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REVUE ROUMAINE DE BIOLOGIE

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REV. ROUM. BIOL.—BIOL. VÉGÉT., TOME 32, p. 73—134, BUCAREST, 1987

AREAL LIMITS IN THE ROMANIAN TERRITORY: *SERRATULA BULGARICA* Acht. & Stoj. 1932 (*S. caput-najae* Zahar. 1946)

G. DIHORU

The paper presents the chorology of the species in danger, moesio-danubial *Serratula bulgarica* Acht. & Stoj. on the Romanian territory. The general area is in the North-East of Bulgaria and the North-East of the Romania Plain, Ghidigeni Station (Gara Ghidigeni) representing its northern point. The places indicated as Dobrogea (2) are considered as uncertain.

From a taxonomical point of view *Serratula bulgarica* was investigated relatively late first in Bulgaria (1932) and then, independently, in Romania (1946). It took a long time because *S. heterophylla* Desf. or *S. radiata* (Waldst. & Kit.) Bieb. (in Bulgaria) and *S. lycopifolia* (Vill.) A. Kerner, could be mistaken for other species (in Romania).

It is true that by its solitary antodia it is similar to other species, but it differs from them by the membraneous appendages of involucral bracts, keeled and wider than the rest of the bracts.

We wonder why the famous botanist C. Zahariadi, who had a good knowledge of the Slavic languages, did not consult the botanical Bulgarian literature and redescribe the species, it is true in an absolutely academic manner, under the name of *S. caput-najae* Zahar. nom. superfl., collected in 1943 between "Buzău and Pogoanele" * (10), (12). For a long time the species has been wrongly appreciated, viz., among the endemites of the Romanian flora (4), (10).

The ecology and cenology of the species *S. bulgarica* were investigated by I. Şerbănescu (10). According to this author, the plant shows an ecological differentiated character in two types of habitats: on the one side, in wet habitats and slighty salted on the surface (such as the area of the forests Frasinu, Spătaru and Birlad Valley) where it grows together with halophils species: *Artemisia santonicum, Aster punctatus, A. tripolium, Beckmannia eruciformis, Chartolepis glastifolia, Elymus elongatus, Iris brandzae, Lepidium ruderale, L. latifolium, Leuzea salina, Limonium gmelinii, Lotus tenuis, Peucedanum latifolium, Plantago schwarzenbergiana, ? Puccinellia distans, Scorzonera parviflora, ? S. austriaca* var. *mucronata, Suaeda maritima.*

On the other side, in dry habitats, evolved from the steppe, with the underground water below 5 m deep, with clay soil in transition to chernosem and the salts in the deep where xerophyte species grow using the surface layer of the substratum: *Artemisia austriaca, Agropyron pectinatum, Eryngium campestre, Medicago falcata, Melica ciliata, Phlomis tuberosa.*

It is therefore a halophyle species (of average salt fields) which could belong to the order of *Puccinellietalia*.

* This is the village of Pogonele, Ţinteşti county and not Pogoanele county.

REV. ROUM. BIOL.—BIOL. VÉGÉT., TOME 32, Nº 2, P. 75—78, BUCAREST, 1987

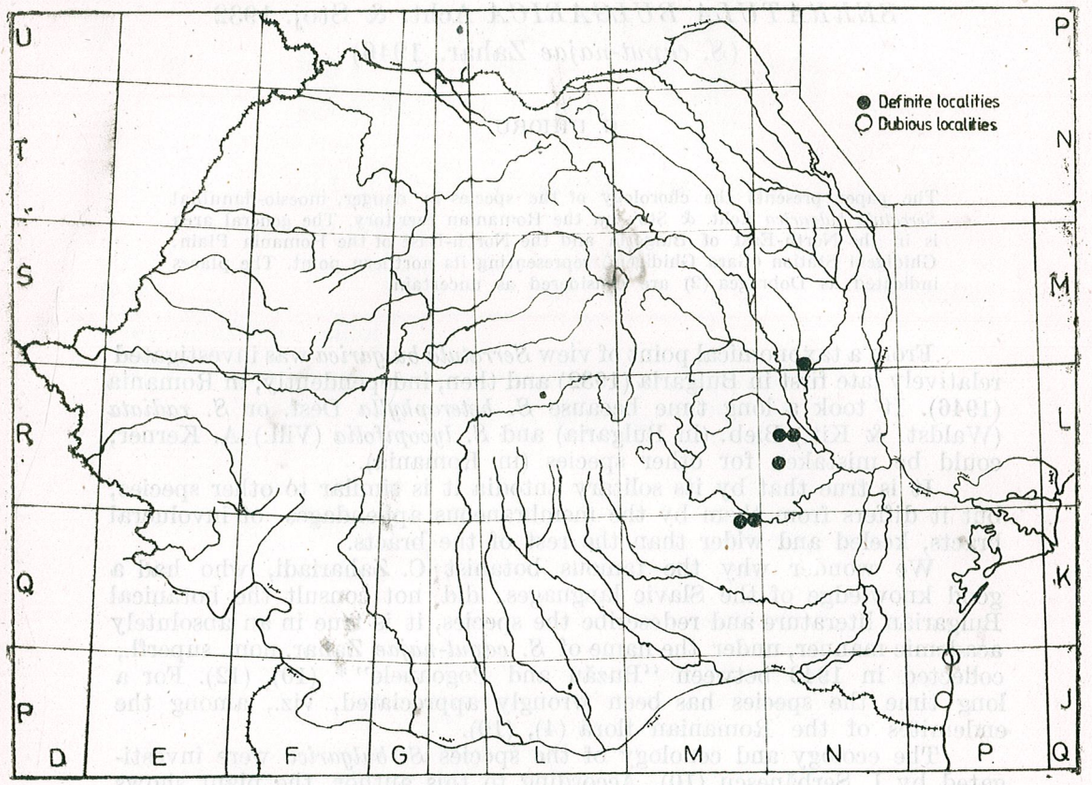

Fig. 1.—Area of *Serratula bulgarica* Acht. & Stoj. in Romania

ENUMERATION OF THE LOCALITIES

1. *Buzău* county

MK 89 — Frasinu Forest (leg. C. Zahariadi et A. Ionescu, 17.V.1949, BUCA 123.363, 123.364, 123.365; leg. G. Negrean 27.VI.1972, BUCM 40.332 and 24.V.1977, BUCM 50.375), (8), (9), (10).

— Forest Spătaru (leg. V. Tutunaru 7.VI.1962, BUCA 121.804; leg. V. Tutunaru et V. Sanda, 9.VI.1963, BUCA 121.801), (8), (9), (10).

— Buzău in Crîng (leg. T. Săvulescu 1.VI.1933, sub *S. tinctoria* BUCM 16.358; leg. C. Zahariadi VI.1943, BUCM 16.337; ? G. Dihoru 1976).

— Between Ţinteşti and Frasinu forest, 70 m. alt. (leg. C. Zahariadi 21.VI.1944, FRE nr. 2.982, BUCA 29.028).

MK 99 — Ţinteşti (leg. C. Zahariadi 21.VI.1944, BUCA 39.606, 123.361, 123.367, 123.368, 123.385; leg. C. Zahariadi 13.VII.1944, BUCA 123.362).

— Between Tăbărăşti and Pogoanele, 70 m. alt. (leg. A. Buia 28.VI.1939, det. ? I. Prodan, BUCA 61.323).

— Pogonele, Ţinteşti county. It is not the village of Pogoanele in the same county it was mistaken for by many botanists. (leg. C. Zahariadi 18.V.1944, BUCA 123.378, 123.379, 123.380, 123.381, 123.382, 123.383, 123.384; leg. C. Zahariadi et A. Ionescu, 11.V.1949, BUCA 123.372, 123.373, 123.374).

2. *Galaţi* county

NM 30 — Ghidigeni Station, 50—60 m. alt. in the Bîrlad valley (leg. M. Răvăruţ 29.VI.1948, BUCA 39.569), (6), (10). Perhaps other authors refer to the same place (1).

3. *Vrancea* county

NL 15 — Coteşti Station (leg. M. Brandza V.1911, sub *S. lycopifolia*: "Coteşti în fineţe", BUCA 39.572), (6), (10). It is the oldest information about *S. bulgarica*.

— Urseşti Forest near Coteşti Station (9), (10).

NL 13 — Proca Mare, Proca Mică forests and Hîria plain, Bogza county (9), (10).

NL 25 — Vernescu forest, Milcovul county (9), (10).

— Hay meadow at Vlăduleasa, Milcovul county (10).

It is also underlined that the ecology of the species *S. bulgarica* is similar to *S. wolffii* Andrae, occupying the place of forests of ash and elm or penetrating in their glades.

As regards its spreading, we notice that it appears in the north-east of Bulgaria and in the north-east of the Romanian plain, practically without intermediary points. In Romania (Fig. 1), three relatively close chorological areas are known: one in the Buzău and Călmăţui flood plain, south of Buzău, (Ţinteşti meadows, Frasinu and Spătaru forests), the low plain of Rîmnicu Sărat (Coteşti Station, Vernescu, Proca Mare, Proca Mică forests, Hîria plain and Vlăduleasa meadow) and the low plain of Bîrlad (Ghidigeni Station).

Besides these chorological points we also mention two uncertain points in south Dobrogea, near the Black Sea coast, recorded on the chorological map of the species in Bulgaria (2). These are uncertain points because we do not know the primary source of information and, moreover, our botanists did not identify it so far in Dobrogea (5). As a matter of fact, in Bulgaria, where the species was described in 1932 by I. Urumov on a material collected in 1889 near the village of Popovo and the town of Târgovişte, it is indicated in the nondetermined sozological category but in danger, last mentioned in the herbarium in 1902 and in literature in 1904.

We notice that the latest information on the species in Bulgaria are very old and therefore we may advance the idea that most plants are to be found in Romania, being confirmed by new chorological investigations (10).

According to the area known so far, it may be appreciated as a moesian-danubian species.

From a sozological point of view *S. bulgarica* in Romania is a species in danger, with relatively poor populations, confined area and habitats seriously affected by soil meliorations (irrigations exsiccations, fallowing) and the intensive grazing practiced on the plain meadows.

Serratula bulgarica is sometimes the host of the fungus *Puccinia schirajewskii* Tranz. (see the material mentioned in BUCM) and in culture of the species *Septoria tinctoriae* Brun. (BUCM 33.754).

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A NEW *LEMBOSIA* (*ASCOMYCETES, ASTERINACEAE*) ON *BRUCKENTHALIA SPICULIFOLIA* (SALISB.) REICHENB. (*ERICACEAE*) IN ROMANIA

A. RICHIȚEANU

Lembosia bruckenthaliae A. Richițeanu occurring on *Bruckenthalia spiculifolia* (Salisb.) Reicherb. (Ericaceae) in Romania is described and illustrated as new.

During our study of the microfungi on Ericaceae in Romania an apparently unknown species occurring on *Bruckenthalia spiculifolia*, a frequent native plant in the mountainous areas of the Carpathians, was collected. The morphological characters of our collection render it a typical *Lembosia*, a genus belonging to the *Asterinaceae*. According to Müller and von Arx (1962), Luttrell (1973), von Arx and Müller (1975), and Barr (1979), this family includes epiphytic or parasitic fungi developing a superficial or partially innate mycelium. The ascomata are dimidiate or scutate, roundish, elongate or linear in outline, opening by crumbling of cells or by irregular slits between rows of cells. The ascospores, mostly 2-celled, rarely with 2, 3 or more septa are hyaline or brown.

As defined by these authors, the members of *Lembosia* are parasitic (usually on leaves) and their mycelia are entirely superficial and lateraly hyphopodiate, sending haustoria into epidermal cells. The ascomata are elongate, linear, x-, y-, or +-shaped, not setose, opening by a longitudinal slit. The ascospores, hyaline when young, become brown with age.

Lembosia has generally been described by Leveillé (Champ. exot., 1845, p. 58) where he included four species. According to von Arx and Müller (1975) there are about 40 species of *Lembosia*, but most of them are found only in the warmer parts of the world (Theissen, 1913 ; Arnaud, 1918 ; Müller and von Arx, 1962). As far as we know, there is not any species of this genus reported from Europe. In the von Arx and Müller interpretation of this family (1975) only *Asterina veronicae* (Lib.) Cooke has a European distribution. There are, however, some European species of *Aulographum* (*A. hederae* Lib. on leaves of various plants), *Aulographina* (*A. pinorum* (Desm.) v. Arx et Müller on needles of *Pinus*), *Morenoina* (*M. epilobii* (Lib.) v. Arx on stems of *Epilobium*), and *Lembosina*, all similar in aspect to *Lembosia*, in having superficial, elongate ascocarps, at maturity opening by rupture, but, according to von Arx and Müller (1975), they belong to *Leptopeltidaceae*, a family of unitunicate ascomycetes which, in Holm and Holm's opinion (1977), should be restricted to the genera *Leptopeltis* s. lat., *Dothiopeltis*, and *Ronnigeria* only.

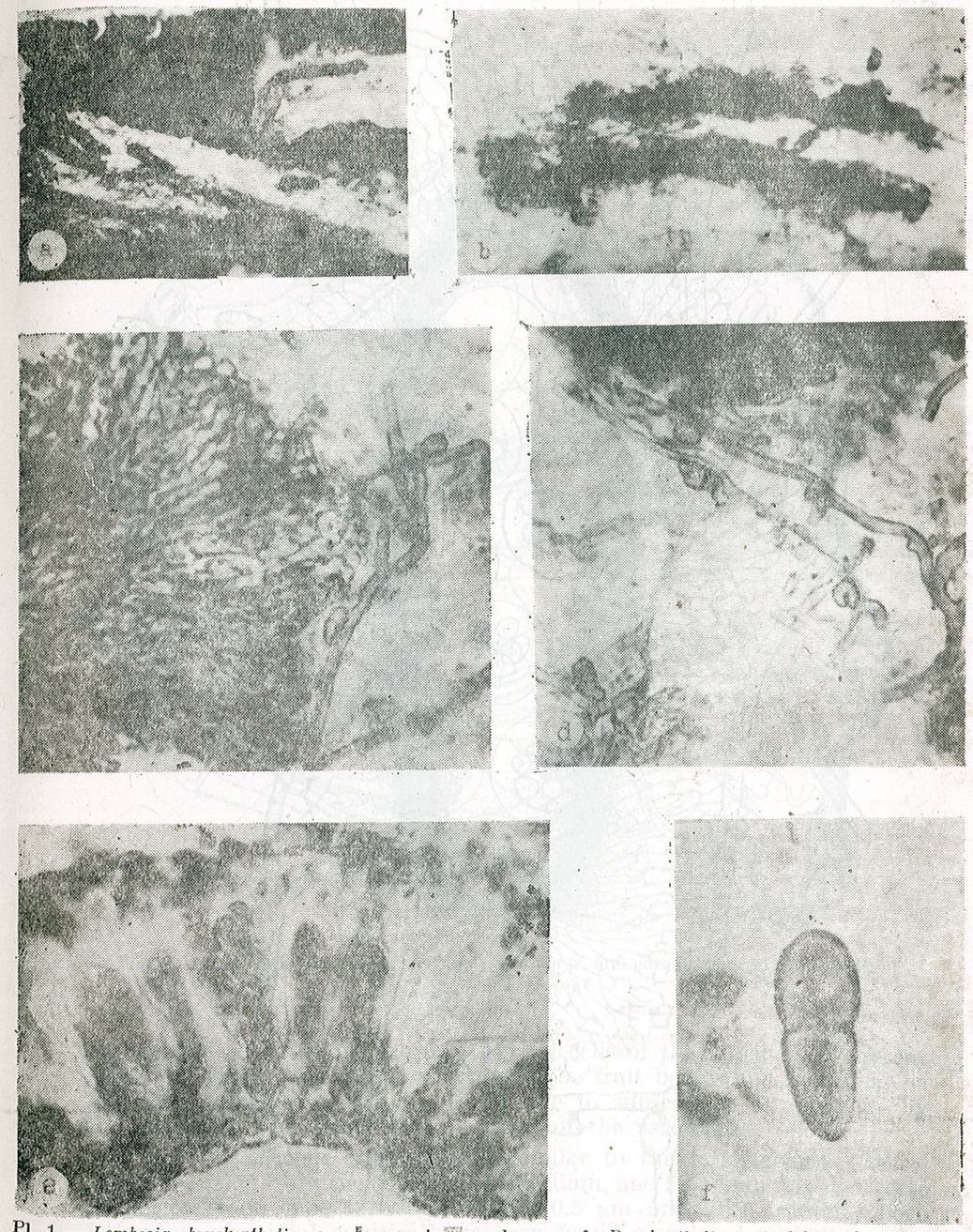
The last genus (*Lembosina*) is represented in Europe by several species, some of them parasitic and saprophytic on *Ericales*. These are : *L. aulographoides* (Bomm., Rouss. et Sacc.) Theiss. on twigs of *Rhododendron* (Müller and von Arx, 1962) and *Arctostaphylos uva-ursi* (Richițeanu and Bontea, 1987), *L. gontardii* Müller on *Arctostaphylos uva-ursi* (Müller, 1963 ; Ericksson, 1974), *L. empetri* B. Erikss. on *Empetrum nigrum* (Eriks-

son, 1974), and *L. ericae* B. Erikss. on *Erica cinerea* and *Erica tetralix* (Eriksson, 1974). This genus differs from *Lembosia* in having a subcuticular or intraepidermal, crustose stroma and a superficial mycelium without hyphopodia. The status of this genus is quite uncertain. Luttrell (1973) and von Arx and Müller (1975) consider it as belonging to *Leptopeltidaceae* while Müller and von Arx (1962), Müller (1963) and Barr (1979) treat it as a true *Asterinaceae*.

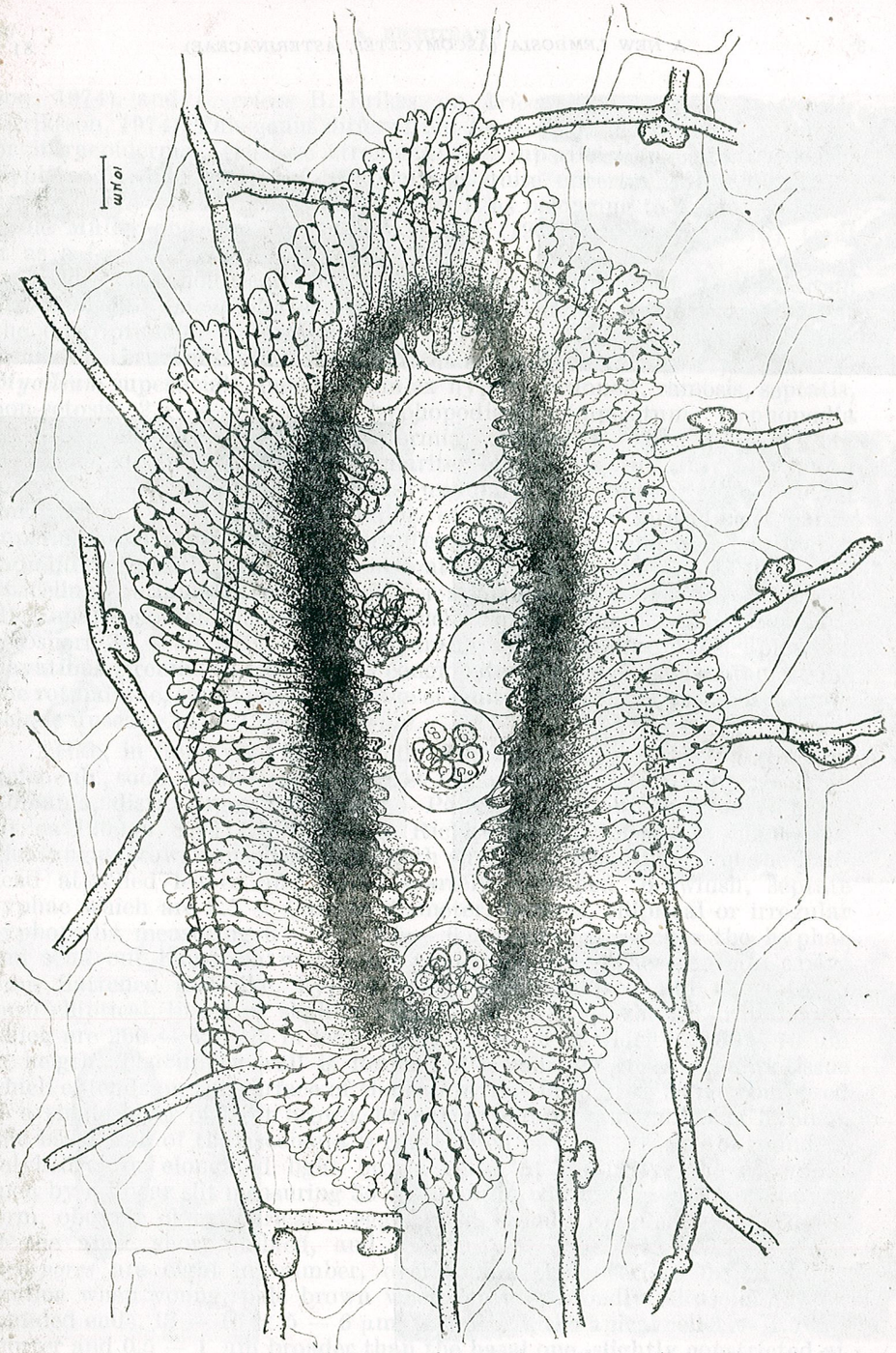
The morphological characters of our specimen of *Lembosia* on *Bruckenthalia spiculifolia* are considered sufficiently distinct to warrant the description of a new species.

Lembosia bruckenthaliae A. Richițeanu sp. nov. Figs. 1, 2; Pl. 1. *Mycelium* superficiale, indistinctum ex hyphis brunneis, ramosis, septatis, non-setosis, 2.5—4 μm crassis, hyphopodiatis compositum; hyphopodia lateralia, elliptica vel irregulariformia, sparsa, 6 — 9 × 4.5 — 6 μm. *Ascomata* atra, carbonacea, irregulariter ellipsoidea, linearia vel ramosa in x-, y-, +-formia, marginalibus undulatis vel lobatis, 200—540 μm longitudine, 70—120 μm latitudine et 50—70 μm altitudine; paries superior ascomatorum densus, brunneus, ex hyphis ramosis agglutinatis, radialiter dispositis, linearibus dehiscentibus; paries basalis 2—3 stratosus, ex cellulis rotundatis, prismaticis vel applanatis, hyalinis vel subfuscis. *Asci* numerosi, dense aggregati, paralleli dispositi, late clavati, bitunicati, octospori, 32 — 40 × 12 — 14 μm, paraphysoidibus filiformibus, apicaliter clavatibus circumdati. *Ascosporae* conglobatae, oblongo — clavatae, utrinque rotundatae, supra medio septatae et leniter angustatae, primo hyalinae, deinde fuscae, 13 — 15 × 5 — 6 μm.

Hab. in foliis vivis et languidis *Bruckenthaliae spiculifoliae* (Salisb.) Reichenb., socia *Clasterosporium* sp. et *Leptothyrium* sp. (stati imperfecti ?), Romania, distr. Argeș, Mts Iezer — Păpușa, Mt. Colții lui Andrei Mari, alt. ca 1200 m, 8.VIII.1982, leg. A. Richițeanu, BUCM 94.353, holotypus. The fungus grows superficially on both surfaces of the living and the half-dead attached leaves and forms sparsely branched, brownish, septate hyphae which are 2.5 — 4 μm in diameter. Lateral, elliptical or irregular hyphopodia measuring 6 — 9 × 4.5 — 6 μm serve to anchor the hyphae and send out haustoria within the epidermal cells. The *ascomata* arrive from flattened disk-like, hyphal knots which radiate and elongate to form elliptical, linear or slightly curved, x-, y- or + -shaped fruit bodies which are 200 — 450 μm in length, 70 — 120 μm in width and 50 —70 μm in heigth. The upper wall is formed of a compact mass of dark tissue which extends on the surface of the leaf in the form of a fringe composed of a single layer of light brown, branched parallel and radiating hyphae. The basal wall of the ascomata is constituted of 2 or 3 layers of rounded, polyhedral or elongated light brown cells. At maturity, the ascomata open by a linear slit measuring 30 — 40 μm in width. The *asci* are obpyriform, obovate or cylindrical — egg-shaped, broadly rounded and thickened at the apex, short stalked, and measure 32 — 40 × 12 — 15 μm. The *ascospores* are eight in number, overlapping three-seriate or crowded, hyaline when young, pale brown when mature, broadly ellipsoidal, with rounded ends, 13 — 15 × 5 — 6 μm, two-celled, the apical cell 1 — 1.5 μm shorter and 0.5 — 1 μm broader than the basal one, slightly constricted at the septum, wall smooth. The *paraphysoids* are filiform, branched, hyaline



Pl. 1. — *Lembosia bruckenthaliae* a. — ascomata on leaves of *Bruckenthalia spiculifolia*; b. — surface view of an ascoma; c. — border part of the superior wall of the ascoma and hyphopodiate mycellum; d. — free hyphopodiate mycelium; e. — asci, ascospores and paraphysoids (stained in lactic blue); f. — mature ascospore.



1. — *Lembosia bruckenthaliae* — front view of the ascoand mycelium sitting on leaf surface. ma

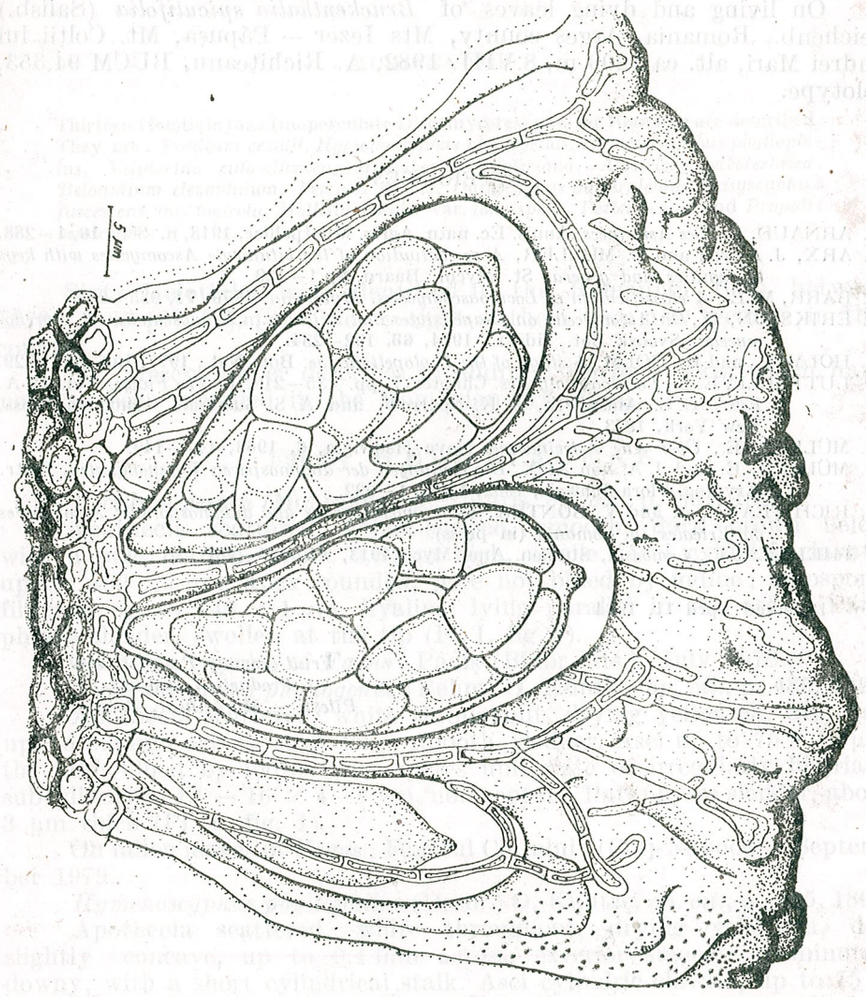


Fig. 2. — *Lembosia bruckenthaliae* — asci, ascospores, and paraphysoids in a longitudinal cut of an ascoma.

and mingle with the light brown hyphal cells of the upper layer present across the linear opening at the top of the fruit bodies and becoming an indistinct brownish gelatinous tissue due to subsequent compression by the enlarging asci upon the maturity of the ascomata.

A dematiaceous hyphomycete similar to the form genus *Clasteosporium* arising from the superficial mycelium, and a pycnidial coelomycete of *Leptothyrium* type forming 3 — 4 × 0.5 µm one-celled conidia, born in scutate pycnidial cavities have been found associated with this ascomycete. They seem to be the anamorphs of our species.

On living and dying leaves of *Bruckenthalia spiculifolia* (Salisb.) Reichenb., Romania, Argeş county, Mts Iezer — Păpuşa, Mt. Colţii lui Andrei Mari, alt. ca 1200 m, 8.VIII. 1982, A. Richiţeanu, BUCM 94.353, holotype.

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CONTRIBUTIONS TO THE STUDY OF ROMANIAN HELOTIALES (ASCOMYCETES)

ADRIANA POP

Thirteen Helotiale taxa (inoperculate Discomycetes) new for Romania are described. They are: *Pocillum cesatii*, *Hymenoscyphus phyllogenus*, *Hymenoscyphus phyllophilus*, *Velutarina rufo-olivacea*, *Hyalopeziza winteriana*, *Albotricha albolestacea*, *Belonidium elegantulum*, *Perrotia abietis*, *Dasyscyphus pulverulentus*, *Dasyscyphu s fuscescens* var. *fagicola*, *Mollisia discolor* var. *longispora*, *Pezicula rubi* and *Propolis versicolor*.

Sixty Helotiales were identified in the Romanian flora between 1979—1985 [6], [7], [8]. Twenty-eight of them were new records in this country.

The present paper describes in addition thirteen taxa, which have not yet been recorded in the Romanian Mycoflora.

Family HELOTIACEAE

Pocillum cesatii (Montagne) de Notaris, *op. cit.*, p. 361, 1864.

Apothecia scattered, goblet-shaped, smooth, dark brown below with a pale margin, about 0.5 mm high, sessile. Asci cylindric-clavate, up, to 150 ×7 μm, apex rounded, pore not blued by iodine; ascospores filiform, up to 140 × 1 μm, hyaline, lying parallel in the ascus. Paraphyses slender, swollen at the tip (Pl. I, fig. 6).

On fallen cupules of *Fagus*; Padiş (Bihor Mts.), July 1979.

Hymenoscyphus phyllogenus (Rehm) O. Kuntze, *op. cit.*, p. 485, 1898.

Apothecia scattered, white throughout, drying yellowish, disc flat, up, to 0.5 mm across, stalk long, smooth, sledner. Asci up to 75× 10 μm, the pore blued by iodine; ascospores uniseriate or irregularly biseriate, subcylindrical 12 — 16 × 4—6 μm, non-septate. Paraphyses slender, about 3 μm thick (Pl. I, fig. 1).

On fallen leaves of *Fagus*; Făgetul Clujului (Cluj-Napoca), September 1979.

Hymenoscyphus phyllophilus (Desm.) O. Kuntze, *op. cit.*, p. 485, 1898.

Apothecia scattered, white throughout, drying yellowish, disc slightly concave, up to 0.4 mm across, exterior smooth or minutely downy, with a short cylindrical stalk. Asci cylindric-clavate, up to 75 × × 9 μm, 8-spored, the pore not blued by iodine; ascospores cylindric-fusiform, 12—14 × 3—4 μm, biseriate, always distinctly 1-septate. Paraphyses cylindrical, 2—3 μm thick (Pl. I, fig. 2).

On fallen leaves of *Fagus;* Făgetul Clujului (Cluj-Napoca), October 1979.

Velutarina rufo-olivacea (Albertini & Schweinitz ex Pers.) Korf, op. cit., p. 476, 1953.

Apothecia solitary or in small clusters, erumpent, cup-shaped with an inrolled margin, subsessile, outer surface covered with rusty-brown or tan-coloured powder formed of thick-walled, loose, irregularly lobed cells, disc up to 3 mm across, olive-green or almost black. Asci cylindric-

clavate, up to 160 × 12 μm, the pore blued by iodine; ascospores uniseriate, elliptical, with obtusely rounded ends, 10—14 × 6—7 μm, hyaline at first but ultimately becoming pale brown, containing one or two large oil drops. Paraphyses 3 μm thick, swelling to 5 μm at the greenish-brown tip (Pl. I, fig. 3).

On dead twigs and branches of *Rubus;* Petroșani (Hunedoara county), August 1979.

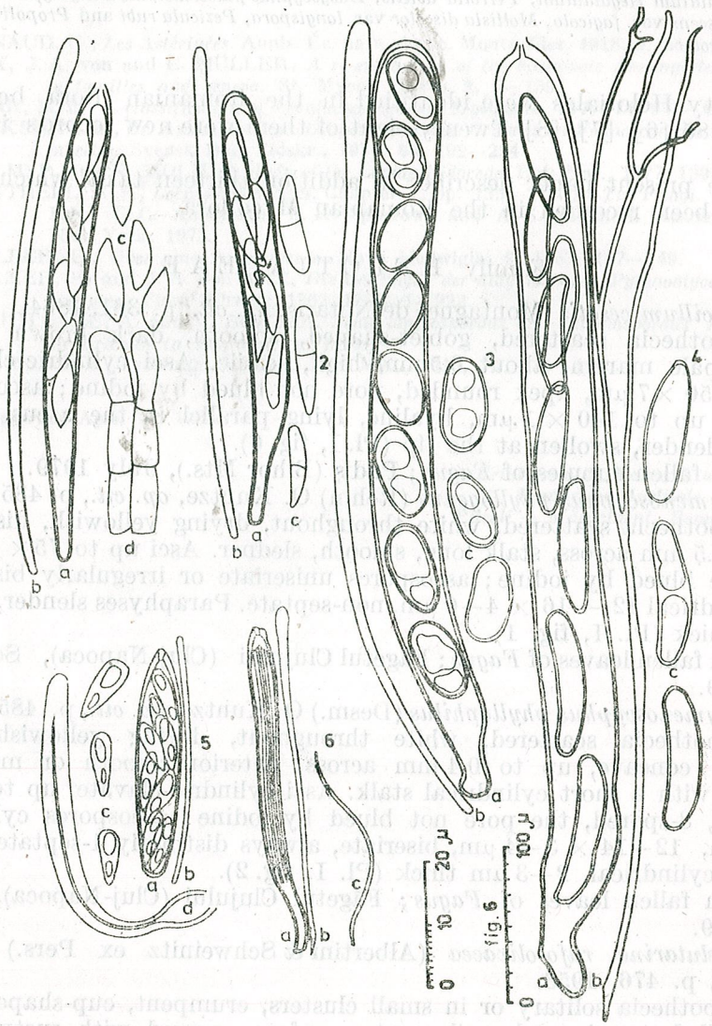

Plate I. — Light microscopy of Helotiales. Fig. 1. — *Hymenoscyphus phyllogenus;* 2. *Hymenoscyphus phyllophilus;* 3. *Velutarina rufo-olivacea;* 4. *Propolis versicolor;* 5. *Hyalopeziza winteriana;* 6. *Pocilum cesatii.* Abbreviations: a — asci; b — paraphyses; c — ascospores; d — hairs.

Family HYALOSCYPHACEAE

Hyalopeziza winteriana (Rehm) Raitv., Scripta Mycol. 1, Tartu, p. 37, 1970.

Apothecia sessile, urceolate, white, 0.2—0.3 mm in diameter, externally covered by short hairs, cylindrical, 25—70 × 2.5—4 μm, with very thick glassy walls, with very small basal lumen. Asci cylindrical, 8-spored, 30—45 × 5—8.5 μm; ascospores clavate, 8—12 × 1.5—2.5 μm. Paraphyses cylindrical (Pl. I, fig. 5).

On dead fronds of *Pteridium aquilinum;* Mărișel, the Apuseni Mts. (Cluj county), June 1981.

Albotricha albotestacea (Desm.) Raitv., Scripta Mycol. 1, Tartu, p. 42, 1970.

Apothecia substipitate, 0.5—1 mm, pink-coloured, covered by whitish hairs. Hairs hyaline to pale ochraceous, 7—11 septate, 116—185 × × 3—5 μm. Asci cylindrical, 38—53 × 4—5 μm; ascospores narrowly fusoid, aseptate, 6.5—11.5 × 1.5 μm. Paraphyses lanceolate, 20—30 μm longer than asci, 3—4 μm in diameter (Pl. II, fig. 1).

On dead grasses *(Calamagrostis);* Reci (Covasna county), October 1980.

Belonidium elegantulum (Karst.) Raitv., Scripta Mycol. 1, Tartu, p. 53, 1970.

Apothecia sessile, 0.5—1 mm in diameter, hemispherical, dark reddish-brown to almost blackish, covered by brown hairs with hyaline walls, their content turning dark purple and dissolving in KOH. Asci cylindric-clavate, 63—71 × 4—5 μm; ascospores elliptic-fusoid, 11.5—15 × × 2 μm, always distinctly 1-septate. Paraphyses cylindrical with acute tips not exceeding the asci, 1.5 μm in diameter (Pl. II, fig. 6).

On dead stems of *Chamaenerion;* Ic Ponor, the Bihor Mts. (Bihor county), September 1979.

Perrotia abietis (Karst.) Raitv., Scripta Mycol. 1, Tartu, p. 63, 1970.

Apothecia externally white, hymenium orange, sessile, 0.5—1.5 mm in diameter, externally covered by hyaline hairs. Hairs cylindrical, with thick walls, multiseptate, usually with smooth walls incrusted by loosely attached granules. Asci cylindrical, with broadly rounded nonamyloid apex; ascospores fusoid, aseptate, 15—24 × 3—5 μm. Paraphyses cylindrical, slender, obtuse (Pl. II, fig. 2).

On dead coniferous wood; Ostra (Suceava county), September 1980.

Dasyscyphus pulverulentus (Libert.) Sacc., Syll. Fung. VIII, p. 463, 1889.

Apothecia subsessile, 0.5—1 mm in diameter, disc yellow, excipulum bright yellow with a fringe of short yellow hairs tipped with red. Hairs with firm walls, up to 70 × 3—4 μm, containing pale yellow oil-drops and bearing irregular lump of resin in tip. Asci cylindrical, 30—40 × × 3—4 μm; ascospores cylindric-clavate, 4—5 × 1 μm. Paraphyses narrowly lanceolate, 5—10 μm longer than asci, 2 μm in diameter (Pl. II, fig. 5).

On fallen needles of *Pinus;* Reci (Covasna county), April 1980.

Dasyscyphus fuscescens (Pers. ex Fr.) Rehm var. *fagicola* (Phill.) Dennis, Mycological Papers, 32, p. 38, 1949.

Apothecia scattered, up to 1 mm across, with short stalk, disbrown, externally covered by brown hairs. Hairs thick-walled, multiseptate, 80 —105 × 3—5 μm. Asci cylindrical, 40—45 × 3—4 μm; ascospores fusoid, 7.5—11 × 1.5—2 μm. Paraphyses lanceolate, 25—30 μm longer than asci (Pl. II, fig. 4).

On fallen cupules of *Fagus;* Muncelu (Alba county), April 1982.

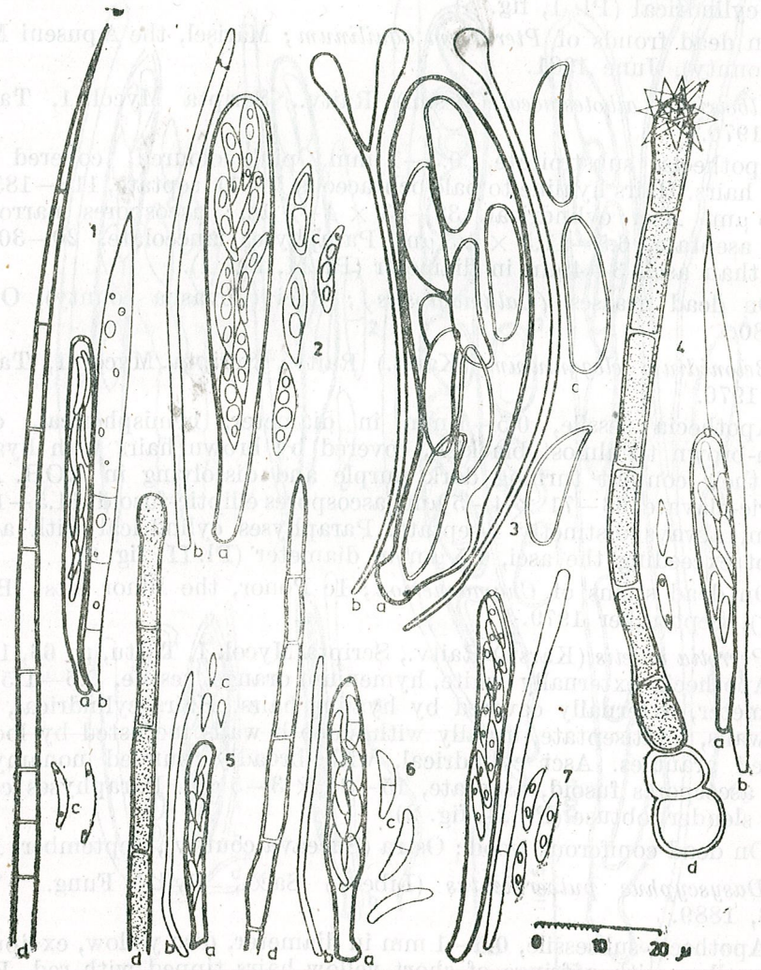

Plate II. — Light microscopy of Helotiales. Fig. 1. — *Albotricha albotestacea;* 2. *Perrotia abietis;* 3. *Pezicula rubi;* 4. *Dasyscyphus fuscescens* var. *fagicola;* 5. *Dasyscyphus pulverulentus;* 6. *Belonidium elegantulum;* 7. *Mollisia discolor* var. *longispora.* Abbreviations: a — asci; b — paraphyses; c — ascospores; d — hairs.

Family DERMATEACEAE

Mollisia discolor (Montagne) Phill. var. *longispora* le Gal in Revue de Mycologie N.S. 4, p. 57, 1939.

Apothecia erumpent in clusters from cracks in bark, cup-shaped, becoming flattened and often undulating, lobed or deformed, externaly dark-brown, disc light grey, drying yellow, about 1.5 mm across. Asci cylindric-clavate, up to 70 × 8 μm, 8-spored; ascospores biseriate, narrowly elliptical, 10—13 × 2—2.5 μm, with two oil drops, nonseptate. Paraphyses cylindrical, about 2 μm thick (Pl. II, fig. 7).

On dead twigs of *Fagus;* Roșioara (Alba county), July 1982.

Pezicula rubi (Lib.) Niessl apud Rabenhorst, Fungi europaei, p. 2122, 1876.

Apothecia erumpent, scattered, sessile, lenticular, very brittle when dry, disc convex, finely pruinose, dull ochraceous yellow or reddish-orange, up to 1 mm across. Asci cylindric-clavate, up to 115—20 μm; ascospores elliptic-fusiform, somewhat pointed at each end, 18—26 × × 6—8 μm, sometimes becoming 3-septate, hyaline. Paraphyses slender, swollen to 5 μm at the tips (Pl. II, fig. 3).

On dead stems of *Rubus;* Petroșani (Hunedoara county), August 1979.

Propolis versicolor (Fr.) Fr., *op. cit.*, p. 372, 1849.

Apothecia scattered, immersed, exposed by shedding of the epidermis or bark and surrounded by a torn margin of host tissue, disc circular or elliptical, flat, up to 5 mm across, hymenium brown but concealed by a white pruina. Asci cylindric-clavate, rounded above, up to 135 × 18 μm, thin-walled, the pore not blued by iodine; ascospores biseriate, hyaline, slightly, kidney-shaped, 20—27 × 6—8 μm, nonseptate. Paraphyses very numerous, thread-like, forked repeatedly near the tip, longer than the asci (Pl. I, fig. 4).

On dead pine cones; Zlatna (Alba county), May 1980.

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LICHEN BIOMASS OF DWARF PINE SHRUBBERIES IN THE RETEZAT NATIONAL PARK

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The paper presents the results of the researches carried out in the Retezat National Park on the lichen biomass of dwarf pine shrubberies. Similar researches have already been carried out on the upper limit and mountain spruce fir forests of the same mountains. Of all these ecosystems, the dwarf pine shrubbery is the forest in epiphytic lichens, both as number of species and biomass (55 $g.m^{-2}$). It is, nevertheless, the richest one in terricolous lichens (193 $g.m^{-2}$). Thus, the biomass of epiphytic lichens is only 0.28% of the total forest biomass, while that of terricolous lichens is 28% of the biomass of subalpine meadows occurring among subalpine shrubberies.

The lichen flora an vegetation are particularly important for the phytocoenosis of a dwarf pine ecosystem. Their distribution in dwarf pine shrubberies is different from that in upper limit and mountain spruce fir forests. As shown in two previous papers (1), (2), in spruce fir forests the lichen flora occurs in almost equal proportions on trees and soil. This peculiar distribution is mostly due to the microclimatic environment generated by the dominant species. In mountain and upper limit spruce fir forests, the trees preserve a high humidity at crown level, while in dwarf pine shrubberies, the shrubs bring about a higher soil humidity. Most of the terricolous lichens of the shrubberies occur in secondary clearings, as a pioneer vegetation that appeared after man had cleared the forest.

MATERIAL AND METHODS

Several methods were used for estimating lichen biomass. Epiphytic lichens were separately collected from dry branches, green branches and boles of dwarf pines. Each of the samples was measured and then related to the total quantity. Biomass estimations were carried out on 3 stands by applying the method of Lang, Reiners and Pike (4).

Terricolous lichens were collected from three 1 m^2 stands, and then from eight 25 × 25 cm stands, together with the upper part of higher plants and with bryophytes.

The shrubbery studied was located at about 2000 m altitude on a gentle westsouth-west facing slope (17°), on oligotrophic, lithic peaty podzol, with moder.

This shrubbery appears like a potential natural formation, with a balanced coenotic structure and floristic composition (3).

RESULTS AND DISCUSSION

The phytocoenoses of the shrubbery in the Retezat Mountains belong to the local association, *Rhododendro-myrtifoliae — Pinetum mughi* Borza 1959 em. Coldea 1984, in which *Pinus mugho* is the dominant species.

R EV. ROUM. BIOL. — BIOL. VÉGÉT., TOME 32, Nº 2, P. 91—96, BUCAREST, 1987

The biomass of epiphytic lichens (Table 1) was estimated to 54.89 g lichens.m^2. Taking into account that the total shrubland biomass was estimated to 19,402.0 g.m^2 (3), it can be noticed that the epiphytic lichens represent only 0.28 % of it.

Table 1

Epiphytic lichen biomass of dwarf pine shrubberies in the Retezat National Park

	Vegetative organs		
	Bole	Braches + brachiblasts	Dried branches
Dwarf pine shrub I (25 m^2)			
Biomass of dried dwarf pine (kg)	263.24	154.63	25.44
Biomass of dried lichen (g)	421.18	1377.27	31.04
g lichen $\cdot$ kg^{-1} dwarf pine. 25 m^{-2}	1.60	20.65	1.22
Dwarf pine shrub II (25 m^2)			
Biomass of dried dwarf pine (kg)	321.52	215.22	77.68
Biomass of dried lichen (g)	353.17	419.57	155.36
g lichen $\cdot$ kg^{-1} dwarf pine. 25 m^{-2}	1.10	3.62	1.99
Dwarf pine shrub III (25 m^2)			
Biomass of dried dwarf pine (kg)	217.27	145.70	37.65
Biomass of dried lichen (g)	297.29	868.07	193.90
g lichen $\cdot$ kg^{-1} dwarf pine. 25 m^{-2}	1.37	5.96	5.15
Average g $\cdot$ kg^{-1} $\cdot$ 25 m^{-2}	1.36	10.08	2.79

Lichen biomass g $\cdot$ 25 m^{-2} = 1372.28
Lichen biomass g.m^{-2} = 54.89

Due to the peculiar shape of a dwarf pine, it is not possible to differentiate lichen distribution on its vegetative organs, as compared to the clear-cut distribution on spruce firs. Nevertheless, it can be noticed that the species *Parmelia physodes* and *Parmelia furfuracea* are mostly present on dry branches and leaves, while the species *Evernia prunastri*, *Cetraria pinastri*, *Cetraria juniperina* and *Parmeliopsis ambigua*, occur on boles and branches. Lichen occurrence on the different vegetative organs of dwarf pines is not influenced by their age, i.e. height or circumference (Fig. 1). The lowest quantity of epiphytic lichens occurs on boles : an averages of 1.36 g lichens . kg^{-1} dwarf pine . 25 m^{-2}.

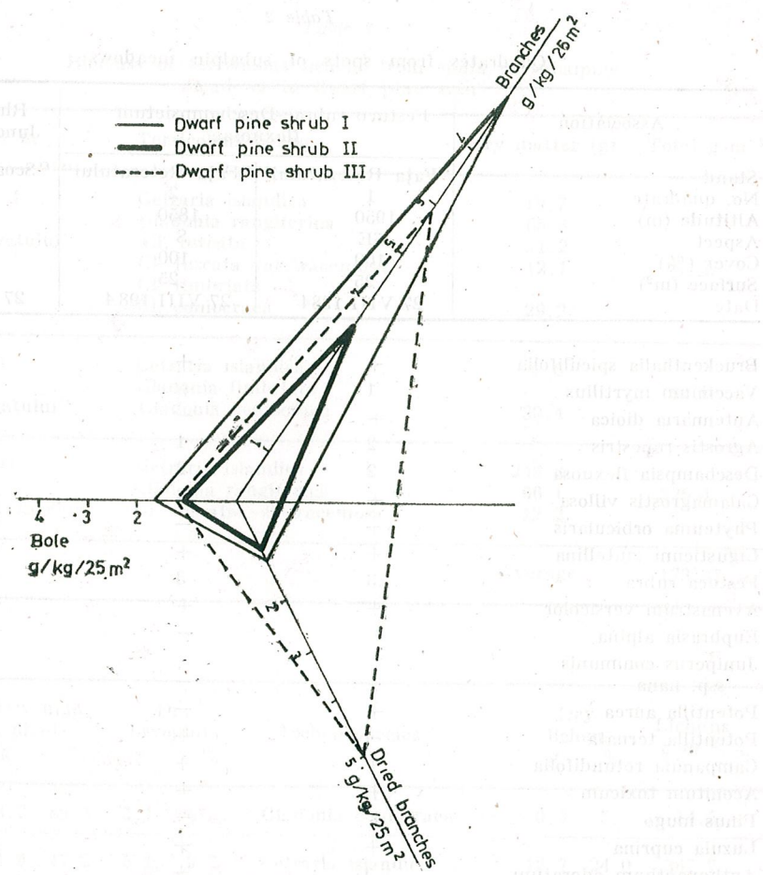

Fig. 1. — Distribution of epiphytic lichens on the dwarf pine shubs vegetative organs.

The branches and leaves are relatively richer in lichens : an average of 10.08 g lichens.kg^{-1} dwarf pine.25 m^{-2}.

Unlike the poor occurrence of epiphytic lichens in shrubland, the share taken by terricolous lichens in subalpine meadows is larger. The values of lichen richness-dominance vary between + and 4 (Table 2). Considering the biomass of all constituent elements (Table 3), we have found that higher plants represent 64 % of the total biomass, lichens 28 % and bryophytes 8 %, which is equivalent to 193.13 g terricolous lichens.m^{-2}.

Figure 2 presents the histogram with the occurrence of different lichen species (in percentages). *Cetraria islandica* of the family *Parmeliaceae* has the highest occurrence, as it represents 32 % of the total lichen

Table 2

Quadrates from spots of subalpin meadows

Association	Festuco rubrae-Deschampsietum flexuosae		Rhododendro-Juncetum trifidi
Stand	"Fața Retezatului"	"Fața Retezatului"	"Scoaba Retezat"
No. quadrate	1	2	3
Altitude (m)	1950	1850	1900
Aspect	SE	S	SW
Cover (%)	100	100	100
Surface (m^2)	25	25	25
Date	27 VIII 1984	27 VIII 1984	27 VIII 1984
Bruckenthalia spiculifolia	+	+	—
Vaccinium myrtillus	1	+	—
Antennaria dioica	+	—	—
Agrostis rupestris	2	1	—
Deschampsia flexuosa	2	2	—
Calamagrostis villosa	+	+	—
Phyteuma orbicularis	+	—	—
Ligusticum mutellina	+	+	—
Festuca rubra	3	3	—
Avenastrum versicolor	+	+	—
Euphrasia alpina	+	—	—
Juniperus communis ssp. nana	+	—	+
Potentilla aurea	+	—	—
Potentilla ternata	—	+	—
Campanula rotundifolia	+	+	—
Aconitum toxicum	+	+	—
Pinus mugo	+	—	—
Luzula cuprina	+	+	—
Anthoxanthum odoratum	1	+	—
Poa alpina	—	+	—
Vaccinium vitis idaea	—	+	—
Geum montanum	—	+	—
Pulsatilla alba	—	+	—
Thymus balcanus	—	+	—
Salix cinerea	—	+	—
Juncus trifidus	—	—	3
Rhododendron kotschyi	—	—	+
Leontodon alpinum	—	—	+
Cetraria islandica	+	+	4
Cladonia rangiferina	2—3	—	2—3
Cladonia furcata	+—1	—	—
Cladonia furcata var. racemosa	+	—	+—1
Cladonia fimbriata	1—2	+	—
Cladonia coniocraea			

Table 3

Biomass of terricolous lichens from spots of subalpine meadows in dwarf pine area

Quadrates (1 m^2)	Terricolous lichens	Dry matter (g)	Total $g \cdot m^{-2}$
I	Cetraria islandica	15.7	
SE aspect	Cladonia rangiferina	85.8	
"Fața Retezatului"	Cl. furcata	21.2	
	Cl. furcata var. racemosa	12.1	164.0
	Cl. fimbriata		
	Cl. coniocraea	29.2	
II	Cetraria islandica	0.6	
S aspect	Cladonia fimbriata		31.0
"Fața Retezatului"	Cladonia coniocraea	30.4	
III	Cetraria islandica	246.8	
SW aspect	Cladonia rangiferina	66.1	325.1
"Scoaba Retezatului"	Cl. furcata var. racemosa	12.2	
		Average	173.37

Quadr. 0.068 m^2	Dry high. plants g	%	Dry bryophita g	%	Lichen species	Dry lichens g	%	Lichens $g \cdot m^{-2}$
I	15.7	89.0	1.1	6.2	Cladonia coniocraea	0.9	5.1	14.5
II	24.9	47.2	5.1	9.7	Cetraria islandica	12.7	24.0	367.7
					Cladonia rangiferina	10.1	19.1	
III	13.7	38.6	2.2	7.8	Peltigera canina	19.6	55.2	316.1
IV	23.9	32.2	19.3	26.0	Cl. cornuto-radiata	13.0	17.5	
					Cl. sylvatica	15.0	20.2	
					Cl. furcata	0.9	1.2	498.4
					Cl. pleurota	2.0	2.7	
V	20.0	63.1	—	—	Cetraria islandica	11.7	36.9	188.7
VI	—	100.0	—	—	—	—	—	—
VII	21.3	48.2	3.2	7.2	Cetraria islandica	3.7	8.4	
					Cl. furcata	15.0	33.9	317.7
					Cl. fimbriata	1.0	2.3	
VIII	—	92.0	—	8.0	—	—	—	—

Average $g \cdot m^{-2} = 212.9$
General average $g \cdot m^{-2} = 193.1$

biomass. The *Cladoniaceae* family, with all its 7 species present, is dominant (53.5%). The *Peligeraceae* family is reprezented by a single species: *Peltigera canina* (14.22%).

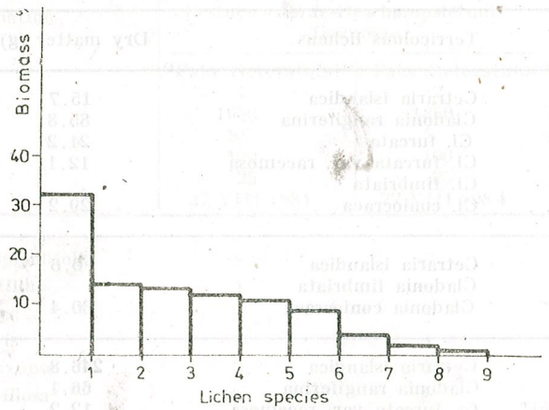

Fig. 2. — Histogram of the distribution of different terricolous lichen species expressed in percentages. 1 = Cetraria irlandica; 2 = Peltigera canina; 3 = Cladonia rangiferina; 4 = Cladonia furcata; 5 = Cladonia sylvatica; 6 = Cladonia cornuta; 7 = Cladonia fimbriata + Cl. coniocraea; 8 = Cladonia pleurota; 9 = Thamnolia vernicularis.

FINAL REMARKS

The researches carried out in the different forest ecosystems of the Retezat National Park allow the conclusions that the shrubbery is the poorest ecosystem in epiphytic lichens, both as species number and biomass. It is, nevertheless, the richest ecosystem in terricolous lichens.

The values obtained were: 54.89 g epiphytic lichens $.m^{-2}$ shrubbery, which represents 0.28% of the total forest biomass; 193.13 g terricolous lichens $. m^{-2}$, which represents 28% of the subalpine meadows biomass.

The lichen species occurring in a shrubbery are most resistant to bad weather, strong winds, intense solar radiations, low atmospheric humidity and other extreme enviromental conditions. This is reflected by the ecophysiological peculiarities of lichen species and by the diversity of their bioforms.

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ASPECTS OF "IN VITRO" CELL DIFFERENTIATION IN WATERMELON (*CITRULLUS LANATUS* (THUNB) MANSF.)

ANA ROȘU, I. ANGHEL, AURELIA BREZEANU

The present study deals with the effect of "in vitro" culture conditions on the ultrastructure of cells from cotyledonary explants of *Citrullus lanatus*, expression of a reprogramming of the cellular function during the dedifferentiation of previously specialised cells from the cotyledonary tissue and the redifferentiation of some cellular types associated with the morphogenetic process. The electronmicroscopic investigations, carried out on the explant cells cultivated "in vitro" after 7 days and 30 days since the culture was started, in comparison with those belonging to the tissue from which the explants were excised, revealed a wide range of ultrastructural features accompanying the regressive transformations of the cotyledonary specialised cells (dedifferentiation), with the appearance of cells having meristematic characteristics, grouped in morphogenetic centres. The ultrastructural details correlated with the metabolic processes characteristic for the differentiation of some specific cellular types (vascular elements) as well as particular aspects at the level of the nuclear apparatus that can be associated with the somaclonal variation phenomenon were also outlined.

The development of higher plants implies progressive cytodifferentiation, division and cellular growth genetically controlled, the development of tissue and organs being the result of some processes of differentiation at the level of the component cells. The morphological peculiarities of various cellular types during development are a consequence of the selective expression, both qualitative and quantitative, of some distinct parts of the genome (9). In the cotyledonary tissue, the synthesis and accumulation of reserve substances represents a complex process of cellular specialisation, whose molecular bases consist in programmes of differentiated expression of genes, implying temporal and spatial coordination in the expression of some parts of genetic information. The "in vitro" culture of cotyledonary explants induces the mitotic stimulation of some specialised cells, accompanied by a wide range of modifications of cellular ultrastructure, expression of reprogramming the cellular functions and therefore of the reorientation of cytological activity (11), (12). The plant tissue cultures, due to their good potential of testing the dynamics of development and their pronounced plasticity as a response to the external treatments, allow to perform interesting investigations regarding the influence of some variable parameters of medium on the process of cellular differentiation (1), (8).

The objective of this work was the electronomicroscopic study of the phenomena taking place in the cells of morphogenetic cotyledonary explants of *Citrullus lanatus* under the influence of "in vitro" culture conditions for a better understanding of the dynamics of cell differentiation and development processes, essential for their efficient control.

MATERIAL AND METHOD

The biological material consisted of explants collected from the cotyledons (with attached testa) of some plantlets of *Citrullus lanatus*

obtained by seed germination under laboratory conditions. After surface sterilisation with a solution of mercuric chloride in a concentration of 0.05% and repeated rinses with distilled sterile water, the cotyledons were cross-sectioned in fragments of 0.2—0.5 cm in width and cultivated on a culture medium represented by the basal medium Murashige and Skoog (1962) supplemented with 6-benzylaminopurine (BAP) in concentration of 10^{-5} M. The transformations at the ultrastructural level under the influence of culture conditions were investigated at intervals of 7 days and 30 days since the moment of culture initiation in comparision with the cells from cotyledonary tissue, at the moment of explanting.

The electronmicroscopic study was carried out by fixing the material with 3% glutaraldehyde in phosphate buffer, followed by postfixation with 2% OsO_4 and inclusion in Araldite, according to the standard method. The sections were stained with uranyl acetate and lead citrate according to Reynolds, and examined under a JEM-7 electronmicroscope.

RESULTS AND DISCUSSIONS

The development of the stationary phase in the cotyledonary cells, that persists until the moment of seed germination, is accompanied by certain morphological aspects at a subcellular level, generally characteristic of all cells in the mature storage tissue of cotyledons, the most evident ones consisting in an accumulation of some large amylipherous inclusions at the plastid level (amyloplasts) as well as the presence in the cytoplasm of a large number of protein bodies and lipid droplets. The studies carried out on the cells of the cotyledonary tissue in numerous species pointed to phenomena of polyploidization and polytenization at the level of the whole genome, fact that does not exclude the possibility of the selective amplification of some genes (8).

In case of the species *Citrullus lanatus* our electronmicroscopic observations pointed out cells without vacuoles, the cellular content being mostly occupied by lipid droplets, collected in compact masses, amyloplasts and protein inclusions with electrondense aspect; the nucleus, centrally displayed, presents finely dispersed chromatin, the plastids and mitochondria are in an incipient stage of development (Plate I, 1). As germination progresses, in the portion of cotyledon released by the testa, the cells acquire mesophyll ultrastructural features; an intense process of vacuolisation is noticed, the cytoplasm is richer in ribosomes, the number of mitochondria increases, the elements of the endoplasmic reticulum frequently present relations of contiguity both with the organelles — plastids, mitochondria — and with the cellular wall (Plate I, 2, Plate II, 1, Plate III, 1, 2). After the metabolisation of the storage substances, necessary to plantlet growth, the cells present ultrastructural characteristics typical for the cotyledonary mesophyll; the central vacuole is large, and in the cytoplasm layer, at the cell periphery, numerous plastids are noticed, with the lamellar system in various stages of organisation (Plate II, 2).

By the cultivation "in vitro" of explants, the cells pass from the stabilised medium into the organism to the new medium of the culture

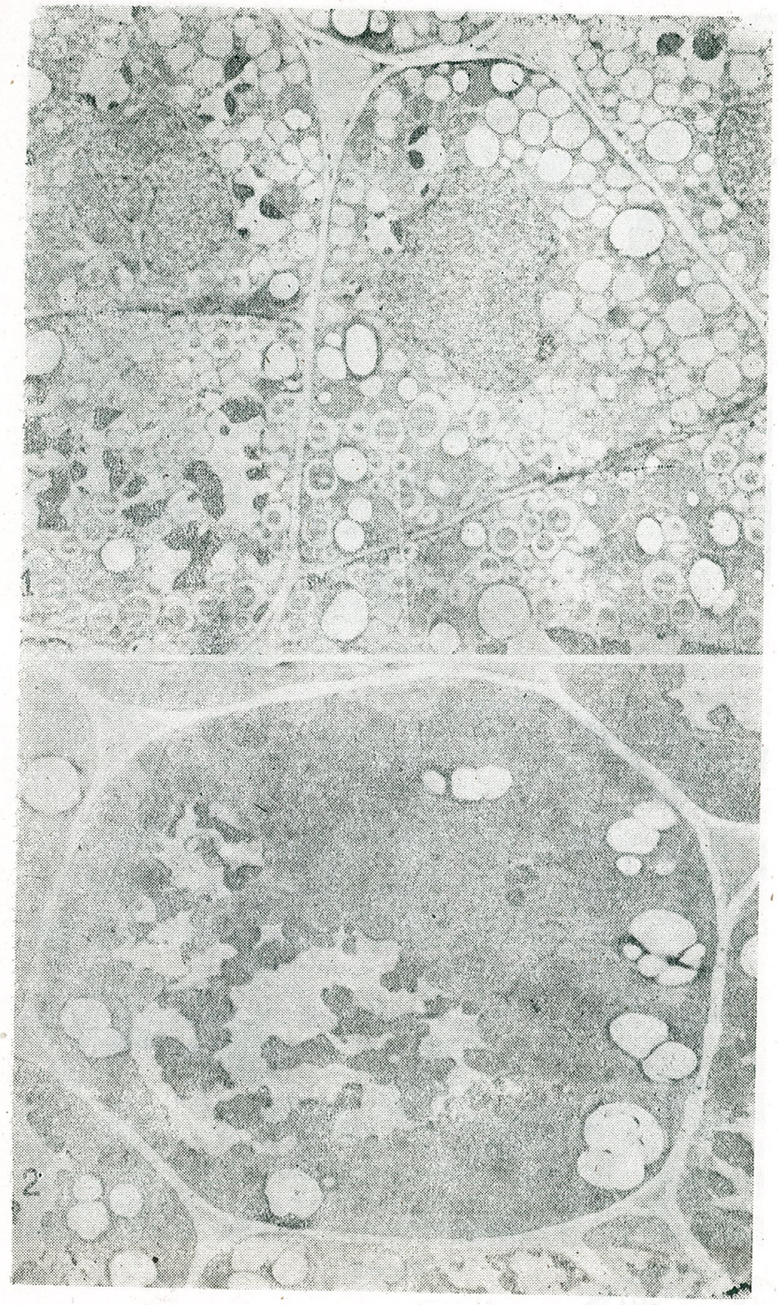


Plate 1

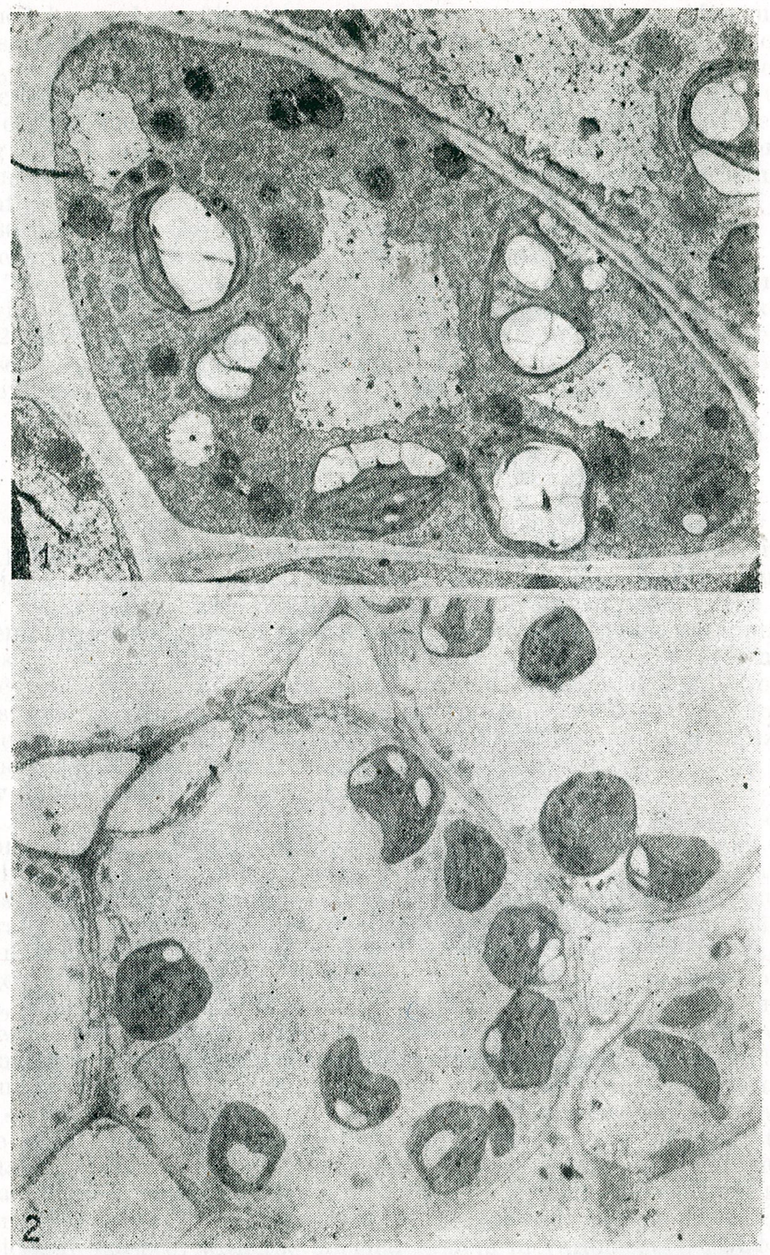
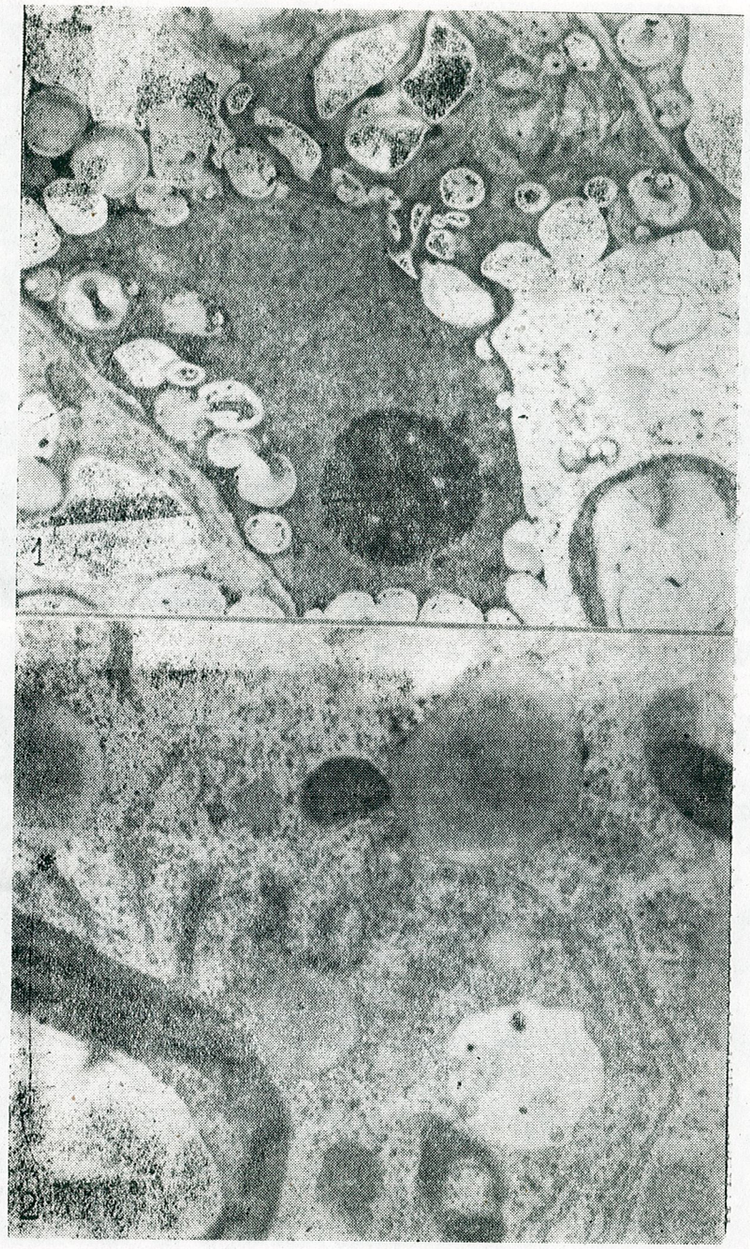


Plate II



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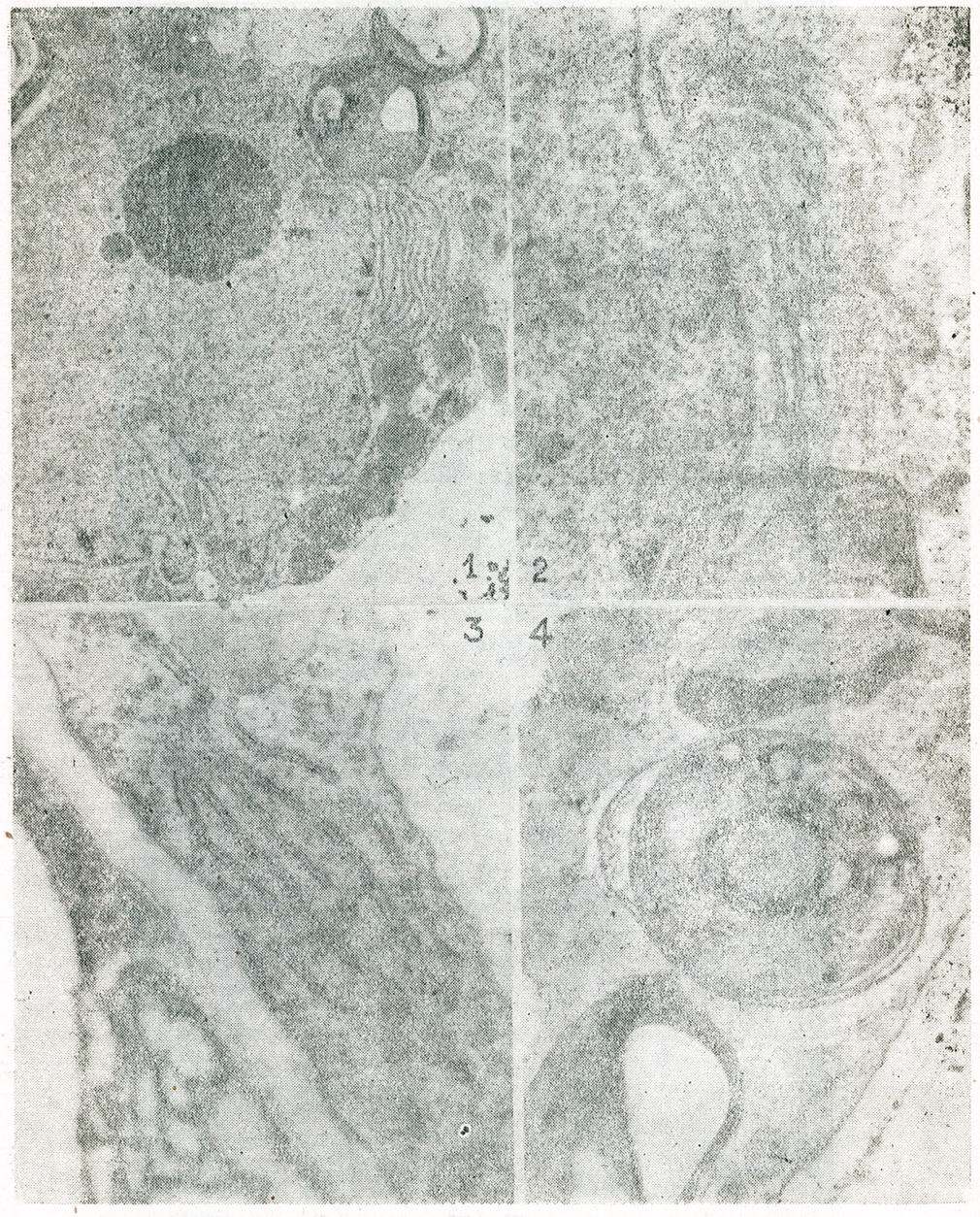

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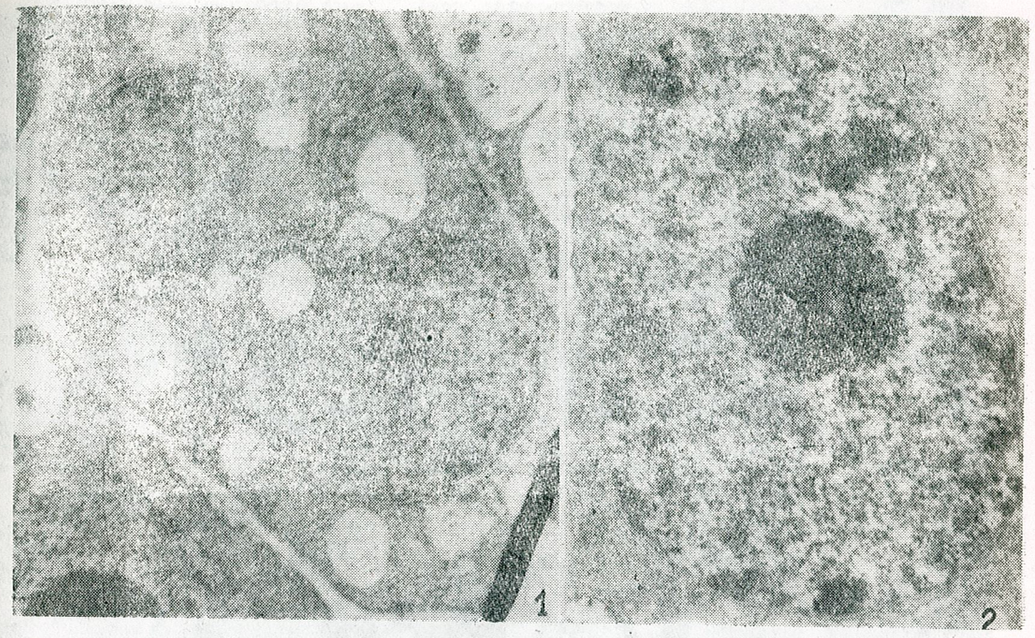

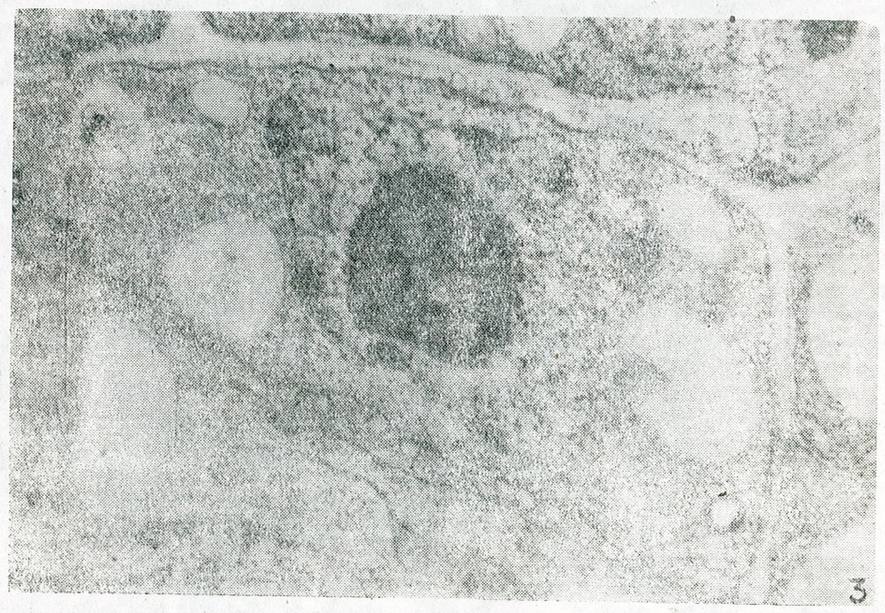

Plate V

Plate VI

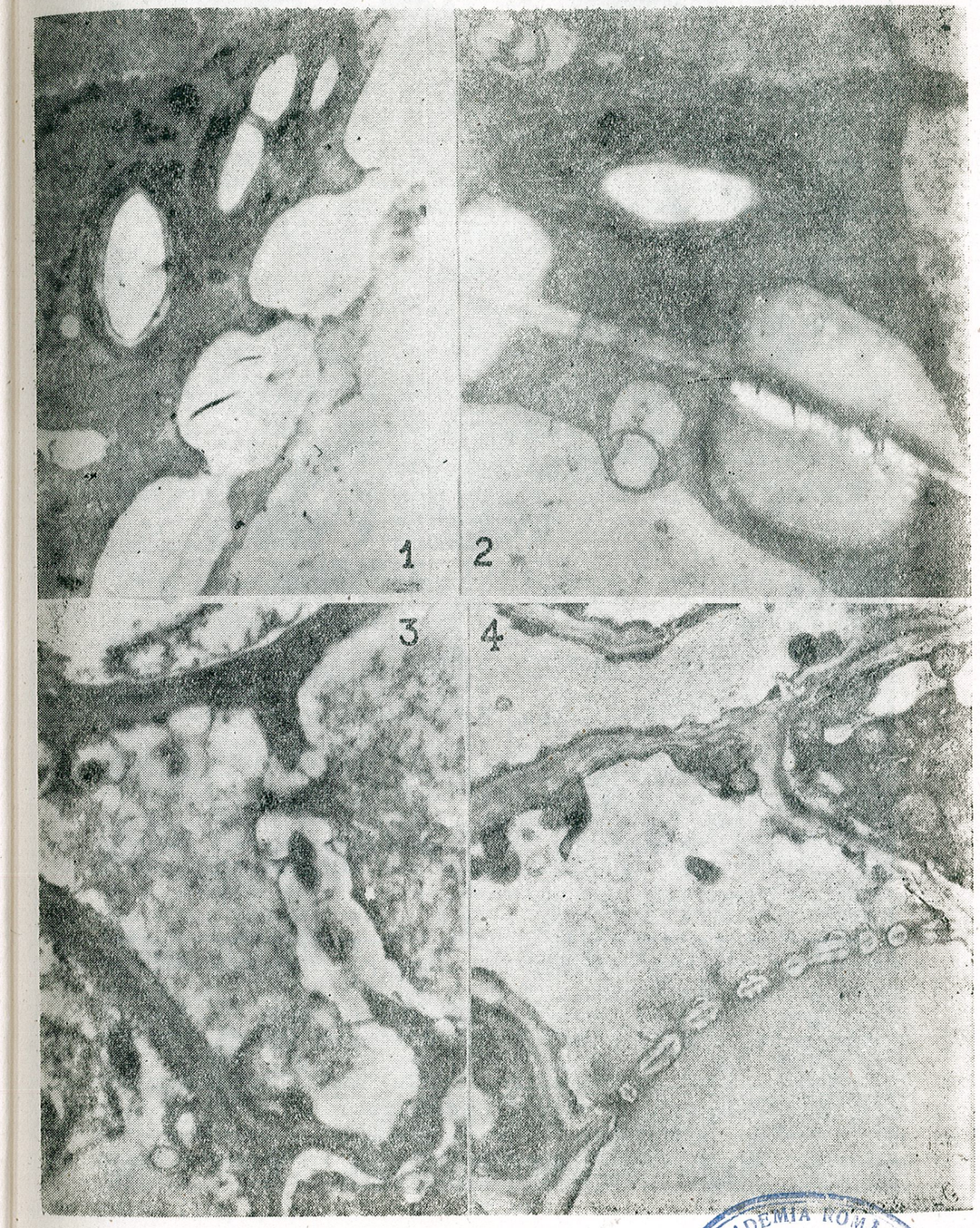

Plate VII

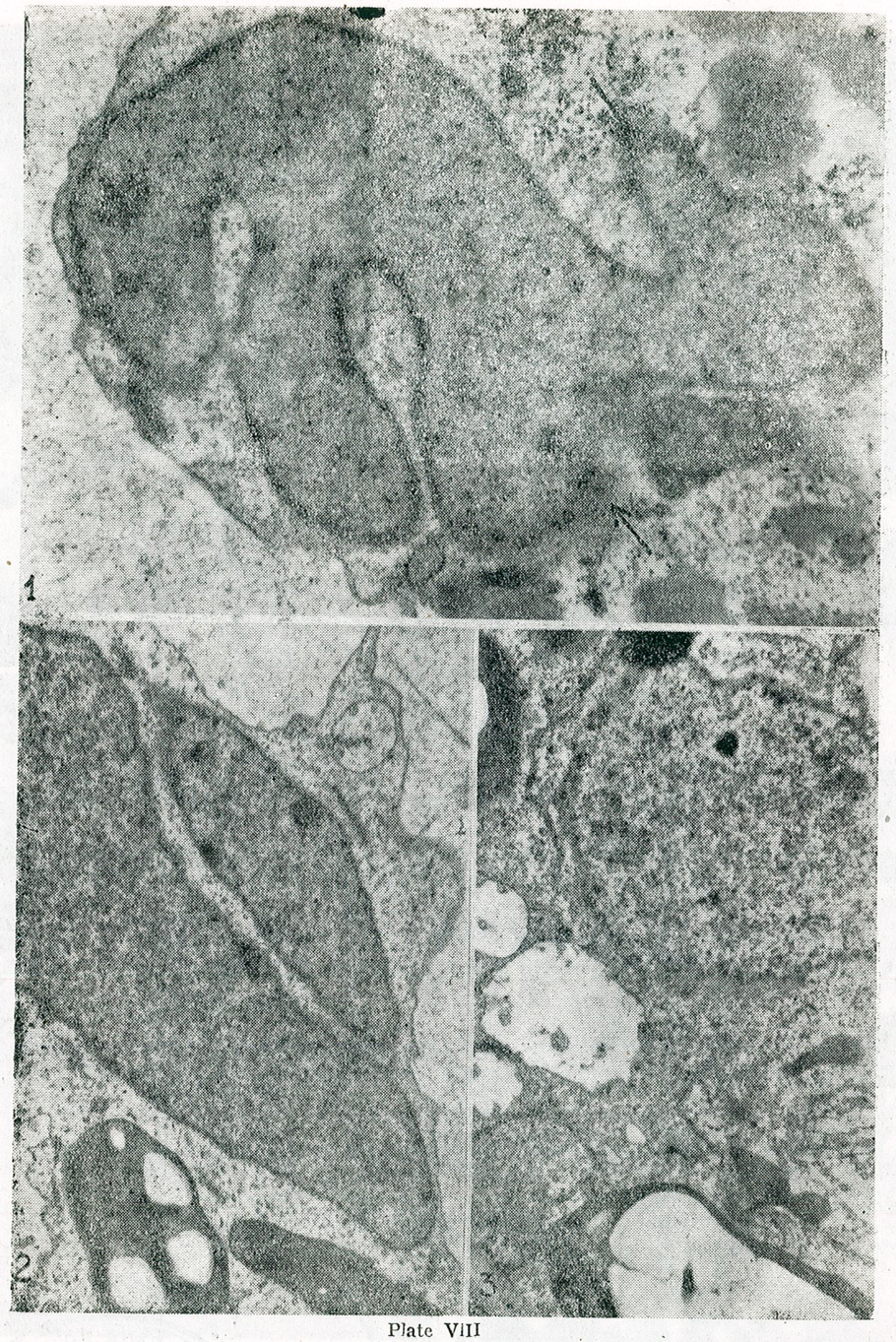

Plate VIII

vial, fact that induces important genetic, metabolic and morphologic modifications, emphasised by the presence of the BAP hormone in the culture medium. The reduction of lipid inclusions is noticed, as well as of protein bodies, correlated with the abundance of endoplasmic reticulum profiles, implied in the transport of substances between various cell compartments. The elements of the endoplasmic reticulum frequently show a tendency to group in parallel, in the vicinity of the cell wall or of the nuclear envelope, expression of the necessities of active transport of substances both in the nucleo-cytoplasmic direction and in the extracellular medium (Plate IV, 1, 2, 3). Sometimes a concentric arrangement of the reticulum is noticed, including inside parts of the cytoplasm with the present organelles (Plate IV, 4).

The revertion of some cells to the meristematic stage by the process of dedifferentiation and implicitly the involvement of these cells in active mitoses (Plate V, 1, 2, 3), leads consequently to the delimitation in the explant body of some wide areas containing actively dividing cells, that evolve toward certain types of organised development. In our experiments, by manipulating the physical, nutritional and hormonal factors in the culture medium, the induced morphogenesis was mainly expressed by the development of adventitious sprouts, from which autonomous plants were regenerated (Plate VI, 1, 2).

Interesting ideas regarding the regulation of "in vitro" differentiation of some specific cell types arise from the study of progressive cytological modifications occurring during the differentiation of vascular elements. The study of these ultrastructural changes, leading in the final phase to the total autolysis of the cell protoplast, may represent a basis for generalizations regarding the cytodifferentiation process in more complex systems.

The deposit of the secondary cellulosic wall, the first visible indicator of xylogenesis is accompanied by the partial autolysis of the cytoplasmic components, a process that begins during the early stages of the vascular element differentiation (6). The existence of some ultrastructural details, characteristic for the accomplishment of some active cytological processes (abundance of mitochondria, reticulum elements, ribosomes, plastids and even nuclei with diffuse chromatin) can be correlated with the intense synthesis of cellulose, necessary to achieve the peculiar morphology of the secondary wall (Plate VII, 1, 2, 3, 4).

During the processes of dedifferentiation and redifferentiation of the different cellular types, the ultrastructural details of the nucleus express the interactions among the nucleus, organelles and cytosol during the various stages of cytodifferentiation. Together with the intensification of the metabolical processes by the activation of some cells previously in a stationary stage, a concentration around the nucleus of the main organelles (mitochondria, plastids, elements of endoplasmic reticulum) and of numerous microvacuoles is noticed. Their presence, as well as other peculiar ultrastructural aspects of the nuclear envelope, expressed by the multiplication of the pore number are certain evidence of an intense metabolism, correlated with a new orientation of the cytological activity (Plate VIII, 1, 3).

Ultrastructural aspects illustrating the active exchange between the nucleus and the cytoplasm during the organisation of meristematic centres were described by some authors using various modern "in vitro" methods: Therefore Nuti Ronchi et al. (10), Martini and Nuti Ronchi (7), studying the phenomenon of gene amplification in the cells of the medullar tissue in *Nicotina glauca* cultivated "in vitro", frequently noticed nuclei abounding in some Feulgen + bodies, similar to nucleoli, which are eliminated in the cytoplasm. At the same time, the nuclei are hypertrophied, lobated and the nuclear envelope disintegrates in certain areas. Nuclear fragmentation follows through, consequently leading to the appearance of some polynucleated cells that are involved in the organization of meristematic centres. Innocenti and Avanzi (4) mentioned the presence of some nuclear extrusions in the cytoplasm of *Allium cepa* cells during xylogenesis. The hybridization experiments demonstrated the ribosomal nature of the extrusionated bodies, suggesting the existence of a succession of phenomena prior to xylogenesis, a necessary stage being the extra-replication at least of the DNA sequences codifying RNAr (1).

The evident furrowing of nuclei increases, on the one hand, the transport nucleo-cytoplasmic surface and, on the other, results in nuclear fragmentation and the appearance of some binucleated or multinucleated cells (amitoses); this phenomenon is relatively frequent in the cells of the cotyledonary explants of *Citrullus lanatus* cultivated "in vitro" (Plate VIII, 1, 2). This phenomenon, followed by the cellulization process may be partly responsible for the appearance of some genetic variants (2), (5); among them, those with an aneuploid number of chromosomes are of a special interest for the breeding programs. The chromatin organization at the level of some nuclei indicates the possibility of some polyploid mitoses, perhaps as a result of the mitotic stimulation of endoreduplicated nuclei already existing in the cotyledonary tissue before explanting. Cionini et al. (3) mention a similar situation in the case of the cotyledonary tissue of *Vicia faba* after approximately 4 days of "in vitro" culture.

By increasing the "in vitro" culture duration (30 days after inoculation) the process of cellular specialisation is more obvious. Together with mesophyllic type cells, cells in various stages of xylogenesis as well as senescent cells were noticed. The latter display a quantitative reduction of cytoplasm and the development of the autophagic vacuolar system, including portions of the cytoplasm with the component organelles, which later on are degradated by the autolytic enzymes present in the vacuolar sap.

Besides the specialised cells, the dedifferentiated cells whose organelles have a morphology and orientation characteristic for the active metabolic cells, organised in morphogenetic meristematic centres are also present.

CONCLUSIONS

1. Under the "in vitro" conditions a genetic reprogramming takes place in the storage cotyledonary tissue, from the stage of differentiated cells of the storage parenchyma, to an active metabolic one, engaged to new types of cellular differentiation.

2. The achievement of cellular dedifferentiation results in the appearance of cells with meristematic characteristics, similar in aspect and function to the cells of the apical meristems, evolving to various types of organised development.

3. The somaclonal variation, often correlated with the adventituous morphogenesis (represented in the case of cotyledonary explants of *Citrullus lanatus* by adventitious sprouts formations from which plants can be obtained) may generate useful genetic variants important for the breeding programmes of this species.

4. A better knowledge of differentiation cytogenetics has important practical implications, since the success of inducing the organogenesis and embryogenesis in cell and tissue cultures, the integration in the breeding programmes of useful genetic variability, all depend on knowing the genetic bases of these phenomena.

PLATE EXPLANATION

Plate I — Ultrastructural characteristics of specialised cotyledonary cells before "in vitro" culture;
1 — Cell, typical for the storage parenchyma of the cotyledon in *Citrullus lanatus* (X 2,150);
2 — Beginning of the metabolisation of the storage materials as the seed germination progresses (X — 4,500).

Plate II — Cells evolving toward the mesophyll type;
1 — Cell displaying cytoplasm richer in ribosomes, having an increased number of the main organelles and with the vacuolar system in the course of organisation (X 4,500);
2 — A complete differentiated cotyledonary mesophyll cell (X 3,300).

Plate III — Details of the ultrastructural changes in cotyledonary cells during seed germination;
1 — Active metabolisation of the lipid droplets and of the starch stored in amyloplasts (X 5,500);
2 — Zone of the cytoplasm rich in ribosomes, free or attached to endoplasmic reticulum prophiles, dictyosomes and large amyloplasts (X — 29,900).

Plate IV — Ultrastructural aspects of cotyledonary cells after "in vitro" culture;
1. — Endoplasmic reticulum prophiles arranged in parallel to the nuclear envelope (X — 6,700);
2. — Detail (X 13,200);
3. — Endoplasmic reticulum elements, parallel to the cell wall (X — 12,100);
4. — Concentric arrangements of the endoplasmic reticulum including parts of the cytoplasm (X 15,500).

Plate V — Cells with meristematic ultrastructural features resulted by the dedifferentiation process;
1 — Actively dividing cell (X 6,600);
2 — Aspect of the nucleus in a cell from meristematic centres (X 9,000);
3 — Cell plate organisation after the mitotic process (X—6,800).

Plate VI — Development of meristematic morphogenetic zones;
1 — Histological aspect in light microscopy showing meristematic centers in the cotyledonary explant cultivated "in vitro";
2 — Plants regenerated by "in vitro" rooting of the adventituous shoots;

Plate VII — Stages of vascular element differentiation;
1 — The deposit of secondary cell wall indicative of xylogenesis (X — 4,300);
2 — Detail (X—7,600);

3, 4 — Advanced stages of the phloematic element differentiation (3—X 13,500; 4—X 4,500).

Plate VIII — Ultrastructural characteristics at the nucleus level during "in vitro" culture;

1 — Pronounced furrowing and abundance in pores in some portions of the nuclear envelope — arrows (X—15,600);

2 — Nuclear fragmentation (X 10,100);

3 — Cell zone showing a nucleus with numerous pores in the envelope (X 9,800).

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LA CROISSANCE DES BOURGEONS AU COURS DE LA VÉGÉTATION DE L'ESPÈCE FETEASCĂ NEAGRĂ. LA LIAISON ENTRE LA CROISSANCE DES DRAGEONS, LA SURFACE DES FEUILLES ET LA CROISSANCE DES BOURGEONS

ANCA ANTOHE

The studies represent the continuation of some previous researches (1, 2, 3, 4) carried out in the north-east of Moldavia on the *Vitis vinifera* type: Aligoté (a type originated from France) and black Feteasca (an autochthonous type). The growth and development of the buds take place in the growing period of the buds and of their leaves. The growing peculiarities of the buds are correlated with the growth of the internodes and leaves, with the glucidic and mineral metabolism, as well as with the intensity of respiration and the activity of oxide-reducing enzymes.
All these show the ecological adaptation peculiarities of the black Feteasca type.

La croissance et le développement des bourgeons de la vigne ont une importance toute particulière étant donné le fait que les bourgeons renferment sous une forme potentielle l'hérédité de toute la plante.

Pour ce qui est de la vigne, il y a plusieurs catégories de bourgeons. Nos recherches ont visé la croissance et le développement des bourgeons primaires ou principaux (5, 6, 7) nommés aussi bourgeons d'hiver (6, 7, 8).

MATÉRIEL ET MÉTHODE

L'étude a été effectuée dans le cadre de la Station expérimentale horti-viticole de Iaşi, dans des conditions d'expérimentation idéntiques à celles rappelées dans les travaux antérieurs (1, 2, 3, 4).

La croissance des bourgeons a été étudiée sur les trois catégories de drageons (de la base, du milieu et du bout) pour l'espèce Feteasca neagră. On a utilisé la méthode des mesurages à la loupe binoculaire, à l'aide d'un oculaire micromètre en établissant ainsi la croissance en longueur et en épaisseur des bourgeons à un intervalle de 15 jours.

On a effectué les mesurages aux dates suivantes: 5V, 18 V, 5VI, 18 VI, 1 VII, 15 VII, 1 VIII, 15 VIII, 1 IX, 15 IX, 15 X.

Les résultats ont été exprimés en unités conventionnelles (u.c.)

RÉSULTATS ET DISCUSSIONS

Pour les drageons situés à la base du sarment (fig. 1), les bourgeons qui apparaissent le 5 mai ont des dimensions relativement petites es la grande période de croissance au mois de mai. Les bourgeons croissent

dans un rythme accéléré jusqu'au premier août, puis la croissance continue plus lentement.

Les internœuds correspondant à ces bourgeons (3), passent par la grande période de la croissance en longueur et en épaisseur en même temps que les bourgeons. Les feuilles (1) passent, elles aussi, par la grande période de croissance au mois de mai. Les surfaces foliées sont réduites.

Les deux ou les cinq derniers bourgeons qui apparaissent sur les drageons le 5 mai, de même que les bourgeons qui apparaissent sur les drageons le 25 mai passent par la grande période de croissance le 5 juin. Ceux-ci sont des bourgeons de plus grandes dimensions.

Les internœuds respectifs passent par la grande période de croissance en même temps que leurs bourgeons, le 5 juin, et par la période de la croissance en épaisseur, plus tard. Les feuilles qui correspondent à ces bourgeons passent par la grande période de croissance au mois de juin et se caractérisent par de grandes surfaces foliées.

Les bourgeons du mois de juin (le 5 VI), ont la grande période de croissance à cette date.

Les bourgeons qui apparaissent le 18 juin passent par la grande période de croissance à la même date et ont les plus grandes dimensions. Les internœuds respectifs passent par la grande période de la croissance en longueur et en épaisseur en même temps que les bourgeons. Les feuilles passent par la grande période de croissance toujours au mois de juin et réalisent de grandes surfaces foliées.

Les bourgeons ont un rythme accéléré de croissance jusqu'au mois d'août et au début du mois de septembre.

Les autres bourgeons qui apparaissent plus tard au mois de juillet ou au mois d'août, passent par la grande période de croissance les mêmes mois, en même temps que la grande période de croissance de leurs internœuds.

Les bourgeons des drageons du milieu du sarment (fig. 2) — les bourgeons 1—6 — passent par la grande période de croissance le 5 mai, de même que les bourgeons des drageons situés à la base du sarment. Ils croissent plus rapidement jusqu'au mois d'août, puis le phénomène continue plus lentement.

Les internœuds respectifs (3) continuent leur croissance jusqu'au mois de juin, en passant par la grande période de la croissance en longueur le 25 juin. Les feuilles (1) passent par la grande période de croissance plus tard (au mois de juin) par rapport à leurs bourgeons et ont des surfaces foliées petites.

Les bourgeons qui apparaissent pendant la deuxième moitié du mois de mai, passent par la grande période de croissance le 5 juin. Ils continuent à croître jusqu'au début du mois d'octobre, mais ne croissent dans un rythme accéléré que jusqu'au mois d'août.

Les internœuds passent par la grande période de croissance le 23 mai, donc une période antérieure à la grande période de croissance des bourgeons et continuent à croître jusqu'au mois de juillet. La grande période de la croissance en épaisseur des internœuds est le 25 juin. Les feuilles passent par la grande période de croissance au mois de juin, en même temps que les bourgeons et réalisent de grandes surfaces foliées.

Les bourgeons qui apparaissent sur les drageons au mois de juin se caractérisent par les croissances en longueur et en épaisseur les plus évidentes. Ils passent par la grande période de croissance soit dans la première moitié du mois de juin (les bourgeons qui apparaissent le 5 juin), soit dans la seconde moitié du mois de juin (les bourgeons qui apparaissent le 18 juin).

Tous ces bourgeons continuent leur croissance jusqu'au début du mois d'octobre. Les internœuds respectifs passent par la grande période de croissance en longueur et en épaisseur en même temps que les bourgeons. Les feuilles passent par la grande période de croissance au mois de juillet, plus tard que les bourgeons et les internœuds respectifs. Leurs surfaces foliées sont assez grandes.

Les autres bourgeons qui se forment plus tard et apparaissent sur les drageons au mois de juillet, passent par la grande période de croissance pendant la première ou la seconde moitié du mois de juillet, en même temps que les feuilles et la grande période de la croissance en longueur des internœuds. La grande période de la croissance en épaisseur des internœuds est le 25 juillet. Les feuilles passent par la grande période de croissance en même temps que les bourgeons et réalisent de grandes surfaces foliées.

Les bourgeons du mois d'août passent par la grande période de croissance le même mois, en même temps que la grande période de la croissance en longueur de leurs internœuds. La grande croissance en épaisseur a lieu le premier septembre. Ces bourgeons ont un rythme lent de croissance.

La croissance de bourgeons sur les drageons situés au bout du sarment (fig. 3) se déroule de la manière suivante : les bourgeons de la base du drageon passent par la grande période de croissance en même temps que la croissance en longueur de leurs internœuds (le 5 mai). Les bourgeons déploient leur croissance sur une grande période jusqu'au début du mois d'octobre, ayant un rythme plus accéléré jusqu'au mois de juillet.

Les deux derniers bourgeons qui apparaissent le 5 mai, de même que les bourgeons qui apparaissent le 25 mai, passent par la grande période de croissance le 5 juin. Leurs internœuds (3) passent par la grande période de la croissance en longueur avant les bourgeons (le 22 mai) et par la grande période de la croissance en épaisseur après les bourgeons respectifs (le 25 juin).

Les feuilles des bourgeons qui apparaissent sur les drageons au mois de mai (1) passent par la grande période de croissance au mois de juin, leurs surfaces foliées ayant des valeurs élevées.

En ce qui concerne les bourgeons qui apparaissent au mois de juin, on remarque les plus grandes dimensions. Ils croissent pendant toute la période de végétation, mais leur croissance a un rythme plus rapide jusqu'à la seconde moitié du mois de juillet. Les bourgeons qui se développent sur les drageons pendant la première moitié du mois de juin, passent par la grande période de la croissance en longueur et en épaisseur le 18 juin. Les internœuds respectifs passent plus tard par la grande période de la croissance en longueur et en épaisseur le 25 juin. Les feuilles de ces bourgeons passent par la grande période de croissance au mois de juillet et ont de grandes surfaces foliées.

Fig. 1 — La croissance des bourgeons chez l'espèce Fetească **neagr**ă. Drageon de la base du sarment.

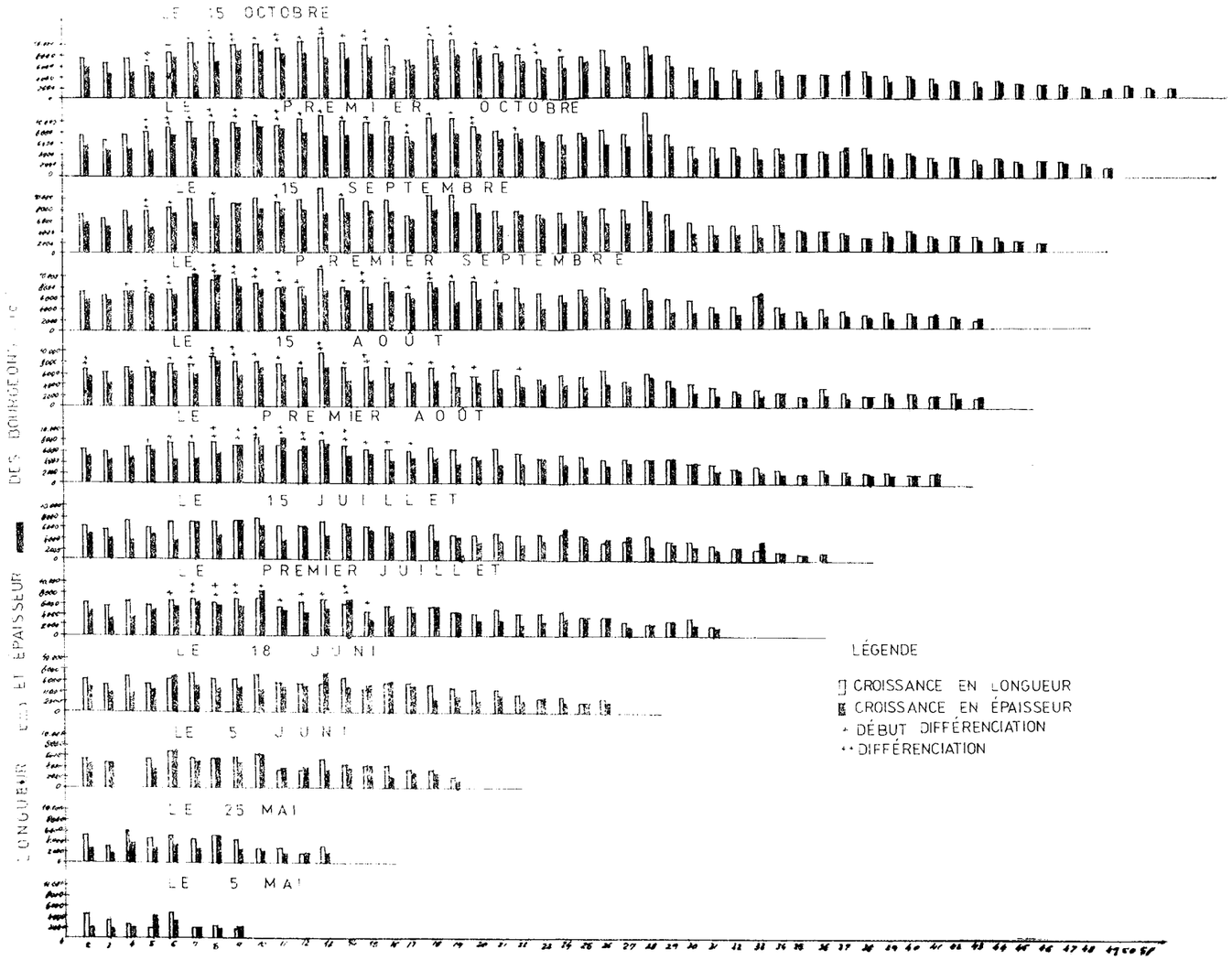

Fig. 2 — La croissance des bourgeons chez l'espèce Feteasca neagră. Drageon du milieu du sarment.

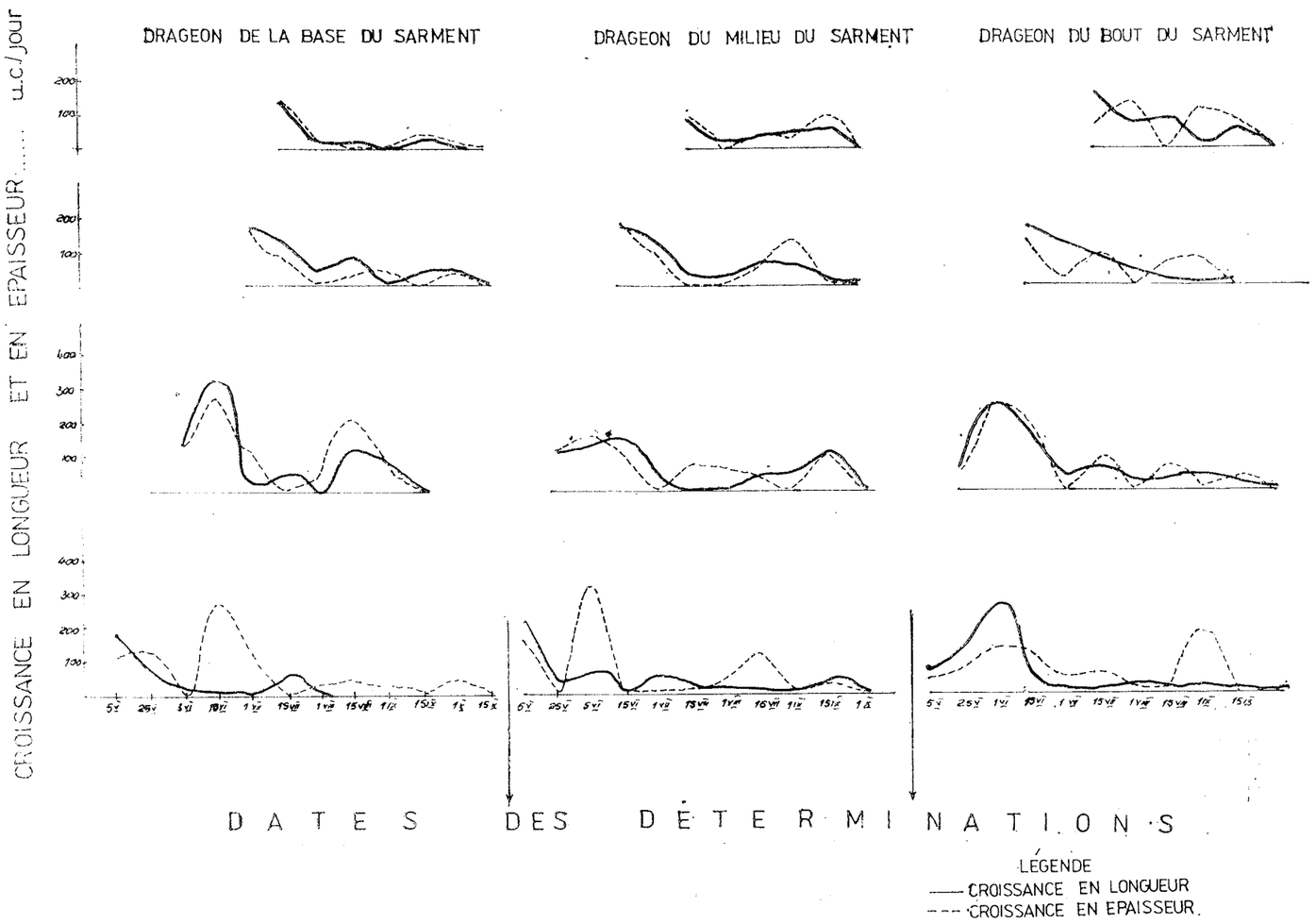

Fig. 4 — La croissance en longueur et en épaisseur de certains bourgeons sur les drageons de l'espèce Feteasca neagră au cours de la période de végétation.

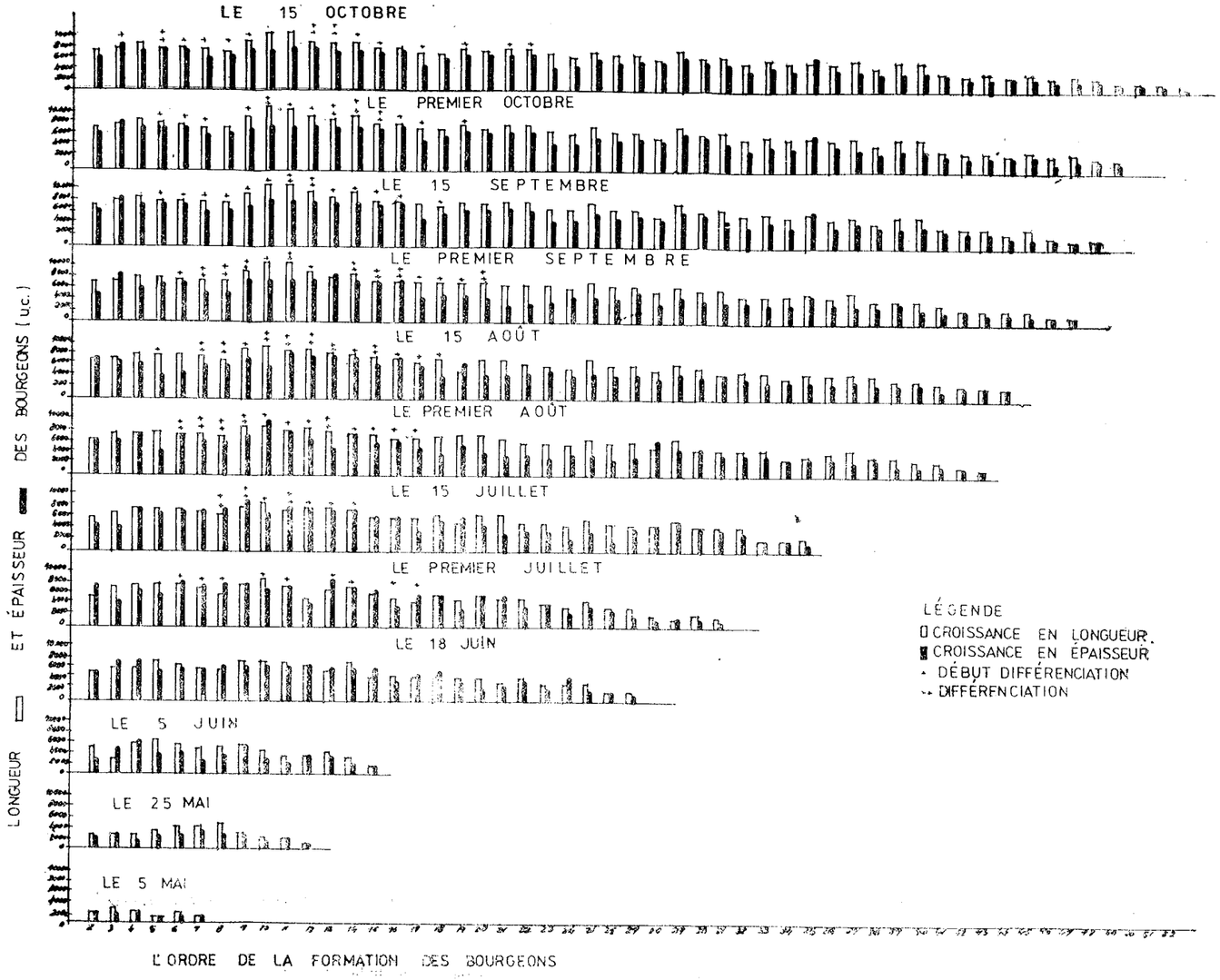

Fig. 3—La croissance des bourgeons chez l'espèce Feteasca neagră. Drageon du bout du sarment.

Les bourgeons du mois de juillet ont des dimensions plus élevées. Ils croissent jusqu'au mois d'octobre. Leur grande période de croissance a lieu au mois de juillet (pendant la première ou la seconde moitié du mois) après leur apparition sur les drageons).

La grande période de la croissance en longueur de leurs internœuds a lieu au mois d'août et la grande période de la croissance en épaisseur a lieu au mois d'août et au mois de septembre. Les feuilles se caractérisent par des surfaces réduites ; elles passent par la grande période de croissance au mois d'août.

Les bourgeons du mois d'août sont petits et croissent dans un rythme plus lent.

Dans les recherches effectuées nous avons remarqué une hétérogénéité bien marquée des bourgeons, la courbe de la croissance sur le sarment étant une courbe cyclique, ondulante (1, 2, 3).

La représentation de la croissance de certains bourgeons en longueur et en épaisseur (fig. 4) met en évidence le fait que le phénomène de la croissance en longueur s'arrête avant l'arrêt de la croissance en épaisseur, fait d'autant plus évident que les bourgeons se trouvent vers la base des drageons.

CONCLUSIONS

1. La formation et la croissance des bourgeons sur un drageon n'ont pas lieu en même temps ; ils diffèrent selon l'âge et les propriétés biologiques.

2. Les bourgeons qui se forment sur les drageons au mois de mai ont un rythme de croissance rapide jusqu'au mois de juillet (les bourgeons de la base du sarment) ou au mois d'août (les bourgeons du milieu du sommet du sarment).

3. Les bourgeons qui apparaissent sur les drageons au mois de juin ont les plus grandes dimensions.

4. Les bourgeons du mois de juillet ont des dimensions plus réduites et croissent plus lentement.

5. A partir du mois d'août, la formation et la croissance des bourgeons ont lieu dans un rythme plus lent.

6. Plus les bourgeons apparaissent tard sur les drageons, plus leur développement se prolonge vers l'automne.

7. L'intensité de la respiration et l'activité des enzymes oxydoréductrices (4) se caractérisent par des valeurs élevées qui, mises en corrélation avec les quantités plus grandes de glucides et d'azote, expliquent les croissances intenses des bourgeons de cette espèce.

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NITROGEN EFFECTS ON *LOLIUM MULTIFLORUM* LAM. CHLOROPLAST AND OTHER CELL-ORGANELLE ULTRASTRUCTURE

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Different amounts of fertilizers with nitrogen as ammonium nitrate were applied to *Lolium multiflorum* Lam under field conditions and their effect upon the ultrastructure of the chloroplast and other cellular organelles has been studied. The micrographs reveal slight ultrastructural changes of the chloroplast for amounts larger than N_{600} kg.ha^{-1} and essential ultrastructural changes of the chloroplast and the other organelles for N_{1000} kg.ha^{-1}. It has been noticed that N_{400} kg.ha^{-1} causes the highest number of photosynthesizing elements (grana per chloroplast), while N_{200} kg.ha^{-1} although decreases the volume of the chloroplast, leads to a high percentage of grana and thylakoids as compared to the volume of the stroma, and thus to the most efficient use of light per chloroplast volume. It follows that the most adequate levels of N fertilizers applied to *Lolium multiflorum* Lam vary between 200 and 400 kg.ha^{-1}, according to ecological conditions. It seems that within these limits the photosynthesizing apparatus functions best. Higher levels of N fertilizers cause ultrastructural changes of the cellular organelles. Such changes are accompanied by metabolic alterations, as, for instance, in the case of chloroplasts the intensity of photosynthesis decreases.

The part played by nitrogen fertilizers in increasing the production of the vegetal biomass is well known, due to several researches carried out both in Romania and abroad [1], [2], [3], [4], [5], [6], [7]. Fodder plants react positively to different nitrogen fertilizers, as reflected by the increase in biomass and protein content. There is even a risk of nitrate accumulation for larger amounts of fertilizers [6]. From a biochemical point of view, an increase of the content in proteins, chlorophylls and carotenes also takes place [7]. Researches carried out so far under the electronmicroscope [8], [9] have revealed ultrastructural changes of the photosynthesis apparatus, for low quantities of nitrogen. The effects of optimal and superoptimal amounts of nitrogen fertilizer upon the ultrastructure of the chloroplast have not yet been completely cleared up.

The present study attempts to observe the effects of nitrogen fertilizer upon the ultrastructure of the chloroplast and of the other cellular organelles in *Lolium multiflorum* Lam.

MATERIALS AND METHODS

The experiment was carried out in Cluj-Napoca in 1983, on the experimental field "Șapca Verde", located on the second terrace of the Someșul Mic river at an altitude of 330 m. The following steps were taken: the limits of a control plot were marked (unfertilized parcel); nitrogen fertilizer was applied as amonium nitrate (35%); the amounts used were the following:

$N = 200$ kg·ha^{-1} variant noted with N_{200};
$N = 400$ kg·ha^{-1} „ „ „ N_{400};

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$N = 600$ kg·ha^{-1} ,, ,, ,, N_{600};
$N = 800$ kg·ha^{-1} ,, ,, ,, N_{800};
$N = 1000$ kg·ha^{-1} ,, ,, ,, N_{1000}.

The fertilizers were administered only once, at sowing time, on a nutritive substrate of P_2O_5 — 50 kg·ha^{-1} and K_2O — 100 kg·ha^{-1}. The experiment was carried out on a loamy alluvial soil, very deep, made up of fluvial clay deposits, arable, slightly alkaline (pH = 7.8), with a reduced content in $CaCO_3$ and an average humus content. Spring was droughty in the year when the experiment was carried out (1983), and had a negative impact upon the growth of seedlings (i.e. they displayed for a long time only two small leaves).

For the electron microscopic investigations, fresh leaves (in a pre-blooming stage) were sampled from the field, and were fixed in 3% glutaraldehyde (2 hrs) buffered at pH = 7.2 with a solution of sodium cacodylate. After fixation, the glutaraldehyde was removed for 1 hour with a buffer solution changed 3—4 times. Postfixation was achieved in 1% osmic acid (1 hr) at a pH = 7.2. All fixation operations took place at + 4°C. After postfixation, the vegetal material was rewashed and dehydrated in acetone solutions of gradually increasing concentrations (up to pure acetone). Inclusion was done in Westopal and sections were obtained with the LKB III ultramicrotome. The contrasting of the sections was performed with uranyl acetate and lead citrate, ultrastructural characteristics being observed under the TESLA BS-500 electron microscope.

The length and width of chloroplasts, as well as the surface of the section (recorded with a Reiss planimeter), were determined on the micrographs. The number of grana per chloroplast section and the number of thylakoids per granum were also recorded. Surface measurements were carried out on 20—25 chloroplast sections for each variant, while other adimensional measurements (number of grana and thylakoids) were determined on 40—45 chloroplasts. Mean values are accompanied by mean error and variation coefficients. The following parameters were determined on the data obtained: number of grana per chloroplasts, total volume of grana in a chloroplast, volume of chloroplasts, and the percentage of grana, thylakoids and thylakoid membranes out of the volume of the stroma. These parameters are displayed in Tables 1, 2 and 3.

Table 1

Sizes of *Lolium multiflorum* Lam. chloroplasts depending upon ammonium nitrate treatment

Treatments kg·ha^{-1}	Chloroplast sizes: Length µm	Variability coefficient %	Width µm	Variability coefficient %	Length/Width	Surface (S) µm^2	Variability coefficient %	Volume (V) µm^3	Variability coefficient %	V/S
Control	4.78±1.02	21.3	2.64±0.94	35.0	1.81	9.89±3.91	39.5	33.3	100	3.37
N_{200}	4.85±1.39	28.8	2.23±0.79	35.0	2.77	8.15±3.40	21.7	26.1	−21.6	3.20
N_{400}	4.96±0.87	17.7	2.44±0.48	19.0	2.03	9.69±2.86	29.5	31.2	− 6.3	3.22
N_{600}	4.77±1.32	27.9	2.74±0.87	31.7	1.74	10.48±4.55	43.5	35.5	+ 6.6	3.39
N_{800}	3.39±1.07	31.6	2.74±0.58	31.0	1.24	9.32±3.05	32.7	23.3	−30.0	2.50
N_{1000}	4.76±1.26	26.5	2.29±0.86	37.0	2.08	7.64±3.02	39.6	26.7	−19.8	3.49

Table 2

Quantified data on the ultrastructure of *Lolium multiflorum* Lam. chloroplast depending upon ammonium nitrate treatment

Treatments kg·ha^{-1}	Number of grana per chloroplast section	Average number of thylakoids per granum	Number of thylakoids: 4	5	8	10	12	15	16	20	>
			Percentage distribution of grana by number depending upon ammonium treatment (%)								
Control	26	11	30	—	34	—	20	—	11	5	—
N_{200}	31	10	24	—	36	—	22	—	11	4	3
N_{400}	33	10	18	—	38	—	22	—	15	6	—
N_{500}	31	10	36	—	26	—	16	—	11	11	—
N_{800}	22	11	—	28	—	39	—	19	—	11	4
N_{1000}	32	8	—	44	—	52	—	4	—	—	—

Table 3

Quantified data on the ultrastructure of *Lolium multiflorum* Lam. chloroplast depending upon ammonium nitrate treatment

Treatments kg·ha^{-1}	Number of grana per chloroplast	Total volume of grana within a chloroplast µm^3	Volume of chloroplasts µm^3	Grana thylakoids, thylakoidal membranes out of stroma volume %
Control	138—250	5.82	33.3	17.5
N_{200}	140—301	6.61	26.1	25.3
N_{400}	161—327	7.32	31.2	23.5
N_{600}	170—296	6.99	35.5	19.7
N_{800}	121—149	4.05	23.3	17.4
N_{1000}	147—305	6.78	26.7	25.4

RESULTS AND DISCUSSION

Lolium multiflorum Lam. is a species that presents two types of chloroplasts: with and without grana. Our study was performed on granal chloroplasts.

(a) Results in control

Plate I, Figs. 1 and 2, present micrographs with the control. All chloroplasts display a typical structure: they are surrounded by a double membrane, and their stroma presents grana provided with granal and intergranal thylakoids, plastoglobules and starch drops. Some of the chloroplasts also have a peripheral reticulum. Chloroplast measurements (length, width, surface, volume) provided data that vary between the limits recorded in Table 1. Tables 2 and 3 display: number of grana per chloroplast, total volume of grana per chloroplast, volume of chloroplasts and the percentage of grana, thylakoids and thylakoid membranes out of the volume of the stroma, as well as the percentage distribution of the number of thylakoids per granum.

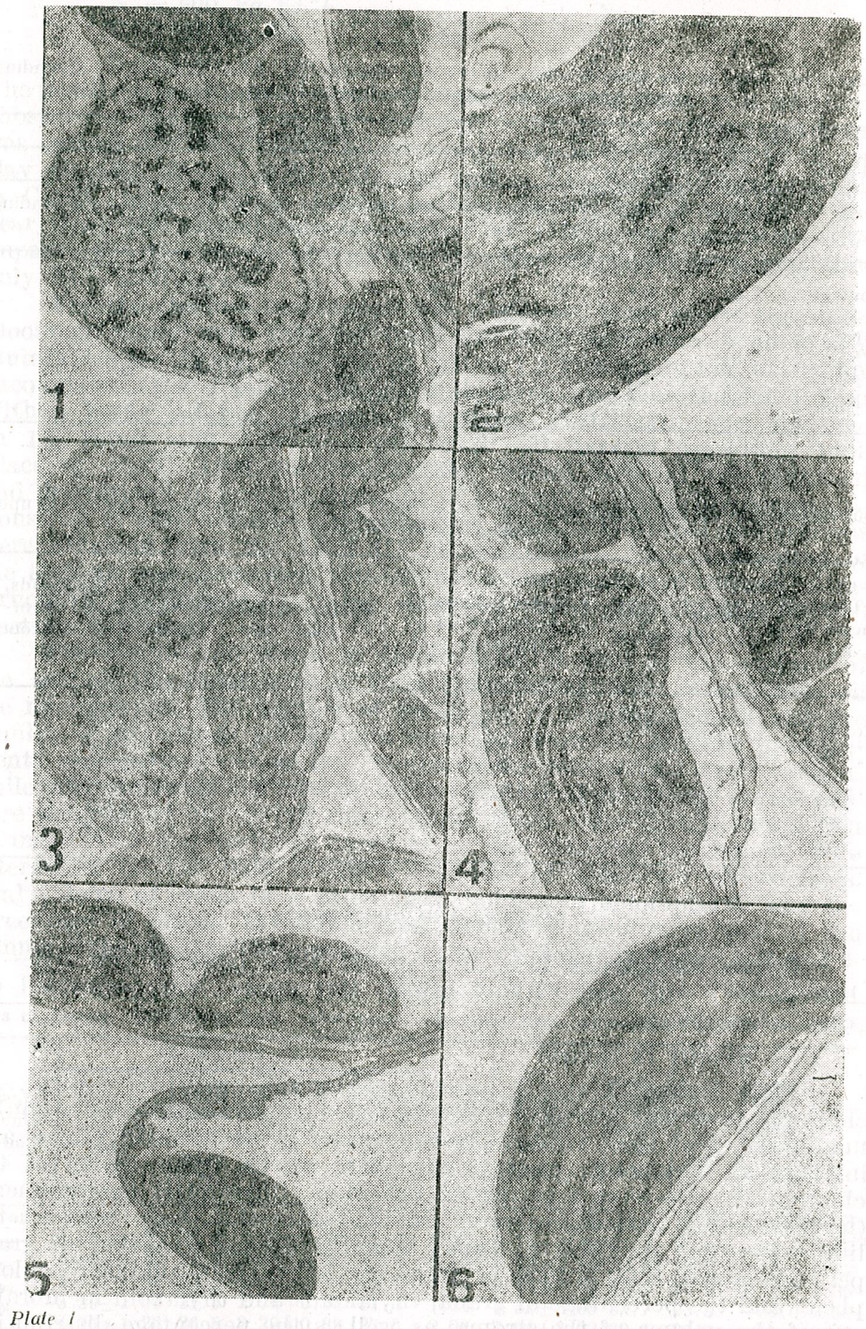

Plate I

Fig. 1, 2 — The ultrastructure of chloroplast and other cellular organelles in *Lolium multiflorum* Lam. (control); Fig. 3, 4 — Effect of N_{200} kg.ha^{-1} (Fig. 3 × 6000; Fig. 4 × 12 000); Fig. 5, 6 — Effect of N_{400} kg.ha^{-1} (Fig. 5 × 6000; Fig. 6 × 12.000)

(b) **Effects of treatments**

Micrographs of the treated variants can be found on Plate I (Figs. 3—6) and Plate II (Figs. 7—12).

The chloroplasts of the variant treated with N_{200} have a generally normal structure, of a lenticular shape, slightly prolonged as compared to control (Table 1), and a rather high density of grana (Table 2). A certain enlargement of the cristae can be noticed in mitochondria (Plate I, Figs. 3, 4). Mitochondrial ribosomes are also prominent. The nuclei reveal no changes. Starch drops can also be noticed in the chloroplasts.

Nitrogen, when applied in low concentrations, N_{400}, causes no marked ultrastructural changes (Plate I, Figs. 5, 6). The chloroplast presents its highest values for length, followed by a slight increase in width. The number of grana per chloroplast section also attains its maximum value (Tables 1 and 2).

For higher concentration (N_{600}), some of the chloroplasts have a somehow rounded shape and dimensions close to control (Table 1). The number of grana per chloroplast section and thylakoids per granum does not differ much from the previous concentration. The other chloroplasts are more rounded, almost spherical, with marked vesicles of the granal and intergranal thylakoids. These pathological alterations are probably due to the toxic effect of the superoptimal N level (Plate II, Figs. 7, 8).

The variant treated with N_{800} (Plate II, Figs. 9, 10) has slightly prolongued chloroplasts, with a much decreased length and an almost unchanged width as compared to control (Table 1). The number of grana per chloroplast section is highest (Table 2) and slight vesicles, more marked than in control, can be noticed in thylakoids, mostly in the distal parts (Plate II, Figs. 9,10). The mitochondria (Plate II, Fig. 10) and the nucleus (Plate II, Fig. 9) are unchanged. No starch drops can be noticed.

The concentration of N_{1000} causes severe changes in the shape of chloroplasts, which become prolonged and deformed, probably also due to dehydration (Plate II, Figs. 11, 12). The nuclear membrane is extremely corrugated, probably due to similar causes. The number of grana and of thylakoids per granum increases in response to the high toxicity of the ammonium nitrate. No changes occur in the structure of mitochondria.

It seems, from the data presented in Table 1, that N_{200} brings about a lengthening and narrowing of the chloroplast, so that it comes closer to the lenticular shape. Length and width are most enhanced by N_{400}. It is interesting to note that the variability of organelle dimensions is lowest in this concentration. This treatment might be a way of levelling chloroplast dimensions. Nitrogen amounts larger than 600 kg·ha^{-1} lead to an increase in the variability of chloroplast dimensions, which is best reflected in the extreme enlargement of the photosynthesizing apparatus.

The data comprised in Table 1 show that small amounts of ammonium nitrate (the variant N_{200}) cause a shrinking of the chloroplasts to about 20% of their volume, as compared to control. The amounts larger than N_{400}, mostly N_{600} lead to an enlargement of chloroplast volume. Even larger amounts (N_{800}) cause a new, sometimes severe, shrinking of the chloroplasts to almost 30% of the control volume. These

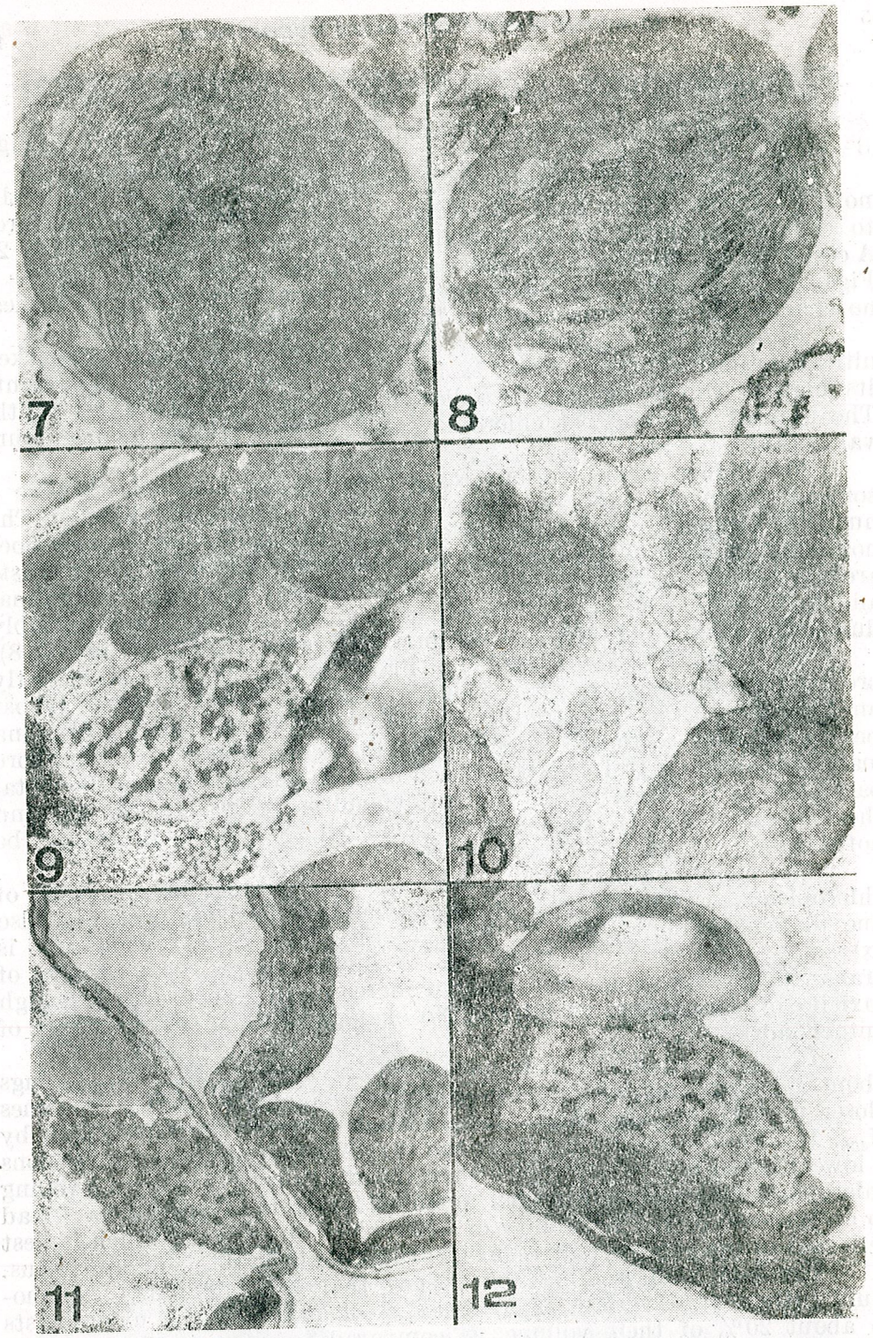

Plate II

Fig. 7, 8 — Effect of N_{600} kg.ha^{-1} (Fig. 7 × 12.000; Fig. 8 × 12.000); Fig. 9, 10 — Effect of N_{800} kg.ha^{-1} (Fig. 9 × 6000; Fig. 10 × 12.000); Figure 9 reveals a chloroplast completely turned into an amyloplast; Fig. 11, 12 — Effect of N_{1000} kg.ha^{-1} (Fig. 11 × × 6000; Fig. 12 × 6000). Figure 12 also reveals an amyloplast.

variations of the chloroplast volume may be due to several physiological processes : (a) the influence of ammonium nitrate upon protein synthesis (probably in variant N_{600}); (b) the effect upon dehydration through perspiration in chloroplasts; (c) the effect upon perspiration itself, which can be very high for N_{200}; (2) the toxicity of ammonium nitrate for N_{800} and N_{1000}.

The data in Table 2 reveal: an increase in the number of grana per chloroplast section, with maximum value for N_{400} (33 versus 26 in control); the lowest number of grana per chloroplast section for N_{800}; a new increase in the number of grana per chloroplast section for N_{1000}, a level considered toxic.

The average number of thylakoids per granum (10) did not vary much, except for N_{1000} where it decreased to 8 thylakoids per granum. Since the average number of grana was not conclusive, a percentage distribution of grana was set up according to the number of thylakoids contained. The following classification of chloroplasts was, thus, achieved : (1) chloroplasts with most of their grana consisting of about 8 thylakoids, i.e. the control and the variants treated with small amounts of nitrogen (N_{200} and N_{400}); (2) chloroplasts with grana consisting of 4 thylakoids (N_{600}) and (3) chloroplasts with nearly all their grana made up of about 10 thylakoids (grana with more than 15 thylakoids were not recorded), as a toxic effect of high levels of ammonium nitrogen (N_{1000}).

The data in Table 3 present an increase in the number of grana per chloroplast, with a maximum value of their total volume for N_{400}. Nevertheless, as the volume of chloroplasts decreases for N_{200}, the highest percentage of grana, thylakoids and membranes of the stroma volume can be recorded at this nitrogen level. It is, therefore, obvious that the most efficient use of solar light per chloroplast volume takes place here. Higher nitrogen levels cause a decrease in the total volume of grana within a chloroplast, except for N_{1000}. Here the relative increase in number of grana and thylakoids per granum might be an adapting effort of certain isolated specimens to the high toxicity of ammonium nitrogen.

FINAL REMARKS

(1) The investigations performed on the ultrastructure of the species *Lolium multiflorum* Lam. reveal several (anatomo-structural) aspects regarding the optimal use of nitrogen fertilizers.

(2) The general ultrastructure of the chloroplast and other cellular organelles underwent severe changes only at levels higher than N_{600}. Large nitrogen amounts also caused a high variability of the parameters studied, which was most obvious in the extreme increase in volume of the photosynthesizing apparatus.

(3) The largest number of grana per chloroplast occurred for N_{400}. Nevertheless, since the volume of chloroplasts decreased for N_{200}, and thus, the highest percentage of grana and thylakoids of the stroma volume was obtained, this nitrogen level seems most fit for an efficient use of light per chloroplast volume.

(4) The highest nitrogen level used (N_{1000}) caused severe ultrastructural changes of the chloroplast and the other cellular organelles. The relative increase in number of grana and thylakoids per granum seems to be an adapting response of certain isolated individuals to the high toxicity of ammonium nitrate.

(5) The most adequate nitrogen levels applied to *Lolium multiflorum* Lam., so that the photosynthesizing apparatus might function to the best, may vary between 200—400 kg·ha^{-1}, depending on ecological conditions.

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PRELIMINARY CHARACTERIZATION OF SOME YEASTS ISOLATED FROM NATURAL PRODUCTS

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In order to select and improve some yeast strains with high capacity of bioconversion of some vegetal sources into ethylic alcohol the biological properties of two strains isolated from natural products in comparison with the properties of 13 reference strains was assessed.
The results of semi-anaerobically and oxidative carbon sources utilizing tests, the yield of bioconversion to ethylic alcohol as well as the vitamin requirements represent a preliminary stage in the selection and improvement of industrial strains.

One of the main research directions is the obtaining of new sources of energy including those of vegetal origin. There are some efficient categories of yeasts, like microorganisms, capable to use certain substrates with production of ethanol.

The identification of some yeast strains isolated from natural sources permits the characterization of their physiological properties in comparison with the properties of some reference strains; it can be a preliminary stage in the selection and improvement of strains with high capacity of bioconversion of substrates from some vegetal sources (e.g. sugar sorg) into ethylic alcohol. Therefore, the present study considers comparative tests of the capacity to utilize semi-anaerobically and oxidatively certain carbon source as well as the vitamin requirements for 15 yeast strains.

MATERIALS AND METHODS

Yeast strains. The 15 years strains investigated, are represented by two strains isolated in IECB from grape juice (P 26) and cherries syrup (SV 3) and 13 strains from the collection of the Laboratory of Microbiology, University of Bucharest, selected according to the principles of identifying isolated yeast strains (Barnett et al., 1983). Several genera and species of the same genus accepted at present as belonging to only one species (Table 1) are included in the research. After purity checking (Rose, 1975) the cultures were kept on Y.P.G. medium (Van der Walt, 1970), under a paraffin layer, at 4°C.

Assay of the biological properties. Considering the identification of strains isolated from the given samples and also the purpose of our investigations some physiological criteria were chosen, keys No. 4 recommended by Barnett et al. (1983) being the common one for all yeasts fermenting D-glucose.

The assay of the ability to utilize fermentatively some sugars (indicated as significant in the above mentioned keys) was based on the method of gas collection (CO_2). There were used large Durham tubes containing

Table 1

List of investigated yeast strains

No.	Genus and species * (Source ***)	Present name **
1.	Saccharomyces cerevisiae I.F.O.0044	
2.	Saccharomyces ellipsoideus I.P.	Saccharomyces cerevisiae ; Kluyveromyces maxianus ; Saccharomyces rosei
4.	,, diastaticus	S. cerevisiae
5.	,, cartilaginosus B.K.M.119	S. cerevisiae
6.	,, carlsbergensis I.F.O.0565	S. cerevisiae on S. cerevisiae uvarum
7.	,, oviformis I.P.	Saccharomyces cerevisiae (bayanus)
8.	Schizosaccharomyces pombe CERIA 109	
9.	Candida tropicalis I.P.150	
10.	Candida 1 polytica N.Y.C.153	Yarrowia lipolytica
11.	Candida guilliermondii I.P.132	Pichia guilliermondii ; Pichia ohmeri ; Hansenula jadinii (C. utilis)
12.	Hansenula subpelliculosa I.P.A.77	
13.	Debaryomyces hansenii I.P.111	
14.	P_{26} — Isolate	
15.	SV_3 — Isolate	

* The nomenclature complies with the original one on the collection tube
** Nomenclature accepted at present (Barnett et al., 1983).
*** I.F.O. = Institute for Fermentation, Osaka, Japan
B.K.M.= Institute of Microbiology, Moscow, U.S.S.R.
I.P. = Institute Pasteur, Paris, France.
N.Y.C.= National Collection of Yeast Culture, Great Britain

insert smaller tubes (Barnett at al., 1983) and a volume of 10 ml yeast extract medium (0,5% w/v) including 50 mM test source.

The assay of the ability to use organic compounds as sole carbon source for aerobic growth was performed with tubes containing liquid media in standard volumes of 5 ml medium YNB (Herlea, 1985), with the carbon source in standard concentration (50 mM). The methods employed the in measurement of the aerobic growth by using certain nitrogen compounds (nitrate and L-lysine) are similar to those described for carbon sources, using liquid media with a carbon base (YCB) instead of nitrogen base (YNB) and also nitrogenous test substrates in concentrations ranging from 2 mM to 5 mM. The yeast requirements for an exogenous supply of certain vitamins (Table 2) representing a specific criterion were analysed by testing the ability to grow in the absence of each single vitamin and also without any of them. The tests were made in liquid medium like for organic compounds, with chemically defined Wickerham growth medium (Lodder, 1970) complete for the vitamins and a variant of complete medium lacking each of the vitamins interesting for the analysed strains. According to some previous results (Herlea, 1971, 1978), which are also in agreement with those of Abadie (1967), hystidine, methyonine and tryptophan were removed from the complete Wikerham medium, using also 2g ammonium sulphate and 5 g D-glucose/l.

The media used for all tests of physiological properties were sterilized by filtering (LABOR-therm 17-G5 glass bacteriological filters) to avoid high temperature action on the tested sources. The inoculation in all

Table 2

The vitamin requirement for chemically defined growth medium (Barnett et al., 1983)

Vitamins	Amount/1
p-aminobenzoic acid	200 µg
Biotin *	20 µg
Folic acid	2 µg
myo-inositol *	10 µg
Nicotinic acid *	400 µg
Pantothenate (ca)	2 mg
Piridoxine HCl *	400 µg
Riboflavin	200 µg
Thiamin HCl	400 µg

* Vitamins of interests to identify investigated yeasts

the tests is made with 100 µl standardised yeast suspension (O.D.=0.2 at 640 nm, estimated with a photoelectric FEK-56 M nephelometer) after bringing the yeast cultures into a state of active development.

The test tubes are incubated at 25°C and shaken daily for 3, 7 and 21 days. The fermentation results are appreciated in comparison with the controls (positive and negative for each strain separately) according to the gas volume (CO_2) collected in the Durham system and also nephelometrically from other tests.

The determination of ethylic alcohol formed after fermentation was performed for strain P 26 (used at present in the pilot stations of IECB in producing ethanol) in comparison with test strains *Saccharomyces cerevisiae* (I.F.O. 0044) and *S. diastaticus* applying the relative method based on monitoring the weight loss.

RESULTS AND DISCUSSIONS

The fermentation results obtained on selected sources were evaluated after a period of incubation of 21 days due to the possibility of forming some adaptative enzymatic systems (Montweher, 1967 ; Van der Walt, 1970 ; Kreger van Rij, 1962 ; Boidin et al., 1964 ; Barnett at al., 1983). These results are represented in Table 3, on a scale of values from — to 4^+ according to the quantity of the gas released. The fermentation of D-glucose can be a positive control, the analysed strain (except on *Candida lipolytica*) being known to ferment this source. During these tests, under normal experimental conditions, *C. guilliermondii* behaves similarly to *C. lipolytica* according to Lodder determination (1970), but as for D-glucose, it differs from those of Barnett a.s.o. (1983). In the case of the negative control (the blank medium, without a fermentative source required for estimation of intensity of fermentation) a certain development of gas is noticed perhaps due to the own intracellular reserves of innoculum and possibly from the composition of the basal medium. As regards the behaviour of the strain isolated, fermenting the three tested sources, SV 3 proved the highest intensity of fermentation on D-glucose and cellobiose.

Regarding the various strains of *Saccharomyces* (number 1—7 in Table 3) belonging to various species according to the original nomenclature, but introduced in a single species — *cerevisiae*, by Barnett et al. (1983), these showed some small differences. Comparing the behaviour of isolated strains with the collection strains — according to fermentation

Table 3

Results of fermentation and oxidative assimilation tests of some sources of certain organic compounds, as the sole major source of carbon

Yeast strain	Fermentation source			Source of C oxidatively utilized									
	D-Glucose	Cellobiose	Inulin	D-Glucose	Maltose	D-Galatose	L-Sorbose	Raffinose	Lactose	Cellobiose	L-Rhamnose	Sucrose	Starch	Glycerol
1. Saccharomyces cerevisiae	+	−	−			+	− +		− −	−	+	−		±
2. S. ellipsoideus	++	−	−	+	+	++	− +		− −	−	+	−		±
3. S. chevallieri	+++	+	+	+	+	+	− +		− −	−	+	−		±
4. S. diastaticus	+++	+	+	+	++	+	− ++		− −	−	+	+	+	−
5. S. cartilaginosus	+	−	−	+	+	+	− +		− −	−	−	+	−	±
6. S. oviformis	+++	+	−	+	++	−	− +		− −	−	+	−		±
7. S. carlsbergensis	++	−	−	+	+	+	− ++		− −	−	+	+	−	+
8. Schizosaccharomyces pombe	+++	−	−	+	+	−	− +		− −	−	+	−		−
9. Candida tropicalis	++	−	−	+	++	+	± −		− +	+		−	+	++
10. C. lipolitica	−	−	−	+	−	−	± −		− −	−	−	−		++
11. C. guilliermondii	−	−	−	+	+	++	+ +			−	+	+	+	++
12. Hansenula subpelliculosa	+++	+	+	+	+	+	− ++		− +	−	+	+	+	++
13. Debaryomyces hansenii	+	−	−	+	++	+	− +		− −	−	+	+	+	+
14. P26	+++	+	+	+	+	+	− +		− −	−	+	−		±
15. SV3	++++	++	+	+	++	++	− ++		− −	−	+	−		±

* Scale of values to appreciats the results is explained in the text

test — their similarities with *S. chevallieri*, *S. diastaticus* and *Hansenula subpelliculosa* are appreciable.

The results of oxidative uptake of C sources are quantitatively appreciated by the degree of growth ; this is determined by the optical density against a negative control (YNB without C source). Its value differs from one strain to another. The assimilation of D-glucose—the best C sources for most yeasts — may be considered a positive control. The more simple notation of results listed in Table 3 implies the transformation of values of optic density (OD) in "+" and "−" in comparison with values obtained for positive control, as follows : control, OD ~1/2 OD positive control = + ; OD > OD positive control = ++ ; OD < 1/2 OD positive control but appreciable = ± and OD ~0.1 = −. According to this scale of values, the analysis of the results on the oxidative utilization of the various 11 sources of C by the 15 strains of yeasts reveals a variability of response among the strains belonging at present to *S. cerevisiae* within limits admitted in taxonomical studies for some sources (eg. D-glucose, starch, glycerol) (Lodder, 1970 ; Barnett et al., 1983) and confirmed for some strains previously studied (Herlea, 1978).

In the experiments mentioned, the isolated strains behave as follows : P26 identical to *S. cerevisiae* and SV3 similar to *S. diastaticus*, according to the assimilation intensity of maltose, D-galactose, raffinose and sucrose.

As regards the oxidative assimilation of nitrate and lysine as significant nitrogen sources, the two yeast isolated behave differently ; P26 similar to strains that can be ascribed as *S. cerevisiae*, while SV 3 assimilates lysine.

The test of vitamin requirements, appreciated by the culture development (determined by OD) in the medium variants, in comparison with the positive control (the same utilized for the assimilation of C sources) leads to the results represented in Table 4. The scale of values from − to ++ implies the transformation of values of optical density as follows : OD − M − (negative control) = − ; OD − M + (positive control) = + ; OD > M + = ++.

Table 4

Results of assessing vitamin requirements *

Yeast strain	Medium without nicotinic acid	Medium without pyridoxin	Medium without biotin	Medium without thiamine	Medium without myo-inositol
Saccharomyces cerevisiae	+	+	−	+	+
S. ellipsoideus	+	+	−	+	+
S. chevallieri	+	+	−	++	−
S. diastaticus	++	−	−	+	+
S. carlsbergensis	−	−	−	+	++
S. oviformis	−	+	−	−	−
S. cartilaginosus	−	−	−	−	++
Schizosaccharomyces pombe	−	+	−	−	−
Candida tropicalis	+	+	−	+	−
C. lipolytica	+	++	−	−	−
C. quilliermondii	−	−	−	+	−
Hansenula subpelliculosa	+	++	−	+	−
Debaryomyces hansenii	++	+	−	−	−
P26	++	−	−	−	+
S.V. 3	++	+	−	−	−

* Notation of the results are explained in the text

Therefore, under the conditions of the experiment, the requirements in vitamins for all tested strains imply the following decreasing order of vitamin requirements : biotin (all strains), inositol (9 strains), thiamine (7 strains), nyacin (5 strains) and pyridoxine (5 strains). In general, for all the strains tested by us the character seems variable in Barnett a.s.o. (1983). The two isolated strains behave differently ; P26 requires for growth pyridoxine, biotin and thiamine, while SV3, biotin, thiamine and inositol. These results are of interest when using yeasts in industry, for their adaptation on economic growth media.

The investigation carried out by us pointed to the ability of the two isolated strains in fermenting D-glucose, cellobiose and inulin, SV3 presenting a higher intensity for the first two sources, and also in using for aerobic growth D-glucose, maltose, D-galactose, raffinose, sucrose

and to a lesser extent, glycerol as well as the requirements for three vitamins. As regards their identification, since we do not have for objective reasons all physiological properties included in key 4 of Barnett et al. (1983), for a correct taxonomical position more morphological, microscopical and sexuate reproduction characteristics are required. The data obtained as such show that the two strains are close to *S. cerevisiae*, revised as including a large number of synonymous species according to the present nomenclature and characterization.

Strain P26 (IECB) identified as *S. cerevisiae* behaved in the bioconversion tests to ethylic alcohol identical with *S. cerevisiae* (IFC 0044). Therefore, after wort fermentation (maltose 10%), strain P26 (IECB) produced 5.95 g ethylic alcohol/150 ml wort alike to strain *S. cerevisiae*, while the test strain *S. diastaticus* yields 6.688 g ethylic alcohol/150 ml wort. Knowing the biological properties (ability of fermentation and assimilation, the yield of bioconversion to ethylic alcohol) one may select potential yeast strains used as such in industrial production or improved by certain techniques as mutagenesis and cellular genetic engineering in order to obtain some superior parameters of economic significance: a better utilization of the culture media used in production, increased ability of bioconversion to ethanol, increased resistance to high concentration of ethylic alcohol.

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THE EFFECT OF LOW TEMPERATURES (– 196°C) ON CELLULAR DIVISIONS AND ON THE SPECTRUM OF RADIOINDUCED CHROMOSOMIAL ABERRATIONS IN THE RADICULAR MERISTEMATIC TISSUE OF *ALLIUM CEPA* L. HV "Stuttgart"

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The effect of low temperatures (– 196°C, liquid nitrogen) was studied on the cellular divisions in the radicular meristem of the *Allium cepa* L. HV "Stuttgart", both in the case of preserving the seeds in liquid nitrogen (3 min – 62 h) and in the case of their irradiation in liquid nitrogen (– 196°C) or at room temperature (+ 20°C). It has been noticed that seed preservation in liquid nitrogen induces the presence of chromosomial aberrations both in the main meristem, and in the secondary meristem of the radicels. Seed irradiation in liquid nitrogen leads to the reduction of the total percentage of aberrant cells and of the number of acentric fragments, caused by the rejoining of the broken ends of the chromosomes, practically in the absence of the cytoplasmic movements. This is an argument in favour of the "rupture-reunion" hypothesis of chromosomal aberrations production.

Studies regarding the effect of very low temperatures on living organisms have been made decades ago. P. Bequerel et al. (1950, 1951) by using the method of "adiabatic demagnetization with iron alaun", have succeeded in preserving seeds of lucerne, clover, tabacco and petunia as well as different other organisms (spores of mushrooms, sporulated bacteria such as *Bacillus subtilis* and *B. mesentericus*, rotifers and tardigrades) at a temperature of – 273°C, for 2 hours. After re-heating and hydration the seeds come back of life, germinating as well as the control (5).

With the present experiment we observed the effect of the temperature produced by the liquid nitrogen (– 196°C) on (a) the mitotic divisions in the radicular meristematic tissue of onion seeds, and (b) on the chromosomial aberrations produced by irradiating the dry seeds with beta or gamma radiations.

MATERIAL AND METHODS

The dry seeds (7.1% water) of *Allium cepa* L. HV "Stuttgart", at about 6 months after harvesting, were used.

In order to establish the effect of low temperatures (– 196°C), dry onion seeds were (a) introduced directly into liquid nitrogen at a temperature gradient of 90°C/min and kept for 3 min, 6, 24, 30 and 62 hours, or (b) frozen in water, in aluminium cups and kept in liquid nitrogen for 3 min or 24 hours (2).

The effect of extreme temperatures on radioinduced chromosomal aberrations was observed by (a) irradiating the onion dry seeds with beta radiations emitted by $^{90}Sr(+ ^{90}Y)$ radionuclid with a 2 mCi activity,

for 70 hours at + 20°C and in liquid nitrogen (— 196°C), the dose received, being of 400 Gy, and (b) by irradiating the dry onion seeds at cobaltron, with gamma radiations emitted by a source of ^{60}Co (1.71—1.33 MeV energy; 20,000 Ci activity, the dose rate being of 89 Gy/h), at a temperature of + 20°C or in liquid nitrogen for 3 min (the received dose being of 44.50 Gy) and 9 min (133.50 Gy) (3).

The effect of the temperatures produced by liquid nitrogen, and that of irradiation at extreme temperatures (+ 20°C and — 196°C) was established by analysing the mitotic divisions in the meristematic radicular tissue (Feulgen stain, squash type preparation).

RESULTS AND DISCUSSIONS

LOW TEMPERATURES EFFECT (—196° C) ON THE CELLULAR DIVISIONS

The analysis of the chromosomial aberrations in the primary radicular meristem revealed the percentage increase of the aberrations with the increase of the thermic stress time, from 1.39% (3 min) to 5.89% (62 h, Table 1).

Table 1

The percentage of chromosomal abberrations induced by the preservation of *Allium cepa* HV "Stuttgart" seeds in liquid nitrogen (— 196°C)

Variant	Total of aberrant cells %	Aberrant cells %		
		metaphase	anaphase	telophase
Primary meristem				
3 min — 196°C	1.39	1.96	0.00	1.71
6 h — 196°C	2.69	3.48	2.82	2.67
24 h — 196°C	3.81	4.35	3.33	1.18
30 h — 196°C	3.85	6.51	5.94	2.65
62 h — 196°C	5.89	6.90	10.71	3.76
3 min H_2O — 196°C	1.83	2.07	1.40	1.89
24 h H_2O — 196°C	4.08	7.49	4.00	2.48
Secondary meristem				
62 h — 196°C	1.65	1.85	7.41	1.12
24 h H_2O — 196°C	2.34	2.82	2.11	1.35

In the water frozen variants the percentage of chromosomial aberrations increases twice because of (a) the ice pressure, (b) and thermic stress. Thermic shocks produced "one-hit" and "two-hit" type ruptures in chromosomes, ruptures that are represented in ana-telophase by acentric fragments, bridges and rings, because of the presence of intra-arm, interarm or interchanges among chromosome fragments (1). The presence of simple bridges points to the fact that some ruptures are produced in G_2 stage of the cellular cycle. In was also observed chromosome depolymerisation, and also a strong heterochromatinization of some regions of the

chromosomes, a phenomenon that can be seen both in metaphases and in prophases. Heterochromatinization is an effect of the asynchronous replication of the DNA in the heterochromatic regions of the chromosomes (4).

The study of the cellular divisions in the secondary radicular meristem reveals certain aspects of the thermic stress that are less unexpected, such as: the percentage of chromosomial aberrations, reduced depolymerization and/or heterochromatinization, the development of cellular divisions which is very close to normal.

LOW TEMPERATURES EFFECT (—196° C) ON RADIOINDUCED CHROMOSOMIAL ABERRATIONS

Onion seeds irradiation in liquid nitrogen (— 196°C) has as a result the decrease of the percentage of chromosomial aberrations, both in the case of radiations with low LET (gamma radiations), and in the case of radiations with high LET (beta radiations, Table 2).

Table 2

The percentage of chromosomial aberrations in the radicular meristematic tissue of *Allium cepa* HV "Stuttgart" induced by a low temperature (liquid nitrogen — 196°C) and by irradiation at extreme temperatures

Variant	Aberrant cells %				Number aberrations at 100 cells			% anaphasic aberrations	
	Total	Meta-phase	Ana-phase	Telo-phase	Meta-phase	Ana-phase	Telo-phase	Frag-ments	Brid-ges
— 196°C, 3 min	1.4	2.0	0.0	1.7	2.0	0.0	1.7	0.0	0.0
— 196 C, 62 h	5.9	6.9	10.7	3.8	4.1	15.0	3.8	5.6	10.0
β^+, + 20°C, 70 h	22.8	29.5	37.8	46.7	43.8	157.8	176.1	35.2	60.6
β^+, — 196 C, 70 h	13.7	12.9	20.0	13.2	32.3	35.0	26.3	28.6	71.4
$\gamma^\times$, + 20°C, 3 min	11.1	14.3	27.3	12.1	25.7	81.8	32.5	44.4	55.6
$\gamma^\S$, + 20 C, 9 min	30.2	31.3	61.5	38.9	56.3	384.6	120.4	56.0	44.0
$\gamma^\times$, — 196 C, 3 min	9.0	3.5	18.6	8.9	6.9	44.2	21.4	26.3	73.7
$\gamma^\S$, — 196°C, 9 min	28.0	10.0	25.0	45.5	40.0	108.3	154.6	38.5	61.5

$^+$ 400 Gy; $^\times$ 44.50 Gy; § 133.50 Gy (1 Gy = 100 rad)

It has also been noticed the decrease of the fragment percentage and the increase of the bridges percentage present at 100 anaphases, when the seeds are irradiated in liquid nitrogen. These effects are caused by the lack of cytoplasmatic movements (5), part of the ruptures produced by the radiations being able to recover. This is an experimental argument that supports "the rupture-reunion" hypothesis regarding the production of chromosomial aberrations.

CONCLUSIONS

1. The preservation of dry onion seeds in liquid nitrogen (— 196°C) induces chromosomial aberrations of the "one-hit" and "two-hit" type (acentric fragments, bridges, rings), and also the depolymerization of the

chromosomes and a strong heterochromatinization, as an effect of the asynchronous replication of DNA in the heterochromatic regions of the chromosomes.

2. The irradiation of the dry seeds at — 196°C with gamma or beta radiations determines a decrease of the percentage of chromosomial aberrations in comparison with their irradiation, with a similar dose, at a temperature of + 20°C. At the same time, there takes place a decrease of the percentage of acentric fragments and a small increase of the precentage of ana-telophase bridges. These experimental observations represent arguments in favour of the "rupture-reunion" hypothesis of producing chromosomial aberrations.

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