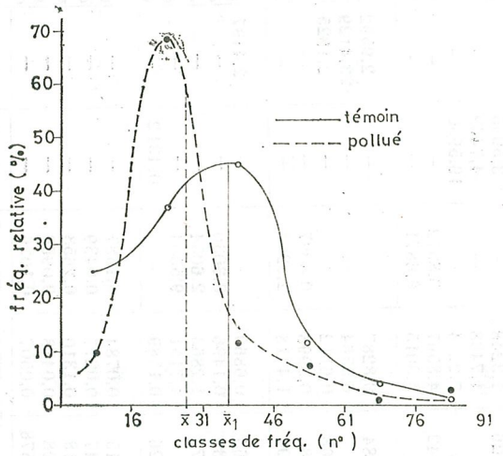

Fig. 6. — Variation du nombre des feuilles d'une tige d'*Elaeagnus angustifolia*.

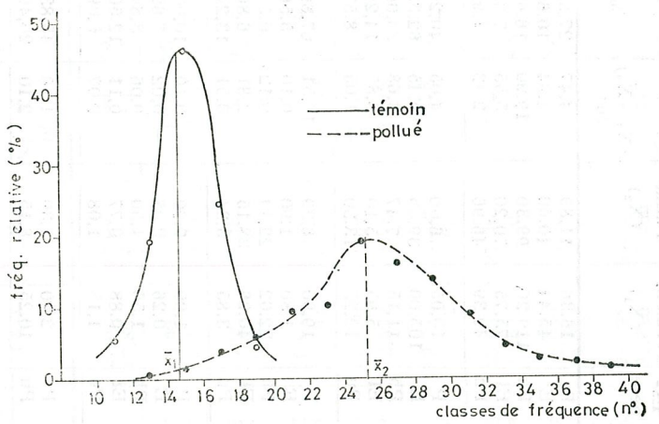

Fig. 7. — Variation du nombre des feuilles d'une tige de *Populus nigra*.

complètement opposée—suggèrent l'établissement d'un indice (A_p) de détermination du degré d'affectation ou de la sensibilité de certaines plantes à la pollution du milieu. On l'obtient en effectuant le rapport entre le nombre des feuilles qui se développent au cours d'une année sur un rejeton (N) et le poids sec de la tige ligneuse (G) entre la zone polluée

(p) et témoin (t).

$$A_p = \frac{\frac{N_p}{G_p}}{\frac{N_t}{G_t}} = \frac{N_p \cdot G_t}{N_t \cdot G_p}$$

Alors que deux groupes d'individus d'une population homogène poussent dans de conditions relativement identiques, la valeur du rapport se situe autour de 1. L'intervention d'un facteur perturbateur, la pollution par exemple, mène à l'augmentation de la valeur de l'indice directement proportionnelle au degré d'affection des plantes. A l'aide des données du tableau nº 1 on obtient pour *Populus nigra* $A_p = 3{,}169$ et pour *Elaeagnus angustifolia* $A_p = 2{,}651$.

Il en résulte que le phénomène d'épaississement des feuilles compense leur chute prématurée *(Populus nigra)* ou la réduction de leurs dimensions *(Elaeagnus angustifolia)*, en maintenant une surface d'assimilation minimale, nécessaire à la survivance de la plante au cours des situations défavorables.

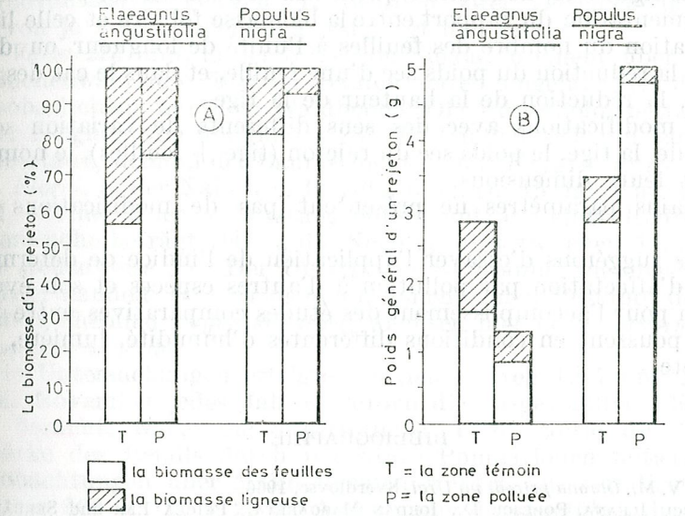

Fig. 8.—Biomasse des rejetons annuels en percentages (A) et en valeurs absolues (B) d'*Elaeagnus angustifolia* et *Populus nigra*.

L'expression du rapport entre la phytomasse foliaire et celle ligneuse en pourcentage (fig. 8-A) et en valeurs absolues (fig. 8-B), indique—pour les exemplaires de la zone polluée — une augmentation considérable de la phytomasse foliaire au détriment de celle ligneuse. L'utilisation des substances photosynthétisées à la formation du surplus de feuilles (organes caducs) contribue parmi d'autres (réduction de l'intensité de la photosynthèse, augmentation de l'intensité respiratoire, modification du rapport eau libre / eau liée, etc.) [1, 2, 5] à la réduction des réserves de

substances dans les organes de base, à l'affaiblissement et même à la mort des arbres [4], comme il est arrivé par exemple à quelques exemplaires de *Populus nigra* de la zone étudiée.

D'autres paramètres sont moins affectés, les différences n'étant pas significatives. Outre ceux nommés plus haut, nous rappellons aussi la longueur et la largeur des feuilles, leur poids sec, le nombre des inflorescences et des branches latérales.

CONCLUSIONS

Etant donnée la similitude des conditions pédoclimatiques des deux zones, les variations constatées peuvent être attribuées principalement à l'action défavorable des noxes. L'influence des agents phytotoxiques est exercée surtout au niveau des processus de croissance et de développement et s'exprime par une série de modifications ayant un caractère constant ou variable dont l'amplitude diffère avec l'espèce ou la concentration des noxes.

Parmi les modifications ayant un caractère constant, dans le cas des espèces ligneuses, nous remarquons : la diminution du poids sec de la tige, l'augmentation du rapport entre la biomasse foliaire et celle ligneuse, l'augmentation du nombre des feuilles à l'unité de longueur ou de poids de la tige, la réduction du poids sec d'une feuille, et dans le cas des espèces herbacées, la réduction de la hauteur de la tige.

Les modifications avec des sens différents de variation sont : la longueur de la tige, le poids sec du rejeton (tige + feuilles), le nombre des feuilles et leurs dimensions.

Certains paramètres ne présentent pas de modifications significatives.

Nous suggérons d'essayer l'application de l'indice de détermination du degré d'affectation par pollution à d'autres espèces et son éventuelle utilisation pour l'accomplissement des études comparatives entre populations qui poussent en conditions différentes d'humidité, lumière, fertilité du sol, etc.

BIBLIOGRAPHIE

1. Babkina V. M., *Ohrona prirodi na Ural*, Sverdlovsc, 1966.
2. Buiculescu Ileana, Popescu D., Iordan Margareta, Peicea I.M. and Şerbănescu G., Rev. Roum. Biol. Vég., Bucarest, 1978, **23**, 2, 187–193.
3. Ceapoiu N., *Metode statistice aplicate în experienţele agricole şi biologice*, Bucureşti, 1968.
4. Giurgiu V., *Studiul creşterilor la arborete*, Ed. Agro-Silvică, Bucureşti, 1967.
5. Sitnikova A.S., *Ohrona prirodi na Ural*, Sverdlovsc, 1966.

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ÖKOLOGISCHE UNTERSUCHUNGEN ÜBER DIE PILZE DER ASSOZIATION *FAGETUM DACICUM*, IM BAIULUI-GEBIRGE, GÎRBOVA (RUMÄNIEN)

VON

VALERIA BARBU

Mycocoenological researches in *Fagetum dacicum* association were made monthly from April till November, during 2 years (1977–1978) on permanent plots. There were identified 114 species of microscopic and macroscopic fungi. Analysing the fungi composition during a year, the greatest development was observed in the preautumnal period. The highest index of abundance (4) was realized by *Hypholoma fasciculare* and *Lycoperdon pyriforme*. The ecological spectrum has been established ; the saprophyte species are the best represented numerically, the lignicole ones being the most numerous. The mycorrhizal species record also large numbers.

Das erforschte Territorium ist auf dem Prahova zugewandten Abhang des Gîrbovamassives gelegen. Es wurden zwei verschiedene Arbeitsflächen von je 1 ha Ausdehnung festgelegt und zwar :

1. *Valea Mărului*, in der Nähe von Sinaia, in einem über 100 Jahre alten Buchenwald (die ältesten Bäume waren ungefähr 130 Jahre alt) auf mesobasischem, braunem Waldboden. Die Meereshöhe beträgt 900 m, bei einer Neigung von annähernd 25°. Es handelt sich um einen reinen Buchenbestand, denn in der Baumschicht ist nur *Fagus silvatica* vertreten.

2. *Florei*, in der Nähe von Posada, in einem 15 Jahre alten Buchenbestand, welcher einer Aufforstung entstammt, die einer Rodung folgte. Die Meereshöhe beträgt 790 m, die Neigung liegt zwischen 5 und 25° und erreicht stellenweise 35°. Der Untergrund ist braune, mesobasische Walderde. Hier handelt es sich nicht um einen reinen Bestand, denn außer der vorherrschenden *Fagus silvatica*, wachsen hier noch *Carpinus betulus* und *Populus tremula*.

Die Untersuchungen erfolgten in den Jahren 1977 und 1978. Von April bis November jedes Jahres wurden die ausgewählten Flächen monatlich besucht, wobei die angetroffenen Pilze, sowie die Häufigkeit und Stärke des Befalls durch parasitäre Populationen notiert wurden. Die Beobachtungen über Makromyceten wurden auf ständigen Arbeitsflächen von 500 m² (20 × 25 m) Ausdehnung durchgeführt, die gleichmäßig über das jeweilige Territorium verteilt waren [3, 4, 5]. Es wurden alle Arten und die Abundanz der Fruchtkörper von jeder Art festgehalten. Auch die Großpilze außerhalb der Arbeitsflächen wurden aufgenommen, wobei das gesamte Territorium auf einem Zickzackkurs durchwandert wurde.

Die Abundanz der Makromyceten wurde nach der Skala von Moser bestimmt (+ = 1 Fruchtkörper ; 1 = 2 – 5 Fruchtkörper ; 2 = 6 – 50 Fruchtkörper ; 3 = 51 – 100 Fruchtkörper ; 4 = 101 – 500 Fruchtkörper ; 5 = über 500 Fruchtkörper.

Aus den Tabellen 1 und 2 geht hervor, daß sich im Laufe des Jahres die Zusammensetzung der Mykozoenose ändert [2]. Für den Frühling sind die *Peronosporaceae*, die Aezidienstadien der *Uredinalen* und einige *Ascomycetes* kennzeichnend. Es wurden auch Makromyceten mit ausdauernden Fruchtkörpern gefunden. Der Vorsommerzustand ist reicher an Arten. Zu den bereits erschienenen gesellen sich noch andere *Uredinalen*, sowie erdbewohnende Großpilze, deren Zahl im Juli-August anwächst (Hochsommer), um im September (Frühherbst) zur höchsten Entfaltung zu gelangen, sowohl als Artenzahl als auch nach der Abundanz. In der Hochsommerphase bestimmen das Bild die Arten von *Lactarius* und *Russula* . Für den vorherbstlichen und herbstlichen Aspekt sind die Gattungen : *Cortinarius*, *Collybia*, *Stropharia*, *Lycoperdon* usw. charakteristisch.

Die Abundanz betreffend zeigt Tabelle 2, daß der höchste Index — 4 — nur von zwei Arten u. zw.: *Hypholoma fasciculare* und *Lycoperdon pyriforme* erreicht wird. Die Mehrzahl der Arten erreicht einen Abundanz-Index von 1—2.

DAS VERHÄLTNIS DER VERSCHIEDENEN ÖKOLOGISCHEN KATEGORIEN ZUEINANDER

Aus der Gegenüberstellung der ökologischen Kategorien aus den beiden Untersuchungsgeländen (Tabelle 3) ersieht man, daß an beiden Standorten die saprophytischen Arten überwiegen (50% in Florei und 32,9% in der Valea Mărului). Unter den saprophytischen Makromyceten liegt der Schwerpunkt in beiden Geländen auf den Lignikolen, gefolgt von den Terrikolen, Humikolen und Foliikolen.

Tabelle 3

Das Spektrum der ökologischen Kategorien

Ökologische Kategorie		Örtlichkeit: Valea Mărului %	Örtlichkeit: Florei-Posada %
Saprophyten		32,9	50,0
davon :	terricole	6,6	11,8
	humicole	3,96	8,8
	lignicole	19,7	25,0
	foliicole	2,64	4,4
Parasiten		25,0	17,7
Lignikole Saproparasiten		22,4	10,3
Mykorrhiza-Pilze		19,7	22,0

Die auf faulendem Holz parasitierenden Arten sind in der Valea Mărului mit 22,4% vertreten, in Florei mit 10,3%. Alle diese Arten wurden lediglich auf faulendem Holz als Unterlage aufgefunden und verhalten sich demnach als Saprophyten. Sie sind aber befähigt zu einer parasitären Lebensweise überzugehen, indem sie lebende Bäume befallen, wo sie verschiedene Fäulnistypen und sogar das Absterben derselben verursachen.

Die Mykorrhiza-Pilze sind an beiden Örtlichkeiten gut vertreten (19,7% in der Valea Mărului und 22% in Florei); sie ermöglichen ein gutes Wachstum der Bäume und steigern die primäre Produktion.

Die auf autotrophen, photosynthetisch aktiven, Pflanzen parasitierenden Pilze (25% in der Valea Mărului und 17,7% in Florei) wirken sich auf die Biozönosen des Waldes schädlich aus. Sie ernähren sich auf Kosten der lebenden Gewebe der Holz- und Krautpflanzen (Blätter, Sprosse, Samen usw.). Die auf Blättern parasitierenden Arten verursachen

Tabelle 1

Mikromyceten aus der Assoziation *Fagetum dacicum*

Pilz-Art	Wirts-Pflanze	Beobachtungsperiode: Valea Mărului v	pe	e	pa	a	Florei-Posada v	pe	e	pa	a
Peronospora cardamines-laciniatae	*Dentaria bulbifera*						++	+	++	++	++
Peronospora dentariae-macrophyllae	*Dentaria glandulosa*								+	+	+
Sphaerotheca balsaminae	*Impatiens noli-tangere*								+	+	+
Sphaerotheca pannosa var. rosae	*Rosa sp.*						+	+	+++	+++	+++
Uncinula aceris	*Acer campestre*										+
Erysiphe galeopsidis	*Galeopsis speciosa*								+	+	+
Phyllactinia suffulta	*Fagus silvatica*			+	+	+	+	+	+++	+++	+++
Hypoxylon fuseum	*Fagus silvatica*			+	+	+					
Rhytisma acerinum	*Acer pseudoplatanus*			+	+	+					
Melampsorella symphyti	*Symphytum cordatum*										+
Puciniastrum epilobii	*Epilobium montanum*										+
Coleosporium campanulae	*Campanula rapunculoides*										+
Coleosporiuna campanulae	*Campanula trachelium*										+
Coleosporium senecionis	*Senecio fuchsii*										+
Coleosporium tussilaginis	*Tussilago farfara*										+
Melampsora rostrupii	*Populus tremula*										+
Tranzschelia fusca	*Anemone nemorosa*										+
Tranzschelia pruni-spinosae	*Rubus caesius*										+
Phragmidium rubi	*Rosa sp.*										+
Phragmidium disciflorum	*Ranunculus ficaria*										+
Uromyces ficariae	*Adoxa moschatellina*										+
Puccinia adoxae	*Aegopodium podagraria*										+
Puccinia aegopodii	*Moehringia trinervia*										+
Puccinia arenariae	*Asperula odorata*										+
Puccinia asperulae-odoratae	*Symphytum cordatum*										+
Puccinia bromina	*Poa nemoralis*										+
Puccinia poarum	*Tussilago farfara*										+
Puccinia prenanthis	*Mycelis muralis*										+

Tabelle 2

Makromyceten aus der Assoziation *Fagetum dacicum*

Pilz-Art	Abundanz										Ökologische Kategorie
	Valea Mărului					Florei-Posada					
	v	pe	e	pa	a	v	pe	e	pa	a	
Daldinia concentrica	+−1	1	1	1	1	·	·	·	·	·	Sl
Aleuria vesiculosa	·	+−1	·	·	·	·	·	·	·	·	St
Stereum hirsutum	1	1−2	1	1	1	1	1	1	1−2	1−2	SP1
Cantharellus tubiformis	·	·	·	·	·	·	·	·	1	·	M
Hydnum repandum	·	·	·	1	·	·	·	·	1	·	M
Polyporellus brumalis	·	+−1	·	·	·	·	1	·	·	·	Sl
Polyporellus melanopus	·	+	1	+	·	·	·	·	·	·	Sl
Polyporellus numularius	·	·	·	1	·	·	·	·	1	·	Sl
Polyporellus varius	·	1	+	+	·	·	1	+	·	·	Sl
Polyporellus squamosus	·	+−1	+	·	·	·	·	·	·	·	SPl
Gloeosporus adustus	·	·	·	·	+−1	·	·	·	·	·	SPl
Fomes annosus	+−1	+−2	2	2	2	+−1	1−2	2	2	2	SPl
Fomes fomentarius	+−1	2	2	2	2	+−2	+−2	+−2	2	2	SPl
Phellinus ribis	·	·	1	·	·	·	·	·	·	·	SPl
Phellinus nigricans	+−1	+−2	+−2	2	2	·	·	·	·	·	SPl
Ganoderma applanatum	1	1	2	2	2	+−1	1	1	1	1	SPl
Coriolus hirsutus	1	2	2	2	2	1	1	1	1	1	Sl
Trametes versicolor	+−1	2	1−2	1−2	2−3	+−1	1−2	1−2	2	2−3	Sl
Trametes gibbosa	·	·	·	1	1	·	·	·	·	·	SPl
Boletus chrysenteron	·	·	·	·	1	·	·	·	·	·	M
Amanita lividopallescens	·	·	+−1	·	·	·	·	·	·	·	M
Coprinus atramentarius	1−2	·	·	·	·	·	·	·	·	·	Sh
Coprinus micaceus	·	·	·	·	·	·	·	1−2	·	·	Sl
Psothyrella gracilis	·	·	1−2	2	·	·	·	1−3	3	·	Sl
Hypholoma fasciculare	·	·	1−2	1−4	1−3	·	·	1−2	1−3	2	Sl
Hypholoma sublateritium	·	·	+−2	1−2	·	·	·	·	·	·	Sl
Pholiota flammans	·	·	·	·	+−1	·	·	·	·	·	Sl
Pholiota squarrosa	·	·	·	·	+−2	·	·	·	·	·	Sl
Conocybe tenera	·	·	+−2	2	·	·	·	1	2	·	Sf
Conocybe pyriodora	·	·	·	·	·	·	·	·	1	·	Sh
Stropharia aeruginosa	·	·	·	·	·	·	·	·	+−2	·	Sh
Cortinarius anomalus	·	·	·	·	·	·	·	·	+−1	·	M
Cortinarius humicola	·	·	·	·	·	·	·	·	1	·	M
Cortinarius hemitrichus	·	·	·	·	·	·	·	·	2	·	M
Cortinarius multiformis	·	·	·	·	·	·	·	·	+−1	·	M
Cortinarius caerulescens	·	·	·	·	·	·	·	·	+−2	·	M
Cortinarius bivelus	·	·	·	·	·	·	·	·	+−1	·	M
Cortinarius bulliardi	·	·	·	2	·	·	·	·	·	·	M
Inocybe pyriodora	·	·	·	·	·	·	·	·	+−1	·	St
Inocybe geophylla	·	·	·	·	·	·	·	·	+−1	·	Sh
Hebeloma testaceum	·	·	·	·	·	·	·	·	+−1	·	Sh
Collybia acervata	·	·	·	·	·	·	·	·	1	·	Sl
Collybia tuberosa	·	·	·	·	·	·	·	·	1	·	Sl
Collybia radicata	·	·	·	+−1	·	·	·	·	+−1	·	Sh-Sl
Collybia atrata	·	·	·	+−1	·	·	·	·	1	·	Sl
Collybia rancida	·	·	·	+−1	·	·	·	·	·	·	St
Collybia longipes	·	+−1	1−2	1	·	·	+−1	+	+−2	·	Sh
Collybia velutipes	·	·	1−2	·	·	·	·	+−2	·	·	Sl
Marasmius alliaceus	·	+	·	1	·	·	+	·	+−1	·	Sf
Marasmius scorodonius	·	·	·	·	·	·	·	1	·	·	Sl
Oudemansiella mucida	·	·	+	+−2	·	·	·	·	·	·	SPl
Delicatula integrella	·	·	·	·	·	·	·	·	+	·	Sl
Mycena alcalina	·	·	+	·	·	·	·	·	·	·	SPl
Mycena crocata	·	·	·	1	·	·	·	·	·	+	Sf

Tabelle 2 (Fortsetzung)

Pilz-Art	Abundanz										Ökologische Kategorie
	Valea Mărului					Florei-Posada					
	v	pe	e	pa	a	v	pe	e	pa	a	
Mycena inclinata	·	·	·	·	·	·	·	·	+−1	·	Sl
Mycena sanguinolenta	·	·	·	·	+−1	·	·	·	·	·	St
Mycena galericulata	·	+−1	·	1	·	·	1	·	1	·	S Pl
Mycena pura	·	1	·	·	·	·	·	·	·	·	Sh
Armillaria mellea	·	·	·	·	1−2	·	·	·	·	1−2	SPl
Lepiota sordida	·	·	·	·	·	·	·	·	+	·	Sl
Tricholoma album	·	+−1	·	·	·	·	·	·	·	·	M
Clitocybe suaveolens	·	·	·	·	·	·	+	·	·	·	St
Clitocybe infundibuliformis	·	·	·	·	·	·	·	·	+−1	·	St
Clitocybe inornata	·	·	·	·	·	·	·	·	1−2	·	M
Clitocybe nebularis	·	·	·	·	+−1	·	·	·	·	·	M
Limacium leucophaeum	·	·	·	·	·	·	·	·	+−1	·	Sh
Camarophyllus virgineus	·	·	·	·	·	·	·	·	1	·	St
Laccaria laccata	·	+	+	1	·	·	+	+−1	1	·	Sh-Sl
Panellus stipticus	·	1−2	2−3	1	·	·	·	·	·	·	Sl
Pleurotus ostreatus	·	·	·	1−2	·	·	·	·	1	·	SPl
Lactarius deliciosus	·	·	+−2	2	·	·	+	+	+−2	·	M
Lactarius piperatus	·	·	+−2	2	+−1	·	+−1	2	+−1	+	M
Lactarius volemus	·	·	·	·	·	·	·	+−2	+	·	M
Russula alutacea	·	·	·	+−2	·	·	·	·	+−1	·	M
Russula olivacea	·	·	·	+−1	·	·	·	·	+−1	·	M
Russula foetens	·	·	1	·	·	·	·	1	·	·	M
Russula ochroleuca	·	·	+−1	·	·	·	·	+−1	·	·	M
Russula caerulea	·	·	1	·	·	·	·	·	·	·	M
Russula emetica	·	·	+−1	·	·	·	·	·	·	·	M
Russula virescens	·	·	1−2	·	·	·	·	·	·	·	M
Russula firmula	·	·	·	·	+−1	·	·	·	·	·	M
Russula xerampelina	·	·	·	·	·	·	·	+	+−2	·	M
Secotium agaricoides	·	·	·	·	·	·	·	+	·	·	St
Lycoperdon echinatum	·	·	·	·	+	·	·	·	·	·	St
Lycoperdon pyriforme	·	·	·	2−3	4	·	·	·	1−4	4	Sl
Lycoperdon gemmatum	·	·	·	+−1	1	·	·	·	+−1	1	St

Erklärung der Symbole: v = Frühjahr, pe = Vorsommer, e = Hochsommer, pa = Vorherbst a = Herbst.
St = terricoler Saprophyt, Sh = humicoler Saprophyt, Sl = lignicoler Saprophyt, Sf = foliicoler Saprophyt, SP1 = lignicoler Saproparasit, M = Mykorrhizapilz

einerseits eine Verminderung der Assimilationsfläche, andererseits Störungen des Stoffumsatzes, welche ihren Ausdruck in einer Abnahme der Photosynthese finden, einer Zunahme der Atmungsvorgänge, einem vorzeitigen Laubfall. Wenn diese Schädigungen nicht den Tod des Wirtes bewirken, führen sie jedenfalls zur Abnahme der Primärproduktion. So wurden z. B. 1978 die Blätter der Buchen (*Fagus silvatica*) im August zu 15 — 20% von *Phyllactinia suffulta* befallen. Im Untersuchungsgebiet Florei-Posada wurde ein starker Befall durch *Phragmidium disciflorum* auf *Rosa sp.* verzeichnet, was schon im August zu einem Laubfall von 40 — 50% führte. In den durch *Phragmidium* befallenen Blättern, sinkt zur Zeit der Sori-Bildung die Photosynthese auf 50% des Wertes für gesunde Pflanzen [1].

Zwischen Pilzen und Gruppen anderer Lebewesen existieren auch nutzbringende Beziehungen. Dazu gehören vor allen Dingen jene Vorgänge während welcher Pilzpopulationen solche Stoffe, die für andere Populationen nicht als Nahrung dienen können, in Verbindungen umwandeln, die für die Ernährung tauglich sind. Diese Rolle spielen die Saprophyten, die in verschiedenen Stadien der Zersetzung komplexer organischer Stoffe und beim Abbau zu einfachen Verbindungen wie CO_2, H_2O und mineralischen Stoffen mitwirken, wodurch diese wieder in den trophischen Kreislauf eingeschaltet werden. In dieser Hinsicht spielen in den bearbeiteten Gebieten folgende holzbesiedelnde Saprophyten eine hervorragende Rolle: *Coriolus hirsutus*, *Trametes versicolor*, *Hypholoma fasciculare*, *Collybia velutipes*, *Lycoperdon pyriforme*; diese nehmen dank ihrer großen Zahl in hohem Maß an der Zersetzung des abgestorbenen Holzes teil.

Was die holzbefallenden Saproparasiten anbelangt, so konnte in den Untersuchungsflächen eine große Zahl jener Arten festgestellt werden, die aktiv am Abbau der Zellulose beteiligt sind (*Stereum hirsutum*, *Fomes annosus*, *Fomes fomentarius*, *Ganoderma applanatum*, *Polyporellus squamosus*, *Armillaria mellea*) und am Abbau des Lignins (*Ganoderma applanatum*, *Armillaria mellea*, *Pleurotus ostreatus* usw.)

Die trophischen Beziehungen der Pilze zu andern Organismen sind höchst verwickelt und ein und dieselbe Art kann an verschiedenen Stellen der Nahrungsketten auftreten und verschiedenartige Bindungen zu Populationen des Ökosystems eingehen.

LITERATUR

1. Barbu, Valeria, Analele Universităţii Bucureşti, Seria biologie, 1967, 135–144.
2. Barbu, Valeria, Acta Botanica Horti Bucurestiensis, 1973, 559–568.
3. Bohus, G., Babos, M., *Coenology of terricolous macroscopic fungi of deciduous forests*, Bot. Jahrb. Syst. Pflantzenges. Pflanzengeogr. Stuttgart, 1969, **80**, 1.
4. Bohus, G., Babos, M., *Mycocoenological investigation of Acidophilous Deciduous Forests in Hunqary* Bot. Jahrb., Stuttgart, 1967, **87**, 3, 304–360.
5. Darimont, F., *Recherches mycosociologiques dans les forêts de Haute Belgique*, Institut Royal des sciences naturelles de Belgique, Mémoire no. 1970, 1973.

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DIE WIRKUNG VON LINDAN UND METHYLCHOR AUF DEN DNS-GEHALT DER ZELLKERNE IM WURZELMERISTEM VON WEIZEN *(TRITICUM VULGARE)* UND ACKERBOHNE *(VICIA FABA)*

VON

GEORGETA LAZĂR-KEUL, V. SORAN, ROZALIA VINTILĂ, AL. POLIZU und M. KEUL

The action of lindane (hexachloridecyclohexane) and methylchloride (1 and 5 kg active substance per ha) on the relative DNA content has been searched using root meristem of wheat (*Triticum vulgare*) and broad bean (*Vicia faba*). The measurement of relative DNA content of Feulgen-stained nuclei has been microspectrophotometrically made. Meanwhile the variation in the nuclear volume has been recorded. With control seedlings the relative amount of DNA has been proved to be constant during growth. With wheat lindane has caused a bulky increase of DNA amount bounded witha larger increase of nuclear size, the decrease of cell divisions and the multiplication of nucleoli number per nucleus. With broad bean lindane has caused an increase of relative DNA amount in spite of nuclear size decrease. Methylchloride has caused a slight decrease of DNA content bounded with a weak decrease of nuclear size. The authors inferred that lindane may cause endopolyploidy and carcinoma of some cells.

Die seit 1943 mit ^{32}P und markierten DNS-Vorstufen (Adenin, Thymidin) unternommenen Forschungen belegten, daß die DNS der Zellkerne unter verschiedenen Bedingungen, mit Ausnahme embryonaler und tumoraler Zellen, eine hohe metabolische Stabilität aufweist (Einzelheiten bei Davidson, 1972 [7]). Biochemische und mikrospektrophotometrische Untersuchungen [2], [18] zeigten, daß die absolute DNS-Menge je nach Art verschieden, in den Zellkernen somatischer Gewebe derselben Art jedoch annähernd gleich ist. Die Ergebnisse dieser Bestimmungen lieferten seinerzeit einen Beweis für die genetische Rolle der DNS und führten zur Anwendung quantitativer Betrachtungen bei der Untersuchung cytogenetischer Prozesse.

Von besonderer Bedeutung für die theoretische und angewandte Forschung ist die Feststellung, daß einige der in der Landwirtschaft verwendeten Pestizide die quantitative Stabilität cytogenetischer Prozesse zu verändern vermögen. Seit 1939 ist bekannt [29], daß mehrere chlorierte Kohlenwasserstoffe, u.a. Lindan, im Wurzelmeristem von Weizen Polyploidie hervorrufen. Diese Feststellungen konnten in der Folgezeit durch Untersuchungen an anderen Pflanzenarten bestätigt und erweitert werden [3], [4], [5], [8], [10], [12], [14], [15], [16], [19], [25], [26], [27], [28]. Da in dieser Richtung nur spärliche quantitative Erhebungen vorliegen [25], soll in vorliegender Arbeit die Wirkung von Lindan und Methylchlor auf den relativen DNS-Gehalt der Zellkerne im Wurzelmeristem von Weizen (*Triticum vulgare*) und Ackerbohne (*Vicia faba*) während früher Entwicklungsphasen vergleichend untersucht werden.

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MATERIAL UND ARBEITSMETHODE

Winterweizenkaryopsen (*Triticum vulgare*, Sorte „Aurora-Turda") und Ackerbohnen (*Vicia faba*) wurden in Linhardt-Schalen auf mit Leitungswasser befeuchtetem Filterpapier zur Keimung angesetzt.

Das verwendete Filterpapier diente gleichzeitig als Unterlage für die Behandlung mit Lindan und Methylchlor. Zu diesem Zweck wurden kreisförmige Filterpapiere, mit Ausnahme der Kontrolle, mit in Aceton gelöstem Lindan und Methylchlor entsprechend einer Aufwandsmenge von 1 und 5 kg aktive Substanz pro ha behandelt. Nach Verdunstung des Lösungsmittels verblieben die Pestizide auf der Filterpapierunterlage relativ gleichmäßig verteilt zurück.

Die Keimung erfolgte unter Laboratoriumsbedingungen bei 20 — 22°C und einem für die Monate März und April charakteristischen zirkadianen Licht-Dunkel-Wechsel.

Als Versuchsmaterial dienten ca. 1 cm lange Wurzelspitzen, die im Falle von Weizen in drei aufeinanderfolgenden Wachstumsphasen der Keimpflanzen abgetrennt wurden : 1. am 2. Tag nach dem Ansetzen ; 2. während der Ausbildung der Koleoptile (am 4. Tag nach dem Ansetzen) und 3. nach dem Erscheinen des 1. Blattes (am 7. Tag nach dem Ansetzen). Bei der Ackerbohne wurden die Wurzelspitzen am 4. Tag nach dem Ansetzen zur Untersuchung herangezogen.

Die Wurzelfragmente wurden 2 Stunden in einem Äthanol-Eisessig-Gemisch 3 : 1 (Carnoy) fixiert, anschließend wiederholt in 70% Äthanol gewaschen und bis zur Färbung in 70% Äthanol konserviert.

Die Bestimmung des relativen DNS-Gehaltes erfolgte cytospektrophotometrisch [1]. Die Hydrolyse wurde in 5 N HCl bei Zimmertemperatur vorgenommen und dauerte 1 Stunde. Anschließend wurde in kaltem Wasser gewaschen. Die Färbung mit dem Schiffschen Reagens wurde 2 Stunden lang im Dunkeln durchgeführt. Danach wurde 3mal jeweils 10 Minuten in SO_2-Wasser (10 ml 1 N HCl + 10 ml 10%ige $K_2S_2O_5$-Lösung + 180 ml destilliertes Wasser) und 2mal jeweils 10 Minuten in destilliertem Wasser gewaschen.

Zur Untersuchung wurden die gefärbten 1—2 mm langen Wurzelspitzen abgetrennt und die Meristemzellen in Quetschpräparaten in einzelliger Schicht auf Objektträgern ausgebreitet [6]. Die DNS-Menge wurde je Variante an ca. 250—300 zufällig ausgesuchten Meristemzellen bestimmt. Aufgrund der gegebenen Meßbedingungen konnten nur Ruhekerne in der Posttelophase (G_1), Synthesephase (S) und Antephase (G_2) des Zellzyklus ausgewertet werden.

Die Messungen wurden nach der bewährten Zwei-Wellenlängen-Methode [17], [21], [22], [32] vorgenommen, deren Meßfehlergrenze unter 5% liegt. Es wurde bei den Wellenlängen 500 und 480 nm gearbeitet. Zur Erzielung monochromatischen Lichtes diente ein Geradsicht-Spiegelmonochromator (Leitz). Zur Messung der Lichttransmission der gefärbten Zellkerne diente ein Ortholux-Mikroskop mit Photometeraufsatz MPE (LEITZ). Aus den Meßwerten wurde der relative DNS-Gehalt in willkürlichen Einheiten anhand von Umwandlungstabellen [17], [22] berechnet. Die Bestimmung des Zellkernvolumens erfolgte nach Messung der kleinen (B) und großen Achse (L) mittels eines Mikrometer-

schraubenokulars (Zeiss) aufgrund der Formel [9], [11] :

$$V = \frac{\pi}{6} \cdot L \cdot B^2 \quad (\mu m^3)$$

Die erzielten Meßwerte wurden statistisch unter Anwendung von Algorithmen [23] verarbeitet.

ERGEBNISSE UND DISKUSSION

In Abb. 1 ist der relative DNS-Gehalt in willkürlichen Einheiten und das Volumen der Zellkerne im Wurzelmeristem von Weizenkeim-

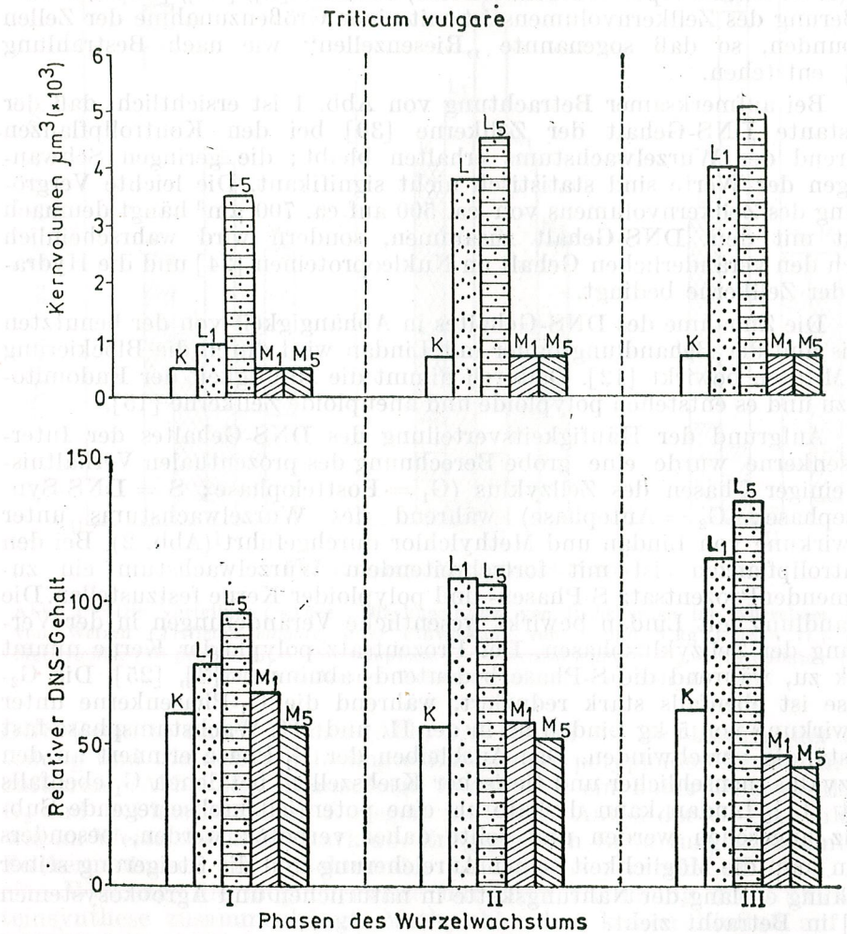

Abb. 1 — Der relative DNS-Gehalt und das Volumen der Zellkerne im Wurzelmeristem von Weizen (*Triticum vulgare*) unter Einwirkung von 1 und 5 kg Lindan(L) und Methylchlor (M) pro ha. Wachstumsphasen der Pflanzen ; I = 2 Tage nach dem Ansetzen ; II = Ausbildung der Koleoptile ; III = Bildung des 1. Blattes.

pflänzchen (*Triticum vulgare*) verschiedenen Alters nach kontinuierlicher Behandlung mit Lindan (Hexachlorcyclohexan) und Methylchlor dargestellt.

Lindan bewirkt eine mit der Dosis und der Behandlungsdauer korrelierte Zunahme des relativen DNS-Gehaltes pro Zellkern. Dieser Befund zeigt, daß Lindan, wahrscheinlich dank seiner Lipoidlöslichkeit, rasch durch die Primärwurzel aufgenommen wird und bedeutende karyologische und cytogenetische Veränderungen induziert. Hervorzuheben ist besonders die Vergrößerung des Zellkernvolumens auf das 4-6fache und die Vermehrung der Nucleolenanzahl von 1—2 auf 8—10 pro Kern gegenüber der Kontrolle. Ähnliche Befunde sind auch von anderen Pflanzen (z. B. *Allium cepa*- Wurzelmeristem) bekannt [8], [10], [12]. Die Vergrößerung des Zellkernvolumens ist mit einer Größenzunahme der Zellen verbunden, so daß sogenannte „Riesenzellen" wie nach Bestrahlung [20] entstehen.

Bei aufmerksamer Betrachtung von Abb. 1 ist ersichtlich, daß der konstante DNS-Gehalt der Zellkerne [30] bei den Kontrollpflanzen während des Wurzelwachstums erhalten bleibt; die geringen Schwankungen der Werte sind statistisch nicht signifikant. Die leichte Vergrößerung des Zellkernvolumens von ca. 500 auf ca. 700 μm^3 hängt demnach nicht mit dem DNS-Gehalt zusammen, sondern wird wahrscheinlich durch den veränderlichen Gehalt an Nukleoproteinen [24] und die Hydratur der Zellkerne bedingt.

Die Zunahme des DNS-Gehaltes in Abhängigkeit von der benutzten Dosis und der Behandlungsdauer mit Lindan wird durch die Blockierung der Mitosen bewirkt [12]. Dadurch nimmt die Häufigkeit der Endomitosen zu und es entstehen polyploide und aneuploide Zellkerne [15].

Aufgrund der Häufigkeitsverteilung des DNS-Gehaltes der Interphasenkerne wurde eine grobe Berechnung des prozentualen Verhältnisses einiger Phasen des Zellzyklus (G_1 = Posttelophase; S = DNS-Synthesephase; G_2 = Antephase) während des Wurzelwachstums unter Einwirkung von Lindan und Methylchlor durchgeführt (Abb. 2). Bei den Kontrollpflanzen ist mit fortschreitendem Wurzelwachstum ein zunehmender Prozentsatz S-Phasen- und polyploider Kerne festzustellen. Die Behandlung mit Lindan bewirkt wesentliche Veränderungen in der Verteilung der Zellzyklusphasen. Der Prozentsatz polyploider Kerne nimmt stark zu, während die S-Phase bedeutend abnimmt [3], [25]. Die G_2-Phase ist ebenfalls stark reduziert, während die G_1-Phasenkerne unter Einwirkung von 5 kg Lindan/ha in der II. und III. Wachstumsphase fast vollständig verschwinden. Das Ausbleiben der G_1-Phase erinnert an den Zellzyklus menschlicher und tierischer Krebszellen, bei denen G_1 ebenfalls fehlt [13]. Lindan kann deshalb als eine potentiell krebserregende Substanz angesehen werden und sollte daher verboten werden, besonders wenn man die Möglichkeit seiner Anreicherung und die Steigerung seiner Wirkung entlang der Nahrungskette in natürlichen und Agroökosystemen [31] in Betracht zieht.

Methylchlor bewirkt im Vergleich zu Lindan z.T. entgegengesetzte Effekte. Es scheint die DNS-Synthese schwach zu hemmen, ohne das Kernvolumen zu beeinflussen (Abb. 1). Diese Wirkung von Methylchlor

geht wahrscheinlich auf die Blockierung des Zellzyklus in der vor der DNS-Synthese ablaufenden G_1-Phase zurück (Abb. 2).

Die Wirkung von Lindan und Methylchlor auf den DNS-Gehalt der Zellkerne im Wurzelmeristem der Ackerbohne (*Vicia faba*) ist mit den bei Weizen erzielten Ergebnissen vergleichbar (Abb. 3). Die Zunahme des

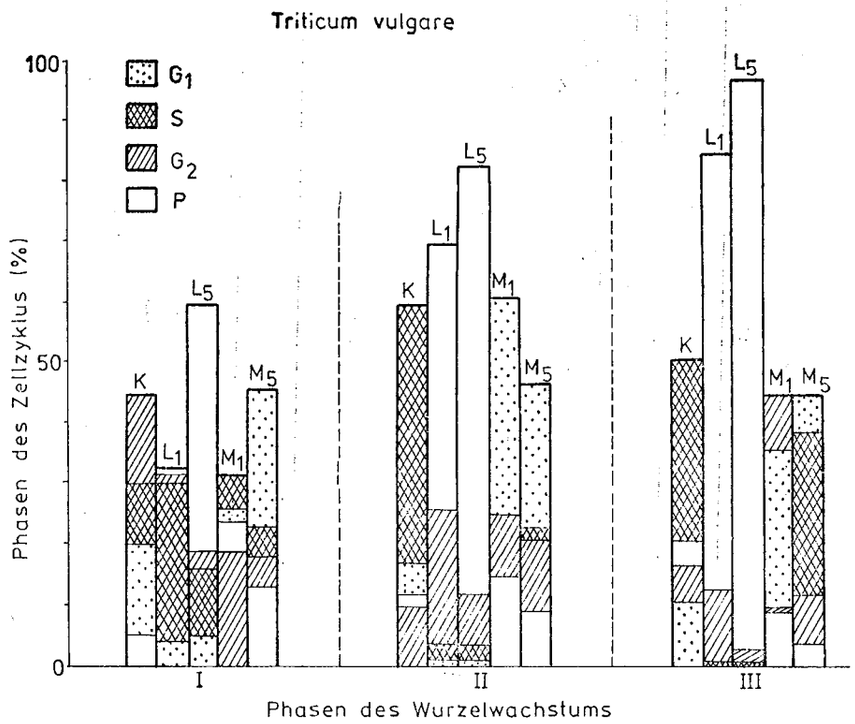

Abb. 2 — Die Verteilung (%) der Zellzyklusphasen von Ruhekernen im Wurzelmeristem von Weizen (*Triticum vulgare*) unter Einwirkung von 1 und 5 kg Lindan (L) und Methylchlor (M) pro ha. G_1 = Posttelophase; S = Synthesephase; G_2 = Antephase; P = polyploide Zellkerne. Weitere Erklärungen in Abb. 1.

DNS-Gehaltes durch Lindan (Abb. 3A) ist auf die Blockierung der Zellteilung und die dadurch bedingte Entstehung polyploider Kerne zurückzuführen (Abb. 3C). Demzufolge findet sich ein hoher Prozentsatz an G_2-und S-Phasenkernen. Dagegen fällt der Anteil der G_1-Phasenkerne wie im Weizenmeristem stark ab. Entgegen den Erwartungen, nimmt das Zellkernvolumen mit zunehmender Lindankonzentration ab (Abb. 3B), eine Wirkung, die möglicherweise mit einer Hemmung der Nukleoproteinsynthese zusammenhängt. Methylchlor hat keinen Einfluß auf den DNS-Gehalt (Abb. 3A); die festgestellte Verringerung des Kernvolumens (Abb. 3 B) ist demnach nicht mit der Hemmung der DNS-Synthese (Abb. 3C), sondern wahrscheinlich mit einer Herabsetzung der Nukleoprotein-Synthese verbunden.

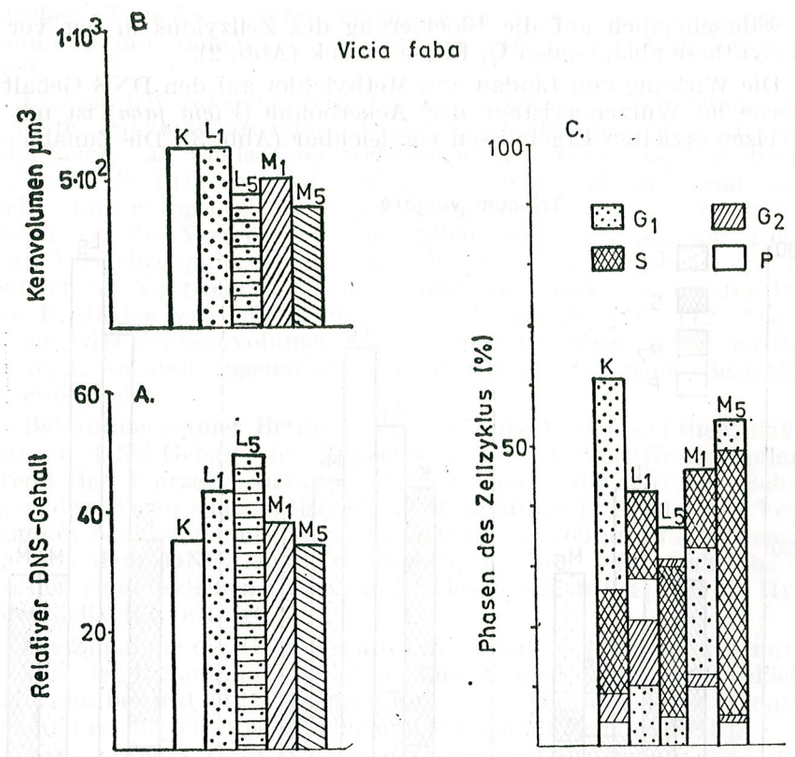

Abb. 3 — Die Wirkung von 1 und 5 kg Lindan (L) und Methylchlor (M) pro ha auf den DNS-Gehalt (A), das Zellkernvolumen (B) und den Zellzyklus (C) im Wurzelmeristem von *Vicia faba* am 4. Tag nach dem Ansetzen. Weitere Erklärungen in Abb. 2.

SCHLUSSFOLGERUNGEN

1. Lindan bewirkt eine durch Endomitosen bedingte anormale Zunahme des Kern-DNS-Gehaltes.

2. Im Weizenwurzelmeristem ist die Steigerung des DNS-Gehaltes mit einer Volumenvergrößerung der Zellkerne verbunden. Bei *Vicia faba* nimmt das Kernvolumen mit zunehmendem DNS-Gehalt unter Einwirkung steigender Lindankonzentrationen dagegen ab.

3. Methylchlor bewirkt bei Weizen eine leichte Abnahme des DNS-Gehaltes und eine Verzögerung des Zellzyklus in der G_1-Phase.

4. Lindan und Methylchlor verändern wahrscheinlich die Dauer der Zellzyklusphasen der Ruhekerne, jedoch in entgegengesetzter Richtung; während Lindan die vor der S-Phase ablaufende Posttelophase (G_1) unterdrückt, wird sie durch Methylchlor verlängert.

5. Durch die Unterdrückung der G_1-Phase ruft Lindan in der Pflanzenzelle eine Art „Krebs" hervor, was sich in der Ausbildung von Riesenkernen und Riesenzellen, sowie durch die tumorartige Anschwellung der Wurzelspitze äußert.

LITERATUR

1. Berlyn G. P., Miksche J. P., *Botanical microtechnique and cytochemistry,* Iowa State Univ. Press, Ames Iowa, 1976.
2. Boivin A., Vendrely R., Vendrely C., C. R. Acad. Sci., 1948, **226**, 1061 — 1063.
3. Borghi H., Puiseux-Dao S., Bonotto S., Hoursiangou-Neubrun D., Protoplasma, 1973, **78**, 99—112.
4. Carpentier S., Fromageot C., Biochim. Biophys. Acta, 1950, **5**, 290—296.
5. Charnetsky W. A., Lichtenstein E. P., Evert R.F., Can. J. Bot., 1973, **51**, 2111—2117.
6. Conger A., Fairchild L. M., Stain Technol., 1953, **28**, 281—283.
7. Davidson J. N., *The biochemistry of the nucleic acids,* 7th ed. Chapman and Hall and Science Paperbacks, Norfolk, 1972.
8. Fernandez-Gomez M. E., Genet. Iber., 1967, **19**, 103—121.
9. Fischer J., Inke G., Acta Morph. Acad. Sci. Hung., 1956, **7**, 141—165.
10. Gimenez M. G., Gonzales-Fernandez A., Lopez-Saez J. F., Fernandez-Gomez M. E., Phyton, 1966, **23**, 11—14.
11. Jakobj W., Roux Arch., 1925, **106**, 124—192.
12. Hervas J. P., Gimenez M. G., Cytobiologie, 1974, **9**, 233—239.
13. Hornsey S., Howard A., Ann. N. Y. Acad. Sci., 1956, **63**, 915—921.
14. Kostoff D., Nature, 1948., **162**, 845—846.
15. Kostoff D., Science, 1949, **109**, 467—468.
16. Lambrev Zh., Adzarova E., Nauchn. Tr. Plodviski Univ., 1974, **12**, 13—22.
17. Mendelsohn M.L., J. Biophys. Biochem. Cytol., 1958, **4**, 415—424.
18. Mirsky A. E., Ris H., Nature, 1949, **163**, 666—667.
19. Nybon N., Knutsson B., Hereditas, 1947, **33**, 220—234.
20. Okada S., *Radiation biochemistry,* Vol. I. *Cells.* Academic Press, New York — London, 1970.
21. Ornstein L., Lab. Invest., 1952, **1**, 250—262.
22. Patau K., Chromosoma, 1952, **5**, 341—362.
23. Plokhinskii N. A., *Biometriya.* 2—3 izd., Izd.-yo Moskovskogo Univ., Moskva, 1970.
24. Pollister A. W., Swift H., Alfert M., J. Cell. Comp. Physiol.. 1951, **38**, Suppl. 1, 101—119.
25. Puiseux-Dao S., Jeanne-Levain N., Roux F., Ribier J., Borghi H., Brun C., Protoplasma, 1977, **91**, 325—341.
26. Sass J. E., Science, 1951, **114**, 406.
27. Scholes M. E., J. Hortic. 1953, **28**, 49—67.
28. Simkover H. C., Shenefelt R. D., J. Ecol. Entomol., 1952, **45**, 11—15.
29. Simon M., Guinochat M., C.R. Hebd. Séances Mém. Soc. Biol., 1939, **131**, 222—224.
30. Wagner R. H., *Environment and man,* Norton and Comp. Inc., New York, 1971.
31. Wied G. L., *Introduction to quantitative cytochemistry,* Academic Press, New York—London, 1966.

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ULTRASTRUCTURE OF THE SCHIZOGONIACEOUS GREEN ALGA *KLEBSORMIDIUM PSEUDOSTICHOCOCCUS* (HEERING) COMB. NOVA : ORGANIZATION OF VEGETATIVE CELLS

BY

L. ŞT. PÉTERFI, N. DRAGOŞ and C. CRĂCIUN

This investigation reveals the ultrastructural cytology of *Klebsormidium pseudostichococcus*, a filamentous green alga, growing mostly as unicells. Its cellular organization resembles to some extent that of *Stichococcus minor* grown in the same conditions, but distinctly differs in the structure of pyrenoid. The vegetative cells agree ultrastructurally with those of *Klebsormidium flaccidum* and *K. substilissimum*, except for the pyrenoid which is bilenticular, traversed longitudinally by a double-thylakoid band, and provided with two large, cup-shaped starch shells. The single-membrane bounded element located between the plastid and nucleus has tentatively been identified with the microbody-like structure (peroxisome) recorded for other species.

A filamentous green alga, growing in vegetative stage mostly as unicells, very similar at first sight with species of *Stichococcus*, was studied in the electron microscope. Although the cells are very close (morphologically) to those of *Stichococcus*, they have been found to possess a more conspicuous spherical pyrenoid with a starch sheath — seen even in the light microscope. Therefore, the authors presumed that the fine structure of this alga might be of interest and possibly differing from that of *Stichococcus*. As such, the plant tentatively identified as *Hormidium pseudostichococcus* Heering [3], [6] was comparatively investigated with a strain of *Stichococcus minor* Naegeli, which was found to show similarities in many respects, when grown in the same conditions. Both algae, herein dealt with, belong to one of the most unnatural orders of the advanced green algae in which two main lines (phragmo/phycoplast) have recently been recognized with great phyletic implications [5], [9].

MATERIALS AND METHODS

Both algal species have occasionally been isolated in our laboratory from samples collected from small meso-eutrophic bogs situated near Sf. Gheorghe (*K. pseudostichococcus*) and Cluj-Napoca (*S. minor*) (Transylvania, Romania) and maintained in soil-water media (with a phosphate source added). The species employed in this paper were identified using classical methods of light microscopy. For electron microscopical observations the cells harvested by gentle centrifugation from the supernatant liquid were fixed in 4% glutaraldehyde for one hour and post-fixed for one more hour at 4°C in 2% osmium tetraoxide in phosphate buffer (pH 7.2), then embedded in Vestopal W. The ultra-thin sections, cut with a

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glass knife on LKB Ultratome, were stained in lead citrate and uranyl acetate, and examined in a BS 613 Tesla electron microscope, at 80 kW, in the Biology Department of the "Babeş-Bolyai' University, Cluj-Napoca.

OBSERVATIONS AND RESULTS

The cells are cylindrical in shape, with broadly rounded poles, having a relatively thin but conspicuous cell wall covered with a layer of slime. The sections revealed clearly that this cell wall consists of randomly arranged microfibrils, embedded in an amorphous matrix, showing a rather uniform appearance (Figs. 2—4). The mucilaginous material outside the cell wall appears as a finely fibrillar deposit resulted from the dehydration of the slime.

The single parietal laminate and unlobed chloroplast of cell length incompletely encircles the lumen and occupies 1/2—3/4 of it, as shown by cross sections (Figs. 4, 6). The lateral thickening of the chloroplast contains mostly a single, large, equatorially located pyrenoid (Figs. 2, 3, 6). Cells with more than one pyrenoid, but at the most three, have occasionally been observed (Fig. 4). The chloroplast has the usual structure recorded in less evoluted green algae, clearly limited by a double-membrane envelope, traversed by extended bands of thylakoids. The bands extending across the whole length of plastid matrix contain usually two to six closely appressed thylakoids. They may be interconnected by single thylakoids. Both longitudinal and cross sections revealed in the matrix, interpolated between the bands, starch grains and osmophilic lipid globules (Figs. 3, 6). The large, spherical pyrenoid exhibits an interesting fine structure. The electron-dense matrix is bisected longitudinally by a double-thylakoid band. The surrounding starch sheath consists of two cup-shaped shells. The boundary of the pyrenoid matrix is apparently not demarcated by a differentiated or membraneous envelope (Figs. 2—7). The whole pyrenoid is lined by a chloroplast band consisting of one or more intimately associated thylakoids (Fig. 7).

The longitudinal sections cut through the cell exhibit the central bridge of cytoplasm separating the two large single membrane-lined end vacuoles as well as the large median nucleus, two mitochondria, Golgi bodies, plenty of Golgi derived vesicles, endoplasmic reticulum and uniformly scattered ribosomes. The nucleus, containing a single nucleolus (Figs. 2, 6), limited by a nuclear envelope, located laterally, is closely pressed to the inner side of the plastid at the pyrenoid level and it is

PLATE I

Fig. 1. — Longitudinal section through a cell of *Stichococcus minor* showing the chloroplast (Chl) containing a "multiple" pyrenoid (Py), nucleus (N), mitochondrial profile (m), Golgi body (G), end vacuoles (V), as well as the cell wall (W). × 20,600.

Fig. 2. — Longitudinal section through a cell of *Klebsormidium pseudostichococcus*, showing the chloroplast (Chl) with a single bisected pyrenoid (Py) covered by two starch caps (sc), both end vacuoles (V), mitochondrial profiles (m), Golgi body (G). The microfibrillar wall (W) is covered with slime (Sl). × 20,600.

Fig. 3. — Longitudinal section through a cell of *K. pseudostichococcus*, showing the chloroplast (Chl) with three pyrenoids (Py), mitochondrial profiles (m), starch granules (sg) and end vacuoles (V) × 20,600.

PLATE I

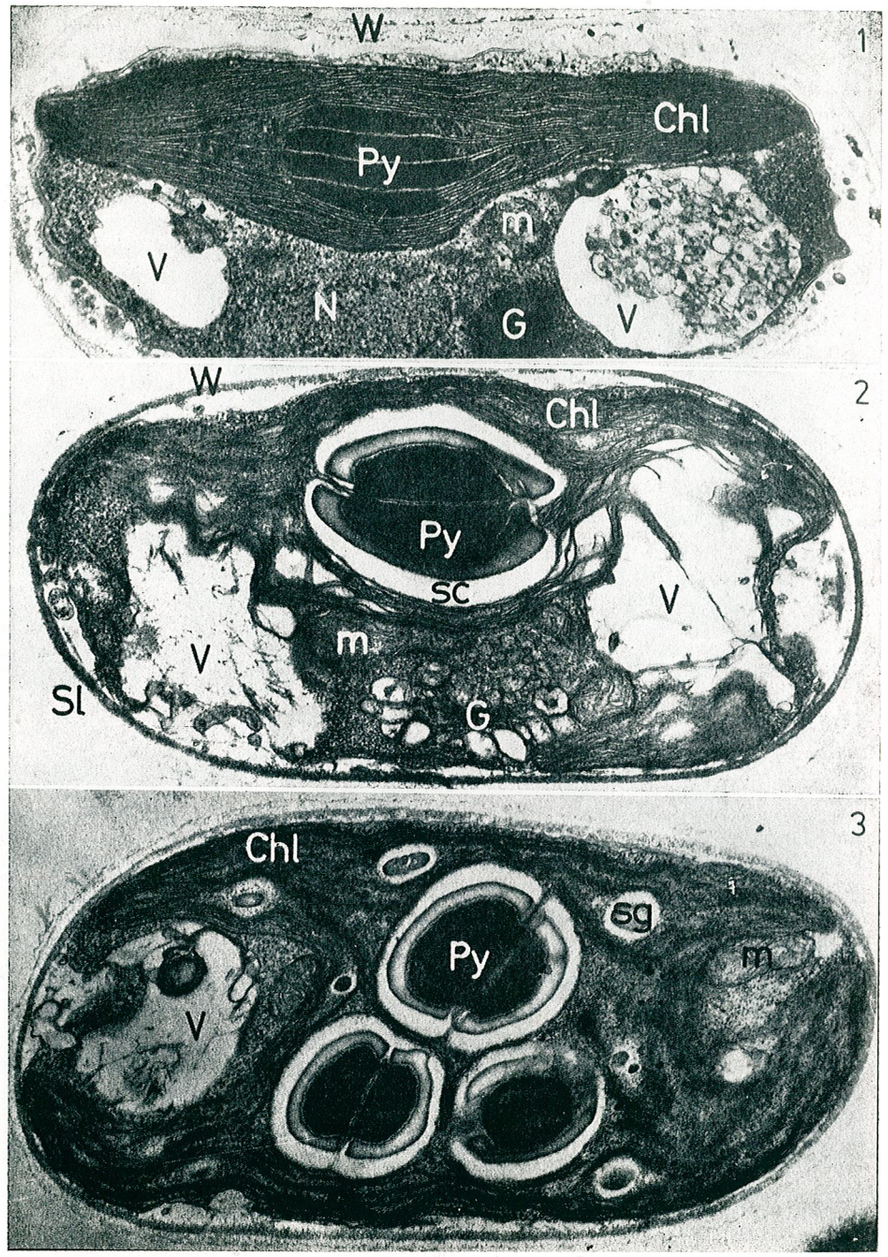
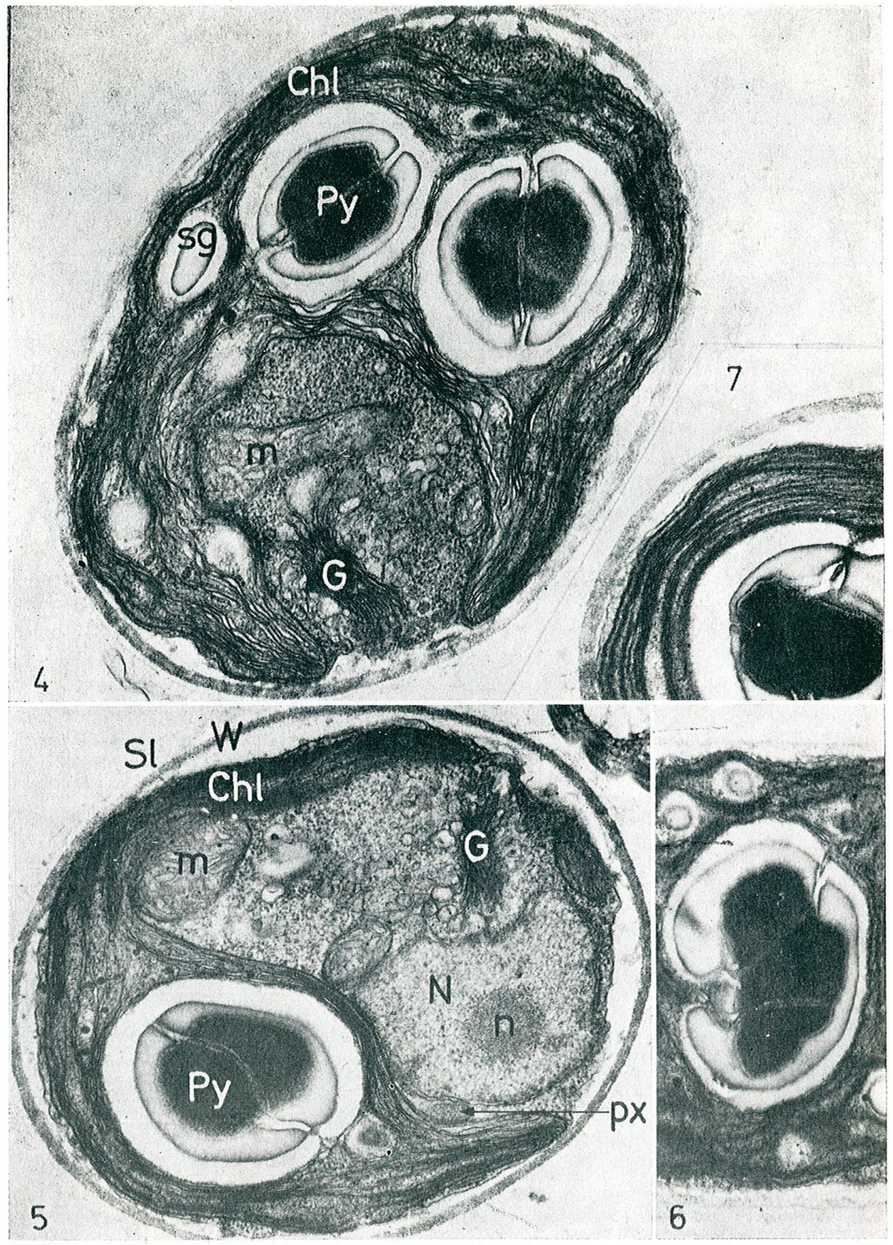


PLATE II



usually flanked by two mitochondria. In one of the cross sections, some of the microtubules situated under the plasmalemma can be seen.

A notable result is the demonstration of an elongated, single-membrane bounded inclusion, located between the chloroplast and the nucleus (Fig. 6), which morphologically fits well with that described previously in *Klebsormidium* [5], [11] as a microbody-like structure and identified as a peroxisome [11].

DISCUSSION

The investigated plant, when inspected by means of the light microscope, looks very similar with *Stichococcus minor*, except that *Klebsormidium pseudostichococcus* grows almost invariably as unicells and has a more conspicuous pyrenoid. *S. minor*, grown in similar conditions, have been found to form also short filaments. Both of these species investigated comparatively in the light and electron microscopes are very similar as concerning their cell measurements too (Figs. 1 — 3).

Ultrastructurally, the bacilliform cells of *S. minor* exhibit the organization and structure already reported [5], [7], [9], [10] and resemble quite closely those of *K. pseudostichococcus*. In both cases the cell contains a large plate-shaped plastid, two vacuoles occupying much of each end of cell, equatorial nucleus flanked by mitochondria and lacking plasmodesmata as well. However, *S. minor* differs in the structural details of chloroplast, the bands containing 2 or 3 thylakoids and having a less elaborated pyrenoid, composed of a central cluster of elongated subunits, separated by single thylakoids (Fig. 1).

It has already been stated that the pyrenoid of *K. pseudostichococcus* is of bilenticular type with two large starch cups. The fine structural differences of the chloroplast bands are less evident, though notable.

The plant (*K. pseudostichococcus*) very closely resembles other species studied in the electron microscope and referred to the schizogoniaceous *Klebsormidium*, a recently erected and defined genus [8] as a substitute for *Hormidium* Kützing. Their ultrastructural features had been described and discussed in detail by previous authors [2], [5], [9], [10], [11], including observations on mitosis, cytokinesis and structure of motile cells, in relation to other filamentous green algae. According to our findings, the ultrastructural organization of our plant agrees very much with that of the already known members of *Klebsormidium* (*K. flaccidum* and *K. subtilissimum*) except for the structure of pyrenoid. There-

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PLATE II

Fig. 4. — Slightly oblique cross section of *K. pseudostichococcus* cell, showing the chloroplast (Chl) with two pyrenoids (Py) and starch grains (sg), cell wall with slime, Golgi body (G) and mitochondrial profile (m). × 33,200.

Fig. 5. — Cross section of *K. pseudostichococcus* cell, showing the slime covered (Sl) cell wall (W), chloroplast (Chl) with pyrenoid (Py), nucleus (N) with a single nucleolus (n), mitochondrial profiles (m), Golgi body (G) and peroxisome (px). × × 28,400.

Fig. 6. — Longitudinal section through a pyrenoid of *K. pseudostichococcus*, traversed by two thylakoid doublets. × 20,600.

Fig. 7 — Detail of chloroplast bands of *K. pseudostichococcus*, × 33,200.

fore, it seems entirely justified to regard this plant as a somewhat different member of the same genus -*Klebsormidium*. It should be mentioned that the pyrenoid of *K. flaccidum* exhibits more than one traversing lamellae and is surrounded by several starch granules [2]. *K. subtilissimum* possesses a so-called "multiple" pyrenoid, divided longitudinally by thylakoid pairs, showing elongated subunits when sectioned [5]. Our plant, *K. pseudostichococcus*, has a typically bisected pyrenoid and is therefore a somewhat outstanding species of the genus.

The pyrenoid structure, over-estimated as a major criterion in the taxonomy and phylogeny of most algal groups, in filamentous green algae seems to provide useful information only at infrageneric level. This statement is firstly based on the fact, that the multiple pyrenoid has been detected in various species and genera of ulotrichalean and chaetophoracean algae, in both phycoplast and phragmoplast exhibiting ones and, therefore, belonging to different evolutive lines [5], [11], [13]. Moreover, this type of pyrenoid has also been found in non-algae, namely in hornwort (*Anthoceros*) [12]. Secondly, it has been demonstrated [13] that species of the same genus (*Pleurastrum*) possess both multiple and bisected pyrenoids. he same seems to be applicable for *Klebsormidium*. One should note that bilenticular pyrenoids, like that of *K. pseudostichococcus* widely occur in many genera of *Cladophorales* and *Siphonocladales* [4], probably unrelated to *Klebsormidium*. Young cells of *Ulothrix* [1] possess a bilenticular pyrenoid too.

There is no doubt that our conclusions are right in connection with the taxonomic position of the plant dealt with ; it belongs to *Klebsormidium* as one of its "unicellular" species and it can readily be identified with Heering's (*Hormidium*) *pseudostichococcus*. It is equally true that its valid name should be *Klebsormidium pseudostichococcus* (Heering) comb. nova (= *Hormidium pseudostichococcus* Heering 1914, in Pascher's Süsswasser-Flora, Heft 6, p. 43, figs. 52, 53 ; basionym, iconotype) and the diagnosis emended by ultrastructural details.

REFERENCES

1. Floyd, G.L., Stewart, K. D. and Mattox, K.R., J. Phycol., 1972, **8**, 68–81.
2. Floyd, G.L., Stewart, K. D. and Mattox, K.R., J. Phycol., 1972, **8**, 176–184.
3. Heering, W., *Chlorophyceae III.* in A. Pascher, *Süsswasser-Flora Deutschlands, Österreich und der Schweiz*, Heft 6, Jena, 1914.
4. Hori, T. and Ueda, R., *The fine structure of algal chloroplast and algal phylogeny.* in J. Tokida and H. Hirose, *Advance of phycology in Japan*, Fischer Verlag, Jena, 1975, 11–42.
5. Pickett-Heaps, J. D. , *Green algae. Structure, reproduction and evolution in selected genera.* Sinauer Assoc. Inc. Publ. Sunderland, Massachusetts, 1975.
6. Printz, H., Hydrobiologie, 1964, **24**, 1–376.
7. Silverberg, B. A., Trans. Amer. Microsc. Soc., 1975, **94**, 417–421.
8. Silva, P.C., Mattox, K.R. and Blackwell, W. H., Taxon, 1972, **21**, 639–645.
9. Stewart, K. D. and Mattox, K. R., Bot. Review, 1975, **41**, 104–135.
10. Stewart, K. D., Mattox, K.R. and Floyd, G.L., J. Phycol., 1973, **9**, 128–141.
11. Stewart, K.D. et al., J. cell. Biol., 1972, **54**, 431–434.
12. Wujek, D. E., Trans. Kan. Acad. Sci., 1966, **69**, 314–316.
13. Wujek, D.E., Trans. Kan. Acad. Sci., 1975, **78**, 133–137.

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EFFECT OF PROCAINE IN THE POWDERY MILDEW CONTROL WITH SOME CULTIVATED PLANTS

BY

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The procaine proved to be a substance which induced the increase of the host plant resistance against the powdery mildew attack. This resistance increase is not proportional to procaine concentration. Generally, the low concentrations of procaine (10 ppm) protected the host plants when aspersions were frequently administered. The procaine concentrations increased to 500 or 1 000 ppm, and the decreased aspersions have not directly proportional raised the anti-fungal inducing effect. Karathane, accepted as a very good chemical product in the powdery mildew control, was very efficient in the lowering of the attack, as compared to procaine. These two substances have different actions ; procaine acts as a systemic substance.

The research of the procaine biostimulating effect on plants rendered evident their stronger resistance to disease. The phenomenon was underlined in literature by Dekker J. in 1961 [4], [5], who mentioned as starting point of his research the experiments of Ark P. A. from California University. The conclusion was that procaine has a systemic action. When the leaves have been aspersed with procaine solution of 1 000 ppm concentration, the infection strongly lowered from 5 in the control to 0.5 in the treated plants.

In vitro, the procaine hydrochloride was inactive with all tested fungi and bacteria.

Niemann G. J. [8] alone or together with Dekker J. [5], [6], continues and develops this research, and in 1964 Niemann G. J. [8] works out his thesis for a doctor's degree concerning the relation between the chemical structure of procaine and its action in the powdery mildew control. The author analyses the antifungal effect of more than 100 chemical compounds related to procaine and concluded that procaine has a general antifungal effect only *in vivo* and not *in vitro* ; it has a maximum efficiency and specificity for the powdery mildew, a disease which cannot be satisfactorily controlled with common fungicides. The procaine induces a special resistance in the host plant against the pathogenic agent, the hyphae of the powdery mildew cannot penetrate the foliar tissues. This uncommon phenomenon could not be explained, it was only experimentally demonstrated [4], [5], [6], [7], [8].

This work aims to test the effect of procaine hydrochloride on the powdery mildew control in some plants, when a diluted or concentrated solution in biostimulating doses is used [1]. It was applied as repeated aspersions during the vegetation period.

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MATERIAL AND METHOD

For testing the resistance of the plants aspersed with aqueous solution of procaine hydrochloride against the powdery mildew we tried the following diseases : —the rose powdery mildew produced by *Sphaerotheca pannosa* var. rosae; — the black current powdery mildew produced by *Sphaerotheca mors*-uvae; — the strawberry plant powdery mildew caused by *Sphaerotheca macularis*.

The experiments have been carried out during 1974—1976, with procaine solutions between 1 — 1 000 ppm concentrations, depending on variants, the treatments being applied from the first symptoms of powdery mildew. The aspersions have been made by means of an AS-14 type aspersion apparatus. Each variant had 4 repetitions with 3 bushes or 5 sqm (for the strawberry plant). To observe the efficiency of procaine aspersions were also administered aspersions with 25 WP Karathane solution (a homologated product used in the powdery mildew control). For a probable synergetic effect a combined variant of procaine +Karathane was set up. All the variants have been related to a lot of control plants (untreated).

The efficiency of the applied substances was observed after each treatment as well as in different stages; the frequency ($F\%$) and intensity ($I\%$) of the attack were registered and the attack degree (AD%) was reckoned both in leaves and shoots.

As for each plant the experiment contained special elements, the results will be separately presented for each experimental category.

RESULTS AND DISCUSSIONS

1. For the control of the *rose powdery mildew* two experiments have been carried out : a) on the "Super Star" sort, cultivated for flowers, a 4 years old plantation, and b) on "Baccara" sort, sensitive to this disease.

a) With the "Super Star" sort the procaine aspersions were : V_1—10 ppm conc.; V_2 — 10 ppm procaine mixed with 200 ppm Karathane; V_3 — 1 000 ppm concentrated Karathane (corresponding to the phytosanitary treatments), and V_0 — the untreated control lot. The first symptoms of powdery mildew have been observed at the beginning of June, when the first aspersion was applied, followed by other aspersions at intervals of 1 — 2 weeks till the end of August (7 aspersions in all). The results are shown in table 1. It is obvious that, as compared to the control, the infection in the plants aspersed with 10 ppm concentrated procaine was considerably reduced, both in leaves and in offsprings, the effect being maintained till the end of the summer.

With the control the attack on the leaves on 13 VII was of 20%, with the procaine aspersed lot it was of 0.85%, and in the one treated with Karathane of 0.22% (solution 100 times more concentrated than the procaine one).

b) The second experiment on roses began in June, with treatments 4 times repeated at intervals of 8,10,11 days (depending on necessity)

till the month of August. The concentration of the procaine solution was increased as follows : V_1 — 500 ppm; V_2 — 1 000 ppm; V_3 — 500 ppm procaine + 500 ppm Karathane-mixture of equal parts; V_4 — 700 ppm

Table 1

Procaine effect of the rose powdery mildew control on "Super Star" sort

Variant substance	Concentration	Powdery mildew attack (AD%)			
		in 13 VII 1974		in 30 VIII 1974	
		on leaves	on offshoots	on leaves	on offshoots
V_0 — Control	—	20.00	3.71	57.19	13.26
V_1 — Procaine	10 ppm	0.85	0.02	22.72	2.65
V_2 — Procaine + Karathane	10 ppm + 200ppm	2.10	0.22	21.95	2.75
V_3 — Karathane	1000 ppm	0.22	0.00	16.98	1.82

Karathane; V_0 — the control (untreated). The results are presented in table 2. Table 2 shows that also in this case the procaine solution lowered the powdery mildew infection, especially in the first two weeks of appli-

Table 2

Procaine effect of the rose powdery mildew control on "Baccara" sort

Variant substance	Concentration	Powdery mildew attack (AD%)				
		4 VI	13 VI	18 VI	25 VI	5V II
V_0—Control	—	24.32	36.90	55.25	62.56	71.25
V_1—Procaine	500 ppm	4.86	22.75	51.66	60.30	59.76
V_2—Procaine	1000 ppm	4.82	13.05	38.19	43.48	41.60
V_3—Procaine + Karathane	500 ppm+ 500 ppm	1.85	6.08	11.52	7.26	6.82
V_4—Karathane	700 ppm	1.08	5.60	5.75	6.72	2.21

cation : afterwards, the anti-fungal effect of the substance weakened. With a 1 000 ppm concentration the infection decreased at about 50% as compared to the control. The procaine and Karathane mixture had good results, close to the ones obtained only with Karathane solutions. Out of the two experiments on roses we observe that the "Baccara" sort is less receptive to the antifungal effect of procaine, though we must underline that only 4 aspersions were administered, but with higher concentrations of procaine.

2. Powdery mildew control in *black current*, the "Big Black" sort, a two-year plantation. Two experiments have been organized (the data of 1974 are shown in table 3, and of 1976 in table 4). The procaine concentrations were : V_1 — 10 ppm; V_2 — 10 ppm procaine + 200 ppm Karathane, equal mixture; V_3 — 1 000 ppm Karathane; V_0 — control (untreated).

The anti-fungal effect of the procaine solution is underlined by the important diminishing of the infection.

In 1976 the experiments have been organized taking into account a higher concentration of procaine and more reduced aspersions. Also in this case the aspersions with procaine (table 4) considerably lowered

Table 3

Procaine effect in the American powdery mildew control on black current, the "Big Black" sort (1974)

Variant substance	Concentration	Powdery mildew attack (AD %)			
		in 14 VII		in 29 VIII	
		on leaves	on offshoots	on leaves	on offshoots
V_0 — Control	—	51.35	2.05	56.85	1.44
V_1 — Procaine	10 ppm	27.31	0.91	20.57	0.68
V_2 — Procaine 10 ppm + Karathane	200 ppm	10.36	0.04	10.97	1.70
V_3 — Karathane	1 000 ppm	2.30	0.00	4.55	0.10

Table 4

Procaine effect in the American powdery mildew control on black current, the "Big Black" (1976)

Variant substance	Concentration	Powdery mildew attack (AD %)			
		in 30 IV	in 14 VII	in 3 VIII	in 15 IX
V_0 — Control	—	6.98	14.15	53.13	23.52
V_1 — Procaine	500 ppm	0.29	0.84	20.00	13.87
V_2 — Procaine	1000 ppm	0.58	1.86	21.91	8.63
V_3 — Procaine + Karathane	500 ppm + 500 ppm	0.15	0.05	0.63	0.36
V_4 — Karathane	1000 ppm	0.05	0.03	0.04	0.05

the powdery mildew attack, but the figures are inferior to those obtained with Karathane. The procaine and Karathane mixture gave intermediate results as compared to each substance tested singly.

3. Powdery mildew control in the *strawberry plants*. The experimental lots have been : V_1 — 500 ppm ; V_2 — 1 000 ppm ; V_3 — 1 000 ppm Karathane, and V_0 — control (untreated). The data are figured in table 5.

Table 5

Procaine effect in powdery mildew control on strawberry. (1976)

Variant substance	Concentration	Powdery mildew attack (AD %)			
		in 16 VI	in 8 VII	in 7 VIII	in 24 VIII
V_0 — Control	—	17.79	14.22	27.70	37.42
V_1 — Procaine	500 ppm	1.92	2.13	9.92	4.91
V_2 — Procaine	1000 ppm	1.33	1.25	7.47	6.14
V_3 — Karathane	1000 ppm	1.54	0.59	0.02	0.00

The attack was much diminished with procaine aspersed plants. If the infection in the control was of 37.42%, at the end of the experiment (24 VIII) the values were between 5 — 6%.

The research rendered evident the following conclusions :

— the procaine proved to be a substance which induced the increase of the host plant resistance against the powdery mildew attack. This resistance increase is not proportional to procaine concentration. Generally, the low concentrations of procaine (10 ppm) protected the host plants when aspersions were frequently administered. The procaine concentrations increased to 500 or 1 000 ppm, and decreased aspersions have not directly proportional raised the anti-fungal inducing effect :

— Karathane, accepted as a very good chemical product in the powdery mildew contol, was very efficient in the lowering of the attack, as compared to procaine.

We think that the two substances have different actions, both in the host plant and the fungus. The procaine actions as a systemic substance, while Karathane annihilates the fungus mycelium, that is to say its hyphae are destroyed by Karathane particles.

Though the mechanism through which procaine induces the host plant increasing resistance against the powdery mildew attack is not yet known, it has been proved that only aspersions on the leaves, without radicular administration, lowered the infection considerably.

The experiments on vegetal tissue cultures [2], [3] pointed out that procaine has its part in the regulation of peroxidase activation, but peroxidases in their turn contribute to the natural immunity of the plant. So, we presume that procaine which penetrates the host plant determines an increase of its immunity against the parasite fungus attack. The anti-powdery mildew character of procaine may be explained by the stimulation of a certain metabolic product in the treated leaves, with an antifungal effect.

REFERENCES

1. Cachiță-Cosma D., *et al.*, Brevet de Invenție R.S.R., nr. 60198.
2. Cachiță-Cosma D., Gaspar T., Negruțiu I., and Jacobs M., Med. Fac. Landbouuw. suniv. Gent. 1976, **41**, *2*, p. 1043—1048.
3. Cachiță-Cosma D., Jacobs M., Negruțiu I., Gaspar T. Med. Fac. Landbouuw Rijksuniv. Gent. 1976, **41**, 2, p. 1521—1526.
4. Dekker J., T. Pl.-ziekten, 1961, **67**, p. 25—27.
5. Dekker J., *Overdruk uit de mededelingen van de landbouwhogeschool en de opzoekingsstations van de staat te Gent.* 1961, Deel XXVI, *3*, p. 1378—1384.
6. Niemann G. J., and Dekker, J., Neth. J. Pl. Path., 1966, **72**, p. 213—221.
7. Niemann G.J., and Dekker J., Ann. appl. Biol., 1966, **57**, p. 53—58.
8. Niemann G. I., *Investigations on the chemical control of powdery mildew*, 1964 (Thesis for a doctor's degree), Utracht (Nederland).
9. Székely I., Intern. Plant. Pat. Congr., III. Chem. Control, part II. 1975. Moscow, p. 653—655.

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NARCISSUS YELLOW STRIPE, A NEW VIRUS DISEASE IN ROMANIA

BY

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Data in connection with Narcissus Yellow Stripe Virus (NYSV), occurring in Romania, are presented. The symptoms of diseased narcissi in the field as well as the reaction of herbaceous host plants, mechanically inoculated for virus identification, are described. The causal agent of the disease, isolated and purified, appears at electron microscope as flexous virus-like particles of 650 — 750 nm in size. Recommendations for control of this virus disease in the Romanian commercial narcissus crops are also given.

Due to it ornamental value, narcissus is one of the flowers cultivated in numerous countries. Among the characteristic diseases of narcissi, those caused by virus infections were described, for the first time, in Holland and Great Britain [3], [4], [5], [6], [17], [18] and afterwards in East Germany [13], Soviet Union [16], Japan [9] and Czechoslovakia [12].

Four kinds of symptoms induced by specific viruses were detected, until now, in diseased narcissi plants such as : *narcissus mosaic*, *narcissus yellow stripe*, *narcissus silver leaf* and *narcissus necrotic stripe* [2], but five other soil-borne viruses have also been found in narcissi, e.g. : "tobacco rattle", "arabis mosaic", "tomato black ring", "raspberry ring spot", and "strawberry ring spot" [2], as well as "tobacco mosaic virus" [12], but neither the symptoms caused by them nor their effect on the yield are known.

As until now no virus disease of narcissi has been described in Romania, our paper presents some data on *Narcissus Yellow Stripe Virus* (NYSV), detected by us in the garden of the Institute of Agronomy in Bucharest.

MATERIALS AND METHODS

The source of virus was represented by naturally infected narcissus leaves with yellow stripe symptoms, harvested either directly from the field, in April—May, or from potted infected bulbs in the greenhouse.

For virus identification, mechanical inoculations with the inoculum obtained by grinding the infected narcissus leaves in 0.065 M, pH—7.2 phosphate buffer, containing 0.06 M 2-mercapto-ethanol, were made on the following herbaceous plants : *Cucumis sativus* L., *Chenopodium amaranticolor* Coste et Reyn., *C. murale*, L., *C. foetidum* Schrad., *C. quinoa* Willd., *Nicotiana clevelandii* Gray., *N. megalosiphon* Heurck et Muell and *Ocimum basilicum* L.

Electronmicroscopic investigations of the virus particles were carried out on the samples obtained by both chemical purification with

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10% polyethylene glycol (PEG) [8] and the dip method [1]. The specimens were negative stained with 1% uranyl acetate and examined in a TESLA BS-500 electron microscope.

RESULTS AND DISCUSSIONS

The main symptoms of the diseased plants are yellow stripes on the leaves (Pl.I, 1), clearly distinguished in April—May; occasionally, on some diseased plants, curling and distortion of leaf lamina can be observed (Pl. I, 2). The infected plants dry earlier, so that a narcissus culture has a typical aspect: among healthy green plants, small areas with dried plants can be seen.

Virus infection has also an unfavourable effect on flowers, which are either smaller than the healthy ones, or if infected fail to bloom.

The symptoms of disease are similar to those described in literature for NYSV, undoubtedly the most harmful pathogen of narcissi [2] [11].

The biological tests corroborated with the electronmicroscopic investigations provided data about the identity of this virus.

The reactions of herbaceous plants, inoculated with the sap from infected narcissi, are presented in table 1.

Table 1

Reactions of the herbaceous host plants inoculated mechanically

No	Host plan ts	Infected / Inoculated	Reaction
1	2	3	4
1	*Cucumis sativus*	0/10	—
2	*Chenopodium amaranticolor*	10/15	+
3	*C. murale*	10/15	+
4	*C. foetidum*	0/10	—
5	*C. quinoa*	3/10	+
6	*Nicotiana clevelandii*	7/10	+
7	*N. megalosiphon*	10/15	+
8	*Ocimum basilicum*	10/15	+

The biological tests pointed out that the following types of symptoms appear in herbaceous infected plants: chlorotic and necrotic spots on the leaves of *C. amaranticolor*, *C. murale*, *C. quinoa*, *N. clevelandii*, *N. megalosiphon* (Pl. I, 3, 4, 5, 6), and necrotic lesions followed by systemic infections on *Ocimum basilicum* (Pl. I, 7), symptoms corresponding to those induced by NYSV [11].

PLATE I. — Symptoms of virus infection on narcissus: yellow stripes *(1)* and curl-leaf and distortion *(2)*, on naturally infected leaves.
Reactions of the herbaceous host plants mechanically inoculated with sap extracted from narcissus leaves: chlorotic and necrotic spots on *Chenopodium murale (3)*, *C. amaranticolor (4)*, *C. quinoa (5)*, *Nicotiana megalosiphon (6)*, and necrotic lesions followed by systemic infection on leaves of *Ocimum basilicum (7)*.
Electron microscopy of *Narcissus Yellow Stripe Virus* particles, isolated from natural infected narcissus leaves by the dip method *(8)*.

PLATE I

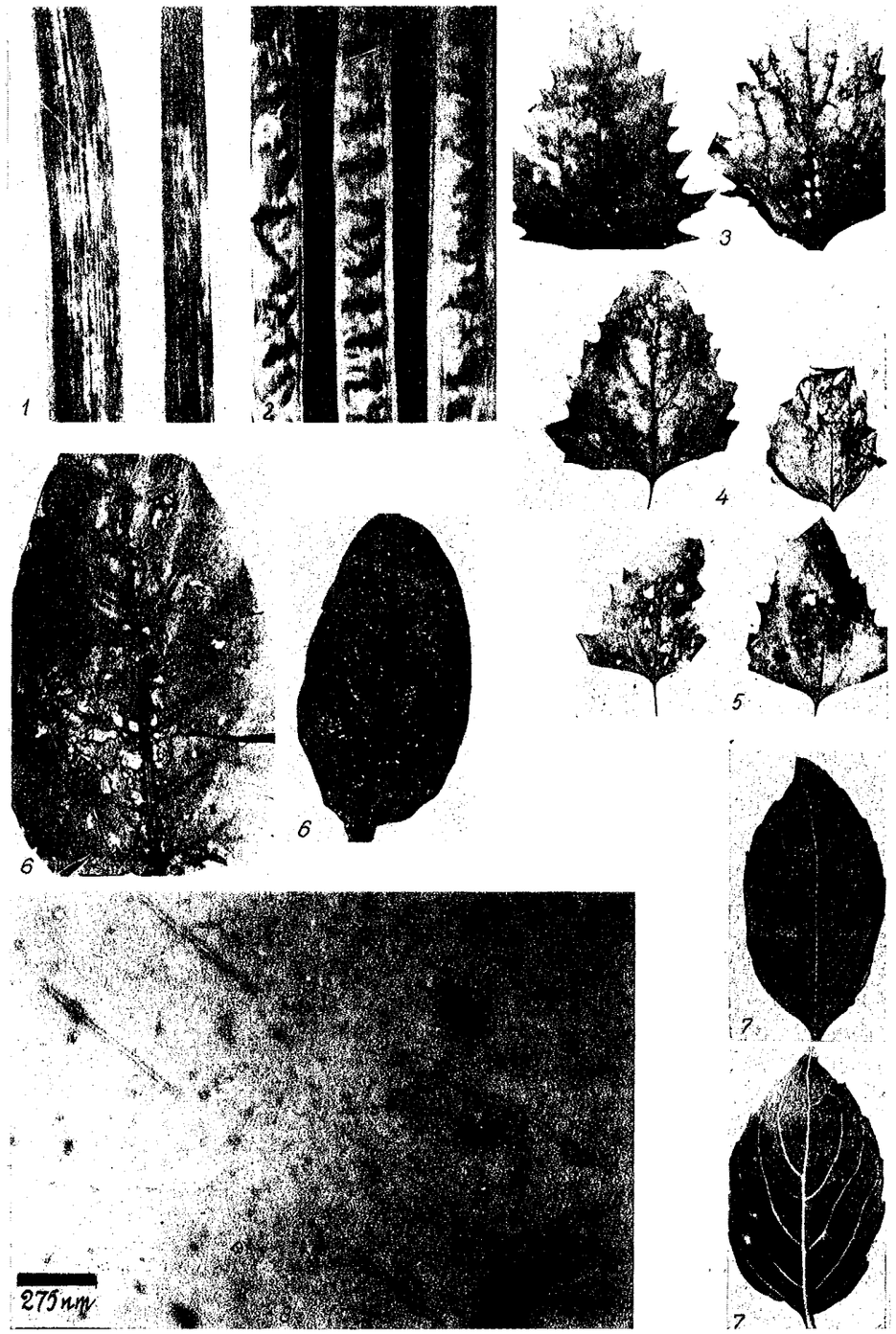
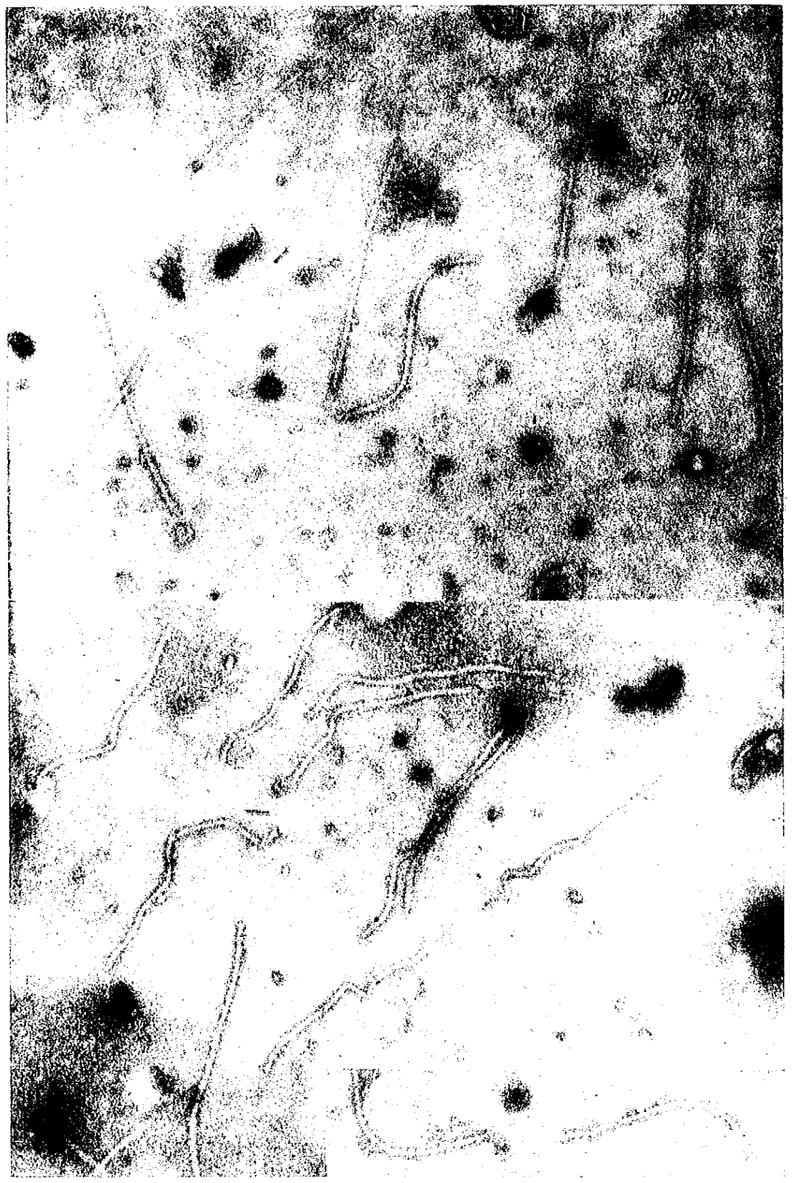


PLATE II



Electron microscopy of the virus particles purified by the chemical method with PEG from *Nicotiana megalosiphon* leaves, experimentally inoculated with *Yellow Stripe Virus* of narcissus.

The presence of this virus disease in our commercial narcissus crops causes considerable economic losses and raises the problem of their protection.The most important methods of control include the application of preventive measures and negative clonal selection [2] : a severe control of the commercial crops, especially during April—May, and the gathering and burning of all plants showing virus-like symptoms. All bulbs, harvested for the purpose of establishing new crops have to come from healthy plants only, and the new plantations should be situated at a minimum distance of 100 m from the old ones.

Because the aphids are the vectors of NYSV [2] it is necessary to control them. Finally, bulbs should not be planted in the soil suspected to be infected with soil-borne viruses.

All the protection measures mentioned should be completed, in the future, by the use of the narcissus plants freed of virus infections either by meristem culture [14], [19], or by heat therapy, although the latter did not give satisfactory results [10].

REFERENCES

1. Brandes J., Mittel. Biol. Bundesanst. f. Land. -u. Forstw., Berlin-Dahlem, 1964, **110**, 130.
2. Broadbent L., Green D. E., Walker P., Daffodil and Tulip Year Book, R. Hort. Soc., London, 1963, 1—7.
3. Brunt A., Ann. Appl. Biol., 1966, **58**, *1*, 13—23.
4. Brunt A., *First Int. Symp. Flowerbulbs* (abstr.), Noordwijk—Lisse, 1970, **2**, 23.
5. Caldwell G., Nature (London), 1946, **158**, *4021*, 735—736.
6. Cremer M.C., Veken J.A., Neth. J. Pl. Pathol., 1964, **70**, 105—113.
7. Hansen H. P., *Contribution to the Systematic Plant Virology*, Ed. D.S. R. Forlag, Kopenhavn, 1970.
8. Hebert T.T., Phytopath., 1963, **53**, *3*, 362—363.
9. Iwaki M., Komuro Y., Ann. Phytopath. Soc. Jap., 1970, **36**, 81—86.
10. Kassanis B., Adv. Virus Res., 1957, **4**, 221—224.
11. Klinkovschi M., *Pflanzl. Virol.*, Akad. Verlag, Berlin, 1968, **II**, *2*.
12. Mokrá V., *Plant Virology* (Bratislava), 1973, 57—62.
13. Oertel C., Arch. f. Gartenb., 1962, **2**, 88—98.
14. Paludan, N., Tidssk. Plantv., 1971, **75**, 387—410.
15. Procenko A.E., *Morfologhia i klasificatia fitopat. virusov*. Akad. Nauk SSSR, Izd "Nauka", 1966, 138.
16. Procenko A.E., Procenko E.P., *Plant Virology* (Prague), 1962, 241—245.
17. Slogteren, van D.H.M., Ouboter, M.P., Meded. Landbhogesch. Wageningen 1940, **45**, 3.
18. Slogteren, van D.H.M., Ouboter M.P., Daffodil and Tulip Year Book, R. Hort. Soc., London, 1946, 25—26.
19. Stone O.M., *Second Symp. Virus Dis. Ornam. Pl.* (abstr.), Lyngby, 1968, 29.

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ELECTRON-MICROSCOPIC STUDIES ON *CHENOPODIUM MURALE* L. PLANTS INFECTED WITH A VIRUS FROM APPLE TREES

BY

MARIA NICOLAESCU and H. TIŢU

The report presents data on the location of spherical virus isolated on *Chenopodium murale* L. from mosaic infected apple trees. The changes occurring in the infection process at the level of cell organites are also described.

Ultrastructure studies carried out on virus infected plant cells provided interesting data on the location of the virus particles as well as on the "de novo" formations appearing in the cells, that are characteristic of infections induced by a certain type of virus particles, irrespective of the host plant from which they were obtained [2], [5].

New data were also obtained with regard to the changes induced by the infection process at the level of cell organites, in particular on the chloroplasts, mitochondria and endoplasmic reticulum [3].

The present studies were carried out with a spherical virus, isolated from apple trees with mosaic symptoms on *Chenopodium murale* with a view to detecting the location of the virus in the cells and to establishing the elements characteristic of the infection with this virus.

MATERIALS AND METHODS

The materials studied were collected from *Chenopodium murale* plants infected mechanically with a virus isolated from apple trees (cvar. Ionathan) showing mosiac symptoms [6]. Small fragments of the infected leaves, 1 — 2 mm in size, were fixed in glutaraldehyde 6%, in phosphate buffer pH = 7,4 at 4°C and finally in osmium tetraoxide, in the same buffer solution, for 6 hours. Dehydration was made in alcohol and acetone, followed by the inclusion of the samples in EPON 812. The ultrathin sections were cut with a TESLA ultramicrotome, stained with uranyl acetate 2% and lead citrate and then examined at the JEM 7 electron microscope.

RESULTS AND DISCUSSIONS

In the plant cells infected by apple mosaic virus the examination of the ultrathin sections revealed the presence of characteristic crystalline bodies in which the virus particles are distributed according to a specific arrangement, conferring them the aspect of crystalline network.

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They follow an almost even geometric arrangement and appear as evenly coloured isometric bodies (Pl. I, fig. 1). As already known, the virus invades gradually the plant cells, ultimately aggregates of virus crystals being formed, characteristic of the infections induced by icosohedral viruses [8]. They are formed only at the moment at which the virus particles reach a high concentration in the host plant, this being also reported for the virus isolated from apple trees on *Chenopodium murale* [7].

The presence of such aggregates plays an important role in establishing an accurate diagnosis of the infections induced by such viruses, since it avoids any confusion with ribosomes [1], which otherwise were very numerous in our preparations too (Pl. I, Fig. 2). The role played by ribosomes in protein synthesis is well known, but they never present crystalline bodies like those detected in virus particles [10].

Studies carried out on viruses with a similar morphology and size showed that the new elements appearing in the infected cells are edifying for diagnosis, although they do not form characteristic filamentous elements in the infections induced by this virus [4], [9].

Changes in the cell organites, in particular at the chloroplast level were detected in the same sections examined by us. In the infected cells they appear slightly rounded, presenting a clear disorganization of the characteristic structures, in the form of distensions of thylakoids; some of the grama appear as compact masses and thus the limit between thylakoids is indistinguishable (P. II, Fig. 3). The above mentioned elements are specific only to infected cells, since they are not present in the normal structure of the chloroplasts (Pl. II, Fig. 4).

The appearance of "de novo" elements as a result of the infection process was not detected in the preparations examined by us, the only specific elements to apple mosiac virus infections being the "crystalline aggregates" of virus particles and the change occurring at the chloroplast level.

REFERENCES

1. Allen T.C., Virology, 1972, **47**, 467–474.
2. Arnott H.J., Smith K.M., J. Ultrastruct. Res., 1967, **19**, 173–195.
3. Krass C.J., Ford R.E., Phytopath., 1969, **59**, *4*, 431–439.
4. Lafleche D., Bové J. M., Progress in Photosynthesis Research, 1969, **1**, 74–83.
5. Lee Y. S., Ross J.P., Phytopath., 1972, **62**, *8*, 839–845.
6. Nicolaescu Maria, St. şi Cercet. Biol. — Bot., 1972, **24**, *6*, 533–536.
7. Nicolaescu Maria, *Cercetări privind mozaicul mărului în România*, (Thesis). Bucureşti, 1976.
8. Robleda S.C., Phytopath. Z., 1973, **78**, 134–140.
9. Ushiama R., Mathews R.E.F., Virology, 1970, **42**, 293–303.
10. Weintraub M., Ragetli H.V.J., Canadian Journal of Botany, 1973, **51**, *5*, 855–861.

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PLATE I

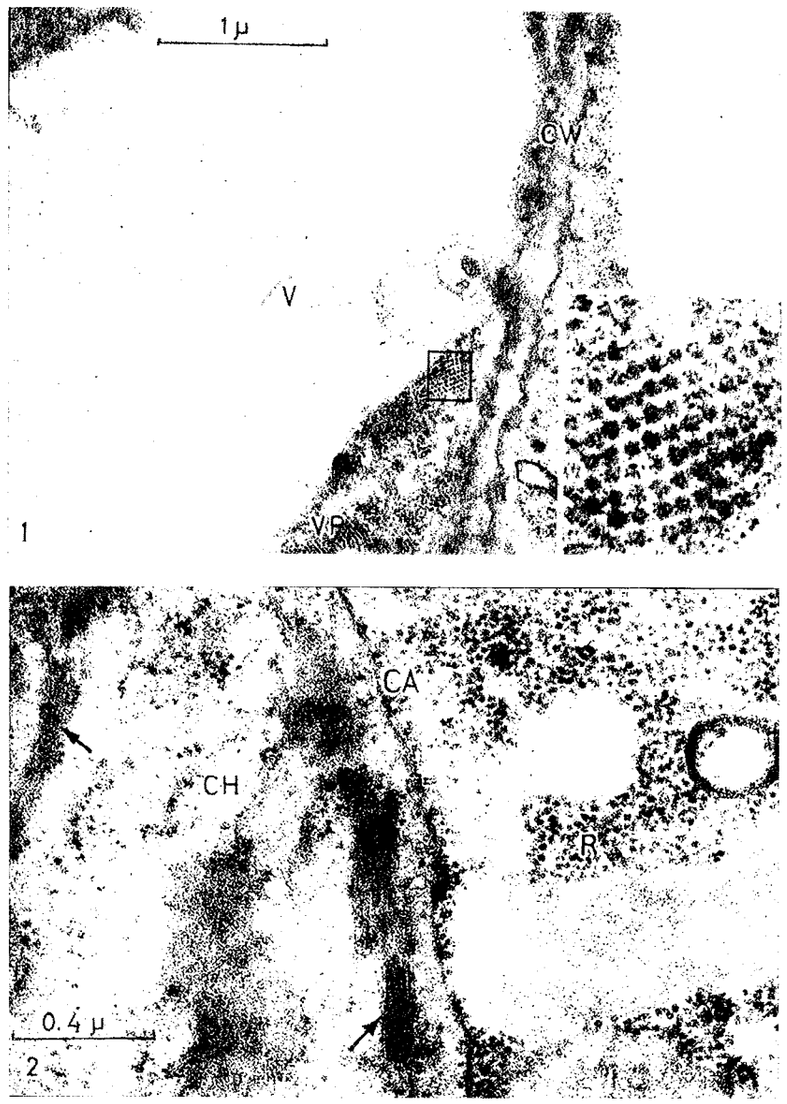
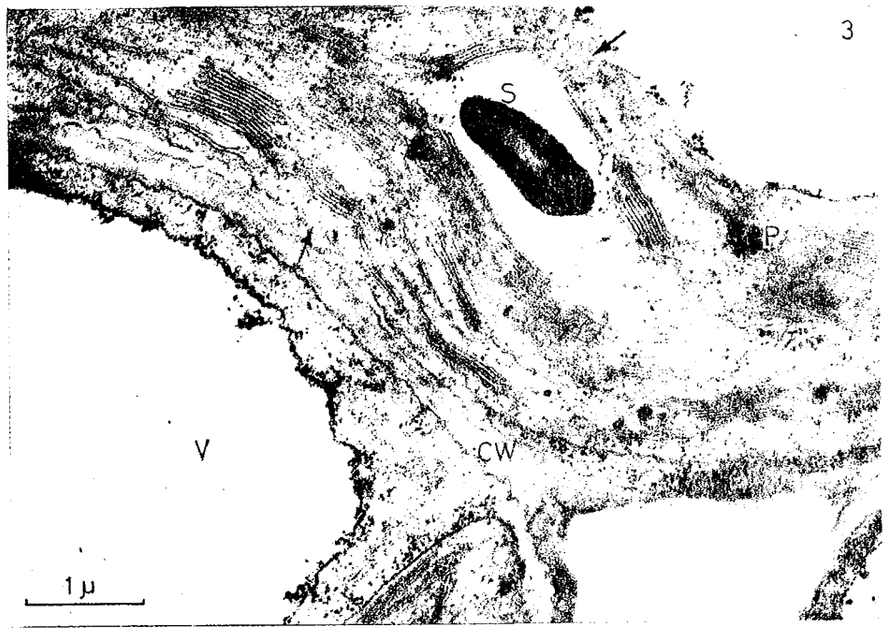


Fig. 1. — Virus particles in the peripheral cytoplasm of cells from *Chenopodium murale* L. infected with apple mosaic virus.
Fig. 2. — Strongly disorganized chloroplast and many ribosoms in the infected cell.
Abbreviations: V, vacuole, VP, virus particles; CW, cell wall; CH, chloroplast; R, ribosomes; CA, chloroplast envelope.



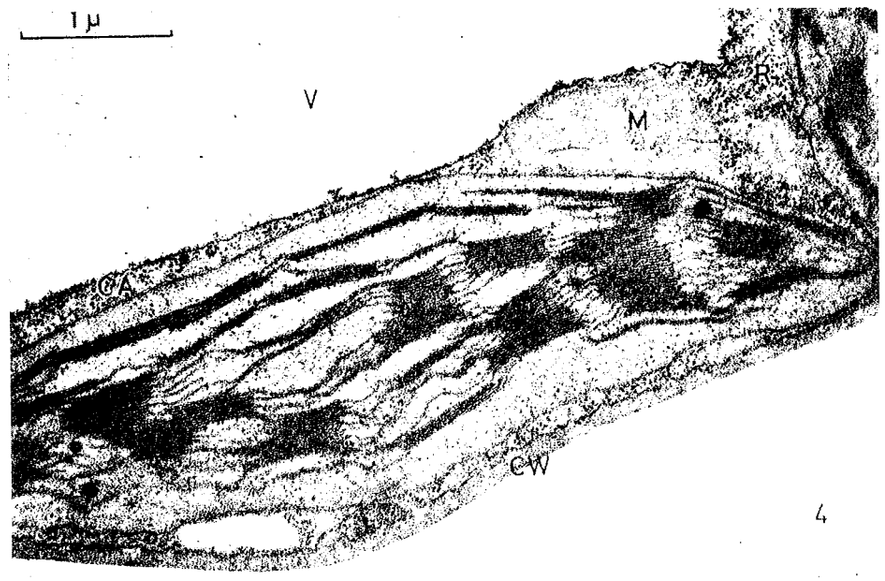


Fig. 3. — Ultrastructure of chloroplasts in an infected cell of *Chenopodium murale*.
Fig. 4. — Ultrastructure of chloroplasts in a normal cell.
Abbreviations : S, starch ; P, plastoglobules ; M, mitochondria.

ALEXANDRU BELDIE, *Flora României — Determinator ilustrat al plantelor vasculare*, I, Editura Academiei Republicii Socialiste România, 1977, 1 — 412.

Die „Flora Rumäniens" ist eine Arbeit, welche vornehmlich den Reichtum der Pflanzenwelt Rumäniens betrifft und sich größtenteils auf eine vertiefte Kenntnis der Pflanzen aus Rumänien stützt.

Es ist eine gelungene zusammenfassende Synthese und zugleich eine natürliche Vervollkommnung seiner langjährigen floristischen und geobotanischen Untersuchungen im Gebiete.

Das Buch kann mit Erfolg für didaktische Zwecke benützt werden. Es ist klar und enthält die wichtigsten Elemente taxonomischer, diagnostischer, ökologischer und chorologischer Ergebnisse der höheren Pflanzen mit ungefähr 3567 Taxonen aus dem ganzen Lande. Zugleich wird die Vegetationsdauer, die biologische Form, das Aussehen und die Blütezeit, sowie die Häufigkeit der Art im Land als auch die Gegenwart derselben in den verschiedenen Phytozönosen, angegeben. Es werden auch die jeweiligen Höhenschichten angegeben, wie das Vorkommen im Niederland, im Hügelbereich und in der montanen, subalpinen und alpinen Schicht, wobei letztere in eine niedere und höhere alpine Stufe geteilt ist. Das Buch behandelt auch die Verbreitung der Pflanzen in der Zone der Steppe, des Waldes und in der alpinen Zone mit der verhältnismäßigen Unterteilung in eine Unterzone der Waldsteppe, der Eiche und Stieleiche, der Buche und der Fichte.

Der Verfasser gibt auch den Sättigungsgrad an Alkalien des Bodens an, indem er eubasische, mesobasische, oligobasische Böden unterscheidet ; die geringste Reaktion in den höheren Schichten, können, wie er angibt, saure, genügend saure, schwachsaure, neutrale und alkaline Böden sein.

In diesem Bestimmungsbuch wird auch der Anspruch der Pflanzen an Licht erwähnt, indem konventionelle Zeichen und ihr allgemeiner ökologischer Charakter als eu-, meso-, oligo- und euritrophe Art angesehen, verwendet werden. Der Verfasser unterscheidet, wie es auch selbstverständlich ist : nitrophile und halophile Arten. Er vermeidet auch nicht die Existenz der erwähnten Arten gegenüber der Feuchtigkeit des Bodens und teilt die Pflanzen in folgende Kategorien ein : in Xerophyten, Mesoxerophyten, Mesophyten, Hygrophyten und Euriphyten.

Auf diese Weise wird in der Spezialliteratur eine Lücke ergänzt und kann so als ein praktisches Bestimmungsbuch, das leicht gehandhabt werden kann, betrachtet werden.

Wir können jedoch nicht verneinen ähnliche ältere Kompendien zu haben, doch ist die Darstellung dieses Bestimmungsbuches vervollkommnet und den jetzigen Verhältnissen entsprechend. Der Verfasser, der heute als einer der besten Kenner der rumänischen Flora betrachtet werden kann, macht dies mit großer Sorgfalt. Er ist zugleich einer der sehr tätigen Botanikern, der auch ein eifriger Mitarbeiter des großen Werkes der „Flora der Sozialistischen Republik Rumänien" ist, nämlich eine wertvolle Sammlung die von der Akademie unseres Landes herausgegeben wird.

Auf jeden Fall wird in dieser Arbeit, mit den nötigen Erklärungen für jedwelche Pflanzenkategorie, auch der Wert der Pflanze in der Natur, besonders aber in der Ökonomie des Menschens, angegeben. Wir finden hier auch eine Fülle von wertvollen informativen Angaben.

Es muß auch erwähnt werden, daß dieser Band genügend und gut illustriert ist, welcher Umstand den Gebrauch desselben erfolgreich und viel erleichtert. In seiner Abfassung kann dieser Band als fast einzige Arbeit angesehen werden.

Die mit großer Sorgfalt und besonderem Anspruch seitens des Verfassers, erschienene Ausarbeitung des Bandes empfiehlt sich von selbst, sowohl den Botanikern als auch den Liebhabern der Natur, als Nachschlagewerk zu einer besseren Kenntnis über den Reichtum der rumänischen Pflanzenwelt zu haben.

Ion T. Tarnavschi

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AVIS AUX AUTEURS

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

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

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

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

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

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