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REVUE ROUMAINE DE BIOLOGIE

SÉRIE DE BIOLOGIE VÉGÉTALE

TOME 25 1980 N° 1

janvier—juin

SOMMAIRE

PHYTOZÖNOLOGISCHE BETRACHTUNGEN ÜBER DIE FICHTENWÄLDER DES RARĂU-GEBIRGES

VON

P. RACLARU

This paper refers to the association *Piceetum carpaticum* Soó 1930, in the Rarău Mountains, understood in a wider meaning, within which three subassociations and two facies were identified. The analysis of the association concerns its spreading, ecology, flower composition, structure, succession and economic importance.

Der Gehölzvegetation kommt eine hohe Bedeutung zu, sowohl in der Wirtschaft als auch in der Natur, wo sie die Auslösung von Erosionsvorgängen verhindert, einen Ausgleich zwischen klimatischen Faktoren vermittelt, und regelnd in den Wasserhaushalt eingreift. Dies veranlaßte uns eine tiefere Kenntnis über die Fichtenwälder des Rarău-Gebirges anzustreben, da hier diese Wälder am weitesten verbreitet sind. Die Literatur ist knapp an Daten über die Gehölzvegetation dieser Berge.

ALLGEMEINE ERÖRTERUNGEN ÜBER DIE VEGETATION

Der gegenwärtige Zustand der Vegetation ist das Resultat der Standorts- und geschichtlichen Faktoren, so wie der Einwirkung des Menschen, der schon immer bestrebt war, sie sich nutzbar zu machen. Die Vegetation der Heuwiesen und Weiden folgt auf eine Waldvegetation und ist anthropogen bedingt; sie entstand vor undenkbar langer Zeit. Die Wälder erlitten auch Umwandlungen durch die Ausbeutung gewisser Arten, durch die Begünstigung der Ansiedlung anderer Arten, durch Anpflanzungen und Aussaaten. Die anthropozoogenen Faktoren haben den meisten Assoziationen einen halbnatürlichen Charakter aufgeprägt.

Die Zonierung der Gehölzvegetation im Rarău-Gebirge gestaltet sich schwierig, denn die Fichte beginnt schonn bei Höhen von 650 m, die Tannen steigen stellenweise bis auf ungefähr 1150 m Höhe — im Gemisch mit Fichten sogar bis auf 1300 m, während der Buchenwald 1350 m Höhe erreicht, und im Gemisch mit Fichten 1400 m.

Der vorherrschende Typus der Gehölzvegetation ist der Fichtenwald, verbreitet von 650 m bis 1600 m Höhe, welcher hier die besten Entwicklungsbedingungen findet. Der Buchen- und Weißtannenwald ist relativ wenig verbreitet; größerer Verbreitung erfreuen sich die Mischwälder aus Buche, Weißtanne und Fichte. In der Vergangenheit waren die Buchenwälder viel ausgedehnter, wurden aber durch die Fichtenwälder

verdrängt, sowohl durch deren Konkurrenzfähigkeit, als auch dank des Menschen.

Einst bedeckte der Wald das ganze Gebiet, wahrscheinlich mit Ausnahme kleiner Teilflächen auf dem Kamm des Rarău und mancher steilen Felsen (Pietrele Doamnei); auch heute kann man an der oberen Waldgrenze nicht bewaldete Felsen beobachten. Allmählich entstanden Lichtungen und Wiesen, welche durch die Tätigkeit des Menschen an Ausdehnung zunahmen und wo eine Grasvegetation Fuß faßte. Infolge umfangreicher Rodungen, insbesondere an der unteren Grenze des Gebietes und gegen die subalpine Stufe hin, zwecks Erweiterung der Weideflächen für das Vieh, entstanden vorherrschend mesophile Gräserassoziationen.

Auf den Bergrücken der höheren Region hat sich als Folge der Rodungen eine subalpine Vegetation ausgebildet, was auf die Umweltbedingungen zurückzuführen ist (niedrige Jahresmitteltemperatur von 2°C, starke Sonneneinstrahlung, reichliche Niederschläge — 926 mm im Jahresdurchschnitt, hohe Luftfeuchtigkeit, häufige und heftige Winde, Skelettböden). Auf einigen Erhebungen und Gipfeln (Rarău-Kamm, Pietrele Doamnei) bestand diese Vegetation wahrscheinlich schon bevor die Rodungen einsetzten. Die Trupps alter Bäume oder vereinzelte Exemplare, die hie und da angetroffen werden, sind Zeugen des ehemaligen Waldes, der noch die höchsten Gipfel bedeckte.

Man darf demzufolge feststellen, daß im Rarău-Gebiet Elemente aus 3 Vegetationsstufen auftreten: Die *Buchenstufe* ist durch eine Interferenz-zone dieser Stufe mit der Fichtenstufe vertreten, die aus Mischwäldern von Buche, Tanne und Fichte mit eingestreuten Reinbeständen aus Buche oder Tanne besteht, und zwar bis zu beiläufig 1350 m Höhe; die *Fichtenstufe* ist weit verbreitet und erstreckt sich von 620 m Meereshöhe bis auf 1550 m, wobei sie von 1350 m ab allein existiert; die *subalpine Stufe* ist zwischen 1550—1653 m schwach vertreten auf Bergrücken und den höheren Gipfeln, und hat durch Rodungen an Boden gewonnen.

Im Rarău-Gebirge nähert sich die Fichtenstufe der östlichen Grenze des Areals, was das Auftreten einer Interferenzzone mit der Buchenstufe erklärt. In den Ostkarpaten sind übrigens die Mischwälder aus Buche, Tanne und Fichte weit verbreitet.

Umkehrungserscheinungen der Gehölzvegetation, die im Rarău-Gebirge häufig beobachtet werden können und darin bestehen, daß die Buchen- und Tannenbestände über dem Fichtenwald stehen, sind zurückzuführen entweder auf das Eingreifen des Menschen oder auf besondere mikroklimatische Verhältnisse (Temperatur, Licht, Feuchtigkeit), welche die Entwicklung gewisser Arten begünstigen bezw. behindern. So ist beispielsweise das Abströmen der Kaltluft in die Täler der niederen Regionen der Entwicklung der Fichte förderlich und ungünstig für die Buche, welche die besonnten, wärmeren Hänge besetzt. Gegen die obere Grenze hin dagegen, gedeihen die Buchen in den geschützten Tälern. Die Entwicklung der Buche wird auch durch das wärmere Kalkgestein begünstigt. Die Temperaturinversion, die für das absolute und mittlere Minimum wirksam ist, welche Werte in Cîmpulung niedriger sind als in der Höhenregion des Rarău, begünstigt ihrerseits die Umkehrung der Vegetation.

DIE ASSOZIATION *PICEETUM CARPATICUM* SOÓ 1930

Die Fichtenwälder des Rarău-Gebirges stellen nach uns eine weiter gefaßte, einzige Assoziation dar, die Charakterarten mehrerer Assoziationen enthält: *Piceetum montanum* Br.-Bl. (1938) 1939 (*P. excelsae normale* Beger 1922), *Piceetum subalpinum* Br.-Bl. 1939, *Piceetum abietis supramontanum* Domin 1933, *Piceetum oriento-carpaticum* Knapp 1942 (von R. Soó [8] *P. excelsae transsilvanicum* Soó 1944 genannt), *Hieracio transsilvanico-Piceetum* Pawl. & Br.-Bl. 1939 em. Borhidi 1957. Da die letzteren drei Assoziationen ökologisch und floristisch einander ähnlich sind, glauben wir, sie als mit *Piceetum carpaticum* synonym betrachten zu dürfen.

Aus dem von uns untersuchten Gebiet beschreibt Tr. I. Ştefureac [9] im Naturschutzgebiet Slătioara eine Assoziation, die er als ähnlich mit *Picea excelsa-Hieracium rotundatum* (Zlatnik 1935) Pawl. & Br.-Bl. 1939 betrachtet (diese wurde von A. Borhidi *Hieracio transsilvanico-Piceetum* genannt); später [10] erwähnt derselbe Autor *Piceetum montanum* Br.-Bl. (1938) 1939 [2] und erkennt sie als vorherrschend. V. Leandru und G. Stanciu [5] melden aus dem Slătioara-Naturschutzgebiet mehrere Gehölztypen, welche als Subassoziationen oder Fazies in das *Piceetum carpaticum* eingereiht werden können.

Verbreitung und Ökologie. Der Fichtenwald ist der am meisten verbreitete Waldtypus im Rarău-Gebirge. Zwischen den Meereshöhen von 650 bis 1550 (1600 m) entwickelt er sich unter optimalen Klimaxbedingungen auf Hängen verschiedener Steilheit, in unterschiedlichen Expositionen, sowohl auf Sedimentgestein (vorwiegend Kalk) als auch auf kristallinen Schiefern. Die Böden sind verschiedener Art, braun, gelblich-braun, sauer, in verschiedenen Podsolierungsstadien, tief oder seicht, vom Skelett-Typus, manchmal auch typische Rendzinen oder verwitterte, mit viel Skelettsubstanz. Die geringen Anforderungen der Fichte an die Tiefe des Bodens beruht auf deren streichenden Wurzeln. Der Ariditätsindex für das Gebiet des Rarău-Gebirges, berechnet auf Grund der Daten der Wetterwarte Rarău, beträgt 63, was den primär podsolischen Böden der Höhenregion entspricht, welche die Entwicklung der Fichtenwälder begünstigen. Der Humufizierungsgrad schwankt; bei der typischen Assoziation herrscht der Moderhumus vor, bei der Subassoziation *vaccinietosum* der torfartige, rohe Humus. Nach dem Feuchtigkeitsgehalt sind die Böden frisch oder feucht-frisch.

Floristische Zusammensetzung und Struktur. Die Anzahl der verzeichneten Arten (einschl. der Thallophyten) beträgt 136, außer den in 1—2 Aufnahmen notierten Arten. Der Artenreichtum ist auf den kalkhaltigen Untergrund zurückzuführen; auf kieselsaurer Unterlage ist die Artenzahl im allgemeinen geringer.

In der Baumschicht herrscht *Picea abies* vor, mit einer Deckung von 60—90%. Die Stämme erreichen eine Höhe von 40 m; sie gehören zur Kategorie des Baumholzes mit Stammstärken bis 50 cm; nur wenige Bestände befinden sich auf der Entwicklungsstufe des Stangenholzes. Das Alter der Bäume erreicht 120—150 Jahre im Naturschutzgebiet Slătioara. Die natürliche Entästung ist an manchen Stellen gering.

Auf den Stämmen und Ästen der Fichten treten oft, manchmal massenhaft, verschiedene Rindenflechten auf (*Usnea florida, U. longissima, Parmelia furfuracea, P. physodes, Physcia stellaris, Alectoria jubata*).

Die Strauchschicht, ausgenommen die Jung-Fichten, -Tannen und -Buchen, ist sehr schwach durch einige zerstreut auftretende Arten vertreten (*Salix silesiaca, S. caprea, Lonicera xylosteum, Sambucus racemosa*).

Die Krautpflanzenschicht (einschl. der Zwerggehölze) weist eine Deckung von 5—70% auf und besteht aus Arten, die verschiedene Ansprüche an den pH-Wert des Bodens stellen, wobei der Prozentsatz der azidiphilen ziemlich hoch ist, selbst in den Fichtenwäldern auf Kalkuntergrund. Die Ursache davon ist die schnellere Mineralisierung der Fichten-Bodenstreu, welche durch das kühle und feuchte Gebirgsklima gefördert wird, und wodurch der höhere Säuregrad des Bodens erhalten bleibt. In seltenen Fällen fehlt die Krautschicht fast ganz, wahrscheinlich wegen des trockenen Bodens der steilen Hänge mit südöstlicher Exposition.

Der Deckungsgrad der Arten in der Krautpflanzenschicht ändert sich im Laufe des Jahres; im Frühjahr überwiegen die frühblühenden geophilen Arten, welche im Sommer nur mit ihren unterirdischen Organen überdauern, und lassen der Entwicklung der Sommerarten Platz. Im Frühling ist die Bodenschicht vor allem durch folgende Arten gekennzeichnet:

Anemone nemorosa, A. ranunculoides, Isopyrum thalictroides, Corydalis solida, C. cava, Cardamine glanduligera, Chrysosplenium alternifolium, Daphne mezereum, Viola sylvestris, Primula leucophylla, Pulmonaria montana, Scilla bifolia, Galanthus nivalis u.a.

Diejenige Art, die in der Krautpflanzenschicht der meisten Fichtenwälder am häufigsten und in großen Mengen auftritt, ist *Oxalis acetosella*, welcher durch seine Blätter vom Frühjahr bis zum Herbst recht auffallend wirkt.

Die Thallophyten kommen auf eine Deckung von 5 bis 80%, wobei die Moose, die im Schatten der Bäume gut gedeihen, an erster Stelle stehen. Die an Nährstoffen ärmeren und seichteren Böden begünstigen die Entwicklung der Bryophyten.

Charakteristisch für die Assoziation sind folgende Arten: *Chrysanthemum rotundifolium, Hieracium transsilvanicum, Campanula abietina, Salix silesiaca.* Man könnte hier noch — unseres Erachtens — als Differentialarten hinzufügen: *Cardamine glanduligera, Symphytum cordatum, Pulmonaria rubra, Ranunculus carpaticus.*

Charakterarten der höheren Vegetationsverbände u.zw. für die Allianz sind: *Picea abies, Luzula sylvatica, Homogyne alpina, Sphagnum quinquefarium*; für die Ordnung: *Melampyrum sylvaticum, Soldanella montana, Sorbus aucuparia, Lycopodium annotinum, Corallorhiza trifida, Listera cordata, Hylocomium proliferum, Entodon schreberi, Ptilium crista-castrensis*; für die Klasse: *Vaccinium myrtillus, V. vitis-idaea, Oxalis acetosella, Ramischia secunda, Moneses uniflora, Monotropa hypopitys.*

Die Begleitarten von grösserer Häufigkeit (III) sind: *Euphorbia carniolica, Anemone nemorosa, Euphorbia amygdaloides, Calamagrostis arundinacea, Luzula luzuloides, Viola sylvestris, Chrysosplenium alternifolium, Daphne mezereum, Rhytidiadelphus triqueter, Thuidium delicatulum.*

Die kalkliebenden Arten, welche auf kieselsaurem Untergrund

fehlen oder nur sporadisch auftreten, sind: *Conocephalum conicum, Fissidens cristatus, Ctenidium molluscum, Tortella tortuosa, Orthothecium intricatum, Neckera complanata, Brachythecium rivulare, Cystopteris montana, Asplenium viride, Gymnocarpium robertianum, G. dryopteris, Polystichum aculeatum, P. lonchitis, Polypodium vulgare, Clematis alpina, Aconitum moldavicum, Spiraea ulmifolia, Cotoneaster integerrimum, Mercurialis perennis, Epilobium montanum, Astrantia major, Primula leucophylla, Veronica urticifolia, Salvia glutinosa, Galium schultesii, Valeriana montana, Hieracium bifidum, Polygonatum verticillatum.*

In den kalten Regionen (im vorliegenden Fall, wo das kühle Klima durch die Meereshöhe bestimmt wird) sind die kalkliebenden Pflanzen nicht an die Kalkbedürfnisse gebunden, sondern an die dem Kalkgestein eigene Wärme.

Außer der typischen Assoziation (Abb. 1) wurden folgende Subassoziationen und Fazies erkannt, welche durch besondere Standortsbedingungen erzeugt werden:

1. Die Subassoziation *luzuletosum sylvaticae* (*Luzulo(sylvaticae)-Piceetum* Wraber 1953) wurde in der höheren Region ausgemacht. Diese zeichnet

Abb. 1 — *Piceetum carpaticum* auf dem Runc-Rüncken.

sich durch eine hohe Abundanz der Art *Luzula sylvatica* aus, was einen sauren Humus vom Modertyp anzeigt [6]. V. Leandru und G. Stanciuc erwähnen sie aus dem Naturschutzgebiet Slătioara als Fichtenwald-Typus mit *Luzula sylvatica* [5].

2. Die Subassoziation *vaccinietosum* (Soó 1930, 1944) [7] wurde ebenfalls in der höheren Region aufgefunden. Diese zeichnet sich durch eine hohe Abundanz der Art *Vaccinium myrtillus*, und manchmal auch von *V. vitis-idaea*, aus. Diese Assoziation entspräche dem *Piceetum subalpinum*

Br.-Bl. 1938 (ohne jedoch mit diesem identisch zu sein), während *Piceetum montanum* Br.-Bl. (1938) 1939 [1] dem typischen *Piceetum carpaticum* näher käme.

3. Die Subassoziation *betuletosum pendulae* (Soó 1944) wurde an einigen Orten festgestellt (besonders im Bistriţa-Tal) und zeichnet sich durch die Abundanz von *Betula pendula* in der Baumschicht aus. Es handelt sich hier um ein Sukzessionsstadium von den krautigen Pionierassoziationen, die sich nach dem Kahlschlag ausbilden, als Übergang zum Fichtenwald (Abb. 2).

4. Der Facies *oxalosum acetosellae* (Soó 1944) wurde häufig an Stellen mit sehr geringer bis mäßiger Neigung, auf tiefen Böden, bei nördlicher und westlicher Exposition.

5. Der Facies *hylocomio-entodontosum* (Leandru 1954, Beldie 1967) enthält in der Moosschicht massenhaft *Hylocomium proliferum* und *Entodon schreberi*.

Abb. 2. — *Piceetum carpaticum betuletosum pendulae* im Bistriţa-Tal

Sukzession. Das *Piceetum carpaticum* stellt einen Basistypus des Waldes dar, der sich auf dem Klimax, also dem Höhepunkt seiner Entwicklung befindet. An manchen Stellen sind die Fichtenwälder von einem abgeleiteten Typus, nämlich dort, wo sie ein Gelände besetzen, das früher einmal mit Buche und Tanne bewachsen war, wobei diese entweder durch das Konkurrenzvermögen der Fichte oder durch Eingreifen des Menschen ersetzt wurden. Die Fichte ist der erfolgreichste Konkurrent für Buche und Tanne, da sie sich leicht an die Schwankungen des Klimas und an die kürzere Vegetationsperiode anpaßt. Die natürliche Regenerierung ist wirkungsvoll; die Jungfichten siedeln sich meist in weniger kompakten Beständen, in Lücken und Lichtungen, an, während sie im Schatten weniger gut aufkommen. In Fichtenbeständen auf geringerer Höhe, benachbart mit Mischwald, wurde beobachtet, daß sich da auch Buchen und Tannen ansiedeln, was auf eine Tendenz zur Bildung eines Artengemisches hindeutet; zu guter Letzt gewinnt aber die Fichte die Oberhand. Als Folge des Kahlschlages bis auf das Bodenniveau bilden sich verschiedene Pionierassoziationen krautiger und verholzter Pflanzen aus (*Senecioni-Chamaenerietum, Deschampsietum flexuosae, Fragario-Rubetum, Salici capreae-Sambucetum racemosae, Alnetum incanae, Piceetum carpaticum*

betuletosum, worauf wieder der Fichtenwald folgt. Das Eingreifen des Menschen, durch Beweiden und Aufräumungsarbeiten innerhalb der Gehölzvegetation hat zum Ersatz des Fichtenwaldes durch dauerhafte Wiesen geführt, welche entweder als Weiden oder als Heuwiesen genutzt werden.

Wirtschaftliche Bedeutung. Die Baumbestände des *Piceetum carpaticum* sind sowohl qualitativ als auch quantitativ wertvoll. Die Produktivität ist bei den meisten Beständen hoch, jedoch mäßig und niedrig gegen die höhere Waldgrenze hin, wo vorwiegend die Subassoziationen *luzuletosum sylvaticae* und *vaccinietosum* gedeihen. Nach P. Ciobanu [3] ist die Aufzucht der Fichtenbestände bis zum Alter der Ausbeutefähigkeit durch die Wirkung der starken Winde in Frage gestellt (Windbruch).

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Eingegangen am 7. März 1979

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LA RELIQUE GLACIAIRE *STELLARIA LONGIFOLIA* MÜHLENB. SUR LE MONT RUNC-VATRA DORNEI

PAR

ION RESMERIȚĂ

The autor presents the results of his researches on the mountain Runc-Vatra Dornei (Bucovina), concerning the species *Stellaria longifolia*, a glacial relic, which probably has immigrated when there still existed a link between Greenland, North America and Europe, respectively at the beginning of the Quaternary period. The resulting general conclusion would be that *Stellaria longifolia* and *Stellaria diffusa* would be the one and the same species, diversified in more populations.

Les reliques glaciaires de Roumanie, qui ont survécu à un climat depuis longtemps disparu, se développent surtout dans des biotopes marécageux eutrophes, ou dans des étangs avec plantes marécageuses [5], où elles se sont réfugiées pendant la période chaude post-glaciaire. Ce groupe, d'une grande importance phyto-historique, comprend aussi les populations de *Stellaria longifolia* (*Stellaria friesiana* Ser., *Stellaria monsquensis* M.B., *Stellaria diffusa* Willd.), qui probablement ont immigrées sur le territoire de la Roumanie pendant qu'il existait encore une liaison entre le Groenland, l'Amérique du Nord et l'Europe, respectivement au début de l'ère quaternaire [3], [10].

L'extension des populations de *Stellaria longifolia* coïncide avec le climat glaciaire, quand beaucoup de plantes boréales-alpines ont été poussées vers le sud de leur aréal mondial, la Roumanie y compris, qui abrittent 44 espèces cormophiles, reliques glaciaires [6]. Elles se sont réfugiées, il y a environ 5000 années, dans des biotopes avec un climat comparé au macroclimat général environnant plus froid et où elles sont restées jusqu'à nos jours, sauf quelques exceptions, parmi lesquelles *Stellaria longifolia*, ce qui nous a déterminé, entre autres, d'entreprendre cette communication.

LA COROLOGIE DES POPULATIONS DE *STELLARIA LONGIFOLIA*

La littérature botanique porte des discussions contradictoires en ce qui concerne la zone de distribution de cette espèce. C'est aussi que la Flore de la Roumanie [8] l'indique comme généralement répandue dans « L'Europe du Nord, rare dans les Alpes et Tatra », tandis que la Flora Europaea [9], l'indique dans l'Autriche, la Tchécoslovaquie, la Finlande, l'Allemagne, la Suisse, l'Italie, le Norvège, la Pologne, la Roumanie, l'U.R.S.S. et la Suède. E. H. Hess et ses collab. [4] précisent la zone de distribution de *Stellaria diffusa* en « l'Eurosibérie, Scandinavie,

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Europe du Nord et Centrale (dans des lieux incertes), les Alpes, les Carpates, la Sibérie, les Ourals. En conséquence, E. H. Hess accepte la dénomination de *Stellaria diffusa*, en considérant toutes les autres dénominations des synonimes, y compris *Stellaria longifolia*.

Il revient à B. Boivin [2] de préciser que « *Stellaria longifolia* Mühlenb. de l'Amérique du Nord n'est pas identique à l'espèce d'Euroasie », c'est pourquoi il utilise la dénomination de *Stellaria diffusa*, ainsi que s'expriment E. H. Hess et ses collab., qui acceptent, en principe, cette séparation et utilisent pour l'Europe (respectivement pour la Suisse) cette dernière dénomination.

On fait mention dans la *Flora Europaea* — et nous sommes du même avis — que les plantes de l'Amérique du Nord et de l'Asie de l'Est ne présentant pas de traits caractéristiques justifient la création d'une espèce indépendante. Il faut pourtant admettre qu'il s'agit, dans le cadre de l'espèce *Stellaria longifolia*, d'une grande diversité de populations. Cette conclusion est renforcée aussi par le fait que toutes les plantes des 20 feuilles de l'herbier du Jardin Botanique de Cluj-Napoca étudiées par l'auteur de cette étude, provenant de : Groenlande, Canada, Scandinavie, Tchécoslovaquie, etc. ont les mêmes traits morphologiques que les plantes récoltées sur le mont Runc.

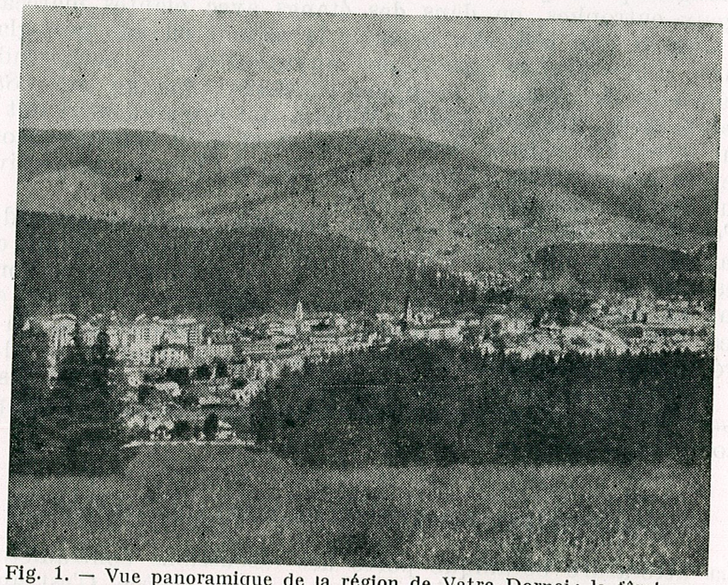

Fig. 1. — Vue panoramique de la région de Vatra Dornei ; la fôret avec l'espèce *Stellaria longifolia* dans le troisième plan (le mont Runc).

En ce qui concerne leur zone de distribution en Roumanie, les populations de cette espèce préfèrent les marais intracarpatiques de Harghita, Ciuc et Gheorghieni, sporadiquement signalées aussi dans les dép. de Sibiu et de Bacău. E. Pop [5] cite cette espèce aussi dans le bassin de Dorna, mais où on ne l'a plus retrouvée, en conséquence, cette localité sera omise dans tous les travaux, y compris la Flore de la Roumanie. Les biotopes du mont Runc sont présentés fig. 1.

ASPECTS ÉCOLOGIQUES ET PHYTOCÉNOLOGIQUES DES POPULATIONS DE *STELLARIA LONGIFOLIA*

Les biotopes de cette région sont placés à une altitude de 850 m, terrain plan, sol ocre monticule podzolique, précipitations 800 mm, température annuelle moyenne 2°, en janvier —7°, en juin 8°. C'est une forêt séculaire d'épicéa rarefiée, les arbres à une distance de 10—20 m l'un de l'autre dont l'entre-espace est occupé par les phytocénoses de l'association *Fragario-Rubetum* (Pfeiffer 1936) Siss. 1946 qui couvrent 90—100% du terrain situé sous les arbres. Ici, autour des souches d'épicéa d'un diamètre de 30—40 cm se développent *Stellaria longifolia* surtout en des biotopes où beacoup d'acicules et de petits rameaux d'épicéa se sont déposés, en divers stades de décomposition. Plus encore, certaines plantes se développent sur les restes des bois attaqués par *Merulius lacrymans* (Wulf.) Schum., une vraie plante saprophyte. Les biotopes respectifs sont fortement acides, et le substrat, sur lequel se développent les plantes, est formé dans sa majorité par des résidus organiques en décomposition, leurs racines n'ayant aucun contact avec le sol proprement dit.

Du point de vue écologique, les biotopes, dont nous nous occupons, ne se superposent pas à ceux des dépressions intracarpathiques mentionnées, ni microclimatiquement, ni macroclimatiquement, ce qui atteste que les populations de *Stellaria longifolia* du territoire de Roumanie ont une amplitude écologique plus large que celle connue jusqu'à présent.

Pour mettre en évidence les exigences écologiques des populations de *Stellaria longifolia*, on cite la Flore de la Roumanie [8] où l'on affirme qu'elles se développent « dans des forêts humides, des marais à tourbe, inherbés», ainsi que la Flore de la Suisse [4] où l'on précise que l'espèce est présente en « subalpin sur des sols humides, riches en humus, dans des leux avec beaucoup de mousses ». I. Pop note sur la feuille de l'herbier du Jardin Botanique de Cluj-Napoca, que ces plantes se développent sur des marais et au bord des lieux plantés d'épicéa. Sur les feuilles du même herbier, E. Pop note que *Stellaria longifolia* se développe en Cariceto-Sphagnetum Soó 1934, 1954, tandis que E. Nyárády indique *Abieto-Sdhagnetum* Borza 1959.

Sur le mont Runc de Bucovina, *Stellaria longifolia* se développe en biotopes mésotréphes, mésophiles, formant de petits faciès dans l'association *Fragario-Rubetum*. Parmi les plantes qui cohabitent avec *Stellaria longifolia*, *Arenaria serpyllifolia*, *Oxalis acetosella*, *Festuca rubra*, etc. mais en certains groupes la première espèce prédomine 100%, en formant des faciès purs.

DISCUSSIONS MORPHOLOGIQUES

Les plantes ont de longs stolons primaires, d'où partent des stolons secondaires et tertiaires et de ces nœuds de fraternité se développent des souches fragiles hautes de 10—20 cm (fig. 2). Il faut souligner que toute cette souche est quadrangulaire avec des papilles en rayure, à l'encontre des affirmations d'autres auteurs. Ainsi Hess et coll. [4] affirment que seulement la partie inférieure est quadrangulaire et porte des papilles, tandis que Al. Beldie [1] et I. Prodan [7] affirment que seulement la

partie supérieure de la souche porte des papilles. Tenant compte que toutes les plantes de l'herbier du Jardin Botanique de Cluj-Napoca provenant de : Canada, Suède, Norvège, Finlande et Europe Centrale, y compris les Carpates de la Roumanie, ont des souches quadrangulaires, avec des papilles sur toute leur longueur, il faut reconsidérer les affirmations des auteurs cités.

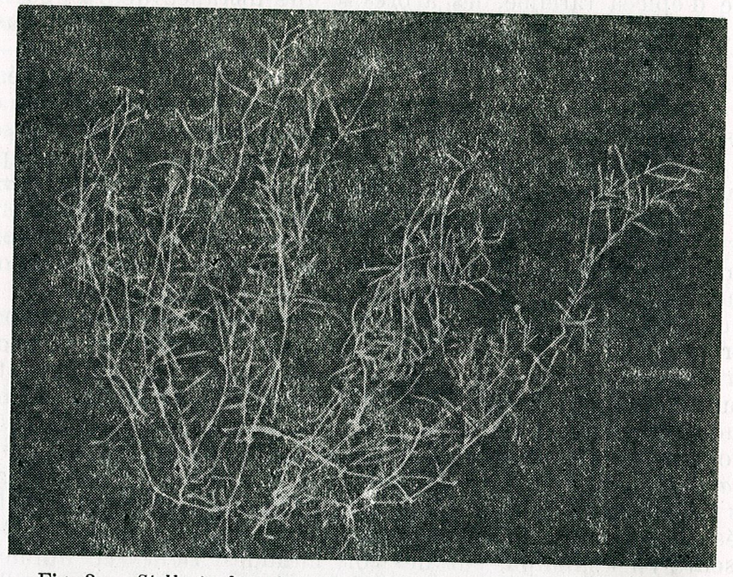

Fig. 2. — *Stellaria longifolia* dans les biotopes sur le mont Runc.

La conclusion générale résultant de nos travaux serait que *Stellaria longifolia* et *Stellaria diffusa* sont une et même espèce, diversifiée en plusieurs populations.

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Reçu le 5 janvier 1979

Centre de recherches biologiques
Cluj-Napoca, str Republicii 48

POLLENMORPHOLOGIE EINIGER *SALICACEEN*-ARTEN UND DEREN BEDEUTUNG FÜR DIE POLLINOSIS

VON

GABRIELA ȘERBĂNESCU-JITARIU und ION T. TARNAVSCHI

27 einheimische und kultivierte Arten der Familie Salicaceae wurden pollenmorphologisch untersucht. Die pollenmorphologischen Daten wurden mit der Bestäubungsart in Beziehung gesetzt. *Salix* wird durch Insekten, *Populus* durch den Wind bestäubt. Die Pollenkörner von *Salix* sind klein und haben in frischem Zustand zahlreiche Fetttröpfchen, sie besitzen Colpi. Die Pollenkörner von *Populus* haben meist sehr kleine Aperturen, die Oberfläche erscheint glatt, ist aber mit äußerst feinen Verrukeln besetzt. Auf die Entwicklung der Pollenkörner wird hingewiesen.

Die Ordnung der *Salicales* ist nur durch eine Familie *Salicaceae* vertreten, welche zwei Gattungen, *Salix* und *Populus*, mit etwa 350 Arten umfaßt und mehr in den nördlichen gemäßigten und subarktischen Gebieten, weniger in den Tropen, verbreitet sind.

In Rumänien ist die Familie durch beide Gattungen mit 26 Arten und 32 Hybriden vertreten, wovon 27 systematische Einheiten pollenmorphologisch untersucht wurden.

Die Anordnung der Arten und deren Nomenklatur ist aus der „Flora Europaea" [2] entnommen und mit jener aus der „Flora R.P.R." [3] verglichen.

Das frische ebenso wie das Herbar-Material ist im Wasser und Chloralhydrat über Farbe, Aussehen und Form beobachtet worden; es wurde auch die Sporodermis-Oberfläche, die Struktur derselben (im optischen Querschnitt) und deren Stärke untersucht.

Allgemein betrachtet haben die untersuchten Arten dieser Familie folgende Kennzeichen :

In der Mehrheit der Fälle sind die Pollenkörner klein gestaltet, die Oberfläche mit äußerst feinen, unregelmäßig verteilten Verrukeln versehen und haben sehr kleine Aperturen als Poren oder Colpi. Frisch aus den Antheren geschüttelt sind diese makroskopisch hellgelb, im Wasser (unter dem Mikroskope) orangengelb, in Chloralhydrat jedoch grünlich bis farblos.

Die Ordnung der *Salicales* hat eine unklare phylogenetische Lage da die Blüten als razemischer Blütenstand amentförmig angeordnet sind und auch als *Amentiferae* behandelt wird.

Die *Salicaceen* nähern sich an manche Vertreter der *Polycarpigenae* oder der *Parietaligenae*.

Die Mikrosporen der *Salicaceae* gehören im allgemeinen zur Pflanzengruppe wo eine große bis mittlere bienenzüchterwirtschaftliche Bedeutung begegnet wird.

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Aus diesem Gesichtspunkt ist die Tatsache zurückzuhalten, daß die Insekten den Nektar ebenso wie den Pollen benötigen.

Wie aus der Fachliteratur bekannt ist, unterscheiden sich die Gattungen der *Salicaceae* durch ihre Bestäubungsart. So ist die Gattung *Salix* entomophil, während *Populus* anemophil ist. Im Zusammenhang mit der Bestäubungsart sind bei den Salicaceen Unterschiede in der Struktur der Mikrosporen festzustellen. So wird *Salix* durch Insekten, besonders durch Bienen und Hummeln, bestäubt, welche Nektar als auch Pollen benötigen ; die Mikrosporen besitzen Colpi, welche bei denjenigen die durch den Wind bestäubt werden sich einschränken ; diese Tatsache deutet auf die Entwicklung der Mikrosporen durch Einschränkung der Furchen.

Hutchinson (1925) zeigte irgendwelche Ähnlichkeit zwischen den *Salicales* und *Tamaricales*, betreffend ihre phylogenetische Beziehungen, aber aus dem Gesichtspunkte der Bienenzüchter und der Wirtschaft ist irgendwelche Ähnlichkeit vorhanden, jedoch jeder erfahrene Palynoge wird keine Ähnlichkeit unter den Mikrosporen der Taxone der oberwähnten Gattungen bemerken [8].

Einige Arten der Gattung *Salix*, wie z.B. *Salix caprea*, *S. cinerea* und *S. alba*, sind für die Bienenzucht besonders wertvoll : erstens wegen einer besseren Entwicklung der Bienenfamilien, die Pollen mit sehr hohen Proteingehalt nützen und zweitens wegen der großen wirtschaftlichen Bedeutung für die Bienenzucht.

Die Erscheinung der Blütenstände vor dem Laube steigert die optische Wirkung in der Insektenanlockung derart, daß bei der Gattung *Salix* im Frühling der Baum in voller Blüte ist, die Insekten anlockt und so den Bestäubungsvorgang erleichtert.

Die Pollenkörner der Gattung *Salix* sind nur im frischen Zustand klebrig und mit zahlreichen Fetttröpfchen versehen, trocken jedoch werden die Pollenkörner pulveratig. Diese Tatsache läßt eine spätere Anemophilie, als zusätzliche Bestäubung, vollkommen möglich zu, wozu die freien langfädigen Staubgefässe in Betracht kommen.

Risch [6] stellt die Frage ob nicht die Gattung *Populus* irgendwie eine reduzierte oder ursprüngliche Familienform darstellt, infolge ihrer Bestäubungsart und das Fehlen der nektarerzeugenden Drüsen, die bei der Gattung *Salix* vorhanden sind.

Für die anemophilen Pflanzen sind die Pollenkörner im allgemeinen klein, kugelförmig und mit glatter Oberfläche, was für die Pollenkörner der Gattung *Populus* kennzeichnend ist, aber grössere Dimensionen als bei *Salix* aufweisen.

In beiden Fällen kann man auch von Pollinosiserscheinungen sprechen, da bei den Arten von *Salix*, deren getrocknete Pollenkörner vom Winde genommen und zerstreut werden, wie bei *Populus* deren Arten von Anfang auf anemophil sind, die Pollinosis bei sehr empfindlichen Personen hervorrufen, besonders wenn die Anzahl der obgenannten Arten in der Gegend groß ist. Diese Erscheinung kann im Frühling, während der Blütezeit, besonders in den botanischen Gärten, beobachtet werden.

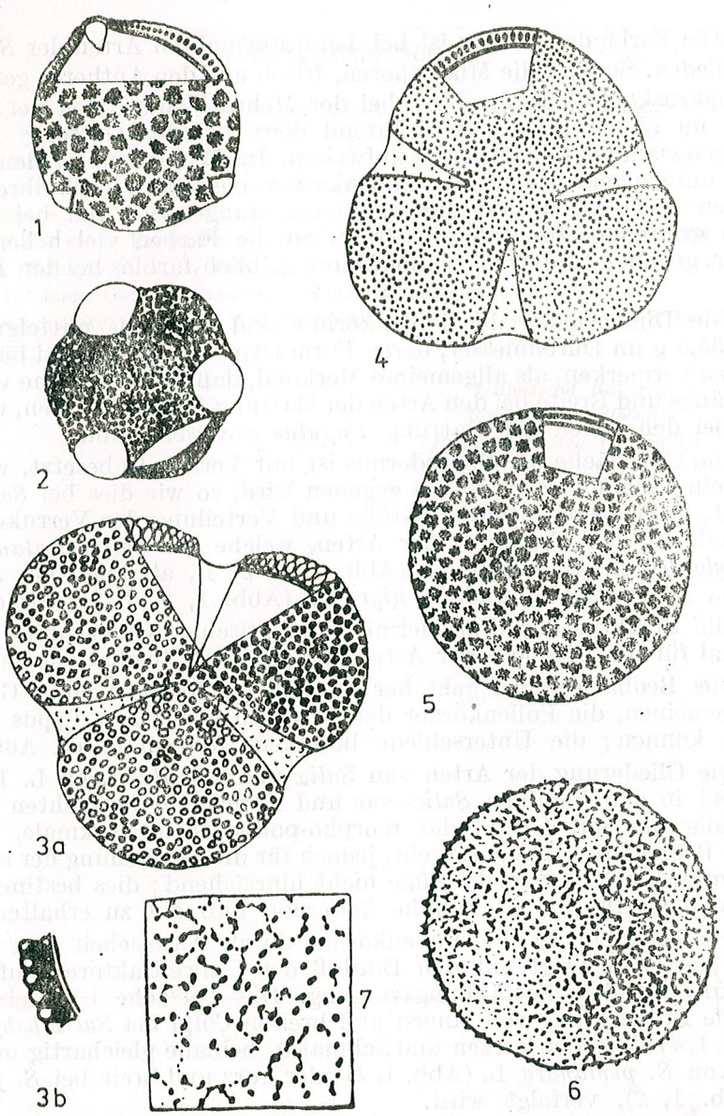

Abb. 1.— *1*, *Salix pentandra* L. : Mikrospor, äußeres Aussehen in Scheitelansicht und Teil der Sporodermis im optischen Querschnitt (550 ×, Original) ; *2*, *Salix fragilis* L. : Mikrospor, äußeres Aussehen in Scheitelansicht und Teil der Sporodermis im optischen Querschnitt (550 ×, Original) ; *3a*, *Salix alba* L. : Mikrospor, äußeres Aussehen in Scheitelansicht und Teil der Sporodermis im optischen Querschnitt ; *3b*, *Salix alba* L. :Teil der Sporodermisoberfläche in vergrößerter Zeichnung (*a* = ca 2525 × ; *b* = freie Handzeichnung ; *a*, *b*, Original) ; *4*, *Salix babylonica* L. : Mikrospor in Scheitelansicht mit Außenansicht und Teil der Sporodermis im optischen Schnitt (2525 ×, Original) ; *5*, *Populus alba* L. : Mikrospor in Scheitelansicht mit Außenansicht und Teil der Sporodermis im optischen Querschnitt (550 ×, Original) ; *6*, *Populus nigra* L. : Mikrospor in Scheitelansicht mit Außenansicht (nach Abbildung Kuprianova) ; *7*, *Populus tremula* L. : Teil der Sporodermis Epistruktur (nach Abbildung Kuprianova).

Die Farbe des Pollens ist bei den untersuchten Arten der *Salicaceen* verschieden. So sind die Mikrosporen, frisch aus den Antheren geschüttelt und makroskopisch beobachtet, bei der Mehrzahl der Arten der Gattung *Salix* im allgemeinen gelb, während diese bei der Gattung *Populus* eine quarzsandige Schattierung aufweisen. Im Wasser (unter dem Mikroskop) unterscheiden sich die Pollenkörner ebenfalls durch ihre Farbe. Bei den *Salix*-Arten sind diese gelb bis orangengelb und bei *Populus* jedoch grün-gelb. Im Chloralhydrat sind die Farben viel heller als im Wasser, grünlich bis farblos bei *Salix* und gelblich-farblos bei den *Populus*-Arten.

Die Dimensionen der Pollenkörner sind klein bis mittelgroß von 15,6—33,8 μ im Durchmesser, deren Form ist prolat-sphäroidal bis prolat. Es ist zu vermerken, als allgemeines Merkmal, daß die Colpi eine verschiedene Länge und Breite bei den Arten der Gattung *Salix* aufweisen, während diese bei den Arten der Gattung *Populus* abwesend sind.

Die Oberfläche der Sporodermis ist mit Verrukeln besetzt, wodurch dieser ein retikuloides Aussehen gegeben wird, so wie dies bei *Salix alba* (Abb. 1, *3*) zu beobachten ist. Größe und Verteilung der Verrukeln sind Merkmale für das Erkennen der Arten, welche bei *Salix pentandra* L., *S. babylonica* L., *S. fragilis* L. (Abb. 1, *1*, *2*, *4*), als auch bei *Populus alba* L., *P. tremula* L. und *P. nigra* L. (Abb. 1, *5*, *6*, *7*), vorkommen.

Die Struktur der Sporodermis, im optischen Querschnitt, ist ein Merkmal für die Diagnose der Arten, als pilat und tegillat-baculat.

Aus Beobachtungen geht hervor, daß im Rahmen einer Gattung, im allgemeinen, die Pollenkörner der Arten zu demselben Typus gestellt werden können; die Unterschiede bestehen wohl in deren Ausmasse.

Die Gliederung der Arten von *Salix* und *Populus* nach L. Kuprianova [4] in den Familien *Salicaceae* und *Populaceae* betrachten wir als notwendig nur auf Grund der morpho-pollinischen Merkmale, so wie aus der Beobachtungen hervorgeht, jedoch für die Darstellung der erwähnten Familien sind nur solche Züge nicht hinreichend; dies bestimmt uns die beiden Gattungen als Familie *Salicaceae* aufrecht zu erhalten.

Die Entwicklung der Pollenkörner durch Anweseheit oder Abwesenheit der Colpi zeigt nur den Einfluß der Umweltfaktoren auf deren Anpassung an den Bestäubungsvorgang. Die Tatsache ist vielsagend, wenn die Entwicklung von langen und breiten Colpi bei *Salix babylonica* L. (Abb. 1, *4*) bis zu den kurzen und schmalen, beinahe gleichartig mit den Poren von *S. pentandra* L. (Abb. 1, *1*) oder kurz und breit bei *S. fragilis* L. (Abb. 1, *2*), verfolgt wird.

In der Pollenmorphologie der *Salicaceen* sind jedoch, wie gesagt, Unterschiede in der Bestäubungsart festzustellen. So bei der Gattung *Salix*, wo die Bestäubung durch Insekten geschieht, haben die Pollenkörner Colpi, welche bei den Arten, die durch den Wind bestäubt werden, reduziert sind, eine Tatsache die nur die Entwicklungsrichtung der Pollenkörner durch die Verminderung der Furchen bei den anemophilen Arten der Gattung *Populus*, anzeigt.

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Eingegangen am 20. April 1979

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THE DYNAMICS OF PHOTOSYNTHETIC PIGMENTS AND THE PRODUCTIVITY OF SOME *SCENEDESMUS* SPECIES

BY

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The correlations between the photosynthetic pigments and the biomass produced were investigated with *Scenedesmus* species grown in experimental laboratory conditions (nutrient mixtures prepared from waste waters — discharged by porcelain and pharmaceutical works, brewery, sugar factory and thermoelectrical works — geothermal water, "Zizin" mineral water enriched with Knop-Pringsheim's or Benecke's medium salts or only with $(NH_4)_2HPO_4$ and urea, respectively.

The correlations vary on a large scale depending on the composition of nutrient media and on the species (strain) of algae, as well. In scant media the pigment content of one and the same species varies to a greater extent than the different species in similar conditions, i.e. divergent adaptation is more developed than specificity. However, the variation of the biomass quantity is less (in similar growth conditions about two times). There is a certain degree of specificity among the *Scenedesmus* species concerning the way they react to unfavourable conditions.

No clear-out correlations could be established between the pigment content and algal biomass and respectively pigment ratio and the same biomass in scant media. In similar conditions and in simultaneous cultures a probably reciprocal relationship between biomass quantities and a/b chlorophyll ratio could be suggested (Fig. 10), which might contradict some data of the literature.

The comprehensive problems involved by photosynthetic pigments could be concentrated (to make a survey easier) in a few fields of investigation (both theoretical and practical) i.e. : a) dynamic and analytical biochemistry dealing with biosynthesis and biodegradation (both *in vivo* and *in vitro*) of pigments. Endowed with the most advanced technical equipment and sophisticated methods the specialists engaged in these researches could reveal striking results [3, [4], [19], [30], [61] ; b) phenomenological biochemistry (plant physiology in its older meaning) which tries to establish the efficiency of photosynthesis and the functions of pigments in various conditions of life (quasinormal, pathological and polluted as well) and ecosystems. Data concerning the variation of pigments, the dynamics of their proportions determined by both external and internal factors represent worthwile indices to the processes appearing in specific biosynthesis and structures [20], [28], [36], [65], [72] ; c) controlled (industrialized) photosynthesis, by means of the biological transformation of wastes into useful materials, is aimed at. The expected organic substances could even be chlorophylls or carotenoids (as Felföldy *et al.*, 1962 [17], and Price and Carell, 1964 [55], have suggested).

Scientists have observed and even experimented, long time ago, variations in colour of algal cells [5], [6], [14], [22], [59], [62]. Despite the knowledge gathered during a century, the phenomenon has continued to

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be studied [10], [11], [15], [31], [35], [37], [54]. It is well known the plasticity of the metabolism of algal cells, and especially that of their plastids [13], [24], [38], [43]. The mean content of chlorophylls in algae ranges between 0.5 — 1.5% [57], but there were rather frequently recorded extremes of 0.03% and 6.0% [36], and moreover, of 8.3% [9]. Thus, differences between the lower and the upper limits [9] could achieve 20,000%, or even 2,000,000%. The typical a/b chlorophyll ratio (considered by Belcher, 1968 [7]) counts 1.0—1.35, but these limits can also be exceeded (Table 1).

The extended variation both in quantity and proportion of the photosynthesis pigments could be considered as an expression of the homeostasis (determined by the self-regulation capacity of chloroplasts) given by the cell and the entire living organism to the environmental factors through the interrelated metabolic processes. This is an evidence, at the

Table 1

Presumptively specific indices of *Scendesmus acutus* and *Sc. acutiformis* based on dry weight, contents of chlorophylls a and b and carotenes

Algae	Parameters / Media	Indices	Dw. mg/l	Protein mg/l	Chl.a. %	Chl.b. %	Carotenoids %	a/b	a+b/c
Scenedesmus acutus	Complete	Minimum	2530	89.40	7.30	2.95	3.00		
		Maximum	5424	281.27	14.17	5.33	6.76		
		Mean	3977	185.14	10.74	4.14	4.88	2.59	3.05
		Diff. %	114	215	94	80	125		
	Scant	Minimum	430	30.05	0.12	0.09	0.45		
		Maximum	5424	419.05	14.17	5.33	6.89		
		Mean	2927	224.55	6.92	2.42	3.41	2.86	2.74
		Diff. %	1006	1294.50	11708.	5822	1431		
Sc. acutiformis	Complete	Minimum	3110	152	2.41	0.85	0.87		
		Maximum	5592	375.30	15.20	5.24	5.14		
		Mean	4351	263.65	8.80	3.04	3.00	2.89	3.95
		Diff. %	80	147	530	516	490		
	Scant	Minimum	820	18.26	0.12	0.11	0.26		
		Maximum	5592	375.30	15.20	5.24	5.53		
		Mean	3206	196.80	5.17	1.90	2.42	2.72	2.92
		Diff. %	582	1955	12566	4664	2027		

same time, of the phylogenetic, physiological, biochemical and genetic heterogeneity of plastids and, consequently, of their adaptability [43], [64]. Though the plastids have a relative autonomy inside the cells, according to the symbiotic origin theory [26], [60], [64], [68], [69], [71], however the dynamics of pigments resulted from the environmental factors in surviving conditions may surpass the internal ones.

The most frequently studied factors affecting the photosynthetic pigments are : *light* (intensity, spectral composition, duration), *nutrients* (composition, deficiency, abundance, disbalance), *biological active specific*

substances (toxins, pesticides, detergents, antibiotics) and *parasites*. Experimentally controlled (in respect of structure and quality) factors result in more correlated and obvious effects (concerning cause and effect) than those "undefined" (residuals, unstable radicals, degrading substances in polluted waters).

a) Undoubtedly the light is the main factor determining the adaptive variation of the chlorophyll content in the plastids of algal cells [24], [27], [43]. Steeman Nielsen *et al.* [cit. 24] have distinguished two types of adaptation determined by light : *Chlorella*-type when the light intensity produces a decrease in the chlorophyll content, and *Cyclotella*-type, when the chlorophyll content seems to be unaffected by the light intensity, but increases the quantity of the enzymes involved in the dark reactions of photosynthesis. It should be mentioned, however, that *Cyclotella* being a diatom does not contain chlorophyll b [8].

The opposite relationship existing between the chlorophyll content and the light intensity has been proved by several experimental data [13], [27], [39], [44], [53], [57], [67]. In a denser culture of *Chlorella pyrenoidosa* the chlorophyll content of the cells becomes higher, thus the population as a whole resembles the shade grown leaves and, conversely, in a less dense culture the cells contain less chlorophylls, hence the population is similar with the sun ungrown leaves [63]. Changes in the a/b chlorophyll ratio depending on light intensity also occur. Brown and Richardson (1968)[10] found (in another strain of *Chlorella pyrenoidosa*) a value of 0.65 at 500 lx and of 0.58 at 1500 lx. Halldal (1970) [24] also concluded that in weaker light more chlorophyll b is synthesized. The synthesis of chlorophylls could be enhanced in *Chlorella pyrenoidosa* by red light, but a significant decrease of the process was recorded in blue light [70]. Long acting light of 670 and 720 nm produced a restructuring of chlorophyll a, which is considered an adaptation similar to that of endozoic *Ostrobium* [47].

b) The relationship existing between the nutrient substances and photosynthetic pigments is manifold, a reason which tempted several research workers to deal with it. In fact even the earlier observations and experiments mentioning the variation of the colour of algae have been based on the effect of sugars [22], [62]. It is quite interesting that this topic remained recurrent during the time ellapsed and happily led to some important results concerning both the biosyntheses and the biodegradation of the pigments controlled by glucose in *Chlorella protothecoides* [1], [2]. Several but sparce data attest the implication (indirect or direct) of other elements (N, S, K, Fe, Mg, Mn) in the control of structure and function of the photosynthetic apparatus [8], [9], [11], [35], [39], [48], [49], [52], [55], [67], [70].

c) Biologically strong active specific substances (natural or synthetic ones) generally injure the plastids. Changes produced by such drugs in the structure of chloroplasts and their pigment content are already considered as indices of effectiveness [33], [73]. Streptomycine and diphenylamine decreased both the chlorophyll and carotenoid contents in *Chlorella pyrenoidosa* ; however, streptomycine acted more strongly on chlorophylls [42]. Ebringer (1972) [16] ascertained 144 antibiotics and concluded that most of them inhibited DNA synthesis, while few of them depressed the

protein synthesis of chloroplasts in *Euglena gracilis*. Actinomycine (which inhibits the synthesis of RNA controlled by DNA) stopped the initial regeneration step of chloroplasts [25]. The chloroplasts of *Chlorella pyrenoidosa* were also damaged by about 15 organo-chlorinated compounds (i. e. phenols, Lindane, DDT- 2,4—D); the sensitivity, depending on the substituted groups, follows this series : $NO_2 > Br > Cl > NH_2 > CH_3 > OH$ [28].

Besides the variations induced (by natural or experimental factors) in the photosynthetic pigment composition, allowed by the physiological state of the cells and chloroplasts as well, other differences may occur among the published data due to the applied experimental technics (either by chronological or optional reasons). Technical uncertainties have already been arisen by Seybold and Egle's (1938) [cit. 56] results, who had attributed Willstätter's et al. [cit. 56] lower values to the dried algal material used from which the pigments could be extracted more quickly. Differences due to methodological choices may count 20—130 % [17], [18]. The more sophisticated are the technics applied the more striking such uncertainties become ; e. g. the absorption spectra determined at low temperatures vary significantly depending on the solvent used for extraction, and differ from those obtained *in vivo* [19], [24].

A source of perpetual and justified uncertainties, which urge to further investigations, will remain the limits to extrapolate the experimental data (especially those of field conditions) and condense them in a precise equation giving then much larger validity. In this respect Bruchet's (1959—1960) [12] results (obtained chromatographically and spectrophotometrically) are interesting. According to them the symbiosis, phylogenetically developed, did not change the pigments of lichengonidia as compared to their supposed partners (green algae). Furthermore, Halldal (1970) [24], in full agreement with Kluyver's idea (1926) [cit. 64] about the unity of biochemistry, proves that the extracted and purified chlorophylls are always identical, no matter the plants they have been obtained from. As long as the environmental conditions allow the algae to grow, or at least to vegetate, their photosynthesis pigments will not necessarily change qualitatively, though they may show changes in quantity and ratio. But under unfavourable conditions which inhibit the growth and promote the development of resting cells, new pigments could be synthesized [67].

In spite of these statements, Aronoff *et al.* (1971) [4] warn that "which is valid for *Chlorella* is not necessarily true for other plants". Thus, it was established that the ribosomes of chloroplasts of the *Chlamydomonas reinhardi* have $266 \pm 32 \times 208 \pm 33$ Å, while those of tobacco $268 \pm 24 \times 219 \pm 20$ Å [69]. The physiological and biochemical diversity of the photosynthetic pigments results from the difficult, but desired chemotaxonomical researches.

The variation of photosynthetic pigments, however complex it may be, constitutes a premise for fruitful investigations. What are the limits of adaptability ? How the plastids respond to favourable and unfavourable effects ? What are the convergence ranges of different species under similar conditions and, conversely, how large the divergence of the same species could be under different life conditions ; how are the metabolic processes assured under such circumstances ? How the dynamics

of pigments is reflected in the productivity of photosynthesis ? Some experiments carried out by us on a few *Scenedesmus* species would contribute to elucidate these questions.

MATERIALS AND METHODS

The *Scendesmus* species studied (*Sc. acutus*, *Sc. acutiformis*, *Sc. falcatus B*, *Sc. obtusiusculus* III. C, *Sc. obtusiusculus* I. D., *Sc. obtusiusculus* S., *Sc. sp.* A) differ both taxonomically and ecologically. They were collected from different habitats at different times, and after that they were cultivated in static and intensive conditions for many years in different artificial nutrient media (Tables 2—5, Figs. 1—9). The polymorphic peculiarity of the genus was more obvious in these culture conditions. *Sc. falcatus* had the most stable characteristic coenobia.

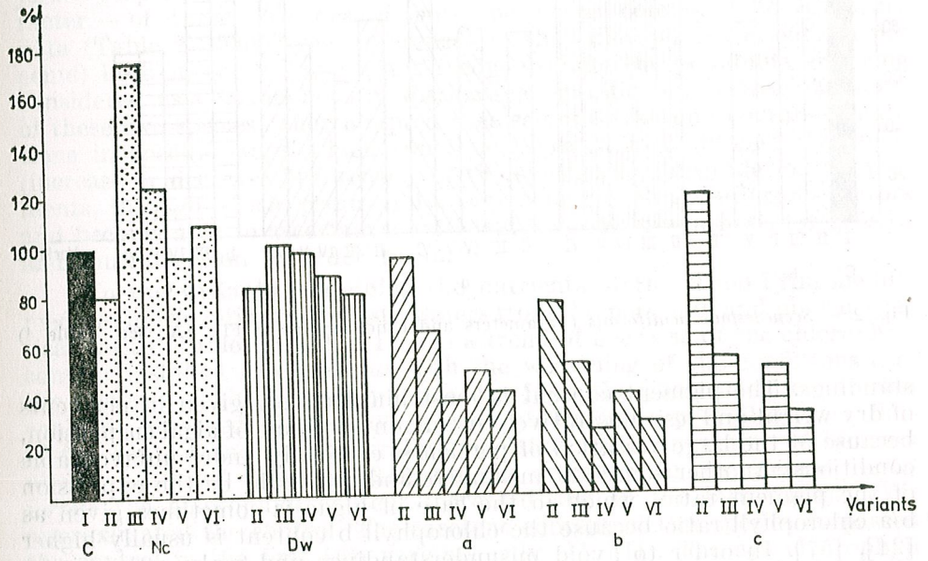

Fig. 1. — Number of cells (Nc), quantity of dry weight (Dw) and pigments (a — chlorophyll a, b — chlorophyll b, c — carotenes) given in per cent by *Scenedesmus acutus* grown in Knop-Pringsheim-Felföldy's medium (I — control, C, considered 100 %) and in mixture of waste waters from porcelain and pharmaceutical works (in ratios of II—4 : 0, III—3 : 1, IV—2 : 2, V—1 : 3, VI—0 : 4) enriched with Knop-Pringsheim's medium salt (see Table 3).

The factors which alternated both simultaneously and subsequently were : the composition of nutrient media, light intensity, the duration of a batch culture set, the intensity and the duration of the bubbling (made by sterilized air containing 3 % of CO_2), initial and optical cell density of the cultures (It is impossible, or at least very difficult, to install

and maintain the uniformity of both cell density and extinction of the culture of different species or strains, because of their different sizes and pigment contents).

All measurements have uniformly been made [51], hence the data recorded do not differ methodologically. It is worth mentioning that the basis to which the pigment content is referred also varies. One could find in the literature different kinds to express the pigment content such as : mg/l (as well as μg/ml, or /mm³), mg/10^6 cells and %/dry or wet weight. Lack of uniformity leads to different conclusions and misunderstandings. The pigment content is most illustratively given in per cents of dry weight and less illustratively in amounts/volume of algal suspension, because of the large variation of pigments, especially under unfavourable conditions. Another source of misunderstandings could be the expression of the pigment ratio, which in the case of algae is sometimes given as b/a chlorophyll ratio because the chlorophyll b content is usually higher [24], [57]. In order to avoid misunderstandings and make comparisons easier in this study, the a/b chlorophyll ratio will be performed in spite of the fact that in a previous work [51] it has been conversely made.

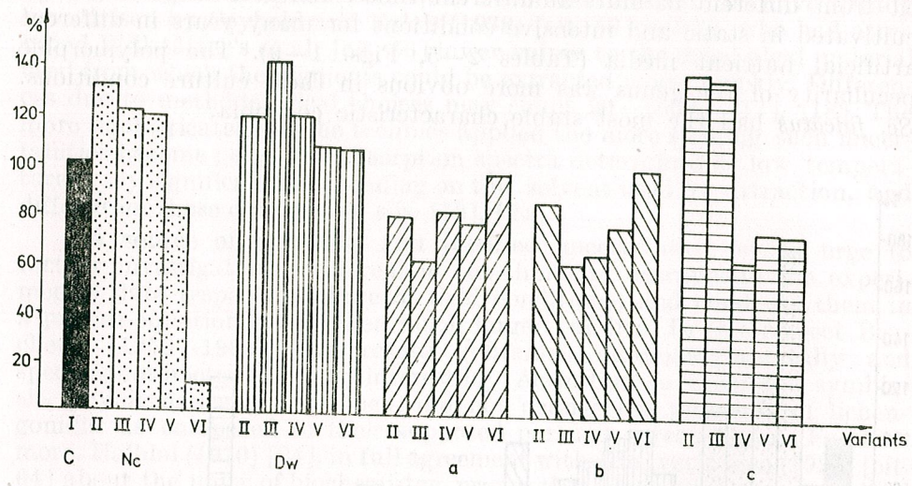

Fig. 2. — *Scenedesmus acutiformis* (Parameters and conditions as in Fig. 1A ; see Table 4)

The pigment (chlorophylls a and b, carotenes, lutein, violaxanthin, neoxanthin) contents were determined spectrophotometrically (Specol spectral colorimeter) according to Hager and Meyer-Bertenrath's (1966) [23] procedure, directly from the cell biomass and also indirectly after their separation by thin layer chromatography and elution of the chromatograms obtained. The proteins (aminic groups) of both cells and culture liquid (supernatant) were determined by Lowry's method applied by Fogg according to Péterfi et al. [51].

RESULTS AND DISCUSSIONS

The data gathered (Tables 1—7, Figs. 1—11) from the experiments disclose so highly complicated relations between the pigments and the factors (either experimental or casual ones) that they are hard or, in some cases, impossible to comprehend and explain. The content of pigments, proteins and photosynthesized biomass in the case of *Scenedesmus acutus* and *Sc. acutiformis* (Table 1) however included in the mean limits of variation (Table 2), reached pretty large extremes (chlorophyll a 11.708%, 12.566%, chlorophyll b 5.822% and 4.664% respectively) expressed by Böger's (1964) (9) rule. It is obvious and at the same time noteworthy that the scanter a medium is, the higher variations arise. This proves, on the other hand, the higher sensitivity of the algae in impaired conditions [50]. The limits between the minimum and maximum have also been enlarged by the relatively higher chlorophyll (both a and b) content in the control populations (mean value of chlorophyll a 10.74% and 8.80%; of chlorophyll b : 4.14% and 3.04% in the case of *Sc. acutus* and *Sc. acutiformis* respectively) — which could be regarded as an obvious shade-character — of these two *Scenedesmus* species as compared to literature data (Table 2). The large differences in the chlorophyll content (in per cents) both in control and scant media exclude the possibility of being considered as a taxonomically distinctive, specific or relative character of these two species. They adapted themselves to the unfavourable conditions in specific ways which could be revealed in a distinct dynamics (increase or decrease in intensity of the synthesis or degradation) of pigments, through which, presumably, they compensate the injurious factors and become able to ascertain a sufficient level of biosynthesis for growth and multiplication in order to survive.

In the variants containing the nutrients of the Knop-Pringsheim's medium in the mixture of waste waters from the porcelain and pharmaceutical works (Tables 3 A, 4 A/I—VI) a trend of decrease of the chlorophyll content installed in *Sc. acutus* with the worsening of the conditions (i.e. the increase of the ratio of waste water from the pharmaceutical works) could de observed). The carotene content, however, prevailed over that of the control and was generally correlated to the quantity of biomass photosynthesized (Figs. 1A, 2A). On the contrary, in *Scenedesmus acutiformis* the chlorophyll content increased and in the last variant (VI) approximated the control. The carotene content of this species surpassed the control in the first two variants only (II, III). The a/b chlorophyll ratio in these two species differed significantly in the mixture containing more waste water from the pharmaceutical works (Table 3A/VI), and it might presumably be attributed to a kind of specific dynamics of chlorophyll b. The ratio correlates pretty well with the rate of biosynthesis which in this variant approached the control in *Sc. acutiformis*, whereas it was less in *Sc. acutus*.

In the subsequent experimental set, considered in this study, *Sc. acutus* revealed almost the same correlations, but in some respects more convincing ones between the pigments and the biomass photosynthesized (Table 3C/I—VI). The mixtures of waste waters were in these variants

Table 2

The pigment contents (% of dry weight) of some algae

Algae	Conditions	Chl.a	Chl.b	Carote-noids	a/b	Diff. %	Refe-rences
Chlorella pyreno-idosa		2.00	0.55	0.045	3.60		[56]
,, ,,		3.00	0.80	0.607	3.75		[17]
,, ,,	KNO_3 2 g/l	3.20	1.50	0.180	2.13		[52]
,, ,,	,, 80 ,,						
	12hrs	1.57	1.07	0.037	1.47		[52]
,, ,,	pH 5.2	1.75	1.30	0.181	1.37		[52]
,, ,,	,, 1.0						
	12hrs	1.06	0.28	0.105	3.78		[52]
,, ,,	,, 12.0 ,,	—	—	0.190	—		[52]
,, ,,	KNO_3 0.2 g/l	2.83					[38]
,, ,,	urea 0.2 ,,	2.51					[38]
,, ,,	weak light	6.60					[8]
,, ,,	strong ,,	3.30					[8]
,, ,,		0.8 —6.3				690	[9]
,, *vulgaris*	young cells	2.80	1.60	0.588	1.75		[17]
,, ,,	old ,,	0.80	0.40	0,360	2.00		[17]
,, ,,		1.0 —8.6				760	[9]
,, ,,	one case	15.0					[53]
,, *ellipsoidea*		1.7 —4.4					[44]
,, ,,		1.5 — 2.5				66	[13]
,, sp.		0.03—6.0				20000	[13]
,, ,,		0.01—0.6		0.002		60000	[13]
				0.16			
,, ,,	direct sun light	1.6					[13]
,, ,,	diffuse ,, ,,	3.5				28	[13]
,, ,,	in green-house	1.2 —5.3				28	[13]
Chlamydomonas reinhardi		1.6 —4.8				200	[9]
Chlorococcum botryoides		3.10	1.10	0.642	2.82		[17]
Chlorocloster terrestris		3.60	1.40	0.769	2.57		[17]
Coelastrum microporum		1.70	0.60	0.300	2.83		[17]
Scenedesmus acutus		2.50	0.90	0.431	2.77		[17]
Sc. acuminatus		2.20	0.80	0.481	2.75		[17]
,, *obliquus*		1.3 —5.1				290	[9]
,, *obtusiusculus*		4.20	1.00	0.747	4.20		[17]
,, ,,	artificial medium	3.80	1.30	0.560	2.92		[18]
,, ,,	marine water 1,0	1.20	0.40	0.420	3.00		[18]
,, ,,	,, ,, 1/4	3.70	1.30	0.610	2.85		[18]
Hydrodictyon africanum	young cells	1.97					*
,, ,,	old ,,	1.12					
Ulva lactuca		0.09	0.07		1.30		[56]
,, ,,		0.33	0.17		2.20		[56]

* Raven J. A., Glidwell S. M., Photosynthetica, 1975, 9, 361—371.

supplemented with the Benecke's medium salts only (in which N and Fe are 10, Mg 5, and P 4 times less than in the Knop-Pringsheim's nutrient solution). It is interesting that the high carotene content in these populations decreases, excepting the mixture of 2 :2 medium to waste waters (Fig. 3C) and it is in good agreement with the biomass quantity, and implicitly with the cell density and protein content. The chlorophylls similarly varied, but their amount did not surpass the control in any variant. Thus the data of this experiment revealed a pretty comprehensive correlation among the cell compartments, and especially in respect of chloroplasts. All particles involved in biosynthesis were inhibited alike by the residuals discharged from the pharmaceutical works.

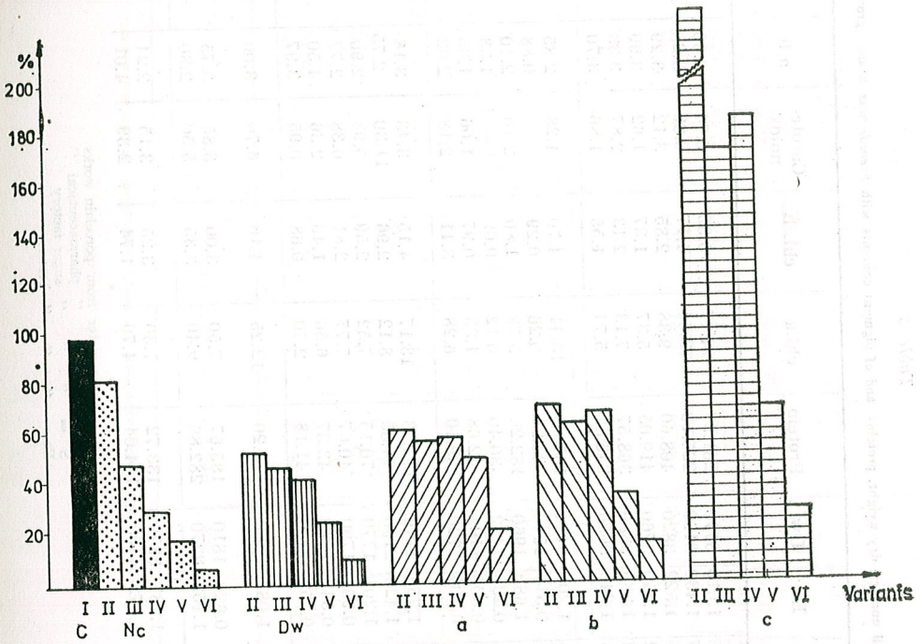

Fig. 3. — *Scenedesmus acutus* grown in a modified Tamiya's medium and in a mixture of waste waters from porcelain and pharmaceutical works enriched with Benecke's medium salts (Further explanations in Fig. 1A).

In impaired life conditions (the only nutrient added to the waste water mixtures being $(NH_4)_2HPO_4$, the amount of N being equivalent with Tamiya's urea EH medium) the correlation of the pigments with other photosynthesized substances becomes confused, due to the more stimulated sensitivity of cells [50] (Tables 3, 4 B/I—VI; Figs. 4B, 5B). The amount of biomass found with the different variants was almost the same and it decreased stepwise the worsened conditions initially installed in the culture liquids. On these grounds the dynamics of the photosynthetic pigments appears more characteristic and specific in respect of the question : how is it assured a relatively correlated substance

Table 3

Variation of production (cell number, dry weight, proteins) and of pigment contents with *Scenedesmus acutus* grown in scant media

Variants \ Parameters	Nc	E	Dw	Protein	chl.a	chl. b	Carote-noids	a/b	µg pigments/10^6 cells			
									chl. a	chl. b	car.	a/b
A/I KPF*	116875	1.610	3770	281.27	14.17	5.33	5.74	2.65				
II I+T 4 : 0 + KP	93437	1.430	3190	225.10	13.64	4.17	6.89	3.28				
III " 3 : 1 "	205625	1.522	3820	408.60	9.38	2.85	3.12	3.29				
IV " 2 : 2 "	146562	1.640	3760	419.05	5.37	1.37	1.62	3.99				
V " 1 : 3 "	114062	1.285	3380	368.57	7.13	2.13	2.87	3.33				
VI " 0 : 4 "	129375	1.240	3050	246.87	5.74	1.55	1.86	3.70				
B/I Tm	86875	1.205	5424	157.86	13.41	4.70	4.28	2.85				
II I+T 4 : 0 $(NH_4)_2HPO_4$	13125	0.315	1540	34.23	0.26	0.29	—	0.88				
III " 3 : 1 "	68750	1.680	1960	182.23	3.78	1.80	2.10	2.10				
IV " 2 : 2 "	10737	0.230	1442	30.40	0.12	0.09	—	1.33				
V " 1 : 3 "	21875	0.305	1104	42.68	1.33	0.97	1.06	1.35				
VI " 0 : 4 "	20625	0.305	720	65.10	6.28	3.11	2.18	2.02				
C/I Tm	123750	1.755	4146	117.73	13.17	4.15	3.43	3.18	0.11	0.03	0.027	3.66
II I+T 4 : 0 + Bk	102604	1.428	2190	74.85	8.12	2.96	11.30	2.75	0.08	0.03	0.110	2.66
III " 3 : 1 "	60100	1.201	1770	70.15	7.52	2.59	5.93	2.90	0.12	0.04	0.098	3.00
IV " 2 : 2 "	39058	0.961	1790	40.67	7.77	2.81	6.38	2.77	0.20	0.072	0.160	2.78
V " 1 : 3 "	23225	0.542	1030	43.37	6.56	1.46	2.36	4.50	0.28	0.063	0.100	2.80
VI " 0 : 4 "	8750	0.252	430	41.18	2.70	0.68	0.95	3.97	0.31	0.078	0.110	3.97
D/I Tm	53300	1.081	2530	174.20	12.25	4.10	6.76	3.00	0.23	0.077	0.130	3.00
II Th+S 1 : 1 + $(NH_4)_2HPO_4$	16375	0.620	1810	185.67	7.50	3.00	3.85	3.75	0.46	0.180	0.240	2.56
III Ge+S 1 : 1 "	46625	1.152	2270	282.86	9.40	3.35	5.50	2.80	0.13	0.045	0.075	2.89
E/I KPF	108281	1.487	3870	133.72	7.30	3.25	3.15	2.24	0.07	0.030	0.030	2.23
II Th+S 1 : 1 + $(NH_4)_2HPO_4$	114270	1.472	4400	144.04	4.70	1.74	2.39	4.01	0.18	0.070	0.090	2.57

*KPF = Knop-Pringsheim-Felföldy's medium salts,
Tm = Tamiya modified medium salts
Bk = Benecke's medium salts

I = waste water from porcelain works
T = " " " pharmaceutical "
S = " " " sugar factory
Be = " " " brewery

Th = thermoelectrical
Ge = geothermal water
Z = "Zizin" mineral water
E = extinction
Nc = number of cells

Table 4

The effects of aggravated nutrient conditions on the growth (cell number, extinction, dry weight production), protein and pigment contents of *Scenedesmus acutiformis*

Variants \ Parameters	Nc	E	Dw	Protein	chl. a	chl. b	Carote-noids	a/b	µg pigments/10^6 cells			
									chl. a	chl. b	car.	a/b
A/I KPF*	87187	1.370	3240	250.32	9.03	2.94	3.99	3.06				
II I + T 4 : 0 + KP	114375	1.440	3860	229.00	7.22	2.52	5.53	2.25				
III " 3 : 1 "	105937	1.610	4570	163.98	5.62	1.78	5.41	3.14				
IV " 2 : 2 "	104062	1.425	3870	140.60	5.91	1.93	2.50	3.05				
V " 1 : 3 "	71250	1.277	3480	201.50	6.94	2.26	2.97	3.06				
VI " 0 : 4 "	94687	1.427	3460	207.45	8.76	2.93	2.95	2.98				
B/ I Tm	92750	2.080	5592	152.00	15.20	5.24	5.14	2.90				
II I+T 4 : 0+$(NH_4)_2HPO_4$	7500	0.433	1820	18.26	0.14	0.11	—	1.27				
III " 3 : 1 "	109375	1.080	2678	132.48	4.81	2.14	2.10	2.25				
IV " 2 : 2 "	91230	0.952	2438	121.02	6.50	3.11	2.54	2.10				
V " 1 : 3 "	20973	0.232	836	30.09	0.42	0.24	0.36	1.75				
VI " 0 : 4 "	20355	0.214	820	38.54	4.10	2.16	1.37	1.90				
C/I Tm	63000	0.900	3460	89.40	2.41	0.85	0.87	2.84	0.13	0.047	0.047	2.77
II Th+Be+S 2 :1 :1+KNO_3	17500	0.570	2200	83.96	1.55	0.61	0.68	2.54	0.19	0.076	0.085	2.50
III Th+Be+S+Z 1 : 1 : 1 : :1 + urea	9680	0.370	1750	79.50	0.31	0.11	0.26	0.81	0.06	0.021	0.046	2.72
D/I Tm	64312	1.200	3110	375.30	5.23	1.72	2.44	3.04				
II Th+S 1 :1+$(NH_4)_2HPO_4$	15000	0.631	1630	160.20	3.62	1.59	1.95	2.27				
III Ge+S 1 : 1 ,'	48062	0.906	2260	185.00	5.25	1.97	2.63	2.66				

* Explanation as in Table 3

production by an uncorrelated pigment quantity. From the experimental mixtures checked as nutrient media, a ratio of 1 : 3 (waste water from porcelain works to that from pharmaceutical works) proved to be favourable in all respects for the algae, although it did not surpass the control.

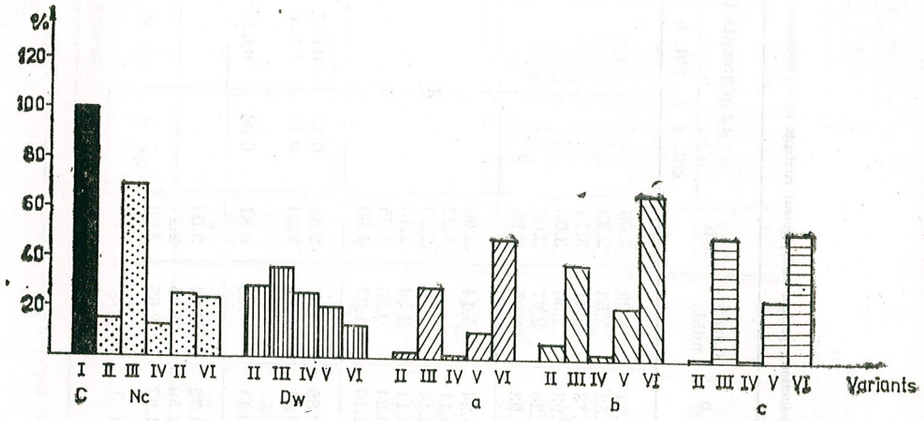

Fig. 4. — *Scenedesmus acutus* grown in the same conditions as those given in Fig. 3C, but the mixtures were supplied with $(NH_4)_2 HPO_4$ (3.30 g/l) only.

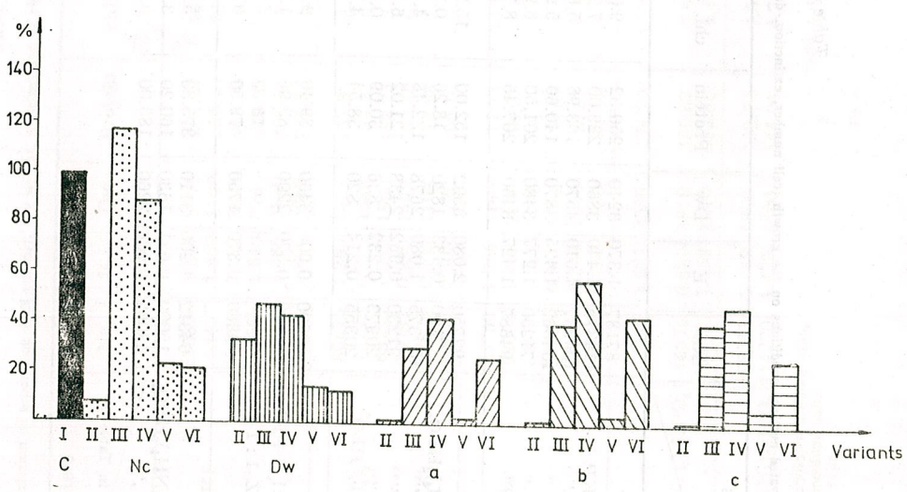

Fig. 5. — *Scenedesmus acutiformis* (See explanation of Fig. 4B).

Differences emerged between those two species in the mixture with a ratio of 2 : 2 of waste waters used in pigment content and biomass quantity. *Sc. acutus* contained less chlorophylls and carotenes than *Sc. acutiformis*, but did not surpass the control in any variant. The a/b chlorophyll ratio was also higher, though not conclusive in all variants.

The effects of various sources of N (KNO_3 and urea) have been assessed on the substrate constituted of waste waters discharged by the brewery, sugar factory and thermoelectrical works,and of "Zizin" mineral water using *Sc. acutiformis* as test organism (Table 4C/I—III). In all variants the

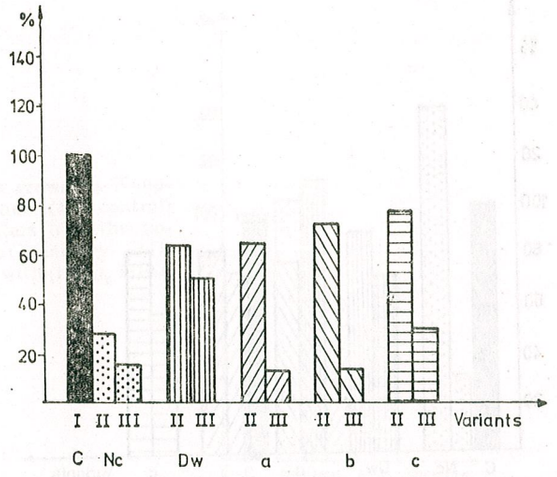

Fig. 6. — *Scenedesmus acutiformis* grown in modified Tamiya's medium (I — control) and in a mixture of waste waters from thermoelectrical works, brewery and sugar factories (II) and from thermoelectrical works, brewery and sugar factory and "Zizin" mineral water (III) (in ratios of 1 : 1 : 1, and 1 : 1 : 1 : 1 as well), enriched with urea (3 g/l).

pigment contents and similarly the biomass quantity and cell density were below the control (Fig. 6C). Undoubtedly the inhibition was produced by the residuals existing in the waste waters poured in the medium and it is tempting to attribute this inhibition to the materials derived from sugar factory, having in mind the particular sensitivity of pigments (chlorophylls) towards sugars. But this assumption is contradicted by lower carotene contents, which according to the available data are increased by sugars. The factors existing in mixtures containing KNO_3 as a source of N have acted more strongly on chlorophyll a than on chlorophyll b and carotenes. In the variant with urea all the pigments are proportionally lowered but not in correlation with the biomass photosynthesized. The C/N ratio so decisive in a well-balanced dynamics of pigments, in these nutrient mixtures, was presumably unfavourable not only for them, but for the entire metabolism of the cells, too.

Noteworthy differences arose between *Sc. acutus* and *Sc. acutiformis* in a set of experiments carried out with mixtures prepared of waste waters from sugar factory, thermoelectrical works and of geothermic water amended with $(NH_4)_2 HPO_4$ only (Table 3, 4, D/I-III, Fig. 7D, 8D). The pigment contents and biomass quantity similarly varied but distinctively in respect of the species. The pigment amount related to the cell number was higher in *Sc. acutus* (consequently *Sc. acutiformis* was strongly inhibited) but in the case it was expressed in per cents of dry matter the difference between the two species was small and *Sc. acutiformis* was only slightly inhibited, if at all. Furthermore, the a/b

chlorophyll ratio was less in this last species which could be accounted for a higher chlorophyll b content resulting from its prevailing plasticity especially under impaired life conditions. As a matter of fact, chlorophyll a proved to be more sensitive in both species, falling more strikingly than chlorophyll b with the worsening of media.

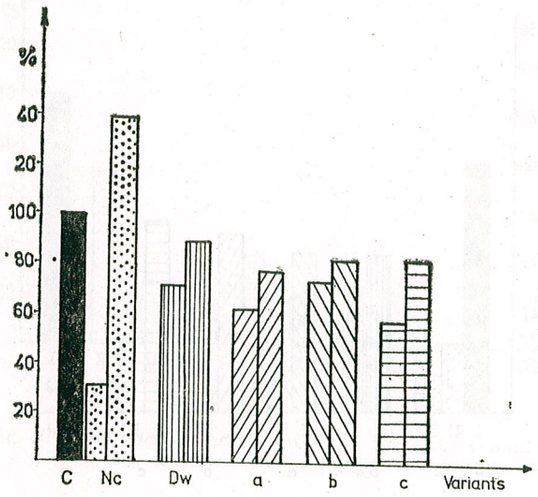

Fig. 7. — *Scenedesmus acutus* grown in modified Tamiya's medium (I — control) and in a mixture of waste waters, from sugar factory and thermoelectrical works (II), and geothermal water (III) as well (in a ratio of 1 :1) enriched with $(NH_4)_2$ HPO_4 (3.30 g/l).

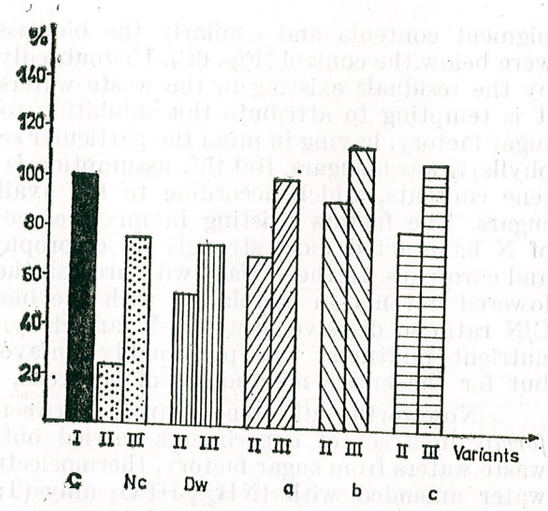

Fig. 8. — *Scenedesmus acutiformis* (The same conditions as those given in Fig. 7D).

The media of waste water obtained from sugar factory and thermoelectrical works, enriched with $(NH_4)_2HPO_4$, were promising for the biomass productivity of *Sc. acutus* (Table 3 E/I—II). The experiment was performed in gas-washing-type vessels. Though the increase in biomass was small as compared to the control, it was reasonably to take into

account the simplicity of the substrate. The pigment content, as against the dry matter, was less than that of the control (Fig. 9E) (may be due to the residuals discharged by the sugar factory), but it was higher if the cell density of the culture was taken into account. The reduced amount of pigments and the moderate increase of the biomass could be explained

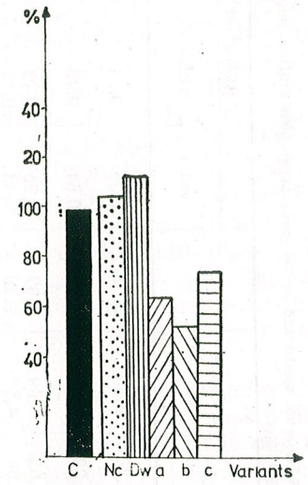

Fig. 9. — *Scenedesmus acutus* grown in Knop-Pringsheim-Felföldy's medium (I — control) and in a mixture of waste waters from thermoelectrical works and sugar factory (II) (in a ratio of 1 : 1) enriched with $(NH_4)_2$ HPO_4 (3.30 g/l)

by the lack, or the presence of Fe in a very small concentration in the medium. Bryan and Bogorad (1963) [cit. 39] have found that the optimum concentration of Fe for the biosynthesis of chlorophylls in *Chlorella vulgaris* lies between 2.0—3.0 ppm, but for the multiplication an amount between 0.6—0.8 ppm was sufficient. On the other hand, this discrepancy points out the possible difference between the two populations in respect of the cell sizes, which could be higher in the mixture than in the control (artificial nutrient medium). This is argued by the pigment contents which were as follows : chlorophyll a 0.067, chlorophyll b 0.03, carotenes 0.03 μg /10^6 cells in the control, and respectively 0.18, 0.07, 0.09 μg/10^6 cells in the mixture. If referred to a single cell, the chlorophyll a content in the control was : 0.67×10^{-10} mg, whereas in the mixture medium : 1.80×10^{-10} mg. These data fairly fit with Meffert and Overbeck's (1968) values established by *Sc. obliquus* ($1.2—2.5 \times 10^{-10}$ mg/cell). However, their values are less if referred to dry weight (0.5—0.8% chlorophylls).

In another set of experiments there were simultaneously and comparatively cultivated six species and strains of *Scenedesmus* (Table 5). Although the algae differed taxonomically and ecologically, their variations in the pigment content and their biomass production were slightly reduced. The values between the lowest and highest limits were as follows : chlorophyll a 81%, chlorophyll b 92%, carotenes 82%, a/b chlorophyll 13%, dry weight 46%. It is worthy mentioning that the pigment content varies about two times more than the biomass. This disagreement could be explained by the idea that the larger variation of pigments is a proper mode of adjustment of the cell metabolism to the actual life conditions.

Table 5

Variation of the cell and optical densities, dry weight, proteins and pigment contents in *Scenedesmus* species and strains grown in complete artificial nutrient medium (Knop-Pringsheim-Felföldy)

Determinations / Algae	Nc	E	Dw	Protein	µg/10⁶ cells chl.a	chl.b	car.	a/b
Sc. acutus	126250	1.780	3900	266,70	12.13	4.36	4.30	2.78
Sc. obtusiusculus I.D	74375	1.040	2820	368.80	16.94	5.38	4.50	3.15
Sc. obtusiusculus III.C	38437	1.140	2680	395.90	17.94	6.05	5.60	2.97
Sc. sp. A.	118750	1.640	3700	236.90	13.76	4.93	4.50	2.79
Sc. falcatus B	103437	1.610	3560	311.00	12.80	4.28	4.16	2.99
Sc. obtusiusculus S	166250	1.790	3120	382.40	9.90	3.15	3.08	3.14

Table 6

The correlations between photosynthetic pigments, cell and optical densities and the biomass quantity in *Scenedesmus acutus* and *Sc. acutiformis* grown in complete artificial media in different culture vessels

Algae	Media	Vessels	Nc	E	Dw	Protein	chl.a	chl. b	Caro-tenoids	a/b
Sc. acutiformis	KPF*	VS	87187	1.370	3240	250.32	9.03	2.94	3.99	3.06
	Tm	T	64312	1.200	3110	375,30	5.23	1.72	2.44	3.04
		Sp	63000	0.900	3460	–	2.41	0.85	0.87	2.84
		VS	92750	2.080	5592	152.00	15.20	5.24	5.14	2.90
	KPF	Sp	108281	1.487	3870	133.72	7.30	3.25	3.15	2.24
		VS	116875	1.610	3770	281.27	14.17	5.33	5.74	2.65
Sc. acutus		T	53500	1.081	2530	174.20	12.25	4.10	6.76	3.00
	Tm	Sp	86875	1.205	5424	153.86	13.41	4.70	4.28	2.85
		VS	123750	1.755	4146	117.74	13.17	4.15	3.43	3.18

* KPF – Knop-Pringsheim-Felföldy's medium; Tm – modified Tamiya's medium; T – vertical columns; Sp – gas-washing type; VS – Vladimirova-Semenenko's type.

The disproportion between cell density (the less among the species cultivated) and the biomass production (which approximates pretty well the other congeners) is more striking with *Sc. obtusiusculus* III.C. The variation could be due to the larger cell sizes which in this case can hold a higher chlorophyll content per cell. Thus, if this species is compared with *Sc. acutus* the chlorophyll a content, given in % of dry weight, is less with 228%, but related to 10^6 cells it is higher with 229% (Table 5). Otherwise, the content of chlorophylls correlates well enough with the biomass quantity and the cell number in all species investigated (unlike the populations grown in the mixture of waste water, obtained from the sugar factory and thermoelectrical works, table 3E/I—II). The observations and determinations obtained prove that, surprisingly, *Sc. obtusiusculus* III.C differs morphologically, physiologically and biochemically from the other two strains, e.g. *Sc. obtusiusculus* I. D., *Sc. obtusiusculus* S).

The decrease of the variations of these *Scenedesmus* species in identical conditions (Table 6) and their increase, in different ones, indicate that their specificity is weaker than their variability, proving, at the same time, their highly evolved convergent and divergent adaptability. The conspicuous polymorphism of *Scenedesmus* species is a revelation of the peculiarities mentioned.

Generally, it is difficult to find a clear correlation between photosynthetic pigments (either their quantity or ratio) and the biomass produced. Though chlorophyll a is used as a standard parameter for the determinations of the phytoplankton primary productivity [72] and, indeed, in some circumstances there is a parallelism between the pigment quantity and the accumulated organic substances [18], unfortunately, such a straight relationship could not generally be found.

Concerning chlorophyll b such a correlation probably does not exist at all [58], notwithstanding the link may be quite tempting. However, in a certain degree the dynamics of chlorophyll b may reflect the connection between the nutrient supply of the cells and the photosynthetic capacity of their pigments. Thus, the protein particles evolved on the outer layer of the thylakoids, involved in photosystem II in which the amount of chlorophyll b is higher [34], [66], disappear during N-starvation, but they reappear after N-supply [42]. On the other hand, lack of chlorophyll b does not exclude the photosynthesis [32]. The fact is also confirmed by those groups of algae in which chlorophyll b is absent (existing in *Euglenophyta*, *Chlorophyta* and *Charophyta* only) [8]. A consequence of such peculiarities might be the reversed ratio of some forms of chlorophyll a (c680, c670) and chlorophyll b ascertained by several algae and Angiosperms, too (Hammans, Thomas 1969 [cit. 58]), and furthermore probably the direct, sometimes almost constant, proportionality between the a/b chlorophyll ratio and the dry weight [58].

Considering the manifold aspects of the possible connections between the photosynthetic pigments and their gross products and looking for some correlations between the data gathered with these *Scenedesmus* species, it can be concluded that they are different and, in some cases, uncertain (Table 7). So, with *Sc. acutus* grown in complete artificial nutrient media (Figs. 10, 11) there exists a reversed correlation between the a/b chlorophyll ratio and the biomass quantity. Similarly the proportions

are reversed among chlorophylls a and b, and carotenes as well, but they are directly correlated to the biomass. But in scant media the connections are hidden. Otherwise there was also recently mentioned the lack of any correlation between the chlorophyll content and the chemical activity

Table 7

The correlation between the pigment and protein content, cell number, extinction and dry weight (relative values *) in some *Scenedesmus* species

Parameters / Algae	Nc	E	Dw	Protein	chl.a	chl.b	Carote-noids	a/b
Sc. acutus	2	2	1	5	5	4	4	6
Sc. obtusiusculus I.D	5	6	5	3	2	2	2	1
Sc. obtusiusculus III.C	6	5	6	1	1	1	1	4
Sc. sp. A	3	3	2	6	3	3	2	5
Sc. falcatus B	4	4	3	4	4	5	5	3
Sc. obtusiusculus S	1	1	4	2	6	6	6	2

* The numbers show the magnitude of estimates, e.g. 1=the best, 6=the worst (the last in the series).

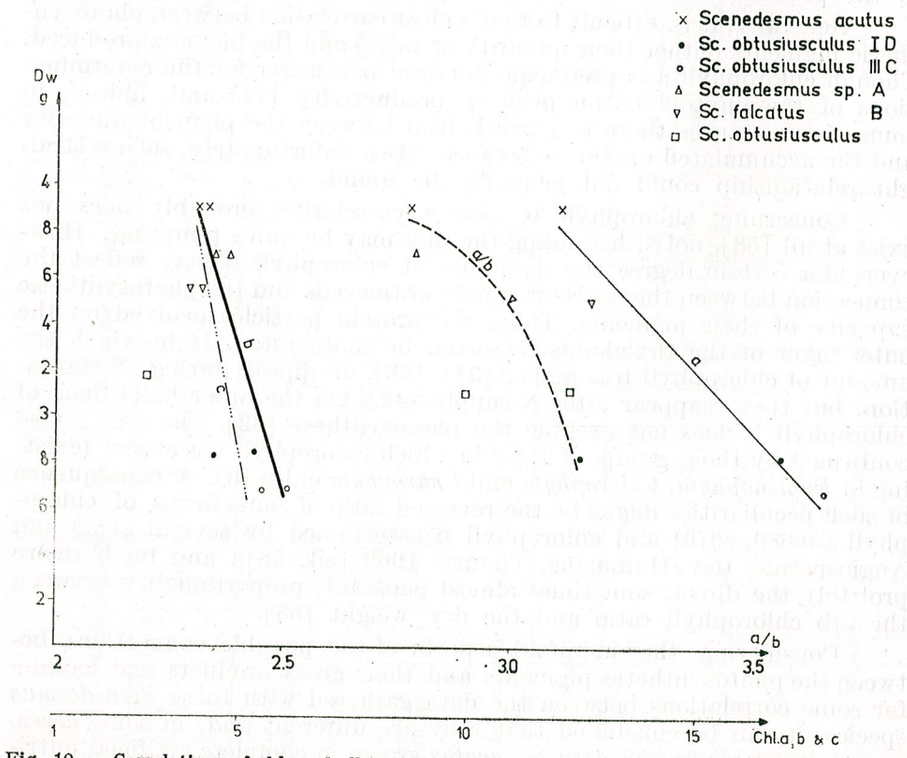

Fig. 10. — Correlations of chlorophyll a (a) and b (b), carotenes (c) and the ratio of chlorophyll a/b to the amounts of dry matter. *Scenedesmus* species grown simultaneously in identical conditions.

of the photosystems in the case of *Chlamydomonas reinhardti* [32]. Positive correlations, fitting well to Sagromsky's (1977) communications [58] were found with *Sc. acutiformis*. Thus, the a/b chlorophyll ratio was proportional with the amount of biomass, moreover, their direct values,

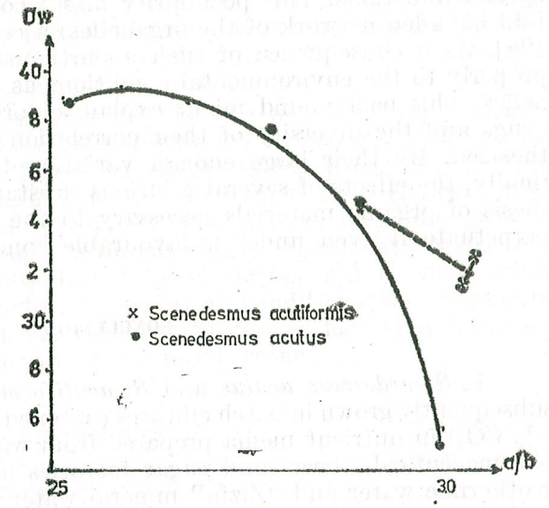

Fig. 11. — A tentative correlation between the a/b chlorophyll ratio and the dry weight of biomass in the case of *Scenedesmus acutus* and *Sc. acutiformis* grown in various complete artificial media and different cultural vessels (Further explanations see in the text).

in most experiments (except the only population grown in mixture of waste waters, from pharmaceutical and porcelain works, supplied with Knop-Pringsheim's medium salts, Table 3A/I—VI, Fig. 2A) considered in this study. The two strains *Sc. obtusiusculus* I.D. and *Sc. obtusiusculus* III.C obtained from The Iron Gate by the Danube river, have had higher chlorophyll a and b contents than the other species, but have differed from those by their pigment and biomass correlations. *Sc. obtusiusculus* I.D. had a higher a/b chlorophyll ratio, but a less production of biomass, meanwhile *Sc. obtusiusculus* III.C had both the ratio and the dry weight lower.

The amount of carotenoids, generally, increases in the algae affected by sugars [15], [31], [54]. According to Halldal (1970) [24] "it thus seems as if a significant increase in carotenoid content takes place whenever certain green algas are exposed to extreme conditions". Some data show, indeed, the increase of carotenoids under N-deficient conditions [37], others, on the contrary, show (even with *Sc. obliquus*) the inhibition of their synthesis by N-starvation [35]. Obviously, there could arise a relative increase due only to the decrease of chlorophylls [21], [37], [67]. Indeed, some unfavourable substances (diphenylamine, streptomycine, tetracycline, actidione) act stronger on chlorophylls than on carotenoids [30], [40]. The carotenes were directly correlated with chlorophyll a in these *Scenedesmus* species in most of the experiments performed; decrease of chlorophyll contents was accompanied by that of carotenes, though it was not proportional in all cases. Unfavourable nutritional conditions

at limits have obviously lowered the whole pigment quantities in *Chlorella pyrenoidosa* [52].

The plastids being part of the plant cells are obviously subjected to some extent by favourable and unfavourable factors. However, besides common processes performed by them they also display some peculiar specific functions. This peculiarity might confer them (within the manifold bounded network of the organelles) a certain degree of independence [29]. As a consequence of such a particular situation they could react properly to the environmental conditions as compared to other cell organelles. This background might explain chloroplast selectivity for several drugs and the diversity of their correlation with the biomass photosynthesized. By their large enough variation the chloroplasts compensate, finally, the effects of several injurious substances and provide the biosynthesis of organic materials necessary to the whole organism in order to perpetuate it even under unfavourable conditions.

CONCLUSIONS

1. *Scenedesmus acutus* and *S. acutiformis* (indigenous species) were subsequently grown in batch cultures (bubbled with sterilized air containing 3% CO_2) in nutrient media prepared from waste waters (from porcelain-, pharmaceutical-, beer- and sugar factories and thermoelectrical works), geothermic water and "Zizin" mineral water enriched with Knop-Pringsheim's or Benecke's nutrient media, or only with $(NH_4)_2HPO_4$ and urea respectively. These two species together with *S. falcatus*, *S. obtusiusculus* III.C, *S. obtusiusculus* I.D, and *S. sp.* A were also simultaneously cultivated under optimum conditions.

The effects induced by external or internal factors (under identical conditions) were estimated function of the growth rate (photocolorimetrically and haemocytometrically determined), productivity (biomass, dry weight), amount of aminic proteins and photosynthetic pigments (chlorophylls, carotenoids).

2. The pigment content of *S. acutus* was correlated with the amount of biomass and decreased gradually with the increasing proportion of waste water from the pharmaceutical works when the nutrient mixtures, coming from waste waters of porcelain and pharmaceutical works mixed in ratios of $4+0, 3+1, 2+2, 1+3, 0+4$, were enriched with Knop-Pringsheim's nutrient media. Although in *S. acutiformis* the amount of pigments decreased similarly with *S. acutus* it could not be correlated with the quantity of biomass.

In the same mixtures, but enriched with the Benecke's nutrient medium (N and Fe 10 times, Mg 5 times, P 4 times less than in Knop-Pringsheim's medium) the amount of biomass produced and the pigment content of the cells was less, although their decrease was similarly controlled by the waste water of the pharmaceutical works.

3. The productivity of *S. acutus* and *S. acutiformis* was more reduced (as compared to the Tamiya's artificial medium) if the mixtures of waste waters from the porcelain and pharmaceutical works were supplemented

with $(NH_4)_2HPO_4$ only. The productivity of these two species gradually decreased depending on the amount of waste water from the pharmaceutical works, but the dynamics of their pigments differed essentially, by large specific variations.

The pigment and the protein contents and the productivity variations of *S. acutus* and *S. acutiformis* with nutrient mixtures obtained from waste waters of sugar or thermoelectrical works or from geothermal water enriched with $(NH_4)_2HPO_4$ proved quantitatively their specificity; all the values recorded were higher with *S. acutus*.

4. The simultaneous, synchronized cultures of *S. acutus*, *S. acutiformis*, *S. obtusiusculus* III.C, *S. obtusiusculus* I.D and *S. sp.* A grown in optimum nutrient medium (Knop-Pringsheim-Felföldy medium containing Hoagland's microelements and 10% soil extract) have shown interspecific differences in respect of pigment and protein contents, growth rate and productivity. All these differences have significantly been less than those interspecifically established by *S. acutus* and *S. acutiformis* grown in different scant media. In identical conditions the pigments varied two times more than the biomass, the latter having a trend of inverse correlation with the a/b chlorophyll ratios.

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Received March 29, 1979

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LES ACIDES AMINÉS ET LES GLUCIDES SOLUBLES DE *SPIRULINA PLATENSIS* (GOM.) GEITER

PAR

L. ATANASIU, LUCIA POLESCU et IOANA SPIRESCU

La présente étude s'occupe des acides aminés et des glucides solubles présents dans la biomasse de *Spirulina platensis*. Parmi les acides-aminés, l'isoleucine, la phénylalanine, la tyrosine, l'alanine, l'acide glutamique, l'acide aspartique et la lysine ont été identifiés.
Le chromatogramme indique parmi les glucides solubles le rhamnose, l'arabinose, le saccharose, le lactose et le raffinose.

Algue bleue d'une grande valeur nutritive, par sa teneur élevée en protéines, la Spirulina a formé l'objet de certaines recherches antérieures qui ont relevé les acides aminés libres et liés y présents. Ayant dans sa composition 60—65% protéines, 18—20% glucides, 2—3% lipides, ainsi que des vitamines B, la vitamine C et le β-carotène, cette algue constitue une précieuse source de nourriture.

L'algue a été cultivée au laboratoire, dans un milieu Zarrouk, en système clos, le barbotage étant assuré par un courant d'air, à une lumière fluorescente de 8000 lx et à la température de 30°C.

La biomasse a été recueillie 5 jours après l'ensemencement du milieu, au moment où la culture a atteint sa densité optimale.

Après fixation à l'alcool méthylique 80%, les échantillons ont été extraits à l'éthanol à diverses concentrations. Les différentes substances ont été séparées à l'aide des résines échangeurs d'ions.

Les substances individuelles ont été identifiées par chromatographie sur papier [2].

RÉSULTATS ET DISCUSSIONS

a) ACIDES AMINÉS LIBRES

La chromatographie unidimensionnelle des amino-acides a été effectuée avec un solvant butanol-acide acétique-eau (4 : 1 : 5).

Le développement du chromatogramme a été réalisé à l'aide de la ninhydrine en solution 0,1%, dissoute en éthanol. La figure 1 représente le chromatogramme des acides aminés libres, extraits de la biomasse obtenue, à savoir l'isoleucine, la phénylalanine, la valine, la tyrosine, l'alanine, l'acide glutamique, l'acide aspartique et la lysine. Quantitativement, l'acide glutamique est nettement prédominant par rapport aux autres acides-aminés libres.

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b) GLUCIDES SOLUBLES

La chromatographie des glucides solubles a été effectuée avec le même solvant qu'à la séparation des acidesaminés. Les glucides ont été révélés sur le chromatogramme par le p-aminophénol en solution alcoolique 1%, les substances étant ensuite identifiées selon le Rf des différents glucides utilisés comme témoins. La figure 2 représente le chromatogramme des glucides solubles extraits de la biomasse de l'algue, notamment : le rhamnose, l'arabinose, le saccharose, le lactose et le raffinose, le saccharose étant quantitativement prédominant.

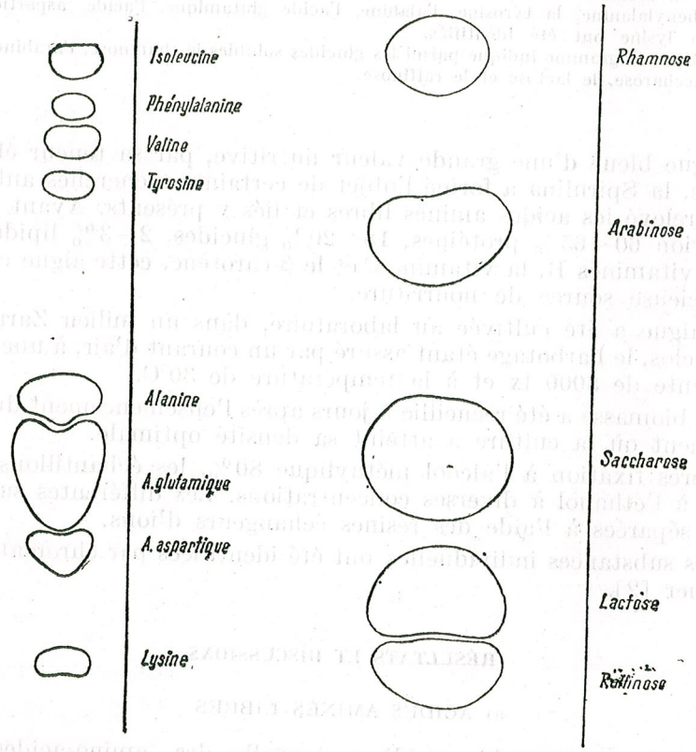

Fig. 1. — Chromatogramme des acides aminés libres.

Fig. 2. — Chromatogramme des glucides solubles.

Les analyses visant la Spirulina ont mis en évidence tous les acides aminés essentiels présents dans la protéine [1], [3] ; seuls les acides aminés à soufre ont été identifiés en quantité insuffisante.

Parmi les 8 acides aminés essentiels et absolument indispensables à l'homme nous avons mis en évidence 5, à savoir : l'isoleucine, la phénylalanine, la valine, la lysine et la tyrosine. La méthode de travail utilisée

ne nous a pas permis d'identifier les acides aminés à soufre. En outre, l'acide glutamique, non essentiel, est quantitativement prédominant.

Les recherches concernant les glucides solubles de la Spiruline sont relativement peu nombreuses. Un pourcentage total des glucides de Spirulina indique des valeurs comprises entre 18 et 20% [4]. Quantitativement, il paraît que tant les mono, que les di-et tri-saccharides y sont représentées, par exemple le saccharose et le raffinose qui sont bien représentés parmi les glucides solubles que nous avons identifiés.

Sans doute, une analyse portant également sur les acides aminés libres et liés serait indiquée en vue de caractériser la composition des protéines dans la biomasse de l'algue. C'est d'ailleurs ce que nous nous proposons. L'investigation et la comparaison des résultats obtenus par rapport aux données connues des plantes supérieures ou à d'autres groupes d'algues, justifient une recherche plus poussée de la teneur chimique de cette algue [5], qui suscite actuellement un intérêt croissant, bien justifié par sa valeur nutritive bien connue.

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Reçu le 5 octobre 1979

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EFFECTS OF LIGHT QUALITY ON THE ACCUMULATION OF BIOMASS, PROTEIN AND BOUND AMINO ACIDS IN *CHLORELLA VULGARIS*

BY

GH. POPOVICI, O. BOLDOR, DOINA STANCA, IOANA SPIRESCU and GABI BARBU

The investigations carried out on two strains of *Chlorella vulgaris* show that the spectrum of light has a different influence on accumulating biomass, protein and bound amino acids. The effect of blue light is more evident in accumulating amino acids and proteins as compared to red and fluorescent light.

The study of light quality on algae growth presents a practical interest, as regards the orientation of metabolism to an increased accumulation of biomass with a high percentage of protein, and a theoretical interest for the display of one or the other of metabolic pathways of glucids and amino acids synthesis. The results in literature point out that in blue light a higher synthesis of amino acids and proteins takes place, while in red light the glucids are mainly synthesized [1], [2], [4]—[9]. Our investigations were aimed towards the effects of light quality on the growth of two strains of *Chlorella vulgaris* in order to increase the efficiency of the production of biomass rich in proteins.

MATERIAL AND METHOD

The investigations were carried out on two strains of *Chlorella vulgaris* collected from different geographical areas. The strain 1 was collected in the north-west of the country, from the dam lake Gilău, district Cluj and strain 2 from the south-east of the country, lake Ciuperca, district Tulcea.

The algal strains were cultivated on the nutritive medium Arnon with microelements, in an installation of the pool type (Fig. 1), with possibilities of stirring the culture medium in order to assure a uniform illumination of algae, keeping the temperature at a constant value (28°C) and bubbling with air as a source of CO_2, necessary in photosynthesis.

The algae were cultivated in fluorescent, red and blue light with a continuous illumination of 2400 lux, along the whole period of the culture cycle. The culture cycle was of 10 days for strain 1 and 6 days for strain 2, considering the culture cycle closed when the algae reached a maximum accumulation of biomass.

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The light spectra were obtained by means of styplex filters, with a percentage of transmitting light of 85%, for red light at the wavelength of 620 nm, and 75% for blue light, at the wavelength of 440 nm, and 85% for colourless filter (Fig. 2).

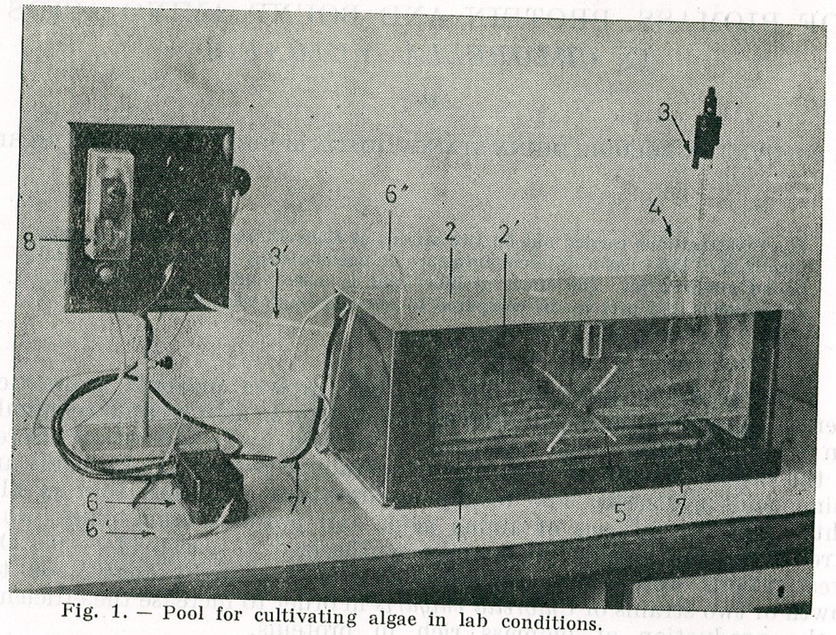

Fig. 1. — Pool for cultivating algae in lab conditions.

1: Tank of pool made of plastic; 2, 2': Styplex screens of various colours; 3, 3': Contact thermometer and electric conducter; 4: Thermometer for control of temperature in algal suspension; 5: Styplex pellets stirring for the algal suspension. 6, 6', 6'': Vibrator with electric conductor and connection tube for barbotating the algal suspension; 7,7': Electric resistance with the conductor. 8: Relay for maintaining the temperature of suspension at a constant value.

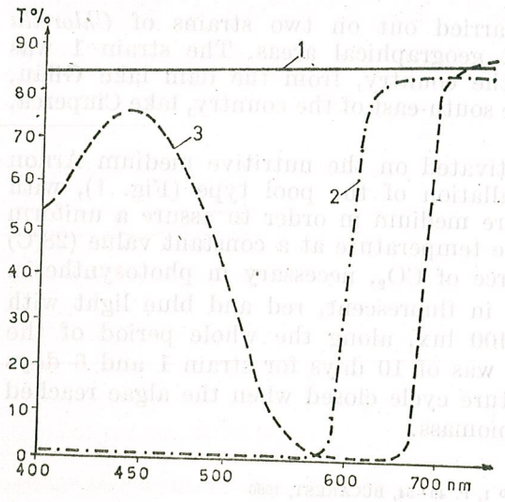

Fig. 2. — Spectrum of coloured filters.

1: Colourless filter; 2: Red filter; 3: Blue filter.

In order to appreciate the effects of light quality on algae growth at the end of the culture cycle the following were investigated: the accumulation of dry biomass in a thermostat, at 75°C, accumulation of protein, calculated by the nitrate determined by the Kieldahal method, as well as the accumulation of bound amino acids, determined by the hydrolysis of dry matter with HCl 4N at 110°C for 24 hours and purified by means of ion exchanges, then separated by chromatography on paper and quantitatively determined by the photocolorimetric method of Moor and Stein [3].

RESULTS AND DISCUSSIONS

Investigating the effect of light quality on the accumulation of biomass in the two strains, one can notice that they present a different

Table 1

Effects of light quality on the accumulation of dry weight, protein and bound amino acids in *Chlorella vulgaris*

Estimation indices	Expressed in:	Strain 1			Strain 2		
		LF*	LR*	LA*	LF	LR	LA
Dry weight	mg/l/24 h	44.64	41.84	45.25	44.19	45.22	61.40
	mg/l/cycle	446.40	418.40	452.50	285.18	291.36	368.70
Protein	%	41.72	35.81	43.92	42.82	37.11	51.89
Bound amino acids	%	33.17	31.27	35.29	34.25	29.69	41.51

LF * = fluorescent light
LR * = red light
LA * = blue light

Table 2

Effect of light quality on the accumulation of dry matter, protein and bound amino acids (% with respect to fluorescent light)

Estimation indices	Strain 1			Strain 2		
	LF*	LR*	LA*	LF	LR	LA
Dry weight	100	93.72	101.37	100	102.17	129.29
Protein	100	85.83	129.84	100	86.17	121.16
Bound amino acids	100	94.27	106.39	100	86.69	121.20

LF * = fluorescent light
LR * = red light
LA * = blue light

behaviour both function of the light quality and their peculiarities. Strain 1, collected in the north-west of the country, has a larger culture cycle than the second strain cultivated in the south-east of the country (10 days

as compared to 6 days). It is possible that this difference between the two strains should be a hereditary one, determined first by the differences in temperature the in areas they grow.

The light quality determines a higher accumulation of biomass only in the case of strain 2, grown in blue light in which 29% more dry matter is accumulated, as compared to those grown in the other light spectra and to the strain 1 (tables 1 and 2).

The algae in strain 1 present, practically, the same capacity of biomass accumulation in all the three light spectra with a slight reduction (2—3%) in red light, as compared to fluorescent and blue light (tables 1 and 2).

The effect of light quality on the accumulation of proteic substances is more evident. It illustrates the role of light spectrum in determining the biosynthesis of either glucids or proteins.

The investigations carried out on *Chlorella pyrenoidosa* in the presence of red and blue light [1] as well as in higher plants [2], [4], [9] point out the fact that the blue light intensifies the protein and chlorophyll biosynthesis. Our investigations show that in both strains a higher accumulation (21—30%) of proteins takes place in blue light as compared to fluorescent and red light (tables 1 and 2). The algae grown in red light present a protein accumulation below the level of those grown in fluorescent light, i.e. with 15% less (tables 1 and 2). In the accumulation of bound amino acids the same variation is noticed function of the light quality as in protein accumulation (tables 1 and 2).

The quality of amino acids and their quantity in the protein composition is of importance, in order to use the algal biomass as food for animals and in food industry.

Table 3

Effects of light quality on the accumulation of bound amino acids in *Chlorella vulgaris* (% from dry matter)

Amino acid	Strain					
	1			2		
	FL*	RL*	BL*	FL	RL	BL
Lysine and arginine	6.30	1.86	1.59	6.02	3.23	5.60
Histidine, aspartic acid, serine and glycine	9.53	8.12	10.87	5.96	7.12	9.63
Treonine and glutamic acid	7.31	13.86	11.62	5.26	7.18	10.94
Alanine	8.50	4.56	8.90	8.03	7.56	10.73
Valine	1.53	2.87	2.31	8.98	4.60	4.61
Total :	33.17	31.27	35.29	34.25	29.60	41.51

FL * = fluorescent light
RL * = red light
BL * = blue light

Analysing the effect of light spectrum on the quality of bound amino acids, the same amino acids are present in both strains, with quantitative variations according to the light quality (tables 3 and 4).

The algae in both strains, grown in fluorescent light, present a higher quantity of essential amino acids (18—20 per cents from their quantity) such as : lysine, arginine and valine, as compared to those grown in red and blue light (table 4).

Table 4

Effects of light quality on the accumulation of bound amino acids in *Chlorella vulgaris* (% from the whole quantity of amino acids)

Amino acid	Strain					
	1			2		
	FL*	RL*	BL*	FL	RL	BL
Lysine and arginine	18.99	5.95	4.50	17.58	10.91	13.49
Histidine, aspartic acid, serine and glycine	28.73	25.97	30.80	17.40	24.05	23.20
Treonine and glutamic acid	22.04	44.32	32.93	15.36	24.26	26.36
Alanine	25.63	14.58	25.22	23.44	25.54	25.85
Valine	4.61	9.18	6.55	26.22	15.54	11.10
	100	100	100	100	100	100

FL* = fluorescent light
RL* = red light
BL* = blue light

From this point of view the algae grown in fluorescent light have a higher nutritive value than those grown in red and blue light.

Investigating the quantitative variation of bound amino acids, in the two strains, according to the quantity of light, one can notice that in fluorescent light the algae contain a closer quantity of amino acids in the analysed groups, excepting valine that accumulates in a reduced quantity in strain 1 (tables 3 and 4).

In red and blue light, in both strains, higher quantities of amino acids of the groups : alanine, treonine, glutamic acid, histidine, aspartic acid, serine and glycine are accumulated (tables 3 and 4).

CONCLUSIONS

Out of our investigations the following can be noticed :

1. The biomass accumulation in strains of *Chlorella vulgaris* is generally influenced by the light quantity.

2. The protein accumulation is evidently influenced by the light quality. In blue light the proteins are accumulated in larger quantities than in red and fluorescent light.

3. The bound amino acids are also accumulated, in larger quantities in blue light than in red and fluorescent light.

4. The essential amino acids — lysine and arginine — are accumulated in larger quantities in fluorescent than in red and blue light.

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Received February 13, 1979

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THE EFFECTS OF TREATMENTS WITH GAMMA RAYS AND HETEROAUXIN ON *HORDEUM DISTICHUM* L.

BY

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The preirradiatory treatments with heteroauxin had a radioprotective effect contributing to the decrease of functional deficiency of plants. In authors's opinion this effect is due to the reduction of seeds radiosensitivity, the concentrations used having a slight inhibitory action on the biochemical and physiological processes investigated. Administered after irradiation heteroauxin stimulated the recovery processes of plants, in this case occurring a direct relation among concentration, the exposure time of seeds in heteroauxin solutions and the effects observed. When the seeds were treated both before and after irradiation, we observed a cancelling of its positive effects in plants and even an increase of disturbances induced by gamma rays.

The disturbances caused by ionizing radiations in the development of some cellular biochemical and physiological processes are felt quite rapidly in the growing phenomena. Among them, the metabolism of nucleic acids has a special sensitivity to radiations. For example, the inhibition of DNA synthesis is noticed even after 10 minutes from the irradiation. In their turn, the injuries induced by radiations in the synthesis of nucleic acids lead to the inhibition of cellular division and even to their death.

It has already been known that the vegetable hormones of auxin-gibberellin type contribute to the growth and development of plants by stimulating their division and length. This phenomenon is based in its turn on the increasing quantity of nucleic acids, therefore on the stimulation of their synthesis.

Starting from the above facts, some authors tried to find out the consequences of the combined treatments with the two agents — ionizing radiations and phytohormones. As to the effects of the irradiation and heteroauxin, Privalov (1974) noticed that the pre-irradiatory treatment with heteroauxin of *Acer negundo* L. seeds led to the increasing of the somatic mutation frequency of seedlings. At a 1% concentration, for example, the frequency of chlorophyllian and morphological mutations in maple leaves grew from 7.5 to 18%. The changes induced by heteroauxin in the frequency of mutations depended on the way the treatments with X-rays and indolyl acetic acid, as well as the hormone concentration succeeded one another. This does not mean that the auxin participated directly in the mutational process, being known that in high concentrations too, auxins do not cause chromosome aberrations. The physiological changes induced by auxin in the irradiated organisms would favour activa-

tion of automutagenic activity in Araratean's opinion (1970). According to other authors [5], [6], the growth regulators would have the function of including or excluding some specific loci from the chromosomes when the genetic information is copied. It is known that the genes in activity are more sensible to the action of mutagens than the repressed ones. Ussuf and Nair (1974) showed that the inhibition of potato germination irradiated with 10 kRad-gamma rays-dose can be removed by heteroauxin treatment. The effect was noticed only when the treatment was applied up to 6 hours after irradiation. The authors established that the indolyl acetic acid administration during irradiation or after maximum 6 hours determined a partial and stable re-establishement of the activity of the heteroauxin synthesis system.

The interesting results of these researches determined us to show how the two categories of agents — the ionizing radiations and phytohormones — act and interact in plants. In this paper we present some results regarding the influence of gamma rays and of heteroauxin on *Hordeum distichum* L.

MATERIAL AND METHODS

Hordeum distichum L. seeds, Elgina variety, were treated in January 1978 pre- or postirradiatory, and even in both ways, with 10 and 25 mg/l heteroauxin. The seeds were firstly immersed for 12 hours in heteroauxin solutions or distilled water (as it was necessary), then they were dried for 20 hours at room temperature up to a water content of 24%. After that, they were irradiated with 7 and 16 kR doses (gamma rays) at a flow capacity of 260 R/min (source 60_{Co}). Immediately after irradiation, the seeds were again immersed in heteroauxin, or distilled water, for 12 hours. For each variant 500 seeds were treated. 100 seeds were grown in Petri dishes for gathering roots for the cytogenetic test (frequency of aberrant anaphases and telophases in the meristem of roots). The other 400 were cultivated in boxes with soil and were kept in a climatized room of our lab (temperature 23—25°C, relative humidity of the air-65%, light intensity 5000 lux-continuous illumination). At ten-day-old plants, grown in these conditions, we determined : the survival percentage, hydric behaviour (transpiration intensity, water content, water deficiency, the water retaining capacity of tissues), the content of soluble sugars, free amino acids, total nitrogen, the catalase and peroxidase activity.

The methods of analysis used have been shown in other papers [2].

In another experiment, the seeds of *Hordeum distichum*, Intensiv-1 variety, were irradiated with 10 kR dose of gamma rays (250 R/min) and then they were postirradiatory treated for 6 or 12 hours with solutions of heteroauxin (10, 20 and 30 mg/l). For each variant 500 seeds were used. These seeds were grown in the climatized room. The same tests were applied on 7-day-old plants, minus the cytogenetic test.

The data obtained from our investigations are presented in tables 1 and 2.

RESULTS

The plants from the single-treated seeds with heteroauxin solutions have a high survival percentage, a lower percentage of aberrant cells in radicular meristems, a higher catalase and peroxidase activity. Anyway, the concentrations and exposure time of the seeds into heteroauxin solutions were probably too high, because they determined a decrease of growing processes, resulting in the smaller dimensions of plants.

Some disturbances were also produced in the hydric behaviour of plants. Though heteroauxin is known as a chemical factor which contributes to the intensification of water absorption by roots, in our case we think that it inhibited somewhat this process ; this is because the water deficiency increased and the hydric content of the plants lowered (Table 1).

The single treatment with gamma rays induced important genetic lesions, which had negative repercussions on the development level of some metabolic and physiological functions. Thus, the water content and the water retaining capacity of the plants were lower, leading to the increase of their hydric deficiency. We also noticed the decrease of the synthetic functions of the organism, reflected by the accumulation of some metabolic compounds such as sugars. These lesions as well as other possible ones, which were not investigated by us, led finally to the decrease of the survival percentage of the plants, as well as to the lowering of their height.

In combined treatments, the indolyl acetic acid shows radioprotective effects, hindering the appearance of more profound disturbances in the development of some metabolic processes in the irradiated organisms. This protecting effect may be due just to the inhibitory action of the solutions used. In the above conditions heteroauxin hindered somewhat the occurrence and development of some metabolic processes which lowered the radiosensitivity of organisms. We do not exclude even the possibility of establishing some complexes between the hormone and other molecules (of vital importance for the organism), phenomenon which would give them a high level of radioresistance.

Whichever the mechanism may be, sure is the fact that : at variants pre-irradiatory treated with heteroauxin (Table 1) the percentage of chromosome aberrations in root meristems lowers ; ameliorations are noticed in the hydric behaviour of plants : their hydric deficiency is reduced as a probable consequence of increasing water retaining capacity of tissues. This permits the achievement under better circumstances of growing processes in the plants of these variants in comparison to those treated only with gamma rays. Consequently, the dimensions of the combined treated plants (with heteroauxin solutions and gamma rays) are evidently bigger than of the plants treated with gamma rays only.

The heteroauxin solutions used in postirradiatory treatments also contributed evidently to the decrease of the functional deficiency of plants (Tables 1 and 2). The positive action of indolyl acetic acid resulted in the behaviour of many investigated indices. In this case the manner in which heteroauxin acts is entirely different from that supposed. At one variant (8 kr $+ H_{10}$ for 12 hours), (Table 1), the stimulation of the recovery

Tab

The effect of the treatment with gamma ra

No.	Variant	Survival after 10 days %	Height of 7-day-old plants $\bar{x} \pm s\bar{x}$		Total A + T analysed	Total A + T aberrants $\bar{x} \pm s\bar{x}$		Transpiration intensity mg/gfr.s/1 min)	Hydric content % fr.s.
1	Control	83.75	169.30	2.10	784	0.76	0.01	5.22	92.34
2	H_{10}	87.00	147.01	2.25	1381	0.36	0.04	4.42	91.22
3	H_{25}	90.25	161.45	2.88	822	0.00	0.00	5.88	91.32
4	8kr	84.25	121.84	2.06	1348	4.90	0.02	5.25	91.15
5	16kr	54.00	93.54	2.56	1292	12.65	0.02	5.65	89.99
6	H_{10}+8kr	85.00	154.78	3.30	1432	2.16	0.01	4.76	91.49
7	H_{25}+8kr	81.50	121.96	3.67	1327	3.84	0.01	5.57	90.98
8	H_{10}+16kr	65.75	107.74	3.00	1335	8.61	0.02	5.25	90.49
9	H_{25}+16kr	45.50	104.82	3.86	1064	15.22	0.03	5.39	90.33
10	8kr+H_{10}	90.75	166.50	2.50	1155	4.24	0.02	4.44	91.69
11	8kr+H_{25}	85.75	136.57	2.46	1462	3.90	0.01	4.10	90.83
12	16kr+H_{10}	46.50	99.34	2.59	1096	11.95	0.03	5.92	90.35
13	16kr+H_{25}	63.25	106.08	3.08	—	—	—	5.09	90.62
14	H_{10}+8kr+H_{10}	85.25	118.50	2.15	1332	2.78	0.01	4.91	91.16
15	H_{25}+8kr+H_{25}	74.00	95.30	2.99	1282	11.70	0.02	4.89	91.04
16	H_{10}+16kr+H_{10}	31.50	98.86	3.90	495	17.17	0.08	—	—
17	H_{25}+16kr+H_{25}	40.75	90.90	4.84	1060	21.51	0.04	4.91	90.46

Table

(*Intensiv— I*

1	10kr	95.00	130.90	1.27	—	—	—	6.80	91.26
2	10kr+H_{10}—6h	75.40	127.62	1.53	—	—	—	5.62	91.59
3	10kr+H_{10}—12h	82.40	130.02	1.49	—	—	—	4.85	91.63
4	10kr+H_{20}—6h	91.00	136.30	1.22	—	—	—	4.97	91.39
5	10kr+H_{20}—12h	89.00	137.90	1.24	—	—	—	5.45	91.51
6	10kr+H_{30}—6h	90.40	140.70	1.15	—	—	—	5.53	91.47
7	10kr+H_{30}—12h	83.40	121.17	1.70	—	—	—	4.79	91.79

H_{10} = heteroauxin 10 mg/l
H_{20} = heteroauxin 20 mg/l
H_{25} = heteroauxin 25 mg/l

processes was so efficient that it assured the development of growing processes in almost normal conditions the dimensions of the plants being nearly the same as those of control plants ($\bar{x}$ = 166.50 mm for the variant and 169,30 for the control).

From Table 2, one can notice that in such combinations, the concentration and wetting time of the seeds with the phytohormone solutions are factors which can influence the results. Better results were obtained using higher heteroauxin concentrations (20 and 30 mg/l) but with a shorter time of exposure (only 6 hours). The participation of auxins to the stimulating processes of DNA, RNA and protein synthesis can be the

1

and heteroauxin in *Hordeum distichum* L.

Hydric deficiency (% water res. at sat.)	Water losses after 6 hours (% tot. water res.)	Total sugars mg/g d.s.	Reduc. sugars mg. g d.s.	Total nitrogen % d.s.	Catalase activity H_2O_2 mg/g fr.s.	Catalase activity Specific activity	Peroxidase activity H_2O_2 mg. g. fr.s.	Peroxidase activity Specific activity
4.27	43.25	82.19	34.28	5.53	71.40	17.41	4.20	1.02
5.83	35.48	81.40	20.28	5.53	79.05	15.50	4.91	0.96
5.38	43.22	69.82	29.10	4.98	72.65	16.70	5.18	1.19
5.08	46.63	64.81	43.20	5.29	71.40	16.60	3.93	0.91
7.25	48.10	174.48	42.62	5.28	81.15	16.23	4.46	0.89
4.92	44.71	97.61	32.38	5.16	72.25	26.27	4.20	1.52
4.86	46.22	59.91	39.54	5.09	82.45	15.85	4.12	0.79
5.94	38.74	104.89	19.07	6.16	74.80	19.94	3.95	1.05
6.48	38.44	59.26	18.62	5.08	74.80	16.62	4.08	0.90
5.32	38.02	48.76	18.85	4.97	73.10	13.92	4.51	0.85
6.47	35.61	202.81	50.28	5.49	78.20	15.95	4.56	0.93
6.68	46.97	169.30	28.80	5.38	79.05	14.77	4.68	0.87
5.58	37.02	143.12	38.16	5.04	79.90	15.66	4.12	0.80
4.85	45.29	96.10	42.71	5.01	63.75	15.17	4.92	1.07
4.85	43.46	127.74	41.38	4.95	64.60	14.59	4.18	0.99
—	—	57.71	26.05	—	—	—	—	—
5.96	39.64	75.20	17.46	5.31	79.05	14.24	5.01	0.90

2

variety)

6.15	45.37	85.20	40.30	5.36	67.55	15.52	4.94	1.13
4.44	46.00	75.38	40.34	6.37	70.10	17.74	4.42	1.11
3.98	42.74	77.46	36.07	5.83	73.50	16.51	4.46	1.00
4.91	44.00	73.02	40.44	4.26	71.40	17.00	4.32	1.02
4.40	43.16	89.89	31.91	5.71	74.80	17.00	4.22	0.92
4.44	41.98	87.30	34.05	6.15	70.55	15.33	4.69	1.01
4.83	42.28	83.03	42.62	6.17	74.80	17.80	4.53	1.07

H_{30} = heteroauxin 30 mg/l
6 h and 12 h = treatment for 6 and 12 hours
A + T = anaphases and telophases

mechanism by which they might contribute to the recovery of the biochemical and physiological processes injured by radiations.

By indolyl acetic acid treatment before and after gamma irradiation one can notice an intensification of genetic disturbances and consequently the physiological and biochemical ones in two-row barley. This led to the inhibition of their growing processes and to reduction of the dimensions. Had the time of exposure of the seeds in heteroauxin solution been shorter (maximum 6 hours), the results might have been different. The evident favourable effects obtained by administration of heteroauxin in low concentrations (either before or after the gamma rays treatment) was annihilated by applying it in double concentration.

CONCLUSIONS

The investigations concerning the effect of heteroauxin and gamma rays treatments of *Hordeum distichum* L., Elgina and Intensiv-1 varieties revealed the following facts :

— The treatment of seeds with heteroauxin solution (10 and 25 mg/l for 12 hours) before irradiation has shown certain radioprotective effect, contributing to the prevention of profounder genetic and metabolic disturbances induced by gamma rays. We suppose that the noticed effect was achieved by reducing the radiosensitivity of seeds as a consequence of the inhibitory action of heteroauxin on some physiological and biochemical processes. The effect could be achieved by creating some complexes between phytohormones and nucleoproteins which gave them a higher radioresistance.

— Postirradiatory treatment with heteroauxin also led to the reduction of the disturbances caused by gamma rays in two-row barley. The effect would be due to the intervention of heteroauxin in stimulating postirradiatory recovering processes. Another fact which has been observed is that, at high concentrations of heteroauxin, the shorter exposures in phytohormone solutions are better and *vice versa*.

— The disturbances induced by gamma rays treatment in *Hordeum distichum* are higher when heteroauxin was administered before and after irradiation.

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Received March 29, 1979

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L'EFFET DE QUELQUES COMPOSÉS CHIMIQUES SUR LA MICROFLORE DE LA BOUE ACTIVÉE GONFLÉE

PAR

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On a testé l'action de quelques substances chimiques organiques et inorganiques fréquemment présentes dans les eaux usées domestiques, vis-à-vis de la microflore de boue activée gonflée et spécialement sur les bactéries du genre *Sphaerotilus*.

Deux des substances organiques testées, l'alcool isoamylique et la glucose en concentration de $1^0/_{00}$ ont montré un effet nettement stimulateur sélectif envers les cellules de Sphaerotilus. Tous les composés inorganiques, excepté le nitrate de sodium, se sont révélés nuisibles à toute la biocénose de la boue.

Le « bulking » des boues activées provoque des perturbations profondes dans le processus d'épuration biologique des eaux usées, influant négativement sur la qualité d'effluent.

Le phénomène consiste dans la détérioration des propriétés de sédimentation et le compactibilité des boues activées déterminées par le développement exagéré de quelques microorganismes qui modifient la structure des flocons de boue.

Parmi les différentes sortes de bulking décrites et rencontrées dans la pratique d'épuration des eaux, les plus fréquentes sont celles déterminées par les microorganismes filamenteux et notamment par ceux du genre Sphaerotilus.

Les investigations concernant le phénomène de bulking ont visé la découverte des conditions qui favorisent le développement de ces microorganismes, afin de les prévenir. C'est ce que nous avons envisagé au cours de nos recherches sur l'action de quelques composés organiques et inorganiques — fréquemment rencontrés dans les eaux usées domestiques — sur les bactéries du genre Sphaerotilus.

La découverte d'une substance à l'effet inhibiteur sélectif pourrait permettre de l'utiliser pour combattre le bulking dû au Sphaerotilus. De même, une substance agissant comme stimulante imposerait l'indication d'un autre procédé d'épuration que celui par les boues activées — dans le cas des eaux contenant la substance respective.

MATÉRIEL ET MÉTHODES

Les substances testées et leur concentration sont les suivantes :

1. alcool isoamylique : $1^0/_{00}$, $2^0/_{00}$, $10^0/_{00}$
2. alcool furfurilique : $1^0/_{00}$, $5^0/_{00}$
3. aniline : $1^0/_{00}$, $5^0/_{00}$
4. phénol : $1^0/_{00}$

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5. *para*-nitro-phénol : 1‰
6. acide trichloracétique : 1‰
7. glucose : 1‰, 5‰
8. lactose : 1‰ 5‰, 10‰
9. nitrate de sodium : 1‰, 2‰
10. fluorure de sodium : 1‰
11. chlorure de sodium : 10‰
12. sulfate de cuivre : 1‰, 5‰
13. sulfate de manganèse : 1‰
14. borax : 1‰.

Le test a été effectué sur la boue gonflée, l'effet étant examiné tant macroscopiquement en observant l'aspect et le volume de la boue comparativement au témoin, que microscopiquement par l'examen des préparations de boue, colorées par la méthode de Gram. Sur ces préparations on a décrit les changements morphologiques des cellules de Sphaerotilus ainsi que des autres microorganismes associés.

Les expériences ont été effectuées sur deux variantes de milieu :

a) Milieu à lait (lait 1 ml ; sulfate d'ammonium 1 g ; eau de robinet 1000 ml).

Tableau 1

Influence des substances chimiques sur le développement de la boue gonflée (examen macroscopique)

Substance testée	Concentration ‰	Effet
Substances organiques		
Alcool isoamylique	1	++
,, ,,	2	+
,, ,,	10	−
Alcool furfurilique	1	±
,, ,,	5	−
Aniline	1	±
,,	5	−
Phénol	1	±
Para-nitro-phénol	1	−
Acide trichloracétique	1	−
Glucose	1	++
,,	5	+
Lactose	1	+
,,	5	±
,,	10	−
Substances inorganiques		
Nitrate de sodium	1	+
,, ,,	2	±
Sulfate de cuivre	1	±
,, ,,	5	−
Fluorure de sodium	1	−
Chlorure de sodium	10	−
Sulfate de manganèse	1	−
Borax	1	−

Légende: ++ Développement supérieur au témoin ; + développement similaire au témoin ; ± inocullum apparemment non modifié quantitatif ; − lyse et diminution d'inocullum.

b) Milieu à caséine (hydrolysat de caseine 2 g ; thiamine 4 mg ; riboflavine 0,1 mg ; pyridoxine 0,03 mg ; nicotinamide 0,66 mg ; levure de bière 66,0 mg ; eau distillée 1000 ml).

Les milieux, stérilisés par filtration, ont été répartis en fioles Erlenmeyer de 200 ml à raison de 15 ml par fiole.

Les substances à tester, sous forme des solutions aqueuses stériles, ont été ajoutées ainsi pour réaliser les concentrations finales indiquées plus haut.

Comme inocullum on a pris une boue gonflée obtenue préalablement sur les milieux décrits, en proportion de 1/10. L'incubation à 25°C pendant 7 jours.

Les résultats des observations sont représentés aux tableaux 1 et 2.

Tableau 2

Effet des substances chimiques sur la microflore de la boue gonflée (examen microscopique)

Substance testée	Concentration %	Effet sur *Sphaerotilus*	Effet sur les bactéries associées
Substances organiques			
Alcool isoamylique	1	++	−
Alcool furfurilique	5	±	−
Aniline	1	−	++
Phénol	1	±	++
Para-nitro-phénol	1	−	++
Acide tricloracétique	1	− Invasion des fungi	
Glucose	1	++	±
Lactose	1	+	±
Substances inorganiques			
Nitrate de sodium	1	±	±
Sulfate de cuivre	1	−	−
Chlorure de sodium	10	−	−
Sulfate de manganèse	1	− Invasion des fungi	
Borax	1	−	−
Fluorure de sodium	1	−	−

Légende: ++ et + Développement supérieur au témoin ; ± développement et morphologie des cellules similaires à celles du témoin ; − altérations profondes des cellules.

RÉSULTATS ET DISCUSSIONS

1. Pour la majorité *des substances organiques* testées l'effet stimulateur, indifférent ou inhibiteur sur la biocénose de la boue activée, est fonction de la concentration.

Des concentrations supérieures à 5‰ se sont révélées dans tous les cas nuisibles pour toute la microflore de la boue.

Les concentrations inférieures à 5‰, notamment celle de 1‰, ont une action variable, fonction de la nature de la substance.

Ainsi, on a pu observer :

— stimulation nette pour : alcool isoamylique et glucose ;
— inhibition pour : aniline, *p*-nitrophénol ;
— effet indifférent pour : phénol, alcool furfurilique, lactose.

Les effets ont été plus évidents dans les milieux à lait que dan ceux à caséine et complexe B.

2. L'action *des substances inorganiques*, à toutes les concentration dans les deux milieux de culture, a été dans la majorité des cas celle d'inhi bition non spécifique.

Le nitrate de sodium 1‰ reste sans effet, tandis que l'acide trichlo racétique et le sulfate de manganèse ont stimulé le développement e exclusivité des champignons.

L'examen microscopique a révélé, des altérations profondes de cellules de Sphaerotilus dans les échantillons où macroscopiquement aussi l'action de la substance s'est montrée inhibitrice. Les cellules morte sont invadées par les bactéries associées qui se développent en abondance.

Sur les frottis effectués en milieux à l'alcool isoamylique 1‰ et à glucose, dans lesquels l'effet a été nettement stimulant, on remarque des groupements abondants de filaments à nombreuses inclusions de poly-hydroxybutyrate, prouvant les conditions favorables, avantageant les bactéries filamenteuses dans leur compétition avec la microflore associée.

CONCLUSIONS

1. Les bactéries du genre Sphaerotilus sont relativement sensibles vis-à-vis des différentes substances chimiques ajoutées dans le milieu, l'effet étant fonction de la nature de la substance et de sa concentration.

Des teneurs supérieures à 5‰ ont agi comme inhibiteurs non sélectifs pour tous les types de microorganismes testés.

2. La composition du milieu a influencé les dimensions de l'effet, le milieu à lait permettant l'exprimation plus claire de l'action de stimulation ou d'inhibition, qu'au milieu à caseine.

3. L'alcool isoamylique et la glucose 1‰ stimulent préférentiellement le Sphaerotilus qui dans ces cas se développe presque en exclusivité.

4. Toutes les substances inorganiques testées, excepté le nitrate de sodium, ont révélé un effet inhibiteur non sélectif.

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Reçu le 30 septembre 1979

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PLANT-MUD-WATER RELATIONS FOR *POTAMOGETON PECTINATUS* AND *CLADOPHORA FRACTA* IN MUDDY ("PELOGENE"), BRACKISH WATER LAKE BALTA ALBĂ (BUZĂU, ROMANIAN PLAIN)

BY

VALENTIN-ALEXANDRU C. BULGĂREANU, VENERA IONESCU-ȚECULESCU, EUGENIA IOANIȚESCU and DIETER HANNICH

This paper is dealing with the physico-chemical relations between the two prevailing taxa — *Potamogeton pectinatus* and *Cladophora fracta* — and their environment: the water and the mud of lake Balta Albă. The chemotaxonomical characterization of the above mentioned plants is followed estimating by statistical methods the ecological factors which are determinant in their growth; among these ones, those referring to the chemical features of mud are comparatively more important from the ecological point of view than the physical parameters of lake water. In the peloïdogenesis processes (=sapropelic mud-forming processes) from this lake, *Cladophora fracta* has a more important contribution than *Potamogeton pectinatus* has.

The relations existing among aquatic vegetation, water and lacustrine sediments have previously been analysed for 4 pelogene, brackish-water lakes from Romanian Plain [1,2,3]: Amara (Ialomița), Balta Albă (Buzău), Cîineni, Tătaru (Brăila) (Bulgăreanu, 1975a, 1975 b; Ionescu-Țeculescu, Bulgăreanu, 1977; 1978).

In this paper, the plant-mud-water relations are examined for the lake ecosystem Balta Albă (pelogene, brackish-water lake, about 1000 ha area) [4], referring to both the chemistry of the two prevailing species *Potamogeton pectinatus* and *Cladophora fracta* and to the influence of some physico-chemical parameters of water and sapropelic mud upon the abundance-dominance indices of the above-mentioned species. Finally, the contribution of the last two plants to the peloïdogenesis was comparatively estimated.

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5 — c. 1790

MATERIAL AND METHOD

The samples of plants, muds and waters were collected from coin cident points (situated on the same vertical). At the reduced maximun depth (2.2 m) and the stratification almost inexistent, the water sample were collected from the lake surface (0—1 m). The collecting depth o mud samples did exceed 30—40 cm, being, generally, coincident wit the extension of *Potamogeton pectinatus* roots.

The plant samples, washed and then dried at room temperature were finally burnt; in the remaining ash there were analysed; SiO_2 (gravi metrically), total Fe (volumetrically), Ca and Mg (complexometrically) Mn (colorimetrically), S and SO_4 (gravimetrically), Na and K (flamephoto metrically) and Cl (volumetrically).

The water and mud samples were analysed following laboratory techniques mentioned in an early paper [5]. The analytical data were pro cessed by statistical means, being calculated the mean values ($\bar{x}$) and Pearson variation coefficient (cv), both for the physical and chemical parameters and for the ratios between those ones. Then, the correlation coefficients (r) were calculated between the abundance-dominance inde and physico-chemical parameters, partly directly determined on the lake, partly analysed in the laboratory.

RESULTS

The comparative chemical composition of *Potamogeton pectinatus* and *Cladophora fracta* is shown in Fig. 1.

Here are mentioned the range of values, mean value ($\bar{x}$) and the Pearson variation coefficient (cv). The latter one shows the stability or the variability of the above-mentioned plant chemistry. The parameters or ionic ratios with minimum values of Pearson coefficient (cv $\leqslant$ 10%) will best characterize the two vegetal species, from the chemotaxonomical point of view. Thus, *Potamogeton pectinatus* is well defined by the HCO_3 : Na ratio; *Cladophora fracta* is best characterized by both his contents in Cl and Na and by the HCO_3 : total Fe, HCO_3 : K and Cl : Na ratios.

The comparison between the range values of both parameters and ionic ratios for the two plants indicates as discriminating criteria between the above-mentioned species, the following ones (see the symbols framed by rectangles, Fig. 1): HCO_3 : Cl, HCO_3 : Na, HCO_3 : K and Cl : Ca (the lines which show the value ranges for these last ratios did not overlap).

For the purpose of estimating the ecological role of physico-chemical parameters which define the biotope of *Potamogeton pectinatus* and *Cladophora fracta* we calculated the Pearson variation coefficient (cv) for the

[4] see footnote 3;
[5] see footnote 1.

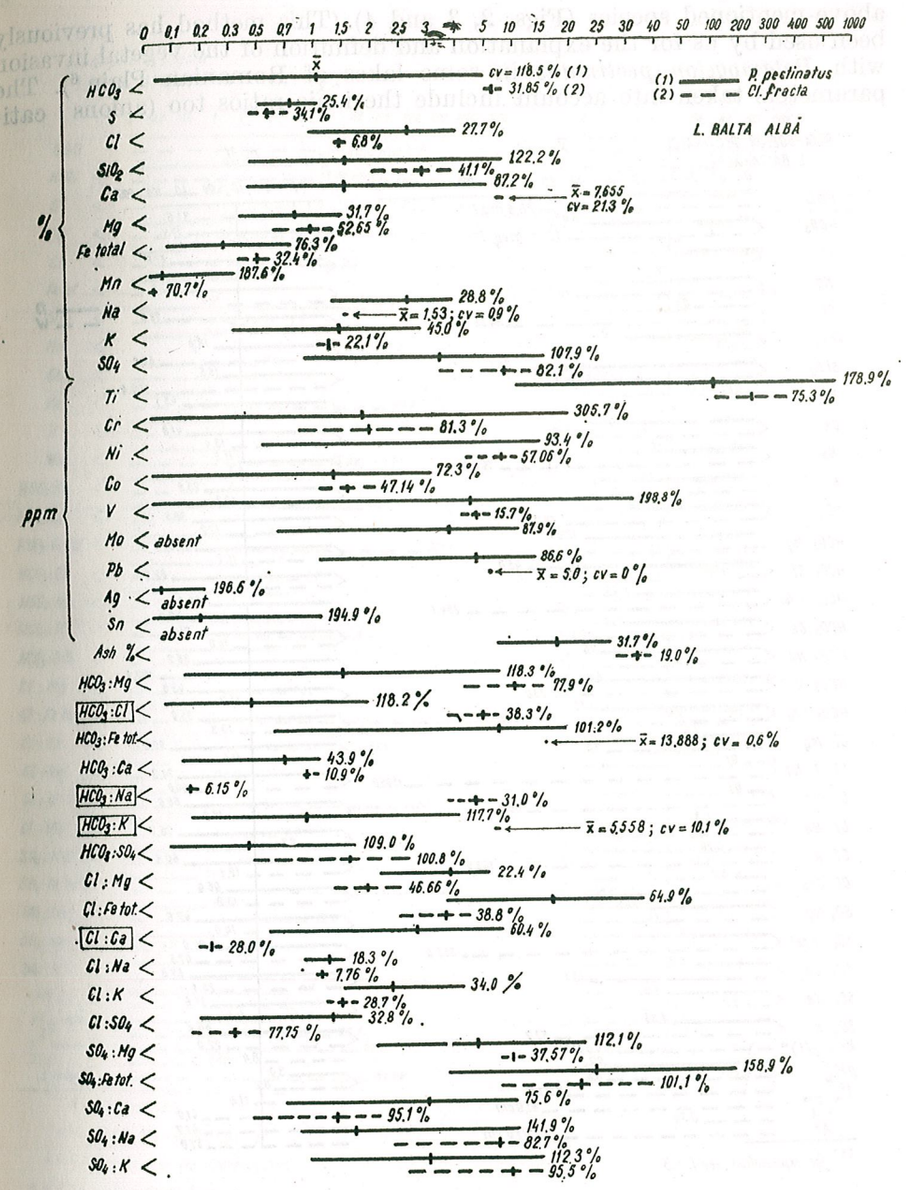

Fig. 1. — Comparative chemical composition of *Potamogeton pectinatus* and *Cladophora fracta*, lake Balta Albă; the horizontal lines (solid or dashed) show the values range of chemical parameters; $\bar{x}$ — arithmetical mean; cv (%) — Pearson variation coefficient (Chemical contents are given for plant samples dried at room temperature).

above-mentioned species (Figs. 2, 3 and 4). (This method has previously been used by us for the explanation and definition of the vegetal invasion with *Potamogeton pectinatus* in some lakes of Romanian Plain [6]). The parameters taken into account include the ionic ratios too (anions : cati-

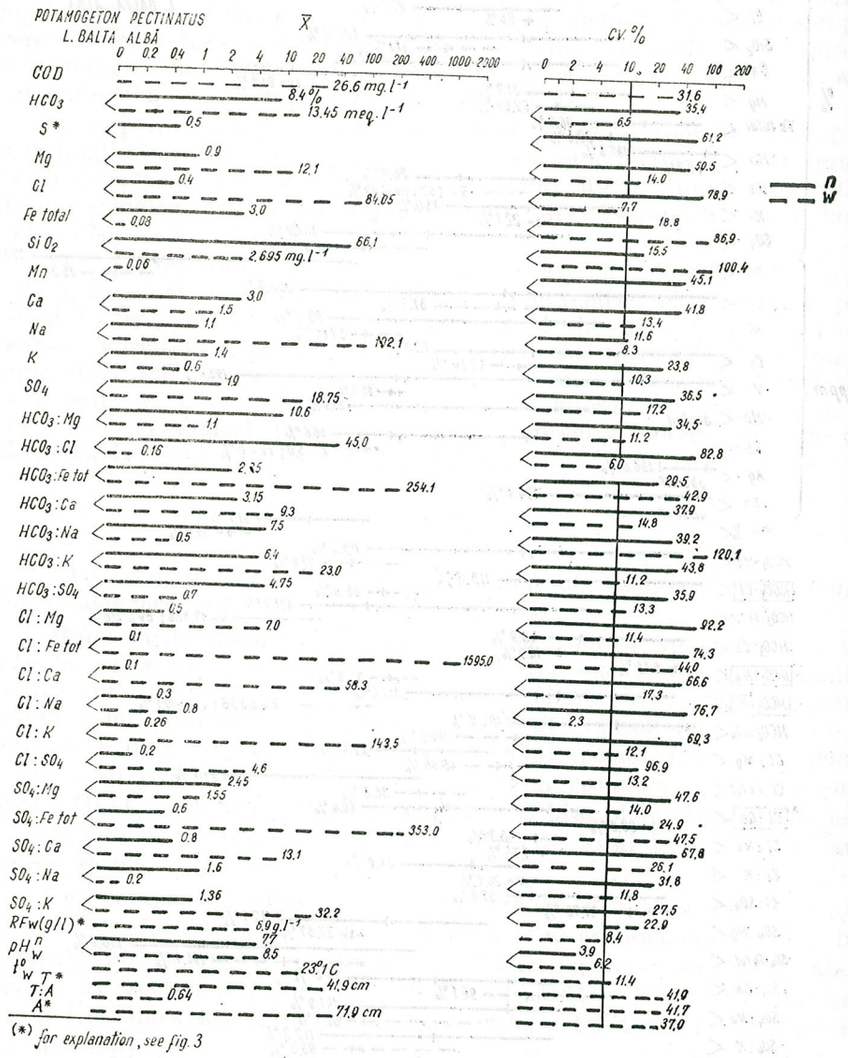

Fig. 2. — Physico-chemical characteristics of the *Potamogeton pectinatus* environment, lake Balta Albă (n — mud ; w — water) ; among the first 10 parameters at the top of the figure, COD and SiO_2 are expressed in $mg.l^{-1}$ and the other ones in $meq.l^{-1}$ (Mud chemical contents are given for the samples dried at room temperature).

[6] see footnote 1.

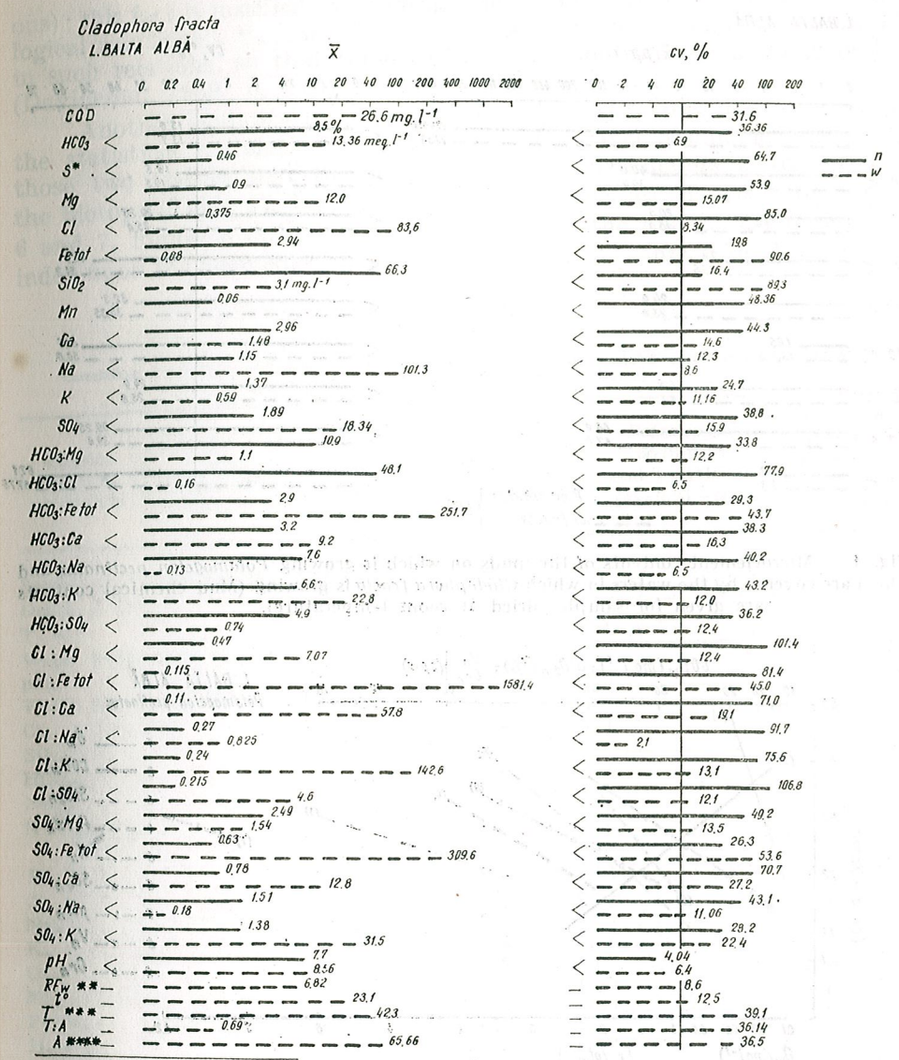

Fig. 3. — Physico-chemical characteristics of the *Cladophora fracta* environment, lake Balta Albă (n — mud ; w — water) ; among the first 10 parameters at the top of the figure, COD and SiO_2 are expressed in $mg.l^{-1}$ and the other ones in $meq.l^{-1}$ (Mud chemical contents are given for samples dried at room temperature).

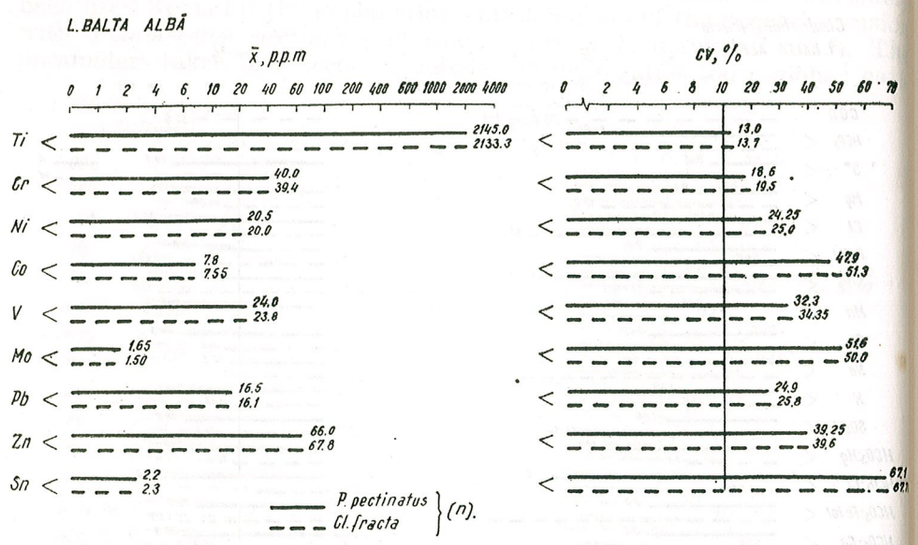

Fig. 4. — Microelement contents of the muds on which is growing *Potamogeton pectinatus* and which are covered by the waters in which *Cladophora fracta* is growing (Mud chemical contents are given for samples dried at room temperature).

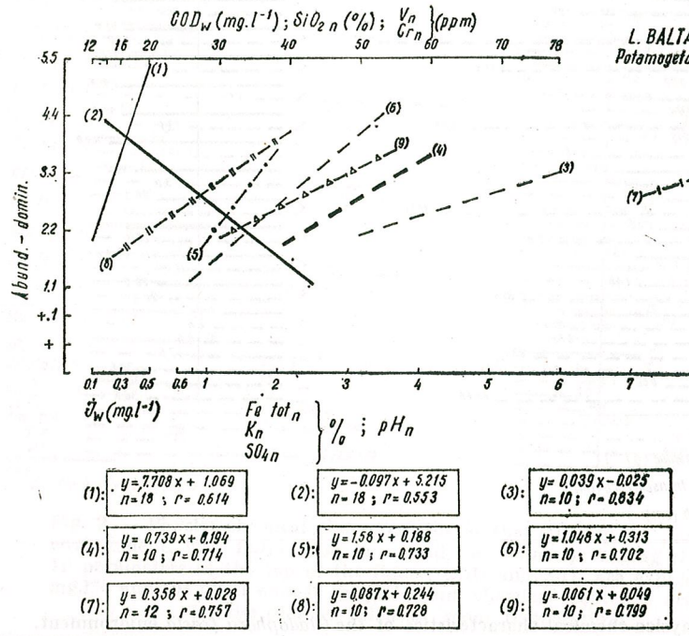

Fig. 5. — Significant statistical correlations between the abundance-dominance index for *Potamogeton pectinatus* and various chemical parameters, lake Balta Albă, see table 1 (Mud chemical contents are given for samples dried at room temperature).

ons); this fact is justified by the principle of so-called "equilibrated physiological solutions", "... in which, the ions of various elements are found in such relations, so that they are most suitable for the plant growth" (Davidescu, 1969, p. 150).

Another modality to estimate the ecological factors was to analyse the statistical correlations between the abundance-dominance index of those two species and the physico-chemical parameters characterizing the biotope (Table 1). The significant correlations are shown in figures 5, 6 and 7. Figure 8 outlines the dependence of the abundance-dominance index upon the chemistry of the above-mentioned plants. The physico-che-

Table 1

Correlations between the abundance-dominance index and the physico-chemical parameters of water (w), mud (n) and plants (p), lake Balta Albă (Buzău)

Physico-chemical parameters	*Potamogeton pectinatus* good correlations (α=0.01−0.05)	*Cladophora fracta* poor correlations α=0.05−0.1
Secchi disk transparency, T(cm)		
Depth, A (cm)		
T : A		
Water temperature ($t°$), °C		
pH_w		
Water dry residue RF_w (g.l^{-1})		
Cl_w (mg.l^{-1})		
SO_{4w} (,,)		
HCO_{3w} (,,)		
NO_{2w} (,,)		
N inorg. tot_w (,,)		
Br_w (,,)		
J_w (,,)	×	
Na_w (,,)		
K_w (,,)		
Ca_w (,,)		
Mg_w (,,)		
$Fe\ tot_w$ (,,)		
H_2SiO_{3w} (,,)		
HBO_{2w} (,,)		
COD_w (,,)	×	
$C\ tot_n$ (,,)	×	×
$C\ org_n$ (,,)	×	×
S_n (by ignition), (,,)		
Cl_n (,,)		
SiO_{2n} (,,)	×	
Ca_n (,,)		
Mg_n (,,)		×
$Fe\ tot_n$ (,,)	×	
Mn_n (,,)		
Na_n (,,)	×	×
K_n (,,)	×	
SO_{4n} (,,)	×	
Ti_n (ppm)	×	×
Cr_n (,,)	×	
Ni_n (,,)		
Co_n (,,)		
V_n (,,)	×	
Mo_n (,,)		
Pb_n (,,)		
Zn_n (,,)		×
Sn_n (,,)		
pH_n	×	
Plant ash, (%)	×	
SiO_{2p} (,,)		
$Fe\ tot_p$ (,,)		
Ca_p (,,)	×	
Mg_p (,,)		
Na_p (,,)	×	
K_p (,,)	×	
Cl_p (,,)	×	
SO_{4p} (,,)		
HCO_{3p} (,,)	×	
S_p (by ignition) (,,)	×	
Mn_p (ppm)		
Ti_p (,,)		
Cr_p (,,)		
Ni_p (,,)		
Co_p (,,)		
V_p (,,)		
Mo_p (,,)		
Pb_p (,,)		
Ag_p (,,)		
Sn_p (,,)		

no data

mical factors with positive ecological role are mentioned synthetically in table 2. For every biotope (water — w; mud — n), the following 4 variants were examined: (a) cv $\leqslant$ 10%; (b) cv $\leqslant$ 33%; (c) good correlations (significance levels, $\alpha = 0.01 - 0.05$); (d) poor correlations ($\alpha = 0.05 - 0.1$). From table 2 and figures 2, 3, 4, 5, 6, 7 and 8, we may draw the following conclusions:

— for both *P. pectinatus* and *Cl. fracta*, the mud characteristics are determining from the ecological point of view;

Table 2

The physico-chemical parameters with positive ecological role upon the growth of *Potamogeton pectinatus* and *Cladophora fracta*, lake Balta Albă (Buzău)

Potamogeton pectinatus						*Cladophora fracta*					
water (w)			mud (n)			water (w)			mud (n)		
(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(d)	(a)	(b)	(d)
RF_w	RF_w	COD	pH_n	pH_n	pH_n	RF_w	RF_w		pH_n	pH_n	C tot.
pH_w	pH_w	J		Fe tot.	Fe tot.	Cl	pH_w			C tot.	C org.
HCO_3	HCO_3			SiO_2	Na	pH_w	HCO_3			C org.	Na
				Na	SiO_2	HCO_3	Cl			SiO_2	Ti
Cl	Cl			K	K	Na	Na			Fe tot.	Mg
Na	Na			Ti	Ti	HCO_3	SO_4			Na	Zn
K	K			Cr	Cr	:Cl	K			K	
HCO_3	Mg			Ni	V		Ca			Ti	
:Cl	Ca			V	C tot.	HCO_3	Mg			Cr	
Cl :Na	SO_4			Pb	C org.	:Na	COD			Ni	
	COD				SO_4	Cl :Na	$t°_w$			Pb	
	$t°_w$										

— except for the ionic ratios which imply HCO_3, Cl and Na (for both plant species), the water influences the plant growth, only by its chemical oxygen demand (COD) (that is referring only to *Potamogeton pectinatus*) and the dry residue($R F_w$), water pH index (pH_w), HCO_3, Cl and Na seem to be of secondary importance (the sole negative correlation is between the abundance-dominance index and COD);

— the main ecological factors for the two species are (n index for mud): pH_n, Na_n, Ti_n; with subordinate role: Fe tot_n, SiO_{2n}, C tot_n, C org_n, K_n, and Cr_n;

— the discriminating ecological factors between the two species are: SO_{4n}, and V_n (for *Potamogeton pectinatus*) and Mg_n and Zn_n, respectively (for *Cladophora fracta*)

— the *Potamogeton pectinatus* biomass increase (expressed by abundance-dominance index) is positively correlated with the plant contents in ash, Na, K, Cl, HCO_3, Ca and S;

— excepting, probably, the water temperature, the other physical parameters (water depth, Secchi disk transparency and the transparency : depth ratio) have, practically, no ecological significance for the lake Balta Albă and the above-mentioned species.

The importance of chemical and granulometrical nature of substratum was emphasized for various Potamogeton species from some British lakes by Pearsall (1920) and Misra (1938). Similar zonations of *Potamogeton pectinatus*, depending on the substratum and on the water movement, were cited by Pearsall and Pearsall (1923) for calcareous muds in Urswick Tarn and by Varga (1931) at lake Fertö (Neusiedler). In lake Balta Albă, *Potamogeton pectinatus* did not prefer the southern littoral

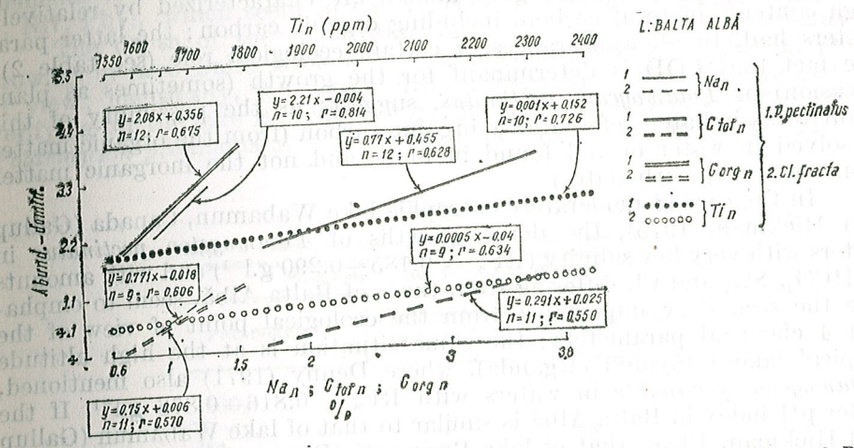

Fig. 6. — Significant statistical correlations among the abundance-dominance index of *Potamogeton pectinatus* and *Cladophora fracta* and some mud chemical parameters, lake Balta Albă; see table 1 (Mud chemical contents are given for the samples dried at room temperature.)

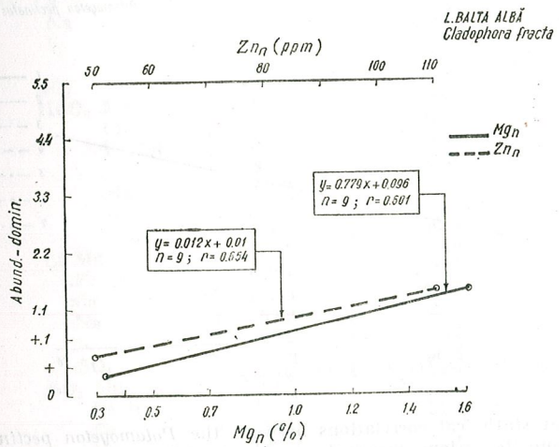

Fig. 7. — Significant statistical correlations between the *Cladophora fracta* abundance-dominance index and the Zn and Ag contents from the mud covered by the waters in which this alga is growing, lake Balta Albă (Mud contents are given for samples dried at room temperature).

zone which consists of sand-prevailing sediments [7] (Ionescu-Ţeculescu, Bulgăreanu, 1977) and the waters are agitated by the northern winds. The sediments on which *Potamogeton pectinatus* is growing, (almost exclusively unctuous, black and gray muds), are characterized by relatively high contents of total carbon, including organic carbon; the latter parameters had, to all appearances, a certain ecological role (see table 2); the fact that COD is determinant for the growth (sometimes as plant invasion) of *Potamogeton pectinatus*, suggests us the possibility of this plant to assimilate preferentially organic carbon (from the organic matter dissolved in water or/and found in mud) and not the inorganic matter from water (bicarbonates).

In the case of moderately eutrophic lake Wabamun, Canada (Gallup and Hickman, 1975), the dense growths of *Potamogeton pectinatus* in waters with very low salinity ($RF_w = 0.185-0.290$ g.l^{-1}) and with amounts of HCO_3, SO_4 and Cl, differing from those of Balta Albă, seem to emphasize the secondary importance from the ecological point of view of the last 4 chemical parameters; the same situation is at the high altitude tropical lake Bunyonyi (Uganda), where Denny (1971) also mentioned, *Potamogeton pectinatus* in waters with $RF_w = 0.816-0.878$ g.l^{-1}. If the water pH index in Balta Albă is similar to that of lake Wabamun (Gallup and Hickman, 1975), that of lake Bunyonyi (Denny, 1971), is very different (4.60—4.65); this fact denotes the non-significant character of pH_w, from the ecological point of view.

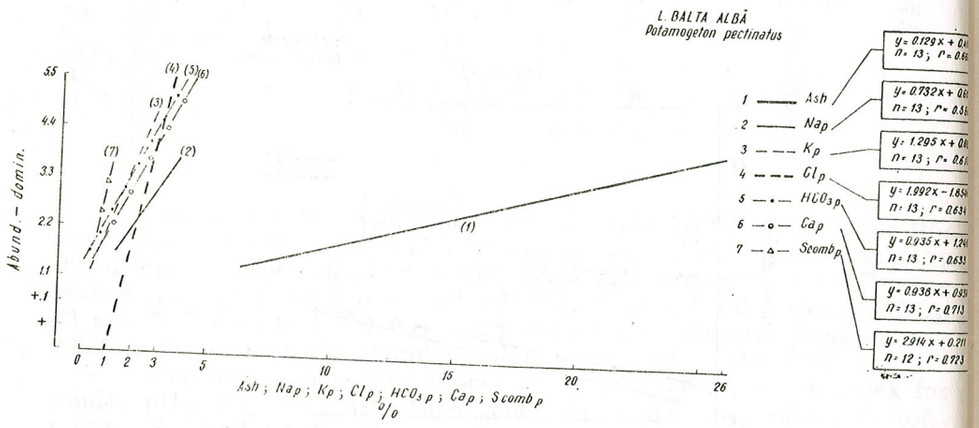

Fig. 8. — Significant statistical correlations between the *Potamogeton pectinatus* abundance-dominance index and its various chemical components, lake Balta Albă (Contents are given for plant samples dried at room temperature).

From the 16 elements, essential for the vegetal life development (Nicholas, 1963, from Goldman, 1965; N, P, Ca, Mg, Na, K, S, Fe, Mn, Cu, Zn, Mo, B, V, Cl, Co) only 9 (Mn, Na, K, Fe, Zn, Ca, S—SO_4, V and Cl), were appreciated as determinant for the development of the above-mentioned species in lake Balta Albă (see Table 2); Nicholas (1963)

[7] see footnote 3.

mentioned, in addition 8 elements, which occur frequently in the ash of plants (Ni, Ti, Pb, Ag, Au, Br, J); the positive effect of these last elements is very little known. From these 8 elements, we found 4 (Ti, J, Ni?, Pb?), which seem to have a certain ecological significance.

The depth of the substratum on which *Potamogeton pectinatus* is growing in lake Balta Albă is not significant from the ecological point of view; identically, for Secchi disk transparency. The first remark is verifying for the lake Bunyonyi Uganda) too, where Denny (1971), found *Potamogeton pectinatus* at very different depths: 0...6 m. But, with this

Table 3

Values of Pearson variation coefficient (cv, %), for various chemical parameters which characterize the hypothetical mixing phase "mud+plant", lake Balta Albă (Buzău)

Parameters	*Potamogeton pectinatus*	*Cladophora fracta*
HCO_3	94.3	36.15
S	45.36	58.8
Mg	41.7	50.9
Cl	81.6	92.2
Fe tot.	97.96	45.1
SiO_2	112.7	50.1
Mn	129.1	58.4
Ca	67.8	60.0
Na	46.4	16.2
K	36.4	24.1
SO_4	102.76	108.25
HCO_3 :Mg	94.8	38.3
:Cl	155.8	91.5
:Fe tot.	103.3	91.5
:Ca	87.1	52.3
:Na	143.9	43.7
:K	105.1	40.4
:SO_4	109.3	50.6
Cl :Mg	75.5	102.6
:Fe tot.	122.1	194.1
:Ca	112.4	63.7
:Na	57.3	89.1
:K	83.5	107.2
:SO_4	74.3	96.4
SO_4 :Mg	107.6	73.1
:Fe tot.	220.3	242.5
:Ca	99.0	73.0
:Na	102.7	95.6
:K	112.1	134.0
Sum :	2822.18	2279.8
Mean :	97.31	78.0

last lake, the light was considered as the foremost factor; this fact seems to gainsay the insignificance of Secchi disk transparency at lake Balta Albă.

The other physical factor — water temperature — to a certain extent contrary to our observations, seems to be, for certain lakes, deter-

minantly from the ecological point of view; thus, in lake Wabamun *Potamogeton pectinatus* occurs only at the mouth of the discharge canal of cooling waters from a thermoelectric power station, i.e. in the region of maximum water temperature and current (Nursall and Gallup 1971, from Gallup and Hickman, 1975).

The comparative estimate of the contribution to the peloïdogenesis processes (= lake mud forming processes), of the *Potamogeton pectinatus* and *Cladophora fracta*, is based on a simplifying working hypothesis : the chemical characteristics of the two phases (plant and mud) should be comparable. That means if a hypothetical mixing phase "mud + plant x" (i.e. a mud formed in a major part from decomposing processes of plant x), will be characterized by very close values of plant x and respective mud chemical parameters (i.e. a minimum value of Pearson variation coefficient, cv), as compared to the other phase "mud + plant y" (for which the variability of the values of the same chemical parameters is greater), the plant x has a more important contribution to the peloïdogenesis than the plant y.

The Pearson variation coefficient (cv), for chemical parameters which characterize the hypothetical mixing phases "mud + Potamogeton" and "mud + Cladophora" are shown in table 3. The comparison between the two ranges of values was made by both addition and calculation of arithmetical means. The results show clearly that "mud + *Cladophora fracta*" phase is characterized by more closed values than "mud + *Potamogeton pectinatus*" phase; this fact proved the more important contribution of alga *Cladophora fracta* to the peloïdogenesis processes.

This result was the expected one; some experimental researches (Edberg, 1977), made with *Cladophora glomerata* (an alga very similar to *Cladophora fracta*) in the moderately eutrophic lake Erken, showed practically a total degradation (90—95%) of this alga, after 100 days. In the same experiment, *Lemna trisulca* (an angiosperm evidently formed of degradation-resistant substances which have no counterpart in alga *Cladophora glomerata*), suffered, after 835 days, a degradation only in a proportion of 50% from the initial weight.

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Received October 5, 1979

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ULTRASTRUCTURAL CHARACTERISTICS OF PALISADE PARENCHYMA CELLS OF THE LEAVES OF NORMAL PLANTS AND OF SOME CHLOROPHYLLOUS MUTANTS WITH *LYCOPERSICON ESCULENTUM* MILL.

BY

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The ultrastructure of palisade parenchyma cells of the leaves of *Lycopersicon esculentum* Mill. was studied in mature normal plants as well as in the chlorophyllous *viridis* mutant, the *sector normal-viridis* mutant and the *albino-chlorine* mutant. The changes of chloroplasts, mitochondria and of the nucleus of these chlorophyllous mutants as against the control are discussed.

Chlorophyllous mutants are found in low percentage both in natural state and also as a result of the action of some mutagen factors. In the specialized literature there are few studies which tackle the ultrastructural characteristics of chlorophyllous mutants [1],[2], [6], [8]. K.—H. von Wangenheim [8] described ultrastructural changes in the primordia of the leaves of wheat plantlets subjected to the action of X-rays. R. Knoth and R. Hagemann [6] described the ultrastructure of the *albostrians* line of *Hordeum vulgare* determined by the presence of the *as as* homozygote gene, insisting on the ultrastructure of plastids. G. C. Corneanu *et al.* [2] described the ultrastructure of palisade parenchyma cells of the leaves from two lines of *Lycopersicon esculentum*, characterized by the presence of the *nv* gene in homozygote (*nv nv*) or heterozygote state (*nv*/+). W. R. Andersen et al. [1] described the chloroplast ultrastructure in the variously colored tissue sectors of the leaves (green, gray, yellow and white tissues) in the *ghost* mutant of *Lycopersicon esculentum*.

In the present paper, the ultrastructure of palisade parenchyma cells from the mature leaves of some chlorophyllous mutants of *Lycopersicon esculentum*, obtained as a result of X-irradiation, is described.

MATERIAL AND METHOD

As a result of the X-radiations treatment of dry seeds of *Lycopersicon esculentum* Mill., the Eurovite, Nemavite and Sonato hybrids (5—35 kr and 400 r/min dose rate) a series of chlorophyllous mutants was obtained in X_1 generation. It was studied the ultrastructure of palisade parenchyma cells of mature leaves from plants grown under the same conditions, belonging to the control variants and to the *viridis* mutants (Sonato, 25 kr; Nemavite, 30 kr), *normal-viridis sector* mutant (Eurovite, 25 kr) and *albino-chlorine* mutant (Nemavite, 35 kr).

REV. ROUM. BIOL. – BIOL. VÉG., TOME 25, Nº 1, P. 79–82, BUCAREST, 1980

Leaf fragments, gathered from the median zone of the same foliole, were prefixed in 3% glutaraldehyde (2 h), postfixed in the 1% Millonig fixing solution $\left(1\frac{1}{2}\text{h}\right)$ and then included in vestopal W. The seriated sections of about 800—900 Å thickness were performed at the ultramicrotome LKB Ultratome III, the contrast of the sections being performed with uranyl acetate and lead citrate. The microscopic analysis of the sections was performed at the electronic microscope TESLA BS—613 of Biology Department in Cluj-Napoca.

RESULTS AND DISCUSSIONS

Control variants. The palisade parenchyma cells from the leaves of control variants present a similar ultrastructure in the three hybrids. The cells are big, having a rich cytoplasm which contains numerous chloroplasts, mitochondria, ribosomes, dictyosomes, elements of the endoplasmic reticulum etc, arranged in the entire cell and along all its walls. The chloroplasts have usually a lenticular or oblong shape and present numerous grana groups arranged orderly on the whole length of the chloroplast. In the grana there are numerous thylakoids (20—50), with no electron clear spaces between them. There are stroma thylakoids between the grana groups which as a rule are undilated and have the same diameter just like the grana thylakoids. In the matrix of the chloroplast there are numerous ribosomes, pyrenoid corpuscles, plastoglobules and accumulating substance (Fig. 2). The mitochondria of different form are found in a great number and present numerous cristae inside. The nucleus has an oblong shape and is found near the plasmalemma (Fig. 1). In the interior it presents numerous electrondense zones of heterochromatin dispersed through the euchromatin as well as on the inner part of the nuclear envelope. The nucleolus presents a central fibrillose zone having a different shape and a peripheric granular zone, in both zones existing electron clear spaces.

Viridis mutants. The palisade parenchyma cells of the mature leaves from the two *viridis* mutants (Nemavite and Sonato hybrids) present a similar ultrastructure. They contain a lower quantity of cytoplasm and only few cytoplasmatic organites, unarranged on all the walls of the cells. The chloroplasts present in section an oval-spherical shape, most of them having disorganized structure (Figs 3, 4). The stroma blades present numerous dilatations, which separate the grana groups in smaller bundles of thylakoids (3)—(8) arranged disorderly in the chloroplast. In the chloroplast matrix there are numerous ribosomes as well as plastoglobules; the quantity of accumulating substance is lower in comparison with the control variants. The mitochondria present generally fewer cristae, they being slightly dilated as well as electron clear zones in the matrix (Fig. 5). The nucleus, usually oblong, and being near the plasmalemma presents fewer heterochromatin zones in its interior (Fig. 5). It is characteristic the fact that the nuclear envelope presents deep invaginations, and in their inside the cytoplasm is crowded with mitochondria. The fibrillose substance of the nucleolus is generally lower in comparison with the control.

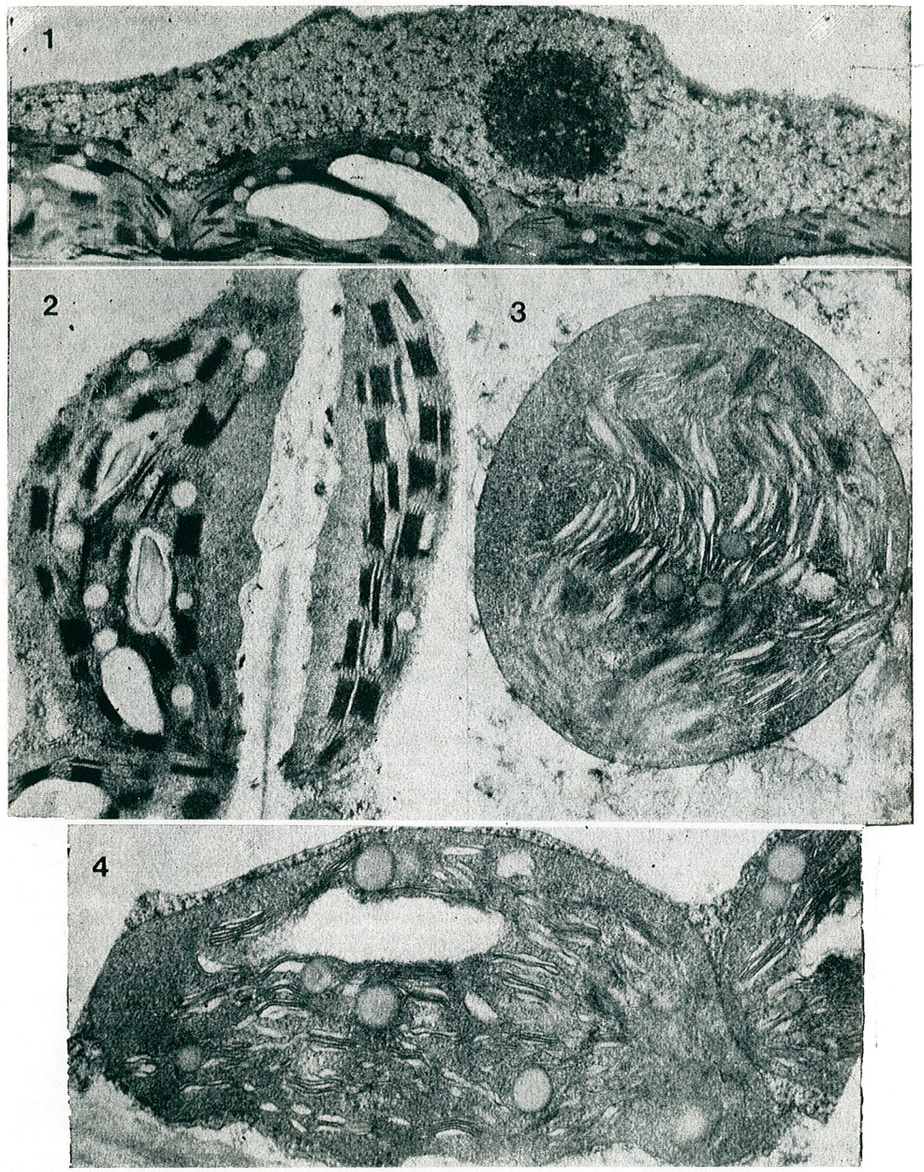

Plate 1

Fig. 1. — The nucleus from the tomato normal leaves (Sonato, 0 kr). × 8000.
Fig. 2. — The chloroplast from the tomato normal leaves (Sonato, 0 kr). × 13,000.
Fig. 3. — The chloroplast from the tomato leaves of the *viridis* mutant (Nemavite, 30 kr). × 18,500.
Fig. 4. — The chloroplast from the tomato leaves of the *viridis* mutant (Sonato, 25 kr). × 24,500.

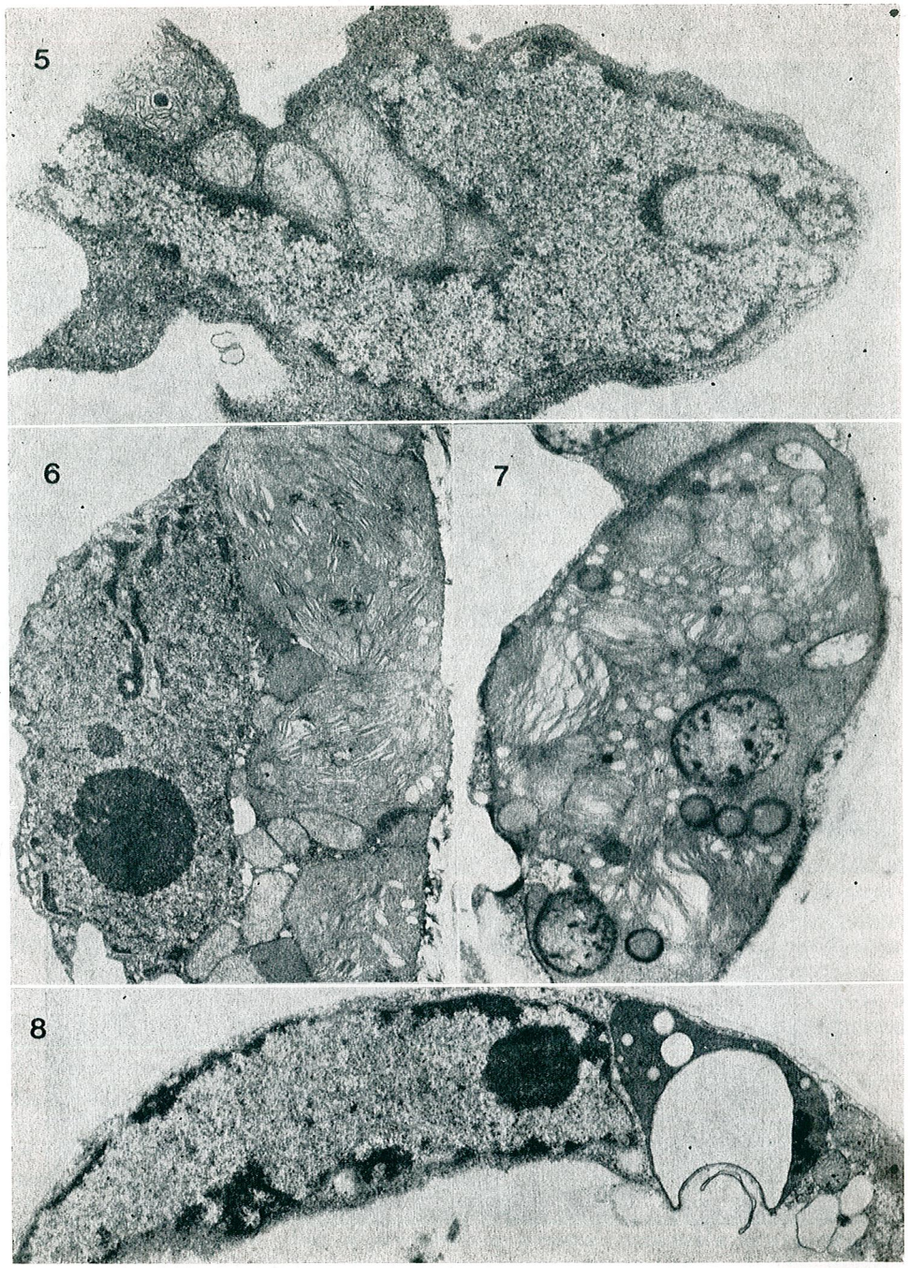

Plate 2

Fig. 5. — The nucleus and the mitochondria from the tomato leaves of the *viridis* mutant (Sonato, 25 kr). × 17,500.
Fig. 6. — The chloroplast, the nucleus and the mitochondria from the *viridis* sector of the tomato leaves of the *sector normal-viridis* mutant (Eurovite, 25 kr). × 8100.
Fig. 7. — The chloroplast and the mitochondria from the tomato leaves of the *albino-chlorine* mutant —(Nemavite, 35 kr), × 16,500.
Fig. 8. — The nucleus and the chloroplast transformed in amyloplast from the tomato leaves of the *albino-chlorine* mutant (Nemavite, 35 kr). × 8300.

Sector normal-viridis mutant. In both sectors of the leaf (the normal and *viridis* ones), it has been found that the structure of cellular organites alters (chloroplasts, mitochondria, nucleus) which is much more marked in the *viridis* sector of the leaf. The cellular organites present the same modified structure (Fig. 6) like that one recorded in the *viridis* mutant. Moreover, we signalize the presence in the caryoplasm, near the nucleolus, of a corpuscle of the "loose body" type, described by E. G. Jordan (1976) and C. Crăciun *et al.* (1979). Their presence is supposed to be linked with virus infection.

Albino-chlorine mutant. The cellular constituents, in small number, are surrounded by a fine film of cytoplasm and are not arranged on all the walls of the cell. The chloroplasts present a very disorganized structure (Fig. 7). The grana thylakoids and the stroma thylakoids are dilated, resulting large electronclear spaces which join and cross the grana. In some chloroplasts there are invaginations in which besides the cytoplasm, penetrate some organites like ribosomes, mitochondria etc. (Fig. 7). Thus in some sections mitochondria appear included in the structure of the chloroplast, presenting or not a link with the cytoplasm. This aspect was also described with the *albostrians* mutant from *Hordeum vulgare* [6]. Some chloroplasts have an aspect similar to that of amyloplasts (Fig. 8). The mitochondria (Fig. 7) present cristae in a small number, at their level there being precipitates of electrondense substance. In their interior there are electronclear zones, due to some dilations of the cristae or of some vacuolization in the matrix. The nucleus having an oblong form is to be found near the plasmalemma (Fig. 8). Sometimes the nuclear envelope presents invaginations. The nuclear chromatin generally presents a homogeneous structure, without zones of heterochromatin in the inside. The heterochromatin is deposited instead in large quantities along the inside membrane of the nucleus (Fig. 8). The nucleolus has a uniform granular structure, the fibrillose zone being not distinct. The tonoplast presents breaking along its length.

CONCLUSIONS

The ultrastructural changes found in the three mutants, as against the control, aim mainly at the chloroplasts, the nucleus and the mitochondria. They reveal the fact that the somatic phenotypic mutation is based on the changes of the structure and of the function of the cytoplasmatic organites implied in the functions of synthesis, assimilation and keeping of hereditary information.

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Received April 21, 1979

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THE EFFECT OF 2.4—DNP ON MITOCHONDRION ULTRASTRUCTURE IN BARLEY ROOT MERISTEM

BY

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2.4—DNP is a well known uncoupler of oxidative phosphorylation which also acts as an inhibitor of protoplasmic streaming in several applied concentrations. The concentration of 25 mg/l 2.4—DNP both alone and mixed with 10^{-3} M $CaCl_2$ or $MgCl_2$ at pH 7 was applied to young meristem of barley root in order to study the changes which occurred within mitochondrion ultrastructure. The treatment lasted for 1h and 30 min. In order to disclose the toxic action of 2.4—DNP some roots were treated with 25 mg/l DNP at pH 7 or 2.5 mg/l at pH 5.5. for 18 hours. The obtained results showed that $CaCl_2$ can preserve to some extent better than $MgCl_2$ the mitochondrion ultrastructure under 2.4—DNP action. The longer treatment of 18 hours with 2.4—DNP has always been destructive for fine plant cell ultrastructure.

2.4—DNP is a well known uncoupler of oxidative phosphorylation both for *in vivo* and isolated mitochondria [2], [9], [10]. The result is the lowering of ATP amount in the living cell after 2.4—DNP treatment.

Our previous researches [4], [5], [6], as well as other papers [1], [2], [8], [11], [12] have shown that the protoplasmic streaming depending on the energy delivered by ATP may be sensitively inhibited by several concentrations of 2.4—DNP.

In order to clear up the 2.4—DNP action on protoplasmic streaming we searched its effect on the ultrastructure of mitochondrion.

MATERIAL AND METHODS

The barley (*Hordeum vulgare* L.) seeds were germinated in Petri dishes on filter paper, daily wetted with tap water. When the seedlings were 3-days old and the primary roots 3—4 cm in length these were carefully cut off and submitted to the following treatments :

1. Several roots were submerged in a solution of 2.4—DNP 25 mg/l at pH 7 for 1h and 30 min ;

2. The same treatment but the pH of the solution was 5.5 ;

3. The roots were submerged for 1 h and 30 min in a mixture of 2.4—DNP 25 mg/l and $CaCl_2$ 10^{-3}M at pH 7 ;

4. A similar treatment with a mixture of 2.4—DNP and $MgCl_2$ 10^{-3}M ;

5. The roots were submerged for 18 hours in 2.4—DNP 25 mg/l at pH 7 or 2.5 mg/l at pH 5.5 ;

The adjustment of pH was made with Na cacodylate.

After treatment the roots were prefixed for 1 hour with 1% buffered OsO_4. The dehydration was performed with acetone and the specimens were embedded in vestopal W [7]. The sections were cut with an LKB ultramicrotome using glass-knives and stained with uranyl acetate and lead citrate. The examination was performed using the Tesla BS—613 electronmicroscope.

RESULTS AND DISCUSSION

1. *Effects of 2.4—DNP.* The short treatment for 1 h and 30 min with 2.4—DNP at pH 7 caused the appearance of many vacuoles within cytoplasm (Pl. I Fig. 1) some of them without tonoplast (Pl. I Fig. 2b) and others with tonoplast (Pl. I Fig. 2c). The mitochondria showed a slight alteration of their ultrastructure ; some disruption of mitochondrion membrane, the disappearance of many cristae and the appearance of small vacuoles inside them can easily be seen (Pl. I Fig. 2a). Similar effects may be noticed at pH 5.5 (Pl. I Fig. 3).

If the treatment at pH 7 lasted for 18 hours, the effect (with 25 mg/l 2.4—DNP) was a destructive one (Pl. I Figs. 4, 5, 6 and 7) but at pH 5.5 (2.5 mg/l 2.4—DNP) the plant cell ultrastructure may be preserved with some modifications (Pl. II Figs. 8 and 9). We have chosen these two concentrations of 2.4—DNP (25 mg/l and 2.5 mg/l) because at these two different pH they actually showed the same inhibitive activity against the protoplasmic streaming. However, the effects on the fine ultrastructure were very different.

On Pl. I Fig. 4 (treatment with 25 mg/l 2.4—DNP at pH 7) we can see the alteration of cell nucleus ultrastructure beginning with anomalous nucleoli and disruption of the nuclear envelope. In more advanced phases (Pl. I Figs. 5, 6 and 7) the nuclear envelope becomes vesiculated and broke here and there so that the content of the nucleus was mixed together with that of the cytoplasm. The mitochondrion ultrastructure was almost destroyed and some osmiophilic (probably lipid droplets) vesicles appeared throughtout the cytoplasm. All these changes show an irreversible state of the cell linked with its death.

The smaller concentration (2.5 mg/l) and the more acid pH (5.5) did not kill the cell. The fine plant cell ultrastructure was preserved but the mitochondrion showed the so-called condensed configuration [3] with soaked cristae (Pl. II Figs. 8 and 9). There are also many vacuoles without tonoplast in the cytoplasm.

2. *Effects of Ca and Mg.* In spite of the fact that in barley root hairs the rate of protoplasmic streaming was slowed down by a mixt treatment with 2.4—DNP 2.5 mg/l at pH 5.5 and $CaCl_2$ [6] the plant cell ultrastructure was well preserved using even 25 mg/l DNP at pH 7 (Pl. II Figs. 10 and 11).The small vacuoles have tonoplast and the mitochondrion ultrastructure is an orthodoxal one [3]. We may assume that Ca^{++} may avoid the destructive action of 2.4—DNP if the treatment is not too long. Mg cations proved to by also protective to some extent (Pl. II Figs. 12 and 13). It cannot avoid the appearance of many small vacuoles without tonoplast but Mg cation may preserve the mitochondrion ultrastructure in

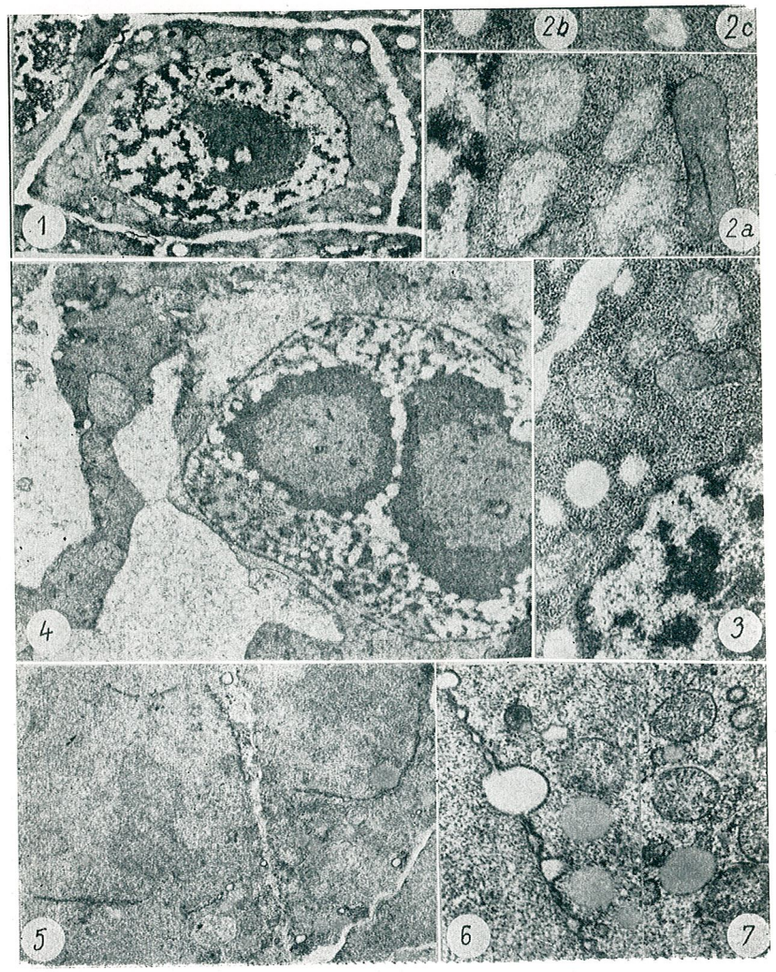

PLATE I Fig. 1. — Effect of 2.4—DNP 25 mg/l at pH 7 on barley (*Hordeum vulgare* L.) root meristem after a treatment of 1 hour and 30 min (× 7250).

Fig. 2. — a) At higher magnification (× 30.000) in order to see the mitochondrion orthodoxal ultrastructure. b) Vacuoles without tonoplast ; c) Vacuole with tonoplast.

Fig. 3. — Effect of 2.4—DNP 2.5 mg/l at pH 5.5 on barley (*Hordeum vulgare* L.) root meristem after a treatment of 1h and 30 min, at higher magnification (× 30.000) in order to see the mitochondrion orthodoxal ultrastructure.

Fig. 4. — The destructive action of 2.4—DNP 25 mg/l at pH 7 after 18 hours of treatment. The disruption of nuclear envelope may be seen (× 7.250).

Fig. 5. — The general view of the disorganisation of plant cell ultrastructure under 2.4—DNP 25 mg/l at pH 7 for 18 hours of treatment. The mixture of nuclear and cytoplasmic contents can be seen by nuclear envelope disruption (× 7.250).

Fig. 6. — An advanced stage of cell disorganization by 2.4—DNP 25 mg/l at pH 7 treatment which lasted for 18 hours. The osmiophilic droplets appeared in the cytoplasm, the mitochondrion ultrastructure was destroyed and nuclear envelope with many vesicles (× 30.000).

Fig. 7. — The same as in Fig. 6 but with several destroyed mitochondrion and lipid droplets (× 30.000).

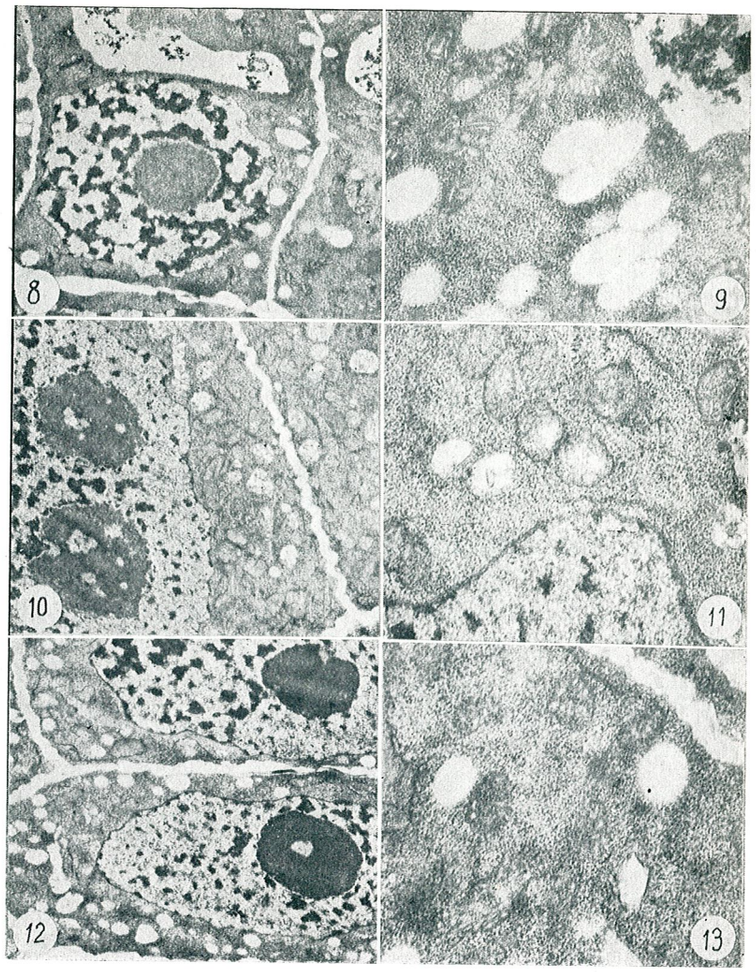

PLATE II Fig. 8. — The action of 2.4—DNP 2.5 mg/l at pH 5.5 after 18 hours treatment. The plant cell ultrastructure is well preserved and the mitochondrion is in the so-called condensed configuration (× 7.250).

Fig. 9. — The same as in Fig. 8 but at higher magnification (× 30.000). The well preserved mitochondrion ultrastructure and many small vacuoles without tonoplast can be seen.

Fig. 10. — The effect of the treatment with a mixture of 2.4—DNP 25 mg/l at pH 7 and $CaCl_2$ 10^{-3}M for 1h and 30 min. The plant cell ultrastructure was well preserved (× 7.250).

Fig. 11. — The same as in Fig. 10 but at higher magnification (× 30.000) in order to see minute ultrastructure of the mitochondrion.

Fig. 12. — The effect of the treatment with a mixture of 2.4—DNP 25 mg/l at pH 7 and $MgCl_2$ for 1h and 30 min. The plant cell ultrastructure was preserved (× 7.250).

Fig. 13. — The same as in Fig. 12 but higher magnification (× 30.000) in order to see the mitochondrion ultrastructure in condensed configuration.

its condensed configuration [3]. Our previous research about protoplasmic streaming [6] showed that a mixture of 2.4—DNP and $MgCl_2$ at pH 7 can re-establish the rate of streaming within barley root hairs near the control values after the inhibition produced by 2.4—DNP. All these to some extent contradictory findings require more careful research on various types of plant cells and under various treatments.

CONCLUSIONS

1. 2.4—DNP treatment leads to a step by step destruction of plant cell ultrastructure.

2. The main alterations of plant cell ultrastructure are the appearance in the cytoplasm of many small vacuoles without tonoplast, the disorganisation of mitochondrion ultrastructure and finally the disrupting of nuclear envelope with the death of the cell.

4. $CaCl_2$ can preserve to some extent the normal plant cell ultrastructure undergoing the inhibitive effect of 2.4—DNP.

5. $MgCl_2$ can preserve only partially the normal plant cell ultrastructure under treatment with 2.4—DNP.

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Recived April 14, 1979

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ULTRASTRUCTURAL ASPECTS REGARDING THE COMBINED EFFECTS OF GAMMA RADIATIONS, CAFFEINE AND FOLCYSTEINE ON CELLULAR DIFFERENTIATION IN THE COTYLEDONS OF *DYGITALIS PURPUREA* L.

BY

AURELIA BREZEANU, FL. TĂCINĂ, I. CIOBANU

The imbibition of *Dygitalis purpurea* L. seeds in solutions of caffeine and folcysteine 1%, before irradiation with doses of 5, 10 and 20 kr, gamma radiations, produced differentiated effects. The caffeine inhibited the metabolization of lipid and protein deposits of the cell and the differentiation of the endomembrane system, producing evident alterations at the plastid level. The folcysteine diminished the destructive phenomenon induced by high doses of radiation, revealing therefore the radioprotective character. As a characteristic element, a strong accumulation of starch was noticed, in the plastidial stroma, indicating therefore some modifications in the metabolism and transport of carbohydrates. Glyoxysomes and peroxisomes showed — under high doses of radiations — a slight hypertrophy and rarefiation of their content. Cytochemically, the presence in active state of the catalase was noticed in all situations.

At present the radioprotective role of some chemical substances of cysteine, cystine and glutathione type is well known. It led to the development of our interest in the field of chemical radioprotection of various biological systems.

The SH-groups of folcysteine, active factors of folcysteine, produced a radioprotective role on animal cells. At the same time the biostimulating role of thyolic groups [8] on plants growth was noticed, creating the premise of testing their effects on plant organisms.

MATERIAL AND METHOD

Three groups of seeds of *Dygitalis purpurea* L. were differentially pretreated for 24 hours, in tap water, folcysteine, caffeine 1%, after which were irradiated with doses of 5, 10 and 20 kr, gamma radiations of ^{60}Co. After irradiation the imbibition continued in the same solutions in which the pretreatment occurred. After 4 days since the beginning of imbibition, samples of cotyledonar tissue were collected and worked for the electron microscopic studies according to the standard method (fixation with glutaraldehyde 3% — 2 hours, postfixation with osmium acid 2% — 2 hours, dehydration in acetone, inclusion in araldite). For cytochemical location of catalase at the microbodies level, the samples were incubated for 1 hour and a half in the 3, 3′, DAB, according to Frederik and Newcomb [5].

The ultrasections were analysed with an electron microscope JEM 7.

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RESULTS AND DISCUSSIONS

ULTRASTRUCTURAL CHARACTERISTICS OF STORAGE CELLS IN COTYLEDONS, AFTER SEED IMBIBITION IN TAP WATER

In this stage of development the cotyledon cells present typical ultrastructural characteristics of a storage tissue in the incipient stage of metabolization (Pl. 1A). Most of the cell content is occupied by lipidic droplets and massive proteic bodies (aleurone grains). As in the case of other species studied by us previously [2], the proteic bodies appear as electrondense formations, delimited by a simple membrane (unit membrane), containing inside an amorphous matrix. Globoidal structures (globoids) and crystalline ones (crystalloid) frequently appear inside. The lipidic deposits are made of electrontransparent drops, delimited by simple membranes, forming a compact mass between the cell wall and the proteic bodies. The other cell organelles (plastids, mitochondria, Golgi bodies) are in an incipient stage of differentiation and generally in this stage they are relatively difficult to identify in structural details, Microbodies are very numerous, well identified after the application of cytochemical reaction, specific of catalase. Their association with the lipid droplets makes us consider them as belonging to glyoxysomes. The metabolization of storage materials, especially of proteic bodies, was also noticed in this stage of differentiation. Round the aleurone grains, areas of lysis appear accompanied by strong vacuolizations of the matrix. As a result of metabolization processes of protein and lipid deposits, the vacuolar system is differentiated.

EFFECTS OF RADIATIONS ON THE ULTRASTRUCTURE OF STORAGE CELLS

Seeds irradiation with doses of 5 kr, previously dipped in tap water, stimulated the enzymatic digestion of proteic and lipid materials as well as the differentiation of cell organelles, first of all of plastids. Many of them present a normal lamellae system. Slight dilatations of grana and intergrana thylakoids are noticed (Pl. 1 B). The microbodies, also very numerous, present associations both with lipid droplets and plastids.

Irradiation of seeds with doses of 10 kr determined modifications in the cell ultrastructure, although the plants presented a normal aspect from the morphological point of view. Anomalies appeared especially at the chloroplasts level, expressed by strong dilatations of intergrana and grana thylakoids (marginal ones) (Pl. 1 C, see arrow). In some cases these anomalies are accompanied by breaks of the plastidial envelope and the whole disorganization of the lamellae system. The microbodies (peroxisomes and glyoxysomes) are slightly hypertrophied and show tendencies of disorganization of their stroma. The nucleus and nucleolus do not show signs of degradation. The only modifications are the dilatations of the perinuclear space (Pl. 1 C) and emission of vesicles from the level of the external membrana of the nuclear envelope (see the arrow).

Doses of 20 kr inhibited the metabolization of lipid and protein deposits (Pl. 1 D) and induced the occurrence of anomalies at the plastidial level. The plastids showed irregular shapes and a slight differentiation

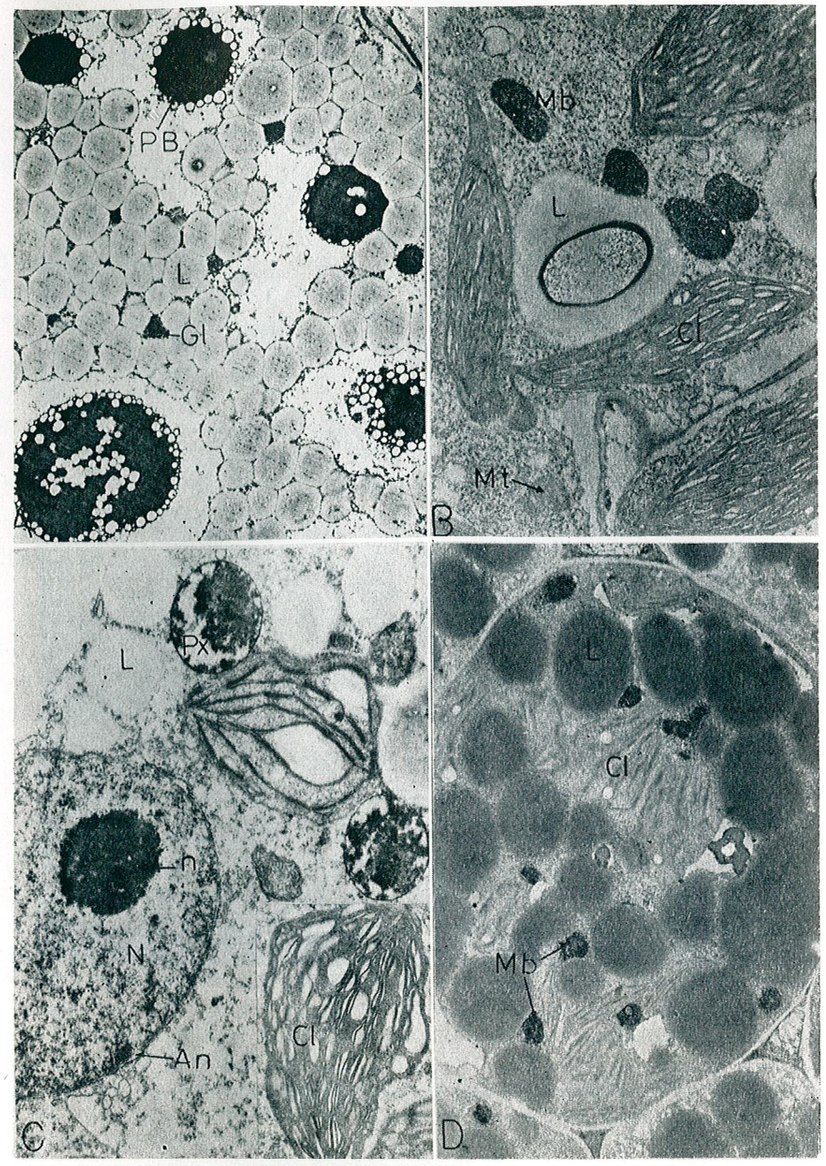

Plate 1 — A. Ultrastructural characteristics of storage cells in the cotyledons of *Dygitalis purpurea* L. after four days of imbibition in tap water; 9,500 × ; B — Part of a cell after irradiation with doses of 5 kr, gamma radiations; × 13,500; C — Modifications induced by doses of 10 kr on cell ultrastructure; × 16,800; D — Inhibition of cellular differentiation under the influence of treatment with doses of 20 kr, gamma radiations ; × 6,700 :

L — lipid inclusions, Gl-glyoxysomes, PB-proteic bodies, Pl-plastids, Px-peroxisomes, Mb-microbodies, N-nucleus, n-nucleolus An-nuclear envelope, Cl-chloroplast.

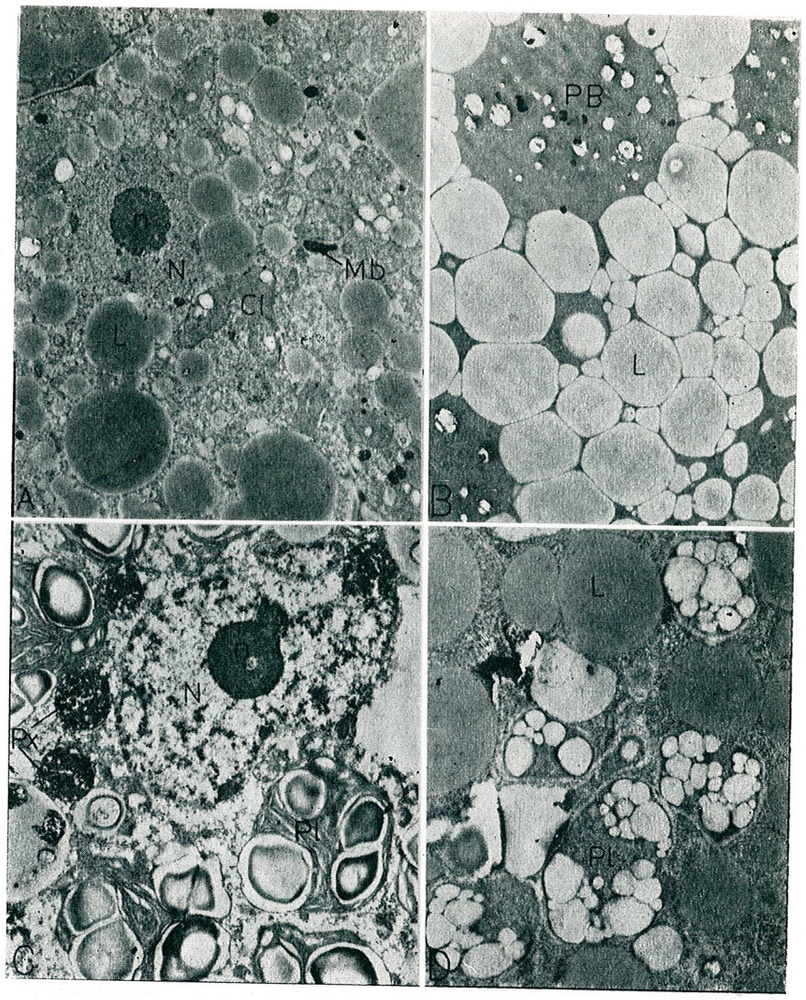

Plate 2 A — Ultrastructural aspect of a cell after caffeine-radiations combined treatment ; × 6,000 ; B — The inhibition of enzymatic digestion of deposits of proteins and lipids and of cellular differentiation processes, as an effect of the combined action of caffeine and radiations in doses of 10 and 20 kr ; × 10,400 ; C — Part of a cell from the material treated with folcysteine and radiations in doses of 5 kr ; × 10,000 ; D — Plastids with multiple amylipherous inclusions, in cells treated with folcysteine and radiations in doses of 20 kr ; 9,800

(The same explanation as in the previous plate)

of the lamellae system. The microbodies, slightly hypertrophied, continue to appear quite frequently. It is remarkable that in all instances they give a positive answer to the cytochemical reaction for catalase proving therefore that the radiations have not affected their content as regards this enzyme.

COMBINED EFFECT OF CAFFEINE AND GAMMA RADIATIONS

The seed imbibition in solutions of 1% caffeine before irradiation with doses of 5 kr produced an inhibition of the cellular differentiation. The cells present typical characteristics of a storage tissue in a latent stage. Protein and lipid bodies are abundant in the cells, and the cell organelles are slightly differentiated. Inside the cytoplasm, relatively reduced quantitatively, a lot of vesicles are found, perhaps born from fragments of the endoplasmic reticulum. The plastids display irregular shapes (Pl. 2 A) and are completely deprived of internal organization. In general, the nucleus does not present important modifications. Only partial alterations of the nuclear envelope are noticed.

Caffeine manifests inhibitory effects on the differentiation of microbodies both as regards their frequency and dimensions.

COMBINED ACTION OF RADIATIONS AND FOLCYSTEINE

The pretreatment with folcysteine before irradiation reduced the range of modifications normally induced by the high doses of gamma radiations. At the same time stimulating effects on the main cellular processes were pointed out, both by the complete enzymatic digestion of proteins and partial enzymatic digestion of lipid deposits and by the differentiation of the vacuolar system. Most of the plastids display a chloroplast structure. The abundance of simple and multiple amylipherous inclusions (Pl. 2 B, C) is a characteristic element. These starch deposits are a characteristic of all variants, as in the case of treatment with folcysteine only. The microbodies are numerous and are associated with both plastids and lipid droplets before their metabolism. Some associations were noticed between the microbodies and the nuclear envelope rarely pointed out. In these variants the peroxisomes and glyoxysomes manifested a positive reaction after DAB incubation for catalase, pointing out that both irradiations and folcysteine do not inhibit catalase activity at the level of these formations.

The experiments called the attention on some more important aspects :

— the various categories of *Dygitalis purpurea* tissues showed a differentiated radiosensitivity ;

— the folcysteine showed double effects, radioprotective and biostimulative, on various categories of plant cells ;

— the effects of gamma ionising radiations showed a specificity character.

As regards the differentiated answer of various categories of tissues when treated with various doses of irradiation, in the case of the species under study, the radiosensitivity of the radicular meristem was noticed as well as the foliar mesophyll and the radioresistance of the cotyledonar tissue. In this last case the effects were not drastic even in the case of

applying doses of 20 kr or the caffeine-radiations combined treatment, although the role of cytoplasmic sensitizer of caffeine is known.

The doses of 5 kr stimulated the cellular differentiation processes and the transformation of storage cells in an assimilatory tissue. In the plant cells treated as such, chloroplasts with a well-differentiated lamellae system were noticed, while in the control samples the structure was typical of a storage tissue. Our observations allow us to suppose that the enzymatic processes which normally are characteristics of the first stages of imbibition—enzymatic digestion of lipid and protein deposits—were stimulated by doses of 5 kr and to a lesser extent by those of 10 kr. In 20 kr irradiation case, the metabolization of storage substances was inhibited; it was revealed by their abundance inside the cell. These effects are much more emphasized by the application of the caffeine—radiations combined treatment. The inhibition of the enzyme digestion of lipid and protein deposits is largely correlated with the reduced differentiation of the cell endomembrane system. Among the cell formations studied, the plastids show the most evident alterations. They appear from the doses of 10 kr and include several modifications ranging from the inhibition of the differentiation of the plastidial lamellae system till its complete disorganization. These phenomena are in fact consequences of the modifications and degradations induced by radiations, at the level of their constitutive proteins. The caffeine administered simultaneously with irradiation emphasized these phenomena.

Folcysteine diminished the alterations induced by the high doses of radiations, pointing out therefore the possibility of its usage in the chemical radioprotection of plant organisms.

As we mentioned above, a first effect of irradiation was the inactivation of various cellular enzymatic systems. Barron [10] considers that this process is due to the transformations of sulfhydryl functional groups in disulfhydryl groups. Therefore, at the level of biological macromolecules, radiosensitive zones are pointed out, the so-called "chemical lesions" being placed there. The radioprotective substances are involved too, as protecting "screens", with the role of taking over the energy from radiations by inactivating the free radicals remoted from water radiolysis. At the same time the radioprotection studies carried out on various macromolecular systems, especially on DNA, pointed out the capacity of these substances to preserve their structure and functional features.

The -SH groups from folcysteine play an important role in the structure and functionality of the mitotic apparatus in the processes of cellular division, getting involved in the processes of growth and development of organisms. They also stimulate the biosynthesis of proteins, glucids, lipids, heteroauxins and enzymes with a role in the REDOX processes.

Finally, the action of -SH groups from folcysteine on the biosynthesis processes is due to the stimulation of aspartic and glutamic acid synthesis, of glutamin — factor which increases the resistance of plant organisms.

The causes of starch deposits at the level of plastids, as a result of treatment with folcysteine, may be determined by several factors such as : an activation of starch synthesis, the inhibition of transport through

envelope as a result of modifications of plastidial envelope permeability, irrespective of inhibition of starch solubilization up to polysaccharides. No matter the cause of this starch deposit it does not lead to the loss of plastid functionality but it has a temporary character.

The experimental data obtained by us allow us to consider that folcysteine by its double character, radioprotective and biostimulative, may be successfully used in the experiments of chemical radioprotection of plant organisms.

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Received April 12, 1979

Institute of Biological Sciences,
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THE STUDY OF MITOTIC CHROMOSOMES AT *MATRICARIA CHAMOMILLA* FROM POPULATIONS ZLOTY LAN ($2n = 32$), CRIŞ IZOLAT ($2n = 18$) AND CRIŞ NEIZOLAT ($2n=18$)

BY

I. I. BĂRA

Investigations on the three populations of *Matricaria chamomilla* have shown the existence of two chromosomial numbers: $2n = 18$ in the Criş izolat and Criş neizolat population and $2n = 32$ in the Zloty Lan population. In all the 3 populations the karyotype is little developed.
The chromosomial dimensions are reduced. The longest chromosomes are in Criş izolat population and the shortest in Criş neizolat population. The origin of the Zloty Lan population as regards the chromosomial number is not very clear yet.

In a previous paper [2] we have studied the mitotic chromosomes of the individuals from Zloty Lan population. The number of chromosomes in somatic cells, the length and the types for each pair of chromosomes were established. Some considerations were made about the basic number of chromosomes as well as their variability limits.

Starting from the reason that the species is not represented by a single population and that the phenotypic differences between populations have a genotypic support (in the number and type of chromosomes), we proposed to extend our investigation on other populations of the species too. Our intention was to make evident some differences between the number, the dimensions and the type of chromosomes.

MATERIAL AND METHODS

The seeds of *Matricaria chamomilla* were obtained (for all populations) from SCPMA — Fundulea. For Zloty Lan population investigations were made in 1977 and for other two populations in 1978, on seeds harvested in 1976 in the first case and in 1977 in the second case. We consider this specification necessary as some differences caused by concrete living conditions in the two consecutive years are possible.

THE GERMINATION

The seeds were put to germinate on 8 February 1978 on moistened filter paper in Petri dishes in a room at 24—25°C. The germination started on the 1st of February 1978 for Criş izolat population, and on 15 February

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1978 for Criş neizolat population. The germination percentage was 11 in the first case and 2 in the second case, for a period of 9 and respectively 5 days.

THE PREFIXATION

When the roots had 0.1—1 cm, the germinated seeds were introduced into a 0.2% colchicine solution for 2 hours, then washed with running water and kept for other 2 hours on moistened filter paper.

FIXATION

The fixation was made in alcohol/acetic acid 3/1. The fixed roots were kept in 70% ethyl alcohol solution.

Staining was assured by Schiff reactive (Feulgen method). The metaphases were obtained by the squash method and examined at the M.C.1—M microscope, with 90 × objective and 15 × ocular. The photographs were realized by Exakta varex RTL 1000 apparatus, with F_2 photo ocular.

RESULTS AND DISCUSSIONS

For the two populations examined in the present investigations, in all metaphases, the number of chromosomes was 18 ($2n = 18$; $n = 9$; $X = 9$). From this point of view, our results are the same with the literature data. Consequently, there is a striking difference between these two populations and Zloty Lan population. In the latter case we registered 32 chromosomes in the somatic cells ($2n = 32$; $n = 16$; $X = 8$).

How can these differences be appreciated?

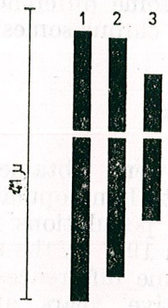


Fig. 1. — *Matricaria chamomilla (Chamomilla recutita* L. Rauchert) The length of the haploid set
1 — Zloty Lan population
2 — Criş izolat population
3 — Criş neizolat population

If $X = 9$ represents the basic chromosomic number, then the diploid must have 18 and the tetraploid 36 chromosomes. Then which was the way in which the 32 chromosomes appeared in the Zloty Lan population? There are two possible interpretations.

I. The diploid $2n = 18$ lost 2 chromosomes. The result was $2n = 16$. The tetraploid of this type is $2n = 32$.

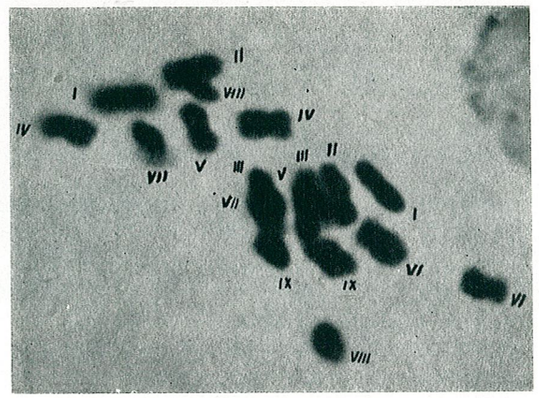
Table 1

The quantitative features at mitotic chromosomes of *Matricaria chamomilla (Chamomilla recutita* L. Rauchert) in populations: Zloty Lan ($2n = 32$), Criş izolat ($2n = 18$) and Criş neizolat ($2n = 18$)

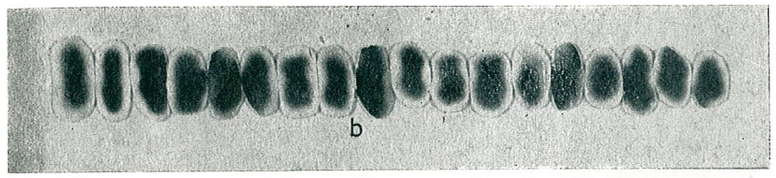
The population	The chromosome pair	The position of centromere	The average length, in microns						The relative length	The centromere index — i—	The ratio of arms — r —
			Total length	Limits of variability	Long arm	Limits of variability	Short arm	Limits of variability			
Zloty Lan	I	m	3.46	4.46 2.35	1.79	2.39 1.07	1.31	1.78 1.00	8.58	37.82	1.37
Criş izolat	I	m	5.04	6.73 3.71	2.69	3.63 2.13	2.35	3.31 1.57	15.47	46.62	1.14
Criş neizolat	I	m	2.72	3.81 2.15	1.54	2.23 1.31	1.18	1.57 0.81	14.22	43.38	1.30
Zloty Lan	II	m	3.20	4.10 2.14	1.57	2.17 0.96	1.31	1.78 0.89	7.92	40.93	1.19
Criş izolat	II	m	4.23	5.42 3.63	2.36	3.05 1.89	1.87	2.36 1.73	13.00	44.20	1.26
Criş neizolat	II	m	2.44	3.34 1.94	1.42	1.76 1.02	1.02	1.57 0.92	12.76	41.80	1.39
Zloty Lan	III	m	3.06	3.89 2.14	1.60	2.07 1.07	1.17	1.46 0.89	7.57	38.32	1.36
Criş izolat	III	sm	3.95	5.50 2.36	2.71	3.78 1.60	1.24	1.84 0.76	12.14	31.39	2.18
Criş neizolat	III	m	2.32	3.26 1.84	1.44	2.07 1.28	0.88	1.18 0.55	12.13	37.93	1.63
Zloty Lan	IV	sm	2.94	3.57 2.42	1.80	2.42 1.35	0.81	1.00 0.64	7.20	27.74	2.21
Criş izolat	IV	m	3.80	4.60 3.44	2.29	3.13 1.97	1.51	1.71 1.05	11.66	39.73	1.51
Criş neizolat	IV	m	2.27	3.05 1.86	1.21	1.65 1,02	1.06	1.42 0.84	11.87	46.69	1.14
Zloty Lan	V	M	2.71	3.50 2.17	1.27	1.60 1.03	1.27	1.60 0.71	6.71	46.87	1.00
Criş izolat	V	m	3.78	5.52 2.89	2.11	3.42 1.60	1.67	2.10 1.28	11.61	44.17	1.26
Criş neizolat	V	m	2.12	2.73 1.86	1.20	1.55 1.05	0.92	1.18 0.78	11.08	43.39	1.30
Zloty Lan	VI	m	2.68	3.50 1.85	1.34	2.07 0.78	1.04	1.35 0.71	6.64	38.85	1.29
Criş izolat	VI	sm	3.36	5.05 1.57	2.49	3.97 1.13	0.87	1.07 0.44	10.32	25.89	2.86

Table 1 continued

The population	The chromosome pair	The position of centromere	The average length, in microns						The relative length	The centromere index —i —	The ratio of arms — r —
			Total length	Limits of variability	Long arm	Limits of variability	Short arm	Limits of variability			
Criş neizolat	VI	m	2.03	2.78 1.81	1.15	1.55 1.05	0.88	1.23 0.76	10.61	43.34	1.30
Zloty Lan	VII	sm	2.64	3.57 1.53	1.70	2.42 1.42	0.71	1.07 0.39	6.53	27.00	2.39
Criş izolat	VII	m	3.03	4.31 2.52	1.67	2.39 1.47	1.36	1.92 1.05	9.30	44.88	1.22
Criş neizolat	VII	sm	1.96	3.02 1.84	1.43	2.23 1.05	0.53	0.78 0.31	10.25	27.04	2.69
Zloty Lan	VIII	sm	2.59	3.21 2.07	1.71	2.00 1.42	0.64	1.07 0.35	6.42	24.72	2.66
Criş izolat	VIII	sm	2.78	3.81 2.42	1.78	2.36 1.39	1.00	1.18 0.55	8.54	35.97	1.78
Criş neizolat	VIII	m	1.73	2.13 1.55	0.95	1.10 0.89	0.78	1.02 0.60	9.04	45.08	1.21
Zloty Lan	IX	m	2.58	3.21 1.78	1.34	1.60 1.07	0.98	1.32 0.53	6.40	38.00	1.36
Criş izolat	IX	m	2.57	2.84 2.10	1.45	1.52 1.26	1.12	1.31 0.84	7.91	43.58	1.29
Criş neizolat	IX	m	1.53	2.10 1.39	0.95	1.36 0.71	0.71	0.73 0.65	8.00	46.40	1.33
Zloty Lan	X	m	2.57	3.03 2.14	1.48	1.71 1.25	0.91	1.32 0.78	6.37	35.44	1.62
„	XI	m	2.56	3.50 1.78	1.27	1.71 0.89	1.01	1.50 0.53	6.35	39.64	1.24
„	XII	m	2.36	3.21 1.89	1.28	1.78 0.82	0.85	1.25 0.42	5.85	36.21	1.50
„	XIII	sm	2.12	2.92 1.50	1.44	1.96 1.03	0.52	0.75 0.35	5.25	24.56	2.77
„	XIV	m	2.05	2.50 1.57	1.08	1.25 0.82	0.76	1.07 0.42	5.09	37.29	1.40
„	XV	sm	1.92	2.28 1.78	1,18	1.60 0.89	0.58	0.82 0.35	4.77	30.30	2.02
„	XVI	sm	1.66	2.46 1.39	1.08	1.46 0.64	0.49	0.71 0.35	4.12	29.40	2.20



a



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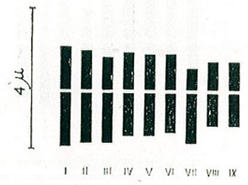
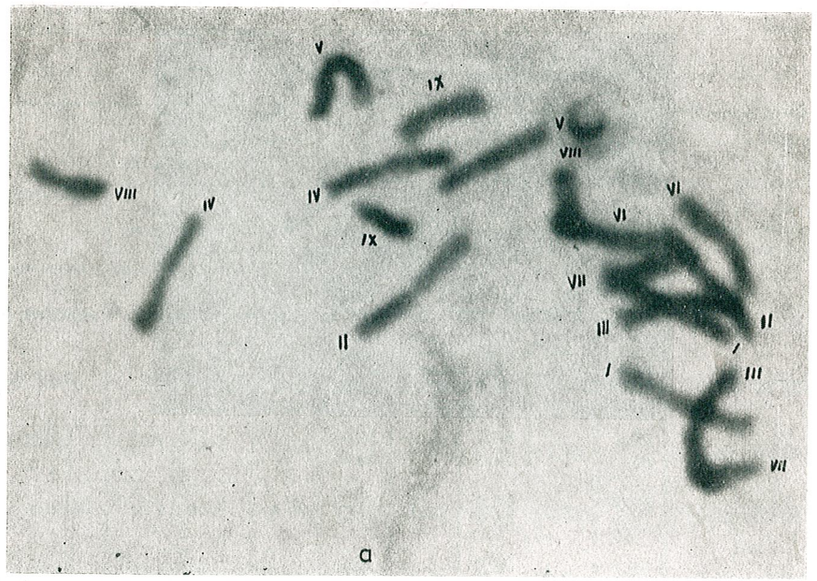
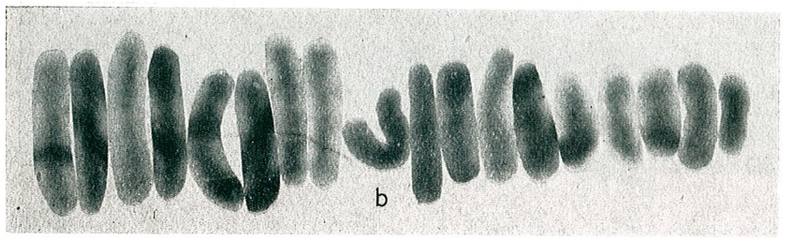


c

Plate 1. — *Matricaria chamomilla (Chamomilla recutita* L. Rauchert) Criş izolat population

a — Metaphase
b — The karyotype
c — The idiogram





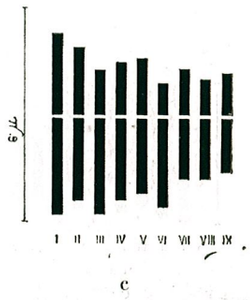


Plate 2. — *Matricaria chamomilla (Chamomilla recutita* L. Rauchert) Criș neizolat population

a — Metaphase
b — The karyotype
c — The idiogram

II. The normal form is $2n = 16$. From this diploid, by tetrasomy, $2n + 2 = 18$ appeared.

The confirmation of one or another from the two hypotheses will be possible only by the study of meiotic chromosomes or by banding. Anyway, from the analysis of morphologic characteristics of chromosomes of the three populations, some clear-cut distinctions could be established between them. Thus, we have found out that the greatest medium length of chromosomes is found at the Criș izolat population (I = 5.04 μ, IX = (2.57 μ) and the least one is registered at the Criș neizolat population (I = 2.72 μ, IX = 1.53 μ) Zloty Lan population is situated, according to the length of chromosomes, between the other two populations (I = 3.64 μ; XVI = 1.66 μ). Though the length of chromosomes of the first pair is inferior to the one registered at the Criș izolat population, the length of chromosomes of the IXth pair comes to exceed that of the similar pair from the Criș izolat population.

Consequently the reduction rate of the length of chromosomes from the first to the ninth pair is less for the Zloty Lan population, than for the other two populations. Besides, for the Zloty Lan population this rate is much slower than the rate registered for the X—XVI pairs, in the sense that if for 9 consequent pairs the length is reduced with 0.88 μ (i.e. 0.09 μ for the pair of chromosomes), for the last consequent 7 pairs the length is reduced with 0.91 μ (i.e. 0.1 μ for the pair of chromosomes).

Accordingly, the first 9 pairs of chromosomes behave more similarly with the 9 pairs of the other two populations.

As to the type of chromosomes for the three populations under investigation, we find out perfect identity in the case of pairs I and II and slight differences between the other ones. The chromosomes from the fifth pair are rather similar and all of them have the centromere in median position. For the rest, the situation is so : Zloty Lan has 7 pairs of chromosomes with the centromere in median position and 6 pairs with the centromere in submedian position; Criș izolat has 3 pairs with the centromere in median position and other three pairs with the centromere in submedian position; Criș neizolat has 5 pairs with a median centromere and only one pair with a submedian centromere. Undoubtedly the position of the centromere, as established by us, may reflect a certain degree of error, that results from measurements and calculations especially in the case of the values that are extremely close. However we cannot help mentioning the fact that all the three populations are characterized by a little advanced karyotype. All the chromosomes are practically registered in two types, except for the Zloty Lan population where we can consider three types. In percentages the situation of the distribution of the chromosomes of the three populations to one or another of the types is as follows.

For Zloty Lan 6.2% of chromosomes belong to the *M* type, 56.2% belong to the *m* type and 37.5% to the *sm* type. For Criș izolat 66.6% of chromosomes belong to *m* and 33.3% to the *sm* type while for Criș neizolat 88.8% belong to *m* and 11.1% to the *sm* type.

The whole length of a haploid set reveals clear-cut distinctions between the three populations. Thus the haploid set is summed up to 41.10 microns for Zloty Lan (22.93 the long arms and 14.36 the short

arm), 32.54μ for Criş izolat population (19.55 on the basis of the long arms and 12.99 on the basis of the short arm) and only 19.12μ for Criş neizolat population (11.29 μ and respectively 7.96 μ).

We can point out that the haploid set of the Zloty Lan population represents, as length, the double of the haploid set of the Criş neizolat population. This can be considered as an argument in favour of the supposition that the Zloty Lan population is the result of a process of aneuploidy where the pairs of longer chromosomes were involved. This would explain the length of the haploid set from this population that is bigger than twice the haploid set from Criş neizolat population. The supposition is sustained by the fact that the sum of the lengths of the first 9 pairs of chromosomes comes up to 25.86 μ — consequently more than the similar pairs of the Criş neizolat population.

The differences between the two Criş populations might be the result of isolation.

As to the variability amplitude of the length of the chromosomes, we find out that the widest limits of variability are found for the Zloty Lan population and the most reduced for Criş neizolat population. Consequently, for the latter, the metaphases studied were much more uniform as to the length of chromosomes. At the same time, the biggest difference between the lengths of the chromosomes of the first and last pair is to be found for the isolated Criş (Criş izolat) population (2.47 μ), and the least is for Criş neizolat population (1.19 μ). For the Zloty Lan population the difference between the first and the last pair of chromosomes is of 1.80 μ, but if we take into consideration only the first 9 pairs of chromosomes, the difference would be of only 0.88 μ. As for the source of the chromosomes of the Zloty Lan population for the moment we can say :

There are no dimensional similitudes between certain pairs of chromosomes belonging to the Zloty Lan population, similitudes that might lead to the conclusion that for example 4 chromosomes belong to the same pair.

As to the other two populations, though it seems abnormally, there are clear-cut distinctions between them concerning the length and the type of chromosomes.

The phenotypic differences materialized in the nonuniform content of active principles find their support in the distinctions existing between the chromosomes of the three populations.

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Received March 26, 1979

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MODIFICATIONS OF GROWTH SUBSTANCES AND REGULATION OF GROWTH PROCESSES IN SOME WOODEN PLANTS UNDER THE INFLUENCE OF ATMOSPHERE POLLUTION

BY

ILEANA HURGHIȘIU and ILEANA BUICULESCU

Comparative investigations were made on growth substances in some wooden species in the polluted and control area, respectively.
An evident action of the pollutants was noticed, expressed by qualitative and quantitative differences of the growth substances.

There are some investigations on growth substances, isolated from different species of plants, under the influence of atmosphere pollution. A reduction of plant growth in certain industrial areas, caused either by the disappearance of some growth substances or their quantitative reduction [1], [4], [5], [6], was noticed.

Recent investigations show the role of auxins in regulating plant growth substances [7].

The investigations carried out by Hurghișiu Ileana in different polluted areas around towns pointed out the characteristic influence of the noxious (polluting) environment on the synthesis of vegetal hormones, expressed by its inhibition or stimulation [3].

The present paper represents a continuation of the investigations related to atmosphere pollution effect on plants, referring to growth substances and their role in regulating the development processes in wooden species, namely *Elaeagnus angustifolia* L., *Populus nigra* L. and *Ulmus procera* L., subjected to the influence of noxae in the industrial area under the conditions of 1977.

MATERIAL AND METHOD

Young leaves from wooden species *Elaeagnus angustifolia* L., *Populus nigra* L., and *Ulmus procera* L. were collected from the industrial area and the control area.

The samples were collected in May, July and September 1977. Qualitative determinations of growth substances were made by means of chromatography on paper [2] and quantitative determinations by means of the biological test Avena [1].

Extractions from 100 g leaves were made in ethyl ether at a pH 5 and respectively 7.5. The chlorophyll was removed by eluting the chro-

matograms with toluene. The migration solvent was n-propanol-ammonia-water 16 : 1 : 3. The identification of growth substances was made with *p*-dimethylaminobenzaldehyde 1% in HCl n.

The biological test for quantitative determinations was made with oats coleoptiles (fragments of 10 mm length) which were kept for 48 hours in elutions of 1 : 10 and respectively 1 : 100 in solution of 2% saccharose. The results are expressed in mm growth rate of oats coleoptiles under the influence of auxins.

The biological test Avena reacts by inhibiting the growth of coleoptiles in the case of some increased concentrations of auxins and *vice versa* by its stimulation, in the presence of small concentrations.

RESULTS AND DISCUSSIONS

The results are presented in figures 1—2.

QUALITATIVE MODIFICATIONS OF GROWTH SUBSTANCES (Fig. 1)

In *Elaeagnus angustifolia* L., the following growth substances: 3-indolyl-butyric acid, 3-indolyl-propionic acid and 3-indolyl-acetic acid were identified during 1977. A qualitative and quantitative accumulation

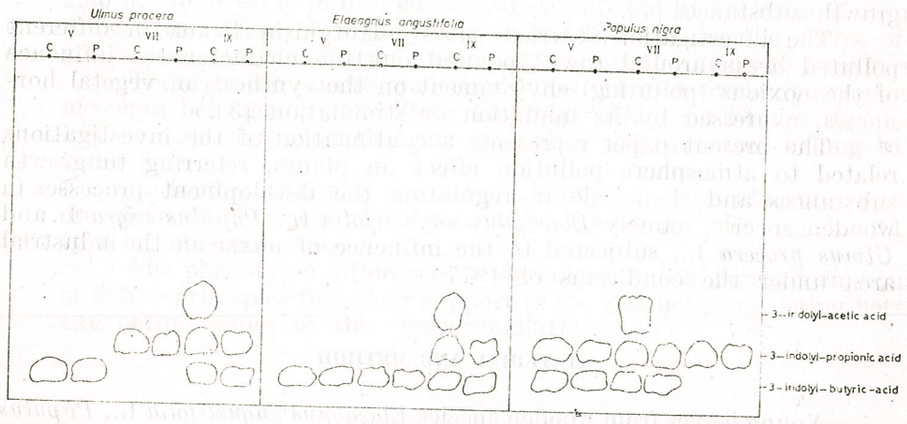

Fig. 1. — Qualitative modifications of growth substances in some wooden plants under the influence of atmosphere pollution during 1977.

of growth substances was found in September. In general, in polluted plants a qualitative and quantitative reduction of growth substances was noticed.

Populus nigra L. was characterized by the presence of the mentioned growth substances, remarking their accumulation in July. The plants subjected to pollution presented a deficiency in growth substances.

As regards *Ulmus procera* L., a similitude as regards the season variation was remarked with that existing in *Elaeagnus angustifolia* L. with an accumulation in the growth substances at the end of the vegetation period. As regards the reaction to noxious factors it is the same as in the previous two species, with an inhibition of the synthesis of growth substances.

QUANTITATIVE MODIFICATIONS OF GROWTH SUBSTANCES (FIG. 2)

In *Elaeagnus angustifolia* L., in plants influenced by noxious factors, higher values were found in the case of 3-indolyl-butyric acid, indicating from the point of view of quantity a lack at the level of growth substances. As regards the 3-indolyl-propionic acid the reaction is the same.

Populus nigra L. presented in July and September a quantitative reduction of 3-indolyl-propionic acid and 3-indolyl-butyric acid.

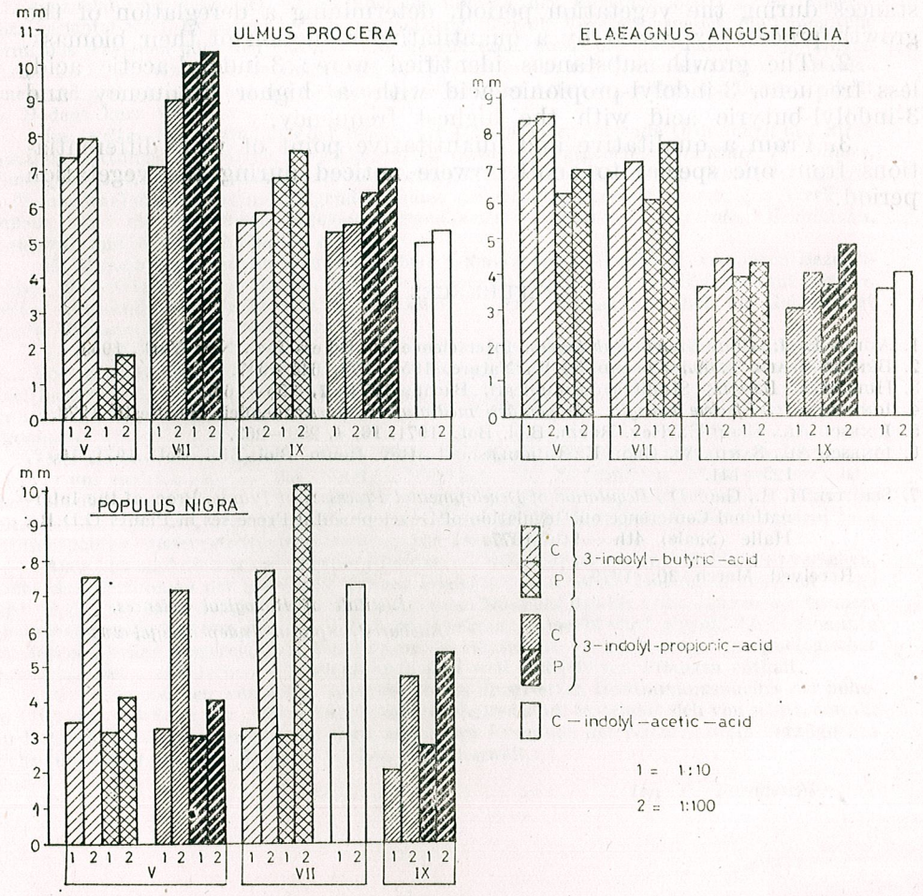

Fig. 2. — Quantitative modifications of growth substances (the biological test Avena) in some wooden plants under the influence of polluted atmosphere in 1977 (mm increase of growth).

As regards *Ulmus procera* L., the plants in noxious environment show quantitative dereglations in the case of 3-indolyl-propionic acid and 3-indolyl-butyric acid.

The qualitative or quantitative lack underlined in all cases as regards the growth substances determines significant modifications expressed by a dereglation of plants growth processes, noticing in general a reduction in their growth, the vegetative biomass being evidently more reduced.

CONCLUSIONS

After the action of noxious factors in atmosphere we may notice that :

1. The wooden plants *Elaeagnus angustifolia* L., *Populus nigra* L. and *Ulmus procera* L., react by inhibiting the synthesis of growth substances during the vegetation period, determining a dereglation of the growth process expressed by a quantitative reduction of their biomass.
2. The growth substances identified were : 3-indolyl-acetic acid, less frequent, 3-indolyl-propionic acid with a higher frequency and 3-indolyl-butyric acid with the highest frequency.
3. From a qualitative and quantitative point of view differentiations from one species to another were noticed during the vegetation period.

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ALEXANDRU BELDIE, Flora României—Determinator ilustrat al plantelor vasculare, **II**, Editura Academiei Republicii Socialiste România, 1979, 406 p.

Im zweiten Bande der „Flora Rumäniens" hat der Verfasser die Möglichkeit den restlichen Teil des großen Reichtumes der Pflanzenwelt unseres Landes den Spezialisten und wohlwollenden Liebhabern des Gebietes vorzulegen.

Seine langjährigen floristischen und geobotanischen Untersuchungen diesbezüglich sind auf diese Weise eine glückliche Vervollständigung seines bedeutenden Werkes über die Pflanzen Rumäniens, die er als einer der besten Kenner unserer Flora, darbringt.

Wie in unserer kurzen Abfassung betreffs des ersten Bandes, wollen wir nachdrücklich auch hier hervorheben, daß in diesem zweiten Bande, in gleichem Maße und in einer konstanten Art, ganz klar und wesentlich wieder über die übrigen der ungefähr 3567 Arten unserer Flora berichtet wird. Er betont hier, in Fortsetzung, über die Vegetationsdauer, die biologische Form, das Aussehen oder den Habitus und die Blütezeit, sowie die Häufigkeit der jeweiligen Pflanze im Lande, als auch die Gegenwart des Taxons in den verschiedenen Pflanzen-Assoziationen und gibt zugleich auch die Existenz der erwähnten Arten gegenüber der Beschaffenheit des Bodens kurz wieder.

Im zweiten Bande sind aus der Klasse der *Dicotyledonen* folgende Ordnungen mit ihren Familien, Gattungen und Arten, sowie Unterarten u.s.w. wiedergegeben : die *Ericales, Primulales, Plumbaginales, Ligustrales, Gentianales* (Contortae), *Tubiflorales* (Lamiales), *Dipsacales, Asterales* (Synandrae) und aus der nachfolgenden Klasse der *Monocotyledonen*, womit das Werk abschließt, sind die Ordnungen *Helobiales, Pandanales, Liliiflorales, Cyperales, Graminales, Gynandrae* und die *Spathiflorales* erwähnt.

Es ist auch bemerkenswert hervorzuheben, daß auch hier neben den allbekannten Bezeichnungen die jetzigen, neuen Namen der angeführten systematischen Einheiten genannt werden, welcher Umstand dem Werke zum Vorteil kommt. Der Verfasser hat auch die Gelegenheit manche Benennungen zu verbessern.

Es sei auch bemerkt, daß wir die Möglichkeit haben einen vollständigen und guten Einblick über die gesammte Gliederung der rumänischen Pflanzenwelt zu erhalten und auch bei leicht bastierenden Gattungen eine soweit mögliche vollkommene Aufstellung der bisher bekannten wichtigsten Bastarde und deren genetische Herkunft zur Einsicht vom Verfasser zu bekommen.

Mit diesem Bande ergänzt er sein Werk und eine Lücke in der botanischen Literatur, indem er uns zugleich als einer der ständigen Mitarbeiter der 13-bändigen „Flora der Sozialistischen Republik Rumäniens" (1952—1976) und so ein ausgezeichneter Kenner unserer Pflanzenwelt, eine kurzgefaßte, erweiterte Wiedergabe dieses großen Standartswerkes in seinem nun veröffentlichten wissenschaftlichen Beitrag, zur Hand legt.

Auch hier sind die merkwürdigsten Pflanzen mit den bedeutendsten Abbildungen versehen, welche das Bestimmen der jeweiligen Taxone erleichtert und so ein Fehlgehen aus dem Wege weist. In den 594 Abbildungen, von der fortlaufenden Nummer 846 bis 1439, können wir vermerken, daß ein großer Teil von diesen zum erstenmal bekannt gemacht wird, obwohl das obgenannte Standartswerk eine ausgezeichnete Wiedergabe des Aussehens, als auch einiger morphologischer Merkmale, aller rumänischen wildwachsenden und weit kultivierten Pflanzen enthält.

Die Fülle von den wertvollen Angaben dieses illustrierten Bestimmungsbuches der höheren Pflanzen, welche in der „Flora Rumäniens" enthalten sind, empfiehlt sich von selbst, sowohl den Botanikern, Studenten und Schülern, sowie den Freunden der Natur, als ein vorzügliches Nachschlagebuch unserer überaus reichen Pflanzenwelt.

Ion T. Tarnavschi

AVIS AUX AUTEURS

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

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

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PRINTED IN ROMANIA