ACADÉMIE ROUMAINE

REVUE ROUMAINE DE BIOLOGIE

TOME 41

2 1996

juilliet – décembre

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REVUE
ROUMAINE
DE BIOLOGIE

SÉRIE DE BIOLOGIE VÉGÉTALE

TOME 41 N° 2 juillet – décembre 1996

SOMMAIRE

THE RECORDING OF SOME NEW ADVENTIVE TAXA FOR ROMANIA IN THE HARBOR OF CONSTANȚA

M. COSTEA

The author presents some new adventive taxa for the Romanian flora, observed during 1993-1995 in the harbour of Constanța. These are: *Amaranthus palmeri* S. Watson; *A. tamariscinus* Nutt.; *Persicaria pensilvanica* (L.) M. Gomez; *Cardiospermum halicacabum* L.; *Senna obtusifolia* (L.) Irwin & Barneby; *Biscutella auriculata* L.; *Ipomoea lacunosa* L.; *Solanum rostratum* Dunal.; *S. carolinenese* L., *Datura stramonium* L., var. *tatula* (L.) Torrey and *Setaria faberi* Herrm. Their origin, the possible sources of introduction and their stage of evolution are discussed. From all these species, *Setaria faberi*, a noxious weed, is already considered naturalised in the Romanian Flora, as it was also found in other places in the District Constanța.

The adventive flora is one of the most dynamic elements, many species being noted for their mobility. Trade and communications at international level are highly effective factors as far as the expansion of the spatial distribution of such species is concerned. Regarding this point of view, harbors are important primary colonization points. Studies have shown that the most common colonization pattern is generally as follows (1):

A. Initial introduction, often only at one spot.

B. May merely survive for a period in a single population.

C. Disjunct population make their appearance.

D. Spreading quickly in unoccupied niches or competing efficiently the already existing species.

The "minor" weeds are restricted by different conditions (for example climatic or edaphic conditions). The "major" weeds are often becoming more abundant in the colonization area than in the native regions. We present a first number of new adventive taxa observed as ruderals during 1993-1995 in the harbor of Constanta, specifying their origin, the probable source of introduction and the stage of evolution in accordance to the already mentioned pattern. It is possible that these taxa be already more widespread in Romania than we consider at the moment.

Specimens were deposited in the Herbarium of Agronomical Sciences Bucharest and duplicates were delivered to BUCA and BUC herbaria.

1. *Amaranthus palmeri* S. Watson (*Amaranthaceae*) – N. America; introduced with soya-bean waste; stage C. (4)

2. *A. tamariscinus* Nutt. – N America; introduced with soya-bean waste and cereals; stage C. (4)

3. *Persicaria pensylvanica* (L.) M. Gomez (*Polygonaceae*)

Syn: *Polygonum pensylvanicum* L.

Usually erect annual to 1(2) m, glabrous below but glandular pubescent above. Leaves lanceolate; ocreae becoming lacerate, but not fringed ciliate. Inflorescence cylindrical, dense; peduncles with stalked glands; perigon eglandular rose or white, scarcely exceeding the achene; tepals 5 with inconspicuous, irregularly forked nerves. Achene concave on both sides 2.6-3.4 mm, 85-100% as wide; 2n = 44. It is very much like *P. lapathifolia* (L.) Gray but distinguished thus:

a. Glands on peduncles evidently stalked; tepals 5, with irregularly forked nerves. . . . . *P. pensylvanica*

b. Glands on peduncles sesile; tepals 4(5) outer ones strongly 3-nerved in fruit, each nerve ending in an anchor-shaped fork. . . . . .*P. lapathifolia*

E, N America; introd with soya-bean waste; stage C.

4. *Cardiospermum halicacabum* L. (*Sapindaceae*)

Annual somewhat woody at base; sparsely hairy through; stem up to 2.5 m strongly ridged, climbing by means of axillary, branched tendrils; leaves alternate, compound, leaflet deeply ternatisect, with a large irregularly incise-dentate terminal lobe and 2 small lateral lobes; flowers in small, long-pedunculate, axillary cymes, often with tendrils intermixed with flowers. Sepals and petals 4-5; sepals ovate-orbicular, 1.5 mm long; petals white 4 mm long; stamens 8 deflexed; ovary 3-locular; capsule up to 3 × 5 cm, trigonous or subglobose, inflated, papery; seeds 3, about 5 mm in diameter, black with a conspicuous white, heart-shaped hilum.

Cultivated as a curiosity in S. Europe, widespread in warmer regions of both hemispheres; source of introduction unknown; stage B.

5 *Senna obtusifolia* (L.) Irwin & Barneby (*Caesalpiniaceae*)

Syn: *Cassia obtusifolia* L.

Annual to 1 m, malodorous; leaves evenly once-pinnate; leaflets 2-3 pairs, especially the terminal pair larger, 4-7 cm long, obovate and broadly rounded above, the others smaller, elliptic to obovate; one large petiolar gland present between the lowest pair of leaflets; flowers solitary or paired in the upper axils, forming a terminal inflorescence; bracteoles 0; petals 5 unequal, yellowish-orange; stamens 10, unlike, the members tending to reduce in size from the abaxial to the adaxial side of the flower; anthers dehiscing through apical pores; pods dehiscent, divaricate, strongly curved, 10-18 cm × 3-6 mm, not segmented. Seeds thick, shining, obliquely truncate at both ends, marked with a diagonal stripe on each side. 2n = 28.

Pantropical (probably originally American); introduced with soya-bean waste; stage C.

6. *Sesbania exaltata* (Raf.) Cory. (*Leguminosae*)

Glabrous annual to 2(4) m high; leaves even-pinnate, without tendrils, with very numerous leaflets (30-70), narrowly oblong, 1-3 cm long; flowers 2-6 in short racemes, 1.5-2 cm long; corolla yellow; standard rotund to reniform, short-clawed; wings short-clawed, obliquely oblong-obovate; keel oblanceolate, clawed, strongly upcurved; stamens 10, diadelphous; pods compressed, linear 10-20 cm × 3-4 mm, subtended by the persistent calyx; the numerous seeds separated by transverse partitions. 2n = 12.

Tropical America; introduced with soya-bean or sun-flower; stage A.

7. *Biscutella auriculata* L. (*Cruciferae*)

Annual up to 50 cm, hispid below, glabrous or glabrescent above; basal leaves, oblong, sinuate-dentate, long-petiolate; cauline leaves auriculate-amplexicaul, sessile; racemes many flowered, remaining compact in fruit; sepals with a short spur; petals up to 15 mm, long-clawed, patent; didymous silicula 7-10 × 12-18 mm cordate, not emarginate at apex, with a diaphanous margin, the wings excurrent with the style. 2n = 16. It is distinguished from *Biscutella laevigata* L. through its totally different ecology and through its winged silicula {characteristic to section *Iondraba* (Med.)DC.}.

W Mediterranean region; source of introduction unknown; stage A.

8. *Ipomoea lacunosa* L. (*Convolvulaceae*)

Annual; stems 1-3 m, glabrous or sparsely hairy, the hairs patent or forwardly directed; leaves ovate, 3-8 cm, deeply cordate at base, short-acuminate, entire or shallowly 3-lobed; peduncles shorter than the substending petioles with 1-5 flowers; sepals lanceolate or lanceovate, (6) 8-13 mm long, shortly acuminate; corolla 1.5-2(2.5) cm, white, occasionally pink or pale purple; ovary bilocular. 2n = 30.

SE, N America; from soya-bean waste; stage C.

Other species of the genus, already grown as ornamentals in Romania, are also very frequent ruderals in the harbour area, probably from the same sources: *I. hederacea* Jacq., *I. purpurea* (L.) Roth. and *I. quamoclit* L. We present a key for their identification:

1a. Corolla hypocrateriform, stamens and style exerted beyond the throat; leaves regularly pinnately divided into nearly thread-like segments. . .*I. quamoclit*

1b. Corolla infundibuliform, stamens and style included in the throat; leaves entire or lobed. 2

2a. Flower-stalks mostly shorter than petioles, with 0-few patent to forwardly directed hairs; ovary 2-celled; stygma 2-lobed; corolla usually white (rarely purple). . . . . *I. lacunosa*

2b. Flower-stalks longer than petioles, with reflexed hairs; ovary 3-celled; stygma 3-lobed; corolla usually blue or pinkish-purple (rarely white) 3

3a. Corolla < 5 cm; sepals abruptly long-acuminate, recurved; most leaves deeply 3-lobed. . . . . *I. hederacea*

3b. Corolla > 5 cm, sepales acute, erect; most leaves entire . . . . *I. purpurea*

9. *Solanum rostratum* Dunal. (*Solanaceae*)

Coarse branching annual to 1 m, stellate-hairy through the stems, calyx and to a lesser extent the leaves; yellow spines, 3-12 mm long also present on these organs; glandular hairs absent; leaves 2-10 cm × 1-8 cm, ovate, deeply pinnately lobed, in the larger leaves the segments lobed again; racemes short pedunculate; calyx with strong spines; corolla yellow, 2-3 cm in diameter; anthers 5, 4 alike yellow, the fifth much longer, curved, purplish; fruit entirely concealed by the spiny calyx. 2n = 24. (*Fig. 1a*)

N. America; from cereals or soya-bean waste; stage A.

10. *S. carolinense* L.

Erect rhizomatous perennial to 1 m; with stellate hairs and yellow spines 2-5 mm long present on stems and leaves, along the main veins; leaves ovate 7-12 × 2-6 cm,

with 2-6 large teeth or shallowly lobed; flowers 3-8; corolla purple to white, 2-3 cm in diameter; anthers equal; fruit yellow, globose, 10-20 mm in diameter, deadly poisonous, subtended (but not enclosed) by the mostly unarmed calyx. 2n = 24. (*Fig. 1b*)

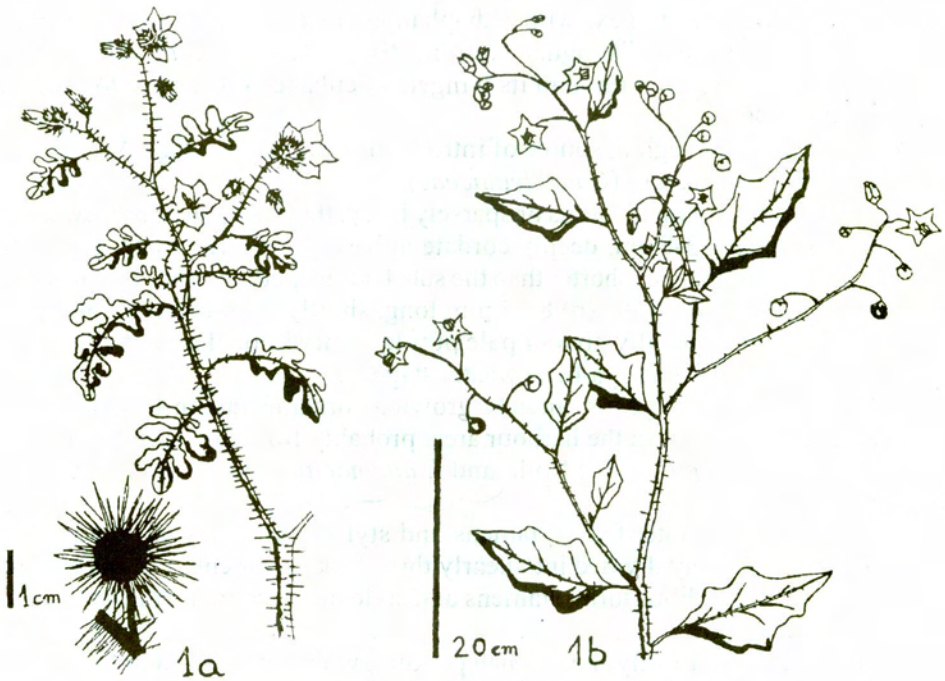

Fig. 1 – 1a *Solanum rostratum* Dunal; 1b *Solanum carolinese* L.

N. America; from soya-bean waste; stage A.

The differences between these 2 species and *Solanum heterodoxum* Dunal. already mentioned in Romania can be synthesized thus:

1a Anthers equal; the calyx mostly stellate-hairy or with 1-2 short spines; perennials. . . . . *S. carolinense*

1b Anthers unequal, 4 alike yellow, the fifth much longer, outcurved, purplish; calyx spiny; annuals. 2

2a Corolla violet or blue; inflorescence glandular pubescent as stellate. . . . . *S. heterodoxum*

2b Corolla yellow, inflorescence stellate hairy but not glandular pubescent . . *S. rostratum*

11. *Datura stramonium L.* var *tatula* (L.) Torrey

Syn: *D. tatula* L.

It is differing *D. stramonium* var *stramonium* through its purple flowers.

America; from soya-bean waste; stage B.

12. *Setaria faberi* Herrm.

Annual; culms 0.5 - 1.5(2) m. Leaves with pubescent sheats on margins and with the lamina about 15-30 × 1-2 cm, at least on the upper side pubescent to sparsely so. Panicle about 6-20 cm long and 1.5-2 cm width, markedly curved to pendent. Spikelets (2.5) 2.7-3 cm long. Below each spikelet 3(4) bristles, up to 10 cm long, with forward-directed barbs. Lower glume about 1 mm long, 5-9 nerved. Upper lemma (lemma of the fertile floret) obtuse, about as long as the spikelet, evidently transversely rugose (Fig. 2) 2n = 36.

E. Asia but most probably arriving from N. America or some European countries; introduced with cereals and soya bean; stage D.

The species had escaped from the harbor area. We could found it in the railway stations Constanta and Medgidia and between these two localities, on the railroad. We consider it is already naturalized in ruderal places, being to become a possible troublesome segetal weed. We emphasize that this species is listed between the quarantine species from Czech Republic and Slovak Republic (9) being also recorded at the moment all over the world as a noxious weed. We present an identification key for the Romanian species of *Setaria*. In the key it was not included *Setaria verticillata* var. *ambigua* (Guss.) Parlatore considered by some authors as a species [*S. ambigua* (Guss.) Guss.; *S. verticilliformis* Dum.] or as a hybrid between *S. verticillata* and *S. viridis*. It differs from *S. verticillata* in having forward-directed barbs.

Fig. 2 – Spiketes and involucral bristles la *Setaria faberi* Herrm: a. spikelet adaxial view, b. bristles, c. spikelet abaxial view.

The number of bristles (1-3 or more) borne on the pedicel is a valuable character but easily to be overestimated where the spikelets have aborted.

IDENTIFICATION KEY

1a Base of lamina with hairs 4-6 mm long. Bristles (4) 6-8(12) below each spikelet; upper glume scarcely longer than lower glume, about 1/2 to 2/3 as long as the spikelet; lower (sterile) floret with palea almost as long as lemma. . . *S. pumila*

1b Base of lamina with hairs ≤ 1 mm long; Bristles 1-3 below each spikelet; upper glume much longer than lower glume, about 2/3 to as long as the spikelet; lower (sterile) floret with palea ≤ 1/2 as long as lemma. 2

2a Bristles with backward - directed barbs (rarely with forward directed barbs); main rhachis hispid with pricklets < 0.2 mm. . . . . *S. verticillata*

2b Bristles with forward - directed barbs; main rhachis densely pubescent with hairs > (0.2) 0.5 mm. 3
3a. Spikelets disculating below the upper lemma which is ± smooth. . . . . *S. italica*
3b Spikelets falling whole; the upper lemma is evidently transversally rugose 4
4a Leaves glabrous; upper glume about as long as the spikelet; spikelets (1.8)2 - 2.5 mm . . . . . *S. viridis*
4b Leaves pubescent to sparsely so; upper glume about 3/4 as long as the spikelet; spikelets (2.5)2.7 - 3 mm long . . . . . *S. faberi*

Besides these unrecorded taxa, we mention the presence in the harbor area of some already cited taxa in Romanian flora: *Sida spinosa* L.; *Salsola collina* Pallas and *Eleusine indica* (L.) Gaertn.

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Received January 20, 1996

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SOME CYTOTAXONOMICAL CONSIDERATIONS ON *PRIMULA AURICULA* SSP. *SERRATIFOLIA*(ROCH.) JÁV

AURICA TĂCINĂ

This paper studies the population of *Primula auricula ssp. serratifolia*(Roch.).Jáv. which lives in Banat, in the Domogled mountains (The White Cross).The karyologic analysis showed the presence of the diploid set of 2n = 22 (x = 11).The karyotype of the species *P. auricula ssp. serratifolia*(Roch.) Jáv. was made and analysed for the first time. The leaf mesophyllum, the limited areal of the taxon studied, are reasons of reflection on its age and taxonomic position.

Primula L. genus includes about 300 species spread in the temperate, subarctic and arctic areas in the whole world and some of them in the mountains in the tropical areas. In the flora of Romania, *Primula* L. is represented by 3 sections : *Vernales* Pax, *Farinosae* Pax and *Auricula* Duby.(5). *Auricula* section includes : *Primula auricula* ssp.*serratifolia* (Roch.) Jáv., *Primula minima* L. and *P. baumgarteniana* Degen et Moesz (4,5).

According to the flora of Romania (4) the species *Primula auricula* (with its leaves having edges intact or sinuously crenate; it has not been signalled yet in our country. This taxon is different from *Primula auricula* L., having its leaves deeply crenated and with a whitish edge in the early stages. This taxon is an endemic or rare species in the flora of Romania.

Hegi (2) mentions it in the Western Carpathians ,completely isolated in the Banat at Baile Herculane and in the South on the opposite side of the Danube, in the North of Serbia.

Phytohistorically, the species of *Auricula* section coming from the populations immigrated from the mountains of Central Asia have their main center of formation in the Eastern Alps and besides are also spread in the Pirinei and the Carpathian mountains. There are tertiary species from which some survived postglacially being diploid species and many others adapted to the new conditions, suffering deep changes of their genetic apparatus. Multiplication of the number of chromosomes twice or thrice as a result of mutagenesis and hybridization processes allowed the development of an active process of speciation of *Primulaceae* (6,7).

According to Scharfetter's opinion (7), the species of *Auricula* section are at a tertiary age with the main development center in the Eastern Alps ,with extension to the Pirinei and the Carpathian mountains. In the flora of Romania this taxon is an endemic or rare subspecies. Most species of *Primulaceae* have been adapted to the extreme postglacial conditions through changes at the genetic level (the increase of the number of chromosomes by polyploidization).

REV. ROUM. BIOL. VÉGÉT., TOME 41, N° 2, P. 97–99, BUCAREST, 1996

MATERIAL AND METHOD

The karyologic analysis was made on material proceeding from the root apexes. Prefixation was made with colchicine 0.2 %, fixation in acetic alcohol 3/l, followed by hydrolysis in the HCl 1N. Coloration was made with the Schiff reactive.

RESULTS

The karyologic analysis of the taxon *Primula auricula* ssp. *serratifolia* (Roch.) Ják. evidenced the fact that it is a diploid species clearly different from *Primula auricula* L., (polyploidic) (3). The chromosomial complement analysed on this occasion is made of 11 pairs of chromosomes (fig.2) among which the pairs 3, 5, 6, 10 are metacentrical,1, 2, 4, 7, 8, 9, 11 submetacentrical.

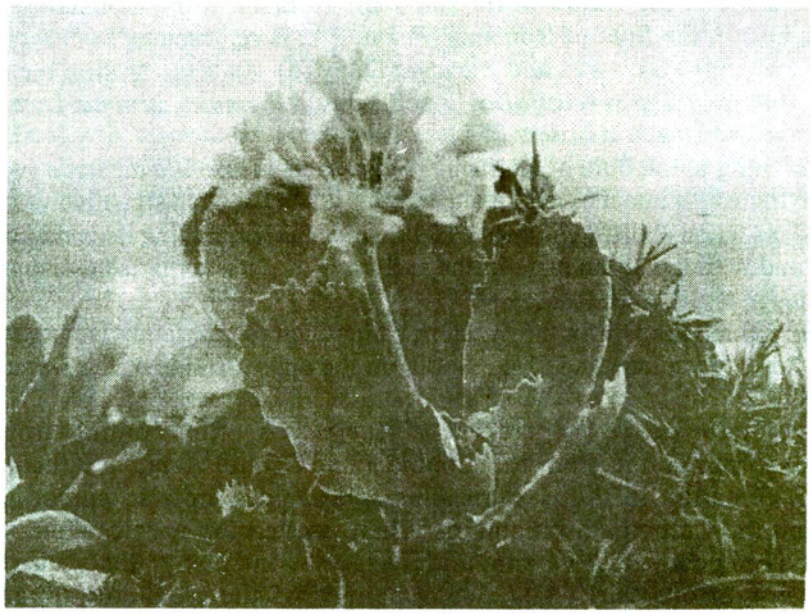

Fig. 1. – *Primula auricula* ssp. *serratifolia* (Roch). Ják. – Crucea Albă (Domogled).

The genetic stability, its rich isolation in Banat, in a geographical region rich in southern species of Balcan-Dacic spreading, moesiac, mediterranean and illiric make us admit there is a tertiary relict form. For the flora of Romania, *Primula auricula* ssp.*serratifolia* (Roch.) Ják. is a rare endemic species.

Ecologically, *P .auricula ssp. serratifolia* (Roch.) Ják. populates the abrupt calcareous rocks, sometimes alpine meadows, rarely in damp places, from the bottom of the mountains up to the alpine area.

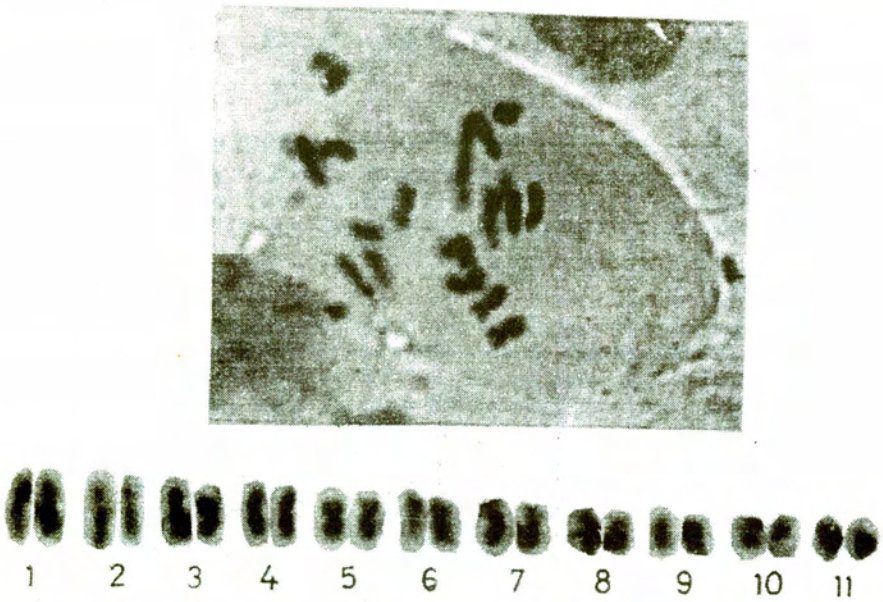

Fig. 2. – Metaphase with diploid chromosomes 2n=22 (x=11).

Phytocenotically, it belongs to *Seslerion rigidae*, *Moehringion muscosae* (l), at *Carici humili - Pinetum pallasinae* (Domin 32) Jáv 55 (8).

In the flora of Romania *P. auricula* ssp.*serratifolia* (Roch.) Jáv. is a rare endemic species under extinction. The old age of the species, proved also by the diploid character, requires a special protection treatment, with the view to its preserving as a valuable component of the Romanian flora genofond.

We thank Dr. Acad. P. Bănărescu for the material and Dr. Acad. N. Boşcaiu for scientific consulting.

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Received August 20, 1995

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ECOSYSTEMIC CHARACTERIZATION OF A *TAMARIX RAMOSISSIMA* SHRUBLAND IN THE DANUBE DELTA (SULINA)

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The article presents the standing structure of a *Tamarix ramosissima* shrubland in an unmanaged field, near the beach Sulina, on the Danube Channel. The influence of soil and macroclimate factors is shown. The maximum height of *Tamarix* is 1.65 m and the density is 62 ind/m^2 the area being intensively grazed and the wood harvested. The herbaceous layer has a large cover (95-100%), 47 species composed especially of annuals, with the most density (1166 shoots/m^2) during the spring period. The canopy fauna but mostly the herbaceous layer fauna has a moderate diversity being dominated by insects. The fauna of invertebrates in the soil which assures the transformation of the organic matter beside the microorganisms is poor enough the enchytreides and lumbricides being nearly absent while an increased part is taken by colemboles, acari and nematodes.

The shrubland of *Tamarix ramosissima* is spread in the Danube Delta along the brooks, channels or less high sand banks area, extends naturally in the Delta and represents the primary stages of wooden vegetation, placed on alluvial deposits or alluvial soil, usually advancing over slightly salty pasture.

From the floristic and phytocenological point of view *Tamarix* shrublands are studied in the Romanian Plain and the Danube Flood Plain by some authors such as: Simon, T. et al. (1962), Şerbănescu, I. (1965), Marin, A. et al. (1967), Sanda, V. et al (1969). From the ecosystemic structure point of view, the investigations are more recent, started by our team (Paucă-Comănescu Mihaela et al., 1995) in Lunca Dunării, Brăila district.

In the present study we intend to perform an ecosystemic analysis of these shrublands in the Danube Delta on the latest collected data.

MATERIAL AND METHODS

The *Tamarix* shrubland studied on the Danube bank, in the Sulina Channel, near the Danube mouth to the sea, is placed at a 200 meters distance by the beach, on the unmanaged land near the free area of the harbour, mostly used as a common pasture field by the inhabitants of the small town and which represents a secondary stage of coenotic evolution.

The surface is plane, with a 0.50 m absolute altitude. The shrubs cover compactly more hectares along a draining channel of the pool flowing into the Danube.

REV. ROUM. BIOL. VÉGÉT., TOME 41, N° 2, P. 101–111, BUCAREST, 1996

The structural determinations of the phytocoenosis were performed by inventories and direct biometrical measurements on areas of 10 m^2 for the shrubs and on areas of 0.25 m^2 for herbs. The measurements were performed in the maximum vernal and estival period of the 1995 year and at the end of winter (after the snow melting but before the vegetation starting).

For the determinations of the canopy fauna of invertebrates, shakings in nets with the diameter of 60 cm were performed, 10 samples each (one sample obtained after 50 shakings) and for the herb layer the net with diameter of 30 cm was used for 10 samples (each sample was obtained after 50 movings) (Vasiliu-Oromulu Liliana et al. 1993).

The fauna of the soil was determined for lumbricides by direct collecting, on 10 cm layers, in blocks 10 × 10 × 40 cm deep and for enchytreides, nematodes, colemboles, oribatid mite and other acari on 3 layers, up to 10 cm deep, with a MacFadyen soil core-borer (Vasiliu-Oromulu Liliana et al. 1993).

The activity of microbiota in soil was determined by enzyme indicators and namely the present and potential dehydrogenase activity, on medium samples collected on the same three layers up to 10 cm depth. The determinations were performed according to the standard technics for the pH of the sample by using running water for T.T.C. and glucose solutions.

The climate analysis was performed on the basis of records from the meteorological station at Sulina.

The physico-chemical determinations on the soil profile and the plant material were performed according to I.C.P.A. proceedings.

RESULTS AND DISCUSSION

Elements of biotope

The substratum consisting of alluvial deposits in a process of soil formation integrates itself with the pedogenetic unit of alluvial salined soil. It is predominantly sandy on the surface (85% middle sand) and under the depth of 30 cm clay and dust are dominant in a proportion exceeding 69%; the soil pH is slightly alkaline (Table 1), moderately carbonated and the salinity (of a chloride type) is high but only below the 30 cm depth, under the level of current life development in soil; the humus content is very low, only on the surface there is active bioaccumulation. The soil oxidant on the surface and moderately reductive is between 10 - 30 cm; it is wholly saturated with Sb and, which varies on the profile; it is high on the surface, decreasing and then increasing again in the depth. The content of mobile nutritive substances (Table 2) is moderate with extremely active Na ionization in soil solution but its effect is partly annihilated by the active ionization of K, P and N while NO_3 assures possibilities of balanced nutrition for plants.

The climate conditions in the year 1995 are characterized by temperatures varying from + 28 to 1.5 °C, and as a monthly average the temperature had positive values all through the year (Fig. 1). The precipitations were low and distributed inconstantly.

Table1
The termodynamic stability characteristics of the soil

Profile	Depth cm	pH	Cl^- %	SO_4^- %	$CaCO_3$ %	Ctx 1.724 %	Sb	Te me/100g	T8.3	Ve %	V8.3 %
$A0_1$	0–3	7.72	–	–	9.9	3.586	51.80	51.80	52.30	100	99
$A0_2$	3–10	8.03	–	–	10.1	0.274	51.80	51.80	55.30	100	99
$A0_3$	10–20	8.38	12	39	9.7	0.137	21.80	21.19	21.69	100	98
ACsc	20–30	8.49	66	60	9.7	0.450	90.50	90.50	90.90	100	100
Csc_1	30–40	8.57	288	56	12.3	1.002	81.15	85.55	81.15	100	100

Table 2
The mobile structure of the soil

Profile	Depth cm	NNO (a)	P*	K	Ca	Mg	Na	Fe ppm	Al	Mn	Zn	Pb	Cd	Cu	H^+ (a)	SiO_2 (a)
$A0_1$	0–3	2.3	5/9	27/40	95/913	21/533	29/107	4/116	0/0	1/19	0.6/2.1	0.6/3.0	0/0.4	0/0	0	20
$A0_2$	3–10	0.9	5/6	27/70	73/986	7/499	25/112	6/118	0/0	0.7/16	0.2/2.0	0.0/3.0	0/0.4	0/0	0	20
$A0_3$	10–20	0.8	5/6	32/100	65/3160	6/499	100/243	16/122	0/0	0.5/8	0.4/0.9	0.0/0.0	0/0.2	0/0	0	40
ACsc	20–30	1.8	6/6	33/100	70/16170	11/546	300/588	23/68	0/0	0.6/114	0.4/4.3	0.0/3.0	0/0.8	0/0	0	50
Csc_1	30–40	0.9	3/4	35/400	95/13860	39/545	605/1015	20/70	0/0	0.6/76	0.3/3.2	0.0/3.0	0/0.7	0/0	0	199

* aquasoluble (a)
mobile

Three periods of drought were recorded, the most important influencing both the growing of vernal species (May) and of the estival ones (July). The snow covering extended up to the end of March and the first snow fell in November. The relative humidity of the air ranged between 70% - 90% on an average, with the lowest level in spring and summer months; that is exactly in the vegetation period. The data of this year are circumscribed to the arid climate of Danubian type where the strong influence of sea diminishes the variation amplitude of temperature and increases the atmospheric humidity.

Elements of biocoenosis

Primary producers

The biocoenosis is edified by the shrubs of *Tamarix ramosissima* together with other 47 species. Among these only one is a shrub too, the others are herbs (Table 3).

The vegetal cover is 90 - 100%. The shrub layer has 84% projective covering of the surface, while the herbaceous layer has a larger extension. In areas with high density of shrub branches herbs are slightly developed, as regards both their covering

(5 - 15%) and their number and biomass and as for the rest the herb covering reaches 100%. It may be noticed here that the vertical stratification is high in the alternating measure and not in the superposed one.

Table 3

The rate of plant species participation to the herbaceous layers of Tamarix shrubland SULINA

Species	Relative abundance (%)			
	Number		Dry biomass	
	May	July	May	July
Cynodon dactylon	24.08	26.06	69.34	60.75
Cerastium glomeratum	31.80	25.97	*8.17	*2.28
Bromus tectorum	12.13	11.70	*3.37	*0.58
Capsella bursa-pastoris	3.70	9.10	–	–
Arenaria serpyllifolia	7.57	5.53	–	–
Agrostis stolonifera	1.78	1.15	5.79	11.19
Medicago lupulina	1.78	1.78	3.16	1.60
Draba muralis	0.96	0.25	*1.64	*0.24
Descurainia (Sisymbrium) sophia	0.27	0.22	*0.09	*0.09
Xanthium strumarium	0.43	0.73	0.13	2.33
Lolium perenne	0.61	0.71	–	–
Senecio vernalis	0.89	3.32	–	–
Hordeum murinum	4.93	2.45	–	–
Plantago coronopus	0.48	0.83	0.52	8.11
Stellaria media	6.79	5.64	–	–
Polygonum aviculare	0.24	0.08	0.04	0.02
Lactuca (Mulgedium) tatarica	0.04	0.22	0.09	0.10
Bromus hordeaceum	0.48	0.77	–	–
Geranium dissectum	0.04	0.01	–	–
Poa annua	0.17	0.02	–	–
Xanthium spinosum	0.08	0.10	1.29	1.66
Anthemis ruthenica	0.08	2.00	–	–
Stenactis annua	0.04	0.72	–	–
Atriplex rosea	0.04	0.01	0.38	1.46
Lappula squarrosa	0.04	0.18	–	–
Cirsium sp.	0.04	0.22	–	–
Chenopodium album	0.04	0.72	0.09	0.07
Trifolium fragiferum	0.04	0.04	0.52	0.29
Centaurium pulchellum	0.04	0.04	0.04	0.09
Lotus tenuis	0.04	0.04	0.09	0.04
Apera spica-venti	0.04	0.04	*7.05	*5.28

* species in the dry period

Existing species out of sample areas: *Eleagnus angustifolia, Althea officinalis, Erodium cicutarium, Geranium pussilum, Gypsophila trichotoma, Juncus acutus, J. gerardi, J. maritimus, Lamium amplexicaule, Medicago minima, Myosotis arvensis, Plango cornuti, Silene conica, Spergularia rubra, Taraxacum officinale, Urtica urens.*

The shrub layer rises up to a maximum of 1.5 m, while its average height is of 0.62 m and the shortest shoots, usually each 0.12 m annually. It should be pointed out that the maximum height of the shrub means the maximum extension of the ecosystem by *Tamarix ramosissima.*

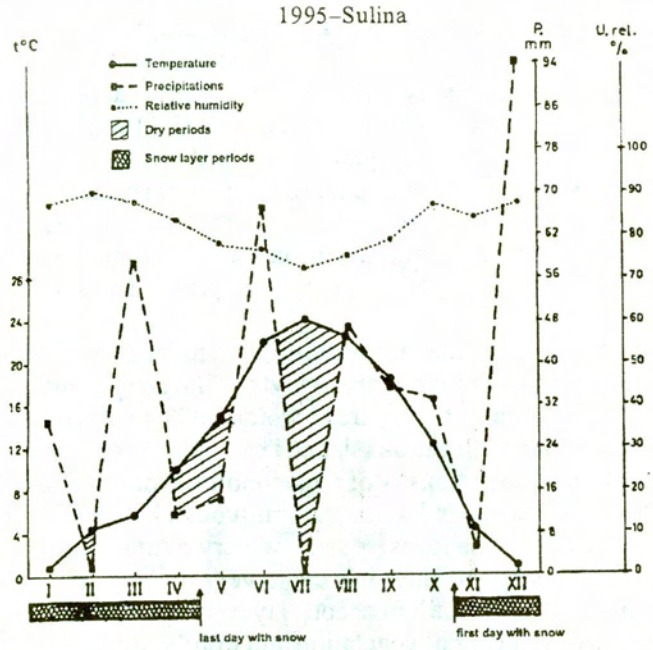


Fig. 1. – Climatical diagram (Gaussen type) for Sulina macroclimate.

The roots of the shrub are developed as a tap type up to 50 cm deep and after that their orientation is only horizontal (they do not penetrate deeply). The density of the shrubs is 9.3 m^2 (930 shrubs/m^2) and the number of branches in each shrub is 6.6 as average. The shrub extension varies between 3 - 15 basal branches. In the case of larger shrubs both a greater number of recently dry branches is always recorded and a greater number of new shoots annually formed from the colet. The density of these basal branches is 62/m^2 (respectively 6200/100 m^2). Among the basal branches only 64.6% are alive as for the rest, though they are dry they still maintain their vertical position at last 1 year. From the total number of alive branches of *Tamarix ramosissima* the annual shoots represent 12.3% and they are in fact the rate of annual rejuvenation of population for the year 1995. The average diameter of all the branches in their basis area (the thickest) is 0.80 cm while the average of the thickest branches in each shrub is 1.16 cm. The maximum diameters of the branches are 3.5 cm while the smallest are 0.30 cm.

Compared to the shrubs of *Tamarix ramosissima* developed in other areas of the Danube flood plain, the number of branches is very large (42 branches/100 m^2 in Insula Mică and 405/100 m^2 at Gura Gârluței) (Paucă-Comănescu, M. et al., 1995); it reflects the intense regeneration in the basal zone after the nearly permanent grazing in spring and summer and after the collection of wooden material in late autumn and winter at Sulina. Also as a result of these causes, the branches reach but very low values of the diameters and heights (compared to 6.5 m height in Insula Mică and respectively 2.4 m at Gura Gârluței) (Paucă-Comănescu, M. et al., 1995).

The annual shoots have an average growing in length of 45.38 cm (varying between 10 - 100 cm) and a weight of 7.76 g of the total weight of the shoot.

The average leaf area is very small, of 0.12 mm^2 (varying between 0.05 - 0.21 mm^2) but the number of leaves is very large, 423,116/m^2. The assimilating surface is much larger because the annual stem is still green.

The herbaceous layer is made of a large number of species, as it is shown in Table 3 but for these only 31, respectively 69% of the total number have a sufficiently large frequency in order to be found again on the permanent sample areas. The Simpson/Pielou diversity index varies for the numerical presence between 0.8035 in spring and 0.5328 in summer.

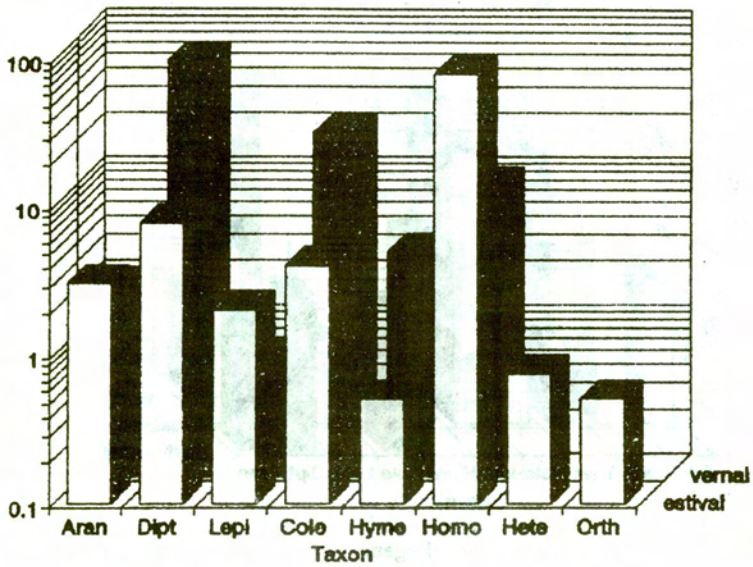
Most of the present species are annual or biennial therophytes (68%) and hemicryptophytes (29%) and microphanerophytes. The geographic area of species is predominantly Euro-Asiatic (56%) from which 13% are Mediterranean-Euro-Asiatic and 12% are continental Euro-Asiatic. The second category of geoelements spread in these vegetal associations is of Cosmopolites species (22%) but Boreal, Circumpolar, Pontic, Atlantic and some Adventivous elements are also present. The annual dynamics of herbaceous species is very active, while some species finish the aboveground stage of their life cycle very quickly. For this reason the inventory of the individuals of the herbaceous layer was performed both in May, in the period of explosive growing of vegetation and in July, the period of maturation of the species existing in the biocoenosis. The density of the individuals is 1166.4/m^2 in spring and 925.2/m^2 in summer. The plants with a short life cycle already have their aboveground part dry 20% of the total number of individuals but remaining in their vertical position during the summer. The species with a reduced life cycle in the vernal period form a special category, consisting both of gramineous species (*Bromus tectorum*, *Apera spica-venti*) and of different families of Dycotyledonous (*Cerastium glomeratum*, *Arenaria serphyllifolia* (*Caryophylaceae*) or *Capsella bursa-pastoris*, *Draba verna*, *Descurainia sophia* (*Cruciferae*)). They take an active part only in the first period of vegetation season, as for the rest of time they represent only the material which enters the geochemical cycle. The other species, of a majority in the total number, have a slower evolution so that they record a gradual increase of their number until July. In the same period a more precise differentiation of fertile and vegetative shoots is observed. They reach their normal dimensions especially with gramineous species.

Consumers and decomposers

The structure of invertebrate fauna, in vertical profile, consists of three main layers: the fauna in the canopy, the fauna of herbaceous layer and the soil fauna.

At the canopy level of *Tamarix ramosissima* shrub the fauna of invertebrates is characterized by individuals belonging to the following superior taxons: Heteroptera, Homoptera, Coleoptera, Lepidoptera, Diptera and Aranea (Fig. 2). In the seasonal dynamics of microarthropodes communities a maximum of the curve is observed in vernal period correlated with a high relative humidity. The differences among taxons, from the point of view of relative values of abundance, are relevant especially in spring, while in summer, the values are more homogeneous. In the

biocoenosis composition of canopy, the primary consumers are preponderant compared to the secondary ones. The coleoptera are characterized by elaterides and curculionides (*Coniatus splendidulus* and *Nanophyes pallidus*) and the Homoptera by cicades (*Opsius stactogalus*), psilides (*Colporcenia osmanica*) and affides (*Brachyunguis tamaricis*).



Legend

Orth = Orthoptera
Hete = Heteroptera
Homo = Homoptera
Hyme = Hymenoptera
Cole = Coleoptera
Lepi = Lepidoptera
Dipt = Diptera
Aran = Aranea

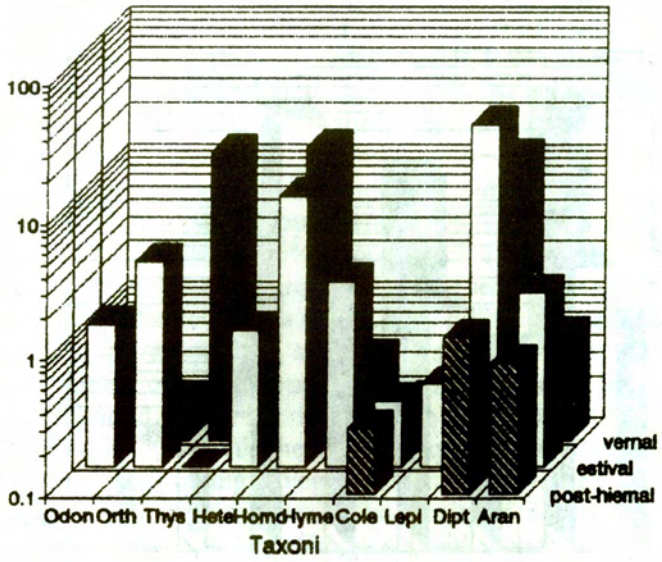
Fig. 2. – Relative abundance of invertebrate fauna in the Tamarix canopy (%).

The zoophagous species belonging to the Heteroptera and the Aranea *Centromerus obscurus*, *Xysticus kochii* complete the community structure of microarthropodes which inhabit the quite reduced canopy of *Tamarix ramosissima* shrubland at Sulina.

The invertebrate fauna in the herbaceous layer forms a coenosis poorly represented qualitatively and quantitatively. The large covering achieved by the shrub and the herbaceous layer constitutes a microclimate favourable to microarthropodes of the hipergaion. (Fig. 3)

The great number of plant species assures a diversified trophic basis for the monophagous microarthropodes and mostly for polyphagous ones which are very numerous. Only a reduced number of phytophagous species: *Anaphothrips obscurus* (Thysanoptera) *Delphacodes venosus*, *Opsius stactogalus* (Homoptera) non-eating adults of *Cryptochironomus defectus*, *Cricotopus sylvestris* (Diptera, Chironomidae), carnivorous ones: *Scatophaga stercoraria*, *S. incola* (Diptera, Scatophagidae) and *Lycosa singoriensis*, *Argiope bruennichi* (Aranea) are quantitatively responsible

for the increased numerical density/m² of microarthropodes in the herbaceous layer. These species represent about 90% of the total number of individuals which inhabit the hipergaion.



Legend

Odon = Odonata
Orth = Orthoptera
Thys = Thysanoptera
Hete = Heteroptera
Homo = Homoptera
Hyme = Hymenoptera
Cole = Coloeptera
Lepi = Lepidoptera
Dipt = Diptera
Aran = Aranea

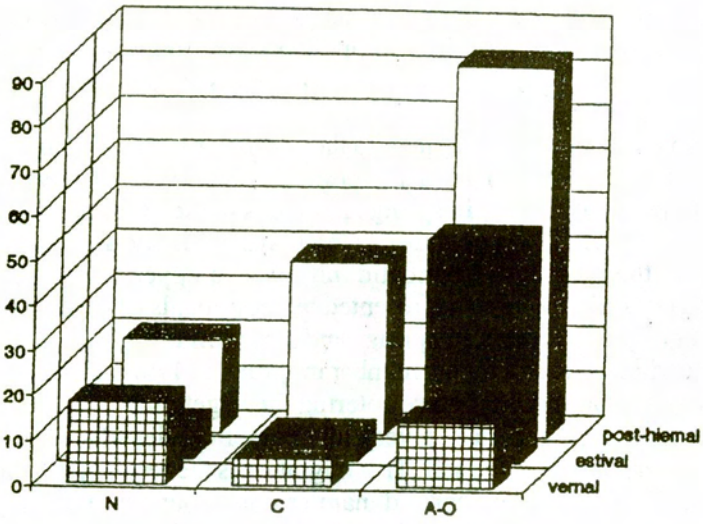
Fig. 3. – Density of invertebrate fauna (individuals nr./ m²).

In the seasonal dynamics in the post-hiemal period, that is before the start of vegetation, the average density/m² of microarthropods reaches the lowest values. The various specializations of the primary consumers (gall, pollinating species) as well as of the secondary ones (carnivorous, hyperparasite species) enables the pronounced integration of the hipergaion in the trophic cycle, thus favouring the efficient use of energy by the upper trophic levels.

The invertebrate fauna in the soil

The fauna of the *nematodes* is characterized by low values of the numerical density/m², comparable to those in sandy littoral areas of the country. The vertical distribution was achieved, in the three studied periods, according to a curve with numerical top in litter and after that it decreased to the deep layers of the soil. In the seasonal dynamics the numerical maximum of the nematodes fauna was recorded before the start of vegetation while the very low values characterized the estival period, with relative humidity of the soil only of 6.19% (Fig. 4). The share of nematodes within the soil fauna is major and it represents 98.6% in the vernal season, various

trophic specializations by detritofagous predacious species as well as their numerical density which is higher compared to the other taxons in edaphic fauna, prove the important function of these worms in the decomposition process in the soil.



Legend

N = Nematoda
C = Collembola
A-O = Acari-Oribatei
Enchytraeidae and Lumbricida see in the text

Fig. 4. – Biomass density of soil fauna (mg dwt / m^2).

Table 4
Numerical density of soil fauna

Group of organisms	Period					
	vernal		estival		post-hiemal	
	nr./m^2	%	nr./m^2	%	nr./m^2	%
Nematoda	360200	98.6	145700	93	409200	96
Enchytraeidae	0	0	0	0	0.1	0
Lumbricida	0	0	0	0	3.2	0
Collembola	2300	0.6	2000	1	1423	0.3
Oribatei	2700	0.8	9300	6	15600	3.7

Enchytreides saprophagous organisms, highly influenced by the scarcity of organic matter and basic pH of the soil, were represented only by a single individual captured in the litter in the post-hiernal period.

Lumbricides. The populations of lumbricids are slightly represented in the sandy soils in Sulina area. Two individuals were captured belonging to *Apporrectodea caliginosa caliginosa* species, a cosmopolitan species which is not able to penetrate deep in the soil and thus it can resist in the upper layers of it, even under dryness conditions. This is the explanation for the identification of this species

in the sandy soil at Sulina, a soil in formation with little organic matter, with humidities varying from prolonged dryness to flood stage. However under the conditions of vernal and estival period no individual was recorded, not even one belonging to this species.

Acari. From the Acari the saprophagous oribatid mite species occupy a preponderant place in comparison with predatorous Gamasida. The oribatid mite species are similar to those recorded in ecosystems which are poor in organic matter and namely in meadows.

The dominant populations belong to the parthenogenetic oribatid mite species *Tectocephaeus velatus* which have a numerical maximum in the estival period. The inhabiting of the oribatid mites in spring is obvious in the litter, reaching a share of 98%, the difference of 10% is be found in the first 3 cm in the soil; the low relative humidity led to the migration of oribatid mites in the upper 3 cm in the soil. The panphytophagous oribatid mites represented by individuals of small size, produces a reduced quantity of biomass in spring, and a maximum in post-hiemal period. The young individuals reach a higher number in spring, of 1700 ind./m^2, 1200 ind./m^2 in spring and only 500 ind./m^2 before entering the vegetation.

Collemboles have generally a very low numerical density, with an obvious presence in the deep layers of the soil. This migration is the result of high temperature on the surface. The curve of seasonal dynamics points out a numerical peak in the vernal period, representing 0.6% of the total number of individuals which belong to edaphic fauna.

Under the soil conditions of *Tamarix* shrublands at Sulina, the process of decomposition and mineralization of organic matter was carried out by the nematodes, acari and partly by colemboles which took the part of the lumbricides and enchytreides practically absent in this shrub ecosystem.

The *community microorganisms* in the soil (microbiota) present a large spectrum of metabolic properties resistant to the fluctuations of environmental factors. The actual and potential dehydrogenase activity (global index of metabolic activity of microbiota in the soil) is reduced in comparison with other shrublands or meadows in the Danube Delta (Vasiliu-Oromulu et al. 1990).

The level of dehydrogenase activity (mg. formazan):

	vernal	period	estival	period	posthiemal	period
layer	Present	Potential	Present	Potential	Present	Potential
Litter	0.120	0.398	0.348	1.660	0.046	0.072
S_1	0.020	0.019	0.135	0.315	0.015	0.026
S_2	0.010	0.009	0.080	0.118	0.006	0.008

The lowest activity is recorded before the start of vegetation season and in the estival period it reaches the highest value for the site, without reaching a usual level for this type of ecosystems.

The dehydrogenase activity has a high diminution in S_1 and S_2 in comparison with the litter layer, during the investigated period. The sandy soil, poor in organic matter, with an increasing salting and an alternative humidity state represent factors which can explain the low level of dehydrogenase activity and its dynamics.

The *macroconsumers* inventoried in this ecosystem, with a very obvious part are represented quantitatively first by the domestic herbivorous species (cow, sheep) highly numerical on these areas, which determines an extremely high pressure upon the primary producers, especially by consuming the edifying species of *Tamarix*. The consuming of annual shoots leads to the above described present structure of the shrub layer, both as dimensions of the old shoots and as the number. This is the form of adaptation for the survival of the primary producer species.

In conclusion, the *Tamarix* shrubland at Sulina with a typical structure for the open phytocenoses, representing successive stages in the evolution of vegetation, is characterized by a high productive capacity but a present reduced development of primary producers due to the intense consuming of domestic macro-fauna and to anthropic directly consuming actions. The canopy fauna but mostly that in the herbaceous layer has a moderate diversity being dominated by insects. The fauna of invertebrates in the soil, which assures the transformation of the organic matter beside the microorganisms, is poor enough the enchytreides and lumbricides being nearly absent while an increased part is taken by colemboles, acari and nematodes. The biotope of this ecosystem presents as limit factors the increased degree of saltness and alkalization the reduced content of organic matter and extreme alternation of soil humidity beside the drought period and strong differences of temperature.

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Received April 15, 1996

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RECHERCHES CONCERNANT LES MODIFICATIONS ULTRASTRUCTURALES DES PLASTIDES ET LA DYNAMIQUE DES PIGMENTES ASSIMILATEURS POUR L'ESPÈCE *ALLIUM SATIVUM* L. SOUS L'ACTION DES RADIATIONS IONISANTES GAMMA DU Co^{60}

RODICA DUMITRESCU

INTRODUCTION

Les radiations ionisantes gamma du Co^{60} agissent sur les cellules du parenchyme foliaire de l'espèce *Allium sativum* L., en déterminant de graves perturbations à ce niveau. Ces perturbations sont dues à un processus d'ionisation des molécules vivantes qui ont accepté l'énergie radiante.

S'y produit le phénomène de radiolyse de l'eau, suivi de la libération des radicaux libres, extrêmement toxiques pour la cellule.

Ces radicaux agissent sur les molécules vivantes inactivant certaines chaînes biologiquement actives. De cette manière on agit sur les lysosomes des formations vésiculaires présentes dans toutes les cellules vivantes, mais qui contiennent des enzymes hydrolithiques inactives. Ils sont doués d'une enveloppe membraneuse qui s'oppose à l'action des différents facteurs physico-chimiques représentée par une double membrane lipoprotéique, doublement revêtue d'une couche de glycoprotéines. Plus les doses de radiations gamma utililsées sont grandes, plus la quantité des radicaux libres formés est grande, donc la lyse cellulaire en est plus forte. Par la lysation des membranes lysosomales se produit une activation brusque des enzymes hydrolithiques et leur libération au niveau du hyaloplasma. A la suite du phénomène de réduction de la perméabilité des membranes des organites cellulaires, celle-ci pénètre dans les organites lésant leur structure et déterminant un dérèglement de leur fonction.

MATÉRIEL ET MÉTHODE

Les bulbilles d'*Allium sativum* L., prétraitées à l'eau, Procaïne 1‰, Tyastime 1‰ et une combinaison de substances chimiques: Procaïne+Tyastime+Cystéamine 1‰, ont été irradiées pendant 24 heures avec des radiations gamma, en doses de 10 Gy, 30 Gy et 50 Gy en flux continu et deux débits: 10 Gy/min. et 3,33 Gy/min. Quarante jour après l'irradiation, on a prélevé des fragments des feuilles qui ont été fixées dans la glutaraldéhyde 1% et postfixées dans OsO_4 1% pendant 24 heures à 4°C. On a fait l'inclusion du matériel dans la laque ME 6600. On a sectionné les blocs à l'ultramicrotome Tesla, et la coloration des grilles a été réalisée avec de l'acétate d'uranyle et le contraste avec de la solution Reynolds.

REV. ROUM. BIOL. VÉGÉT., TOME 41, N° 2, P. 113–119, BUCAREST, 1996

PLATEAU I

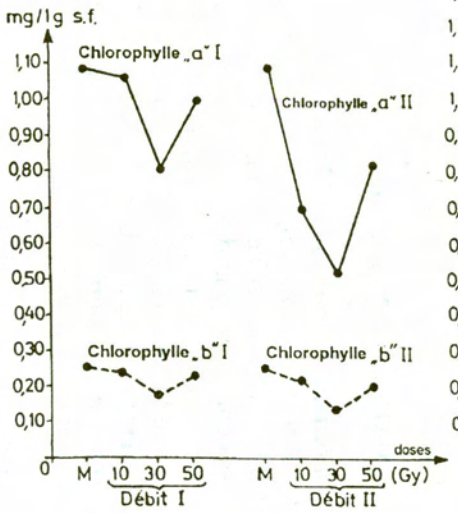

Fig. 1

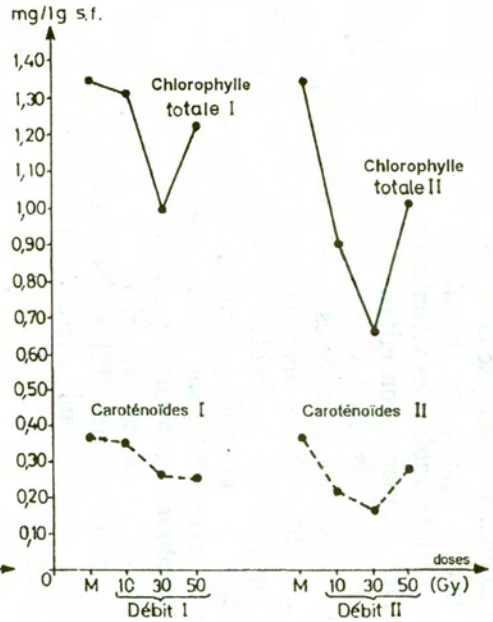

Fig. 2

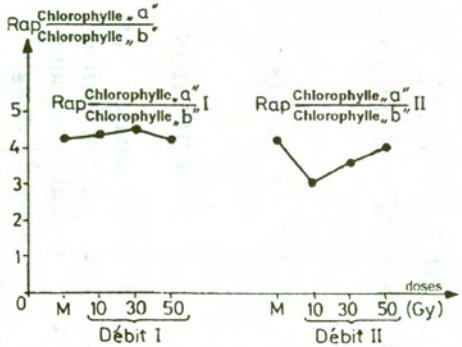

Fig. 3

Fig. 1. L'effet direct des radiations ionisantes gamma, deux débits, sur les quantités des chlorophylles «a» et «b».
Fig. 2. L'effet direct des radiations ionisantes gamma sur la quantité de chlorophylle totale et des pigments caroténoïdes.
Fig. 3. L'effet direct des radiations ionisantes gamma sur le rapport chlorophylle «a»/«b». D I = 10 Gy/min. D II = 3,33 Gy/min.

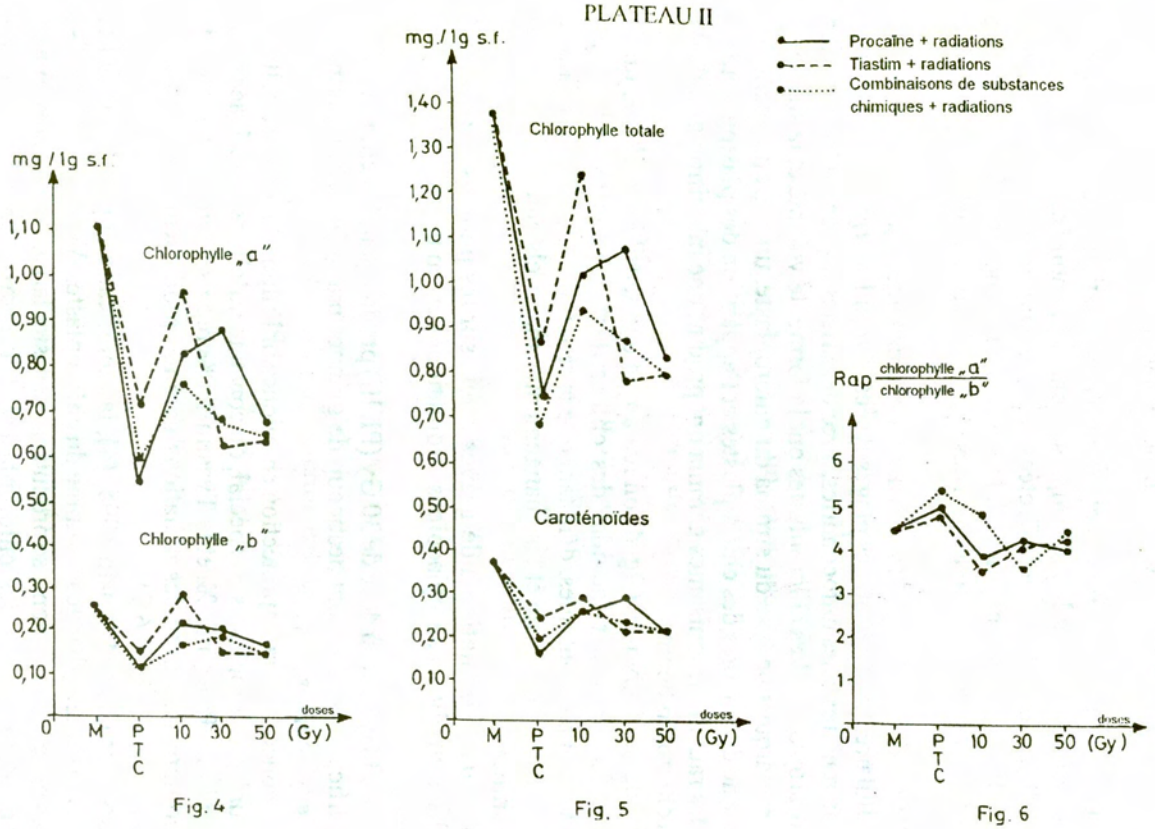

Fig. 4

Fig. 5

Fig. 6

Fig. 4. L'action combinée des radiations ionisantes gamma et des substances chimiques sur la quantité de chlorophylle «a» et «b».
Fig. 5. L'action combinée des radiations ionisantes gamma et des substances chimiques sur la chlorophylle totale et sur les pigments caroténoïdes.
Fig. 6. Le rapport chlorophylle «a»/«b» dans les conditions des traitements combinés des radiations gamma avec des substances chimiques.

On a fait l'examen au microscope électronique Jem 7.

L'extraction des pigments assimilateurs a été faite avec de l'acétone 80% et pour calculer la chlorophylle on a employé la formule de Holm (10).

RÉSULTATS ET DISCUSSIONS

Les plastides sont des organites cellulaires caractéristiques au règne végétal, au niveau desquelles se produit le plus impressionnant phénomène de la nature, la photosynthèse. Elles sont gravement affectées par les radiations ionisantes gamma. Au niveau des cellules du parenchyme foliaire on constante une réduction du nombre des plastides, leur déformation, la réduction des grannes et du nombre des tylacoïdes/ grannes.

Dès 30 Gy, débit 10 Gy/min. (Pl III.) on constante une grande lyse du cytoplasme cellulaire, l'apparition des plages lysées. Parmi les organites cellulaires présentes ici, on remarque des mitocondries, avec des cristes dilatées et leur matrice lysée et des chloroplastes. Les chloroplastes ont la forme, le volume et la structure modifiés. On remarque une lyse du stroma du chloroplaste, un regroupement des grannes vers l'une des marges des chloroplastes et l'apparition des plastoglobules. À cette dose, les radiations ionisantes gamma ont produit une inhibition grande de l'assimilation chlorophylienne.

À 50 Gy, débit 10 Gy/min (fig. 4) on remarque une sensible amélioration de l'ultrastructure du système tylacoïdal des chloroplastes, mais les tylacoïdes stromatiques ne sont pas continues, dénotant que la répartition des grannes dans le système tylacoïdal est chaotique et les changementes matériels entre tylacoïdes et stroma sont diminués.

Le processus d'irradiation a de graves effets sur les photosystèmes PS I et PS II, localisés au niveau des tylacoïdes stromatiques et, en même temps, sur la photosynthèse.

Le débit 3,33 Gy/min., à dose de 30 Gy (Pl. III) produit dans les chloroplastes de petites modifications. Elles ont beaucoup de grannes mais elles ont un nombre remarquable de tylacoïdes/grannes.

En ce qui concerne la radioprotection chimique sur l'ultrastructure des cellules en général et sur les chloroplastes en spécial, on constate qu'elle est inefficace pour *Allium sativum* L. La Procaïne 1‰ et la Tyastime 1‰ représentent des facteurs de stress et, en combinaison avec des radiations gamma, produisent l'amplification des négatifs dans la cellule (5.6.7).

Selon Douglas C. et M. Chain 1995, (4), la modification de la perméabilité de l'enveloppe plastidiale change le volume du chloroplaste. Walne P. (11) précise qu'à de grandes doses de radiations, se produisent la vésicularisation des enveloppes et la désorganisation interne des chloroplastes. La destruction des tylacoïdes stromatiques signifie la destruction des deux centres de réaction PS I et PS II localisés à l'intérieur des membranes tylacoïdales stromatiques. Entre les couches des lipides et les protéines des membranes tylacoïdales se trouvent localisés, sous la forme d'un film monomoléculaire, les pigments assimilateurs. Leur rôle est

d'accepter l'énergie lumineuse (la chlorophylle «b» et les pigments caroténoïdes) et de la transférer au pigment actif (la chlorophylle «a»). Les radiations ionisantes gamma ont produit une diminution quantitative des pigments assimilateurs, donc une réduction de la quantité d'énergie lumineuse absorbée. L'inhibition du transport photosynthétique d'électrons par la destruction des deux centres de réaction détermine une inhibition de la photosynthèse. Si l'on regarde la figure 1, Pl. I, on constante que les doses de radiations utilisées produisent une diminution de la quantité des pigments assimilateurs. Dans le cas des deux débits, on enregistre les moindres valeurs à la dose de 30 Gy (Chl. «a» - 0, 78 mg/lg s.f., débit 10 Gy/min. et 0,52 mg/lg s.f. au débit 3,33 Gy/min).

La chlorophylle «b» à cette dose, débit 10 Gy/min. - 0, 18 mg/lg s.f. et au débit 3,33 Gy/min. - 0,14 mg/lg s.f. À 50 Gy; on remarque des augmentations sensibles des chlorophylles, mais sans atteindre les valeurs du témoin. Dans la figure 2, Pl I, on constate que les doses de radiations ont affecté de la même manière la quantité totale de chlorophylle et les pigments caroténoïdes.

On peut affirmer que la dose de 30 Gy représente pour cette espèce une dose critique, un seuil inexplicable vu qu'on remarque de légères augmentations des valeurs à 50 Gy. Le débit 10 Gy/min., bien que fort, a affecté moins la quantité de chlorophylle comparativement au débit 3,33 Gy/min. Le rapport chlorophylle «a»/ «b» (fig. 3, Pl. I.) confirme l'existence de certaines perturbations des processus physiologiques, de métabolisme, qui se passent à ce niveau.

Les substances chimiques utilisées dans le traitement se sont manifestées comme des radioprotectrices partielles contre l'action nocive des radiations gamma. L'effet des substances chimiques dépend du type de la substance utilisée et de la dose de radiations. Généralement, autant la Procaïne 1‰, que la Tyastime 1‰, employées seules, sont trop fortes pour cette espèce, produisant des décroissements de la chlorophylle «a» et «b» (fig. 4, Pl. II) et de la chlorophylle totale et des carotenoïdes. (fig. 5, Pl. II).

La dose 10 Gy + Procaïne 1‰, ou 10 Gy + Tyastime 1‰, prouve que les radiations gamma stimulent la quantité de pigments assimilateurs.

La dose 30 Gy + Procaïne 1‰, détermine des augmentations des chlorophylles «a» (0,86 mg/lg s.f.) par rapport à la 10 Gy + Procaïne 1‰ (0,82 mg/lg s.f.).

La dose 30 Gy + Tyastime 1‰ (Chl. «a» - 0,62 mg/lg s.f. et chl. «b» - 0, 15 mg/lg s.f.) et la combinaison de substances chimiques 1‰ (chl. «a» - 0,67 mg/lg s.f. et chl. «b» - 0,19 mg/lg s.f.) produisent des décroissements des chlorophylles «a» et «b».

Les valeurs de la chlorophylle totale et des pigments caroténoïdes à 30 Gy sont proportionnelles à celle obtenue dans le cas de la chlorophylle «a». La dose de 30 Gy présente en ce cas aussi, des curiosités.

Les substances chimiques utilisées dans le traitement ont eu une action de stabilisation des membranes des lysosomes par rapport à l'action des radicaux libres, empêchant ainsi l'activation et la libération des enzymes hydrolithiques (2, 3, 6). Les substances chimiques utilisées dans les traitements ont des effets limités (7).

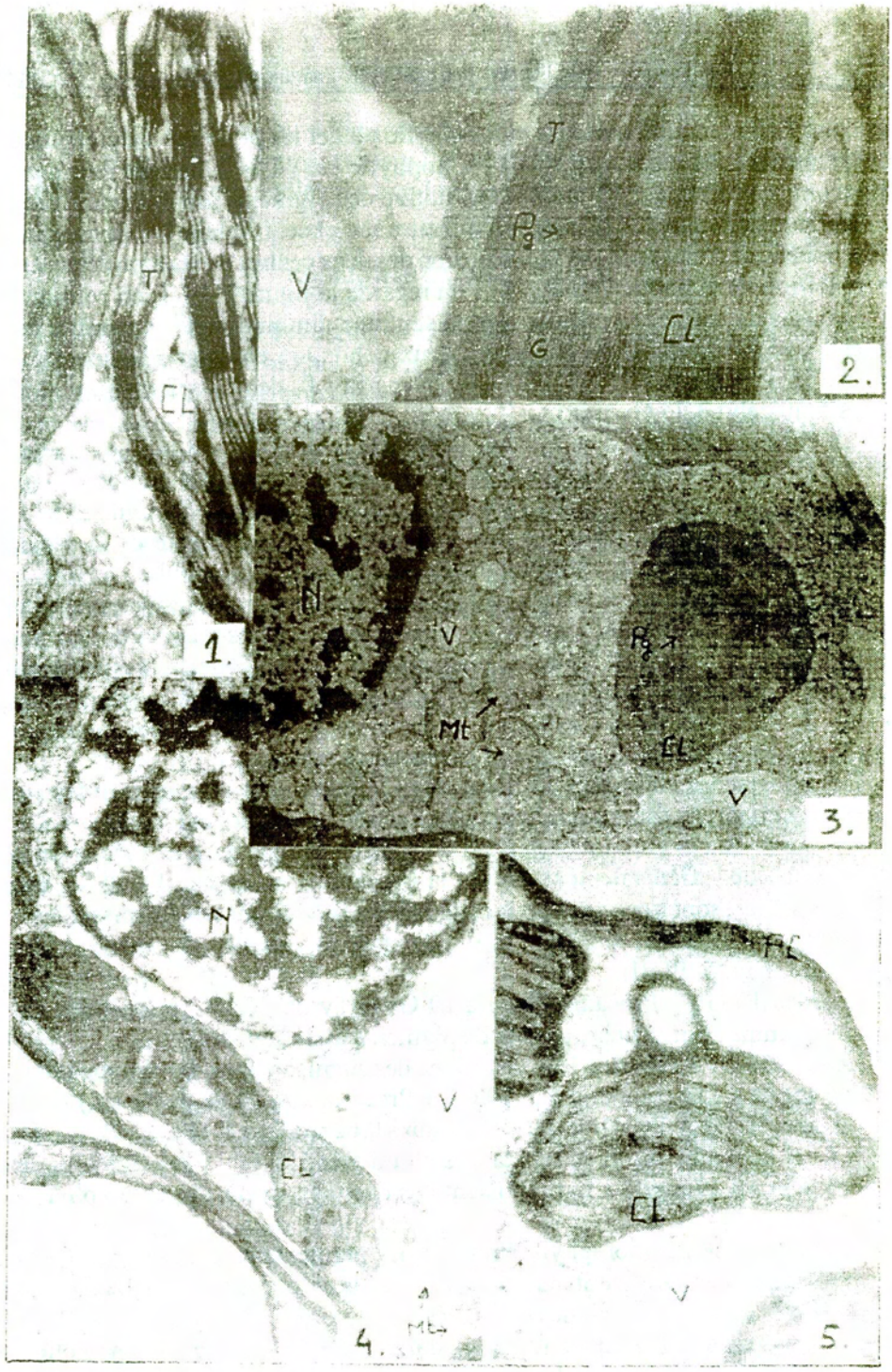

Fig. 1. Aspect ultrastructural d'un chloroplaste de la cellule du témoin.
Fig. 2. Aspect ultrastructural d'un chloroplaste irradié avec 10 Gy débit 10 Gy/min.
Fig. 3. Aspect ultrastructural d'une cellule irradiée avec 30 Gy débit 10 Gy/min.
Fig. 4. Aspect ultrastructural d'une cellule irradiée avec 50 Gy débit 10 Gy/min.
Fig. 5. Ultrastructure d'un chloroplaste irradié avec 30 Gy débit 3,33 Gy/min.

CONCLUSIONS

1. La corrélation de l'action des radiations ionisantes gamma sur le chloroplaste avec celle liée à la radioprotection chimique détermine un élargissement du champ d'utilisation de l'énergie nucléaire dans les processus d'amélioration des plantes, ainsi que dans le but d'annihiler les effets de la pollution radioactive accidentelle.

2. Les doses de radiations gamma de 10 Gy à 50 Gy sont trop fortes pour l'espèce *Allium sativum* L. Après deux mois, toutes les plantes irradiées sont mortes.

3. Au niveau ultrastructural, les modifications induites par les radiations ionisantes gamma sont proportionnelles à la dose d'irradiation.

La dose de 10 Gy produit les moindres lésions au niveau du chloroplaste.

4. On a remarqué que la concentration de 1‰ des substances chimiques utilisées est trop forte, produisant une diminution de la quantité des pigments assimilateurs.

5. La combinaison de substances chimiques avec la dose de 10 Gy, détermine une augmentation de la quantité des pigments assimilateurs.

6. Dans toute les situations on constate une similarité dans le processus d'assimilation chlorophyllienne.

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Reçu le 15 Mars 1996

Institut de Biologie Bucarest
Splaiul Independenței nr. 296, sector 6

FACTORS AFFECTING "IN VITRO" EMBRYO GERMINATION AND PLANT DEVELOPMENT, IN SOME *PRUNUS* GENOTYPES

GABRIELA COSTEA*, ANTONIA IVAŞCU**, VIORICA BĂLAN**, ŞTEFANIA TOMA**

Embryo culture of embryos ≥ 4 mm long of some *Prunus* genotypes was investigated. The effects of various media on embryo enlargement, germination and development into plantlets were compared. The germination of the embryos presents significant differences depending on the medium and genotype. The culture media beneficial for the germination of embryos had not always the same effect on their development into plantlets but all the media who were beneficial for the development contributed also in their further survival during acclimatisation phase.

INTRODUCTION

The difficulties encountered in the development of improved early-ripening cultivars in *Prunus* sp., due to the embryo abortion stimulated "in vitro" embryo cultures in the seventies (7). As a result embryo culture became a very useful instrument in shortening the breeding cycle of peach, and in increasing germination of embryos from early-ripening cultivars and from interspecific crosses (2, 3, 4). In this case the inability of the hybrid zygote to produce a viable embryo is due to the degeneration of the endosperm which is unable to ensure proper nutrition for the embryo. The excision of immature embryos and their culture on a synthetic medium can provide the nutrients that are lacking in situ and, thus, hybrid plants can be obtained. There is generally accepted that the stage of embryo development is the key factor on embryo survival and germination in vitro (3, 4, 7, 8).

The Smith, Bailey and Hough (SBH) medium was one of the first used, but it proved inadequate for the survival of small immature embryos < 10 mm length (6).

Murashige and Skoog (1962) medium with the addition of potassium succinate and glutamine allowed embryos as small as 5 mm to be grown successfully (7). Embryos < 10 mm length are less likely to grow successfully even though they are grown on a more complex medium. During embryo culture complex morphological and physiological changes occur accompanied by continuous changes in the requirements of the embryos as they grow in the medium.

Other media used recently for peach embryo culture were: Woody Plant Medium (WPM), Chee Medium (Chee & Pool 1987) and Murashige & Skoog modified. The best germination and plant development were achieved on WPM (2).

Our objectives were to evaluate the effects of 3 medium formulations in correlation with the genotype on embryo germination and plant development and also the possible influences during the acclimatisation phase.

REV. ROUM. BIOL. VÉGÉT., TOME 41, N° 2, P.121–128, BUCAREST, 1996

MATERIALS AND METHODS

The material used was represented by 6 genotypes obtained at the Institute for Fruit Tree Research, Băneasa, Bucureşti, through hybridisation between some early-ripening peach cultivars (*Prunus persica Batsch*) and among *Prunus persica* * *Prunus armeniaca*.

The media used were: Hough (H), Murashige & Skoog (1962, MS) modified by doubling the concentration of microelements and reducing to half the major salts and iron and Quoirin-Lepoivre (1977, LP) basal medium modified in the same way, also adding 20 g/l sucrose and 7 g/l agar. Final pH was adjusted to 5.7-5.8 and the media were autoclaved at 121 C for 20 min. at pres. = 1 atm.

For embryo extraction the fruit were split open and immature seed removed and placed into Petri dishes. Any further handling of material was done within a laminar flow cabinet. Seeds were dipped into 95% ethyl alcohol and flamed by positioning the micropylar end of the seed into the flow of air. After that the embryo was removed and placed into the test tube, in vertical position.

Embryo length varied between 1-8 mm for P1, P2, P3 genotypes and 5-10 mm for R1p34, ARK 65 and NJC'89 genotypes.

Embryo < 4 mm degenerated or remained in the same stage. That is the reason why all the measurements and recordings were made only for the embryos ≥ 4 mm length.

Cultures embryos were stratified in the dark at 0.5 – 1 C, for 30 days in the genotypes P1, P2, P3 and for 20 days in the genotypes R1p34, ARK 65, NJC'89. The length of the embryo before and after stratification was measured.

The cultures were placed in the dark at 16 C to permit germination. Embryos were considered germinated when their radicles had extended into the medium at least 2 mm. For further development into plants the embryos were maintained at 22-25 C with a 16 h photoperiod.

For the acclimatisation the plantlets passed through three phases: maintained on the medium on the test tube uncovered for the 5 days, 10 days on the test tube with water and then transferred in pots and placed in high humidity level conditions.

RESULTS AND DISCUSSIONS

For the six studied genotypes it was evident much variability of each genotype response during stratification, germination and development of the embryos into plants.

The enlargement of the embryos (*Table 1*) measured by the difference between the length after stratification and the initial embryo length, was relatively constant for all three media and for all the genotypes.

The germination of the embryos presents significant differences, depending on the medium and genotype (*Fig. 1*).

For the embryo germination of NJC'89 and ARK 65 the best media were H and MS (80-100%). For the genotypes P1, P2, P3 the same media proved to be less efficient (0-40%), meanwhile the LP medium allowed a good germination (16-80%).

Table 1

Change in embryo length from culture initiation to the end of stratification

Genotype	L = embryo length after stratification-initial embryo length (mm)		
	H	LP	MS
R1p34	4	5	6
NJC'89	6	4	6
ARK 65	9	7	4
P1	1,45	1,5	2
P2	3,25	2	2,5
P3	2,67	3,76	4,67

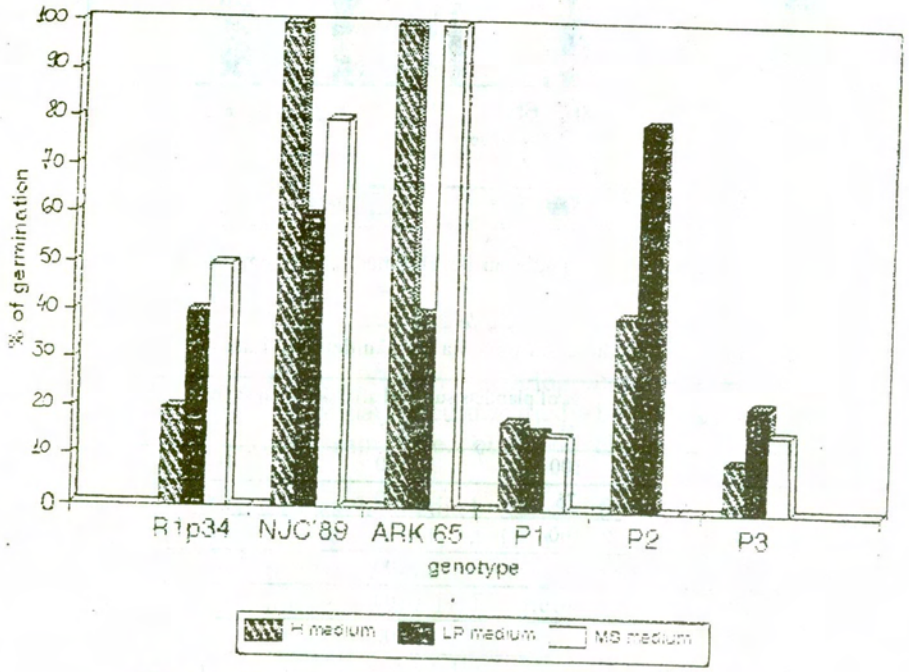

Fig. 1. – The effect of culture media on embryo germination.

The development into plants of the germinated embryos was found to be influenced by the same factors (*Fig. 2*).

For NJC'89, ARK 65 and R1p34 on all the media the percent of germinating embryos developing into plants was 100%. The embryos of P1, P2 and P3 recorded the same percent on LP medium but the H and MS media were inadequate.

The survival of the plantlets during acclimatisation phase is illustrated on *Table 2*.

It appears that all media which were beneficial for the development of the embryos into plantlets also contributed to their further survival.

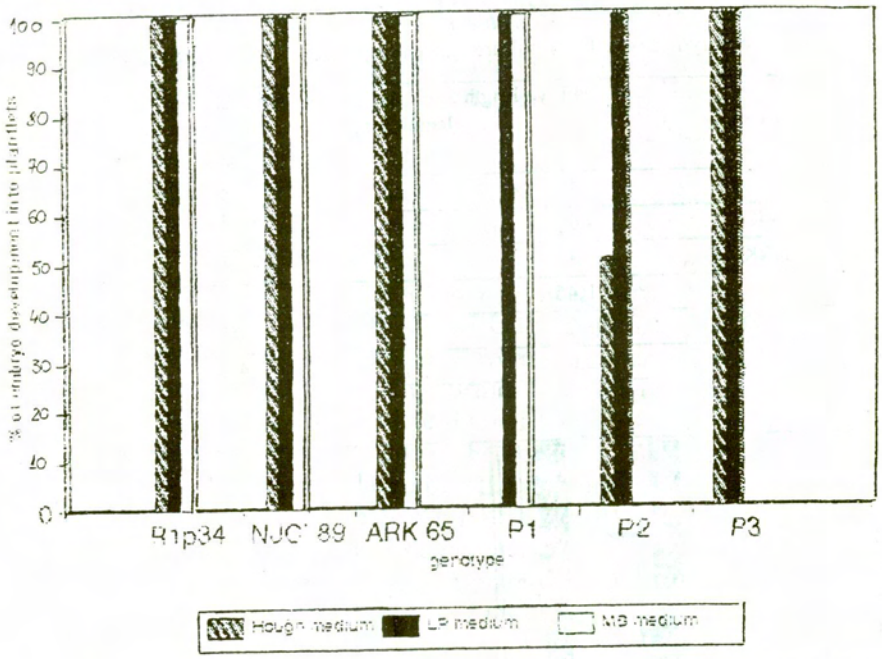

Fig. 2. – The effect of culture media on development of the embryos into plants.

Table 2

The influence of the culture media on plantlets acclimatisation

Genotype	% of plantlets survival after acclimatisation		
	H	LP	MS
R1p34	100	100	100
NJC'89	75	100	100
ARK 65	100	–	20
P1	–	100	–
P2	–	100	–
P3	–	100	–

The medium LP was poor for the genotype ARK 65, maybe due to its poor effect on embryo germination.

The average length of the main roots measured in the first day of acclimatisation reflects the influence of the culture media (*Table 3*). The lower salt concentration of the media had a positive influence on root development and subsequently on the growth of the plantlets.

The size of the plantlets after 15 days of acclimatisation evaluated by the length of the stem and the number of leaves is presented in Table 4.

The high number of leaves for a relative small length of the stem for R1p34, NJC'89 and ARK 65 may be explained by the appearance of the lateral shoots, in 54% of plantlets, having or not necrotic shoot tips.

Table 3
The growth of the root depending on culture media

Genotype	The mean length of the main root (cm)		
	H	LP	MS
R1p34	16	13,5	8,5
NJC'89	15	11,5	14
ARK 65	14,5	6	10
P1	–	8	–
P2	–	10	–
P3	–	12,5	–

Table 4
The length of the stem and number of leaves varying with the genotype after 15 days of acclimatisation

Genotype	The mean length of the stem&Number of leaves		
	H	LP	MS
R1p34	1.5/12	0.75/6	1.75/9,5
NJC'89	2.2/7.3	1.5/14.6	2.5/14
ARK 65	3.4/8.6	–	1.5/7
P1	–	2.5/10	–
P2	–	3.8/5.5	–
P3	–	4/9.6	–

The genotypes P1, P2 and P3 do not develop lateral shoots.

It appears from this study that the culture media beneficial for the germination of embryos had not always the same effect on the development of the embryos into plantlets.

For the studied genotypes the media beneficial for germination were: H, MS - for NJC'89 and ARK 65, MS - for R1p34, LP for P2, P3 and H - for P1.

For development into plants all the tested media proved beneficial for: R1p34, NJC'89 and ARK 65, while for P3 the H, PL media, for P2 - LP and for P1 - LP, MS media.

According with the literature data, the lower salt and iron concentration and the doubling of the microelements concentration in MS and LP media were beneficial on germination and development into plants, but only for embryos ≥ 4 mm (3, 7, 8).

The conditions during the germination phase may have an impact on the plantlet survival during the acclimatisation phase probably due to a poor development of the radicle during germination or an insufficient stratification period.

Recent research regarding the influence of cold for removing embryo dormancy indicates that the peach embryos present a dormancy localised at the gemula-epicotylar level, without radicular dormancy (1). So the germination may occur without stratification period, but the resulted plantlets are weak (*Fig.* 3.4).

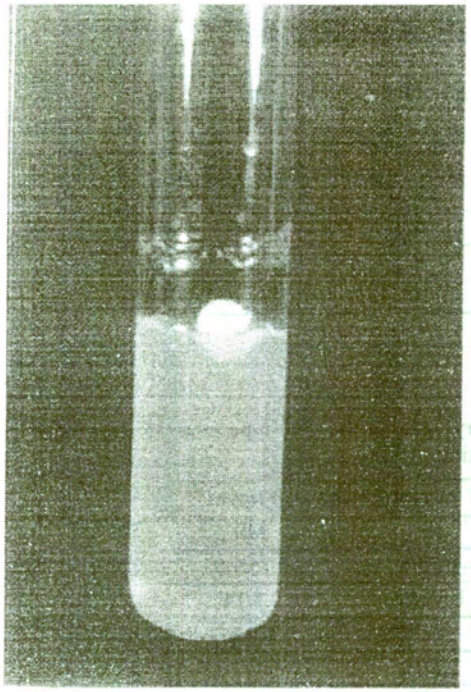

Fig. 3. 1

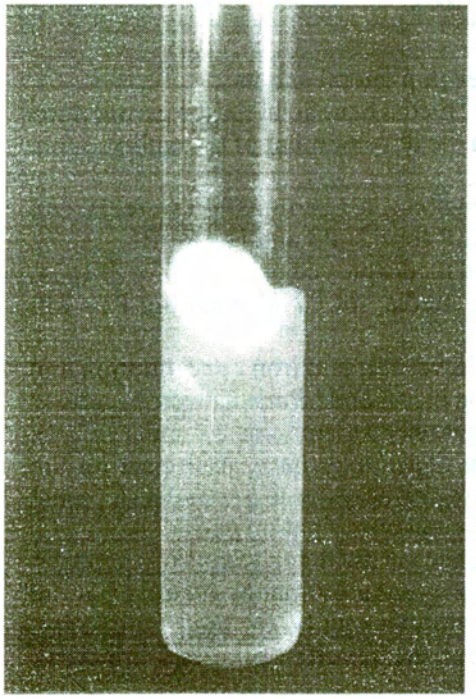

Fig. 3. 2

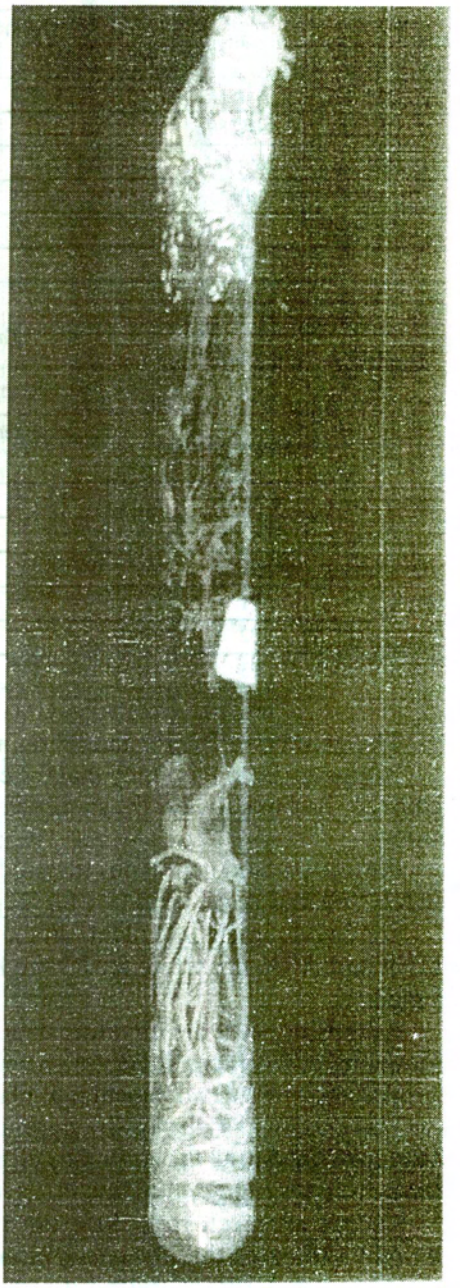

Fig. 3. 3

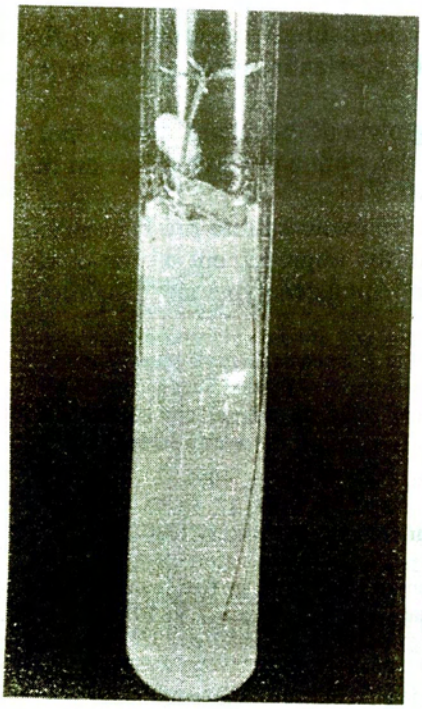

Fig. 3. 4

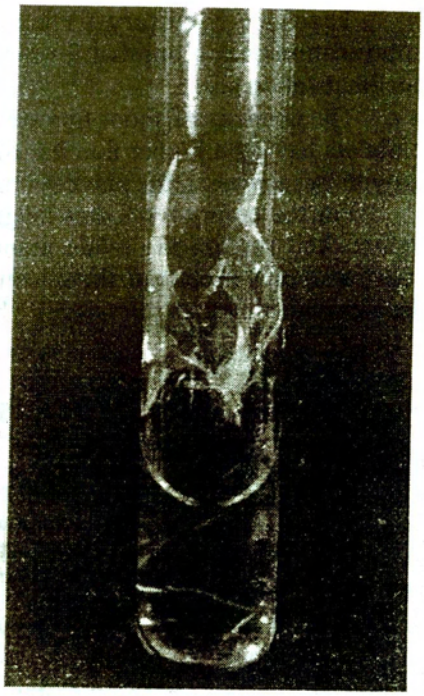

Fig. 3. 5.

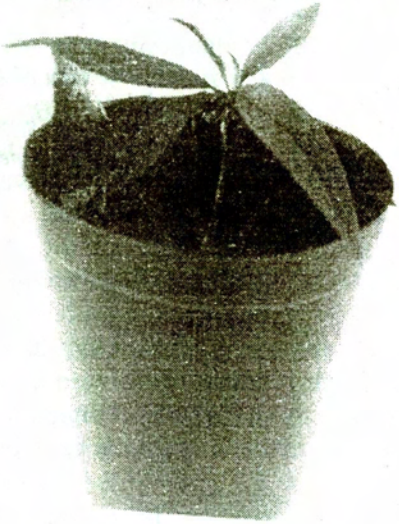

Fig. 3. 6.

Fig. 3. – Stages in embryo culture: excised embryo in test tube (1);
– enlarged embryo after stratification period (2);
– completed developed plantlet–R1p34 on H medium (3);
– plantlet with weak development (4);
– acclimatisation phase: in test tube (5) and in pot (6).

There are necessary further studies for establishing the duration of the stratification period optimal for the stem growth and plantlet survival during the acclimatisation phase.

The necrosis of shoot tips stimulated the growth of the lateral shoots, these plantlets having a higher number of leaves/plantlet, with a positive effect on the growth by increasing the photosynthetic capacity.

For the embryo < 4 mm length, the use of in ovulo culture for 1-2 weeks, before their rescue, the ovule manipulation and the improvement of the ovule substrates, may accelerate the selection of early-ripening genotypes and also allow the survival of Prunus interspecific hybrids (6).

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Received February 12, 1996

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SEASONAL OCCURRENCE OF FILAMENTOUS FUNGI ASSOCIATED WITH *CLADOPHORA* MASS FROM TECHIRGHIOL LAKE

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Strains of *Penicillium*, *Cephalosporium*, *Cladosporium*, *Aspergillus*, *Fusarium*, *Stemphylium*, *Oospora variabilis* and *Alternaria alternata* were found on *Cladophora mass*. They are able to degrade the mucilage around cells but not the cell walls from *Cladophora mass* and represent significant decomposers in the Techirghiol lake. *Penicillium sp.*, *Alternaria alternata* and *Cladosporium strains* can produce, in vitro, cellulase, xylanase and arabanase as a proof of their terrestrial origin.

The marine medium is unique by its large surface and volume, low content of inorganic nutrients (except for the estuaries), high salinity and ionic content, low temperature and high pressure, depending on the depth. In these conditions microorganisms (both bacteria and fungi) elaborated their own strategies for survival.

According to Rheinheimer (13) there are 149 species of Ascomycetes, 91 species of Pyronomycetes, 51 species of Loculoascomycetes, 56 species of Deuteromycetes, and only 4 species of Basidiomycetes.

The carbon photosynthetically fixed by microphytes and macrophytes goes into insoluble structural polymers unavailable to the food web in salt lake or salt marsh. Microorganisms are able to convert this material into microbial biomass. The resulting organic detritus serves as either a direct or indirect food source for much of the salt lake biota (7, 10).

As producing of degrading enzymes, fungi might be considered as potentially significant decomposers of the salt terrestrial or aquatic microbial community.

Gessner's studies (1-4) utilizing purified wall components as inducers or substrates for cell wall components demonstrated that it is not the same as enzyme induction by native cell walls.

Cladophora is the main alga from the Techirghiol lake and its decomposition is exceptionally important in providing a base for the food web on the shore (6).

We report herein our attempts to establish the main fungi grown on *Cladophora* from spring to autumn and their ability to degrade its cell walls. We also studied polysaccharidases produced by them.

There are studies on the seasonal occurrence and distribution of fungi in the marine environment inhabiting submerged and interdidal wood (11), mangrove seedlings (9), turtle grass (8) and *Spartinia alterniflora* (1, 2). No information has been reported in the literature on the seasonal occurrence of filamentous fungi associated with *Cladophora*.

REV. ROUM. BIOL. VÉGÉT., TOME 41, N° 2, P. 129–132, BUCAREST, 1996

The purpose of this paper was to study the seasonal occurrence of filamentous fungi on growing, senescing and dead *Cladophora*. We also report their ability to degrade *Cladophora* cell walls and to produce some degradative enzymes: xylanase, cellulase, arabanase.

MATERIAL AND METHODS

Cultures of each of the isolated fungal species were obtained from *Cladophora mass* by homogenization and filtration. Pure cultures were maintained on slants DPA medium.

The degradation of cell walls measured by incubation of 3 ml of culture filtrate and 10 mg of cell walls, at 30°C for 72 h. After the removal of the residual cell walls, supernatants assayed for reducing sugars using dinitrosalicylic acid (DNSA).

Cellulase, xylanase and arabanase activities were determined spectrophotometrically using DNSA reducing sugar (5).

The culture medium for cellulase, xylanase and arabanase content sunflower bran, maize flour, glucose and NaCl - 0; 5; 6; 7%.

Units forming colonies (ufc) were calculated according to the successive dilution method.

RESULTS AND DISCUSSION

The data presented in Table 1 include occurrence of filamentous fungi on growing, senescing or dead *Cladophora mass* from the Techirghiol lake collected from May to November.

The highest ufc on the dead *Cladophora* was found in November. In August and September there were found the same values of ufc on the growing or senescing *Cladophora*.

Filamentous fungi isolated from *Cladophora* are known as terrestrial fungi. Although some of them were found in marine muds, Tubaki (12) described two *Cephalosporium* species from marine muds in Japan and Davids and Choist (12) described another two from saline lake muds in the United States. Also *Alternaria alternata* was found on *Spartinia alterniflora* (salt marsh cordgrass). Regarding the vertical distribution of fungi on *S. alterniflora*, Gessner (3) isolated marine fungi from the lower portion of the plant which was submerged twice a day by the tides and terrestrial fungi from the upper part of the plant which was not generally inundated during high tide. In contrast we isolated all filamentous fungi from *Cladophora* mass frequently bound on the stone near the shore at 5-20 cm depth.

The cell wall degrading activities for 6 fungal strains were significantly different: 425 nm glucose reducing equivalent/ml culture filtrate/24h for *Penicillium sp.* and 0 for *Stemphylium* (Table 2).

Three fungal strains can produce xylanase and four are able to produce arabanase in our testing conditions (Table 3).

Table 1
The occurrence of fungi on *Cladophora* from Techirghiol lake

Sample	Time	ufc/ml	Fungi
1	May	2×10^3	*Penicillium sp., Cephalosporium, Cladosporium sp.*, Aspergillius sp., Alternaria alternata.
2	August	3×10^2	*Fusarium sp.*, Cladosporium sp., Penicillium sp., Alternaria alternata.
3	August	3×10^2	*Stemphylium*, Oospora variabilis, Alternaria alternata.
2	September	5×10^2	*Cephalosporium, Spicaria* sp. Aspergillus sp. Penicillium sp. Alternaria alternata.
2	November	30	*Alternaria alternata*, Cephalosporium, Aspergillus sp., Cladosporium

1 = dead *Cladophora* mass
2 = growing *Cladophora* mass
3 = senescing *Cladophora* mass

Penicillium sp., Alternaria alternata and *Cephalosporium sp.* can produce cellulase, xylanase and arabanase. *Cladosporium sp.* and *Stemphylium* can produce only xylanase and arabanase and *Oospora variabilis* none of them.

Comparing the rates of sugar release from cell wall (Table 2) with those from polysaccharidases activity and microscopical analyses we found the highest rates enzymic activities, but in this case we worked with purified substrates.

These enzymic activities are additional proofs of the terrestrial origin of those fungi involved in the decomposition of the cell walls with cellulose and xylan content. They have not ceased yed these particular activities.

The reducing sugars are released from the mucilage around the *Cladophora* cells and not necessarily from the cell walls.

A possible explanation emerges from the microscopic notice that showed that 90% of the cell walls were untouched.

Table 2
Release of reducing sugar from cell walls of Cladophora

Fungal strain	Reducing sugar (nmol glucose/ml)
Penicillium sp.	425
Alternaria alternata	172
Cephalosporium sp.	320
Cladosporium sp.	150
Stemphylium sp.	0
Oospora sp.	203

Table 3
Polysaccharidases produced by fungi isolated from *Cladophora* mass

Fungus	Cellulase[x]	Xylanase[x]	Arabanase[x]
Penicillium sp.	425	1420	220
Alternaria alternata	375	1850	175
Cephalosporium sp.	318	680	130
Cladosporium sp.	0	390	198
Stemphylium sp.	0	430	0
Oospora variabilis	0	0	0

x = nmol. glucose reducing equivalents liberated/ml culture filtrate/h.

CONCLUSIONS

1. Fungi occuring on dead *Cladophora* are in greater number then fungi occuring on senescing and growing *Cladophora* mass.

2. *Penicillium sp.*, *Alternaria alternata* and *Cladosporium strains* can produce, in vitro, cellulase, xylanase and arabanase.

3. Filamentous fungi isolated from *Cladophora* mass, can degrade the mucilage around cells but not the cell walls.

4. Filamentous fungi isolated from *Cladophora mass* are significant decomposers in the Techirghiol lake.

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Received March 12, 1996

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RESEARCH ON THE INFLUENCE OF THE ACORGA TYPE ORGANIC EXTRACTANT M-5640 ON THE GROWTH OF *THIOBACILLUS FERROOXIDANS* POPULATIONS

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In this paper the experimental results regarding the bacterial capacity of Fe^{2+} oxidation and the bacterial number/ml after the contact between the bacterial populations of *Thiobacillus ferrooxidans* and the ACORGA type reagent M-5640, as well as their component parts (ACORGA reagent and kerosene) are presented. It was found that the growth rate of bacterial populations diminished until complete absence after 14 days of experimentation on rotary shakers, although pH was acid and the Fe^{2+} was entirely oxidized. The ACORGA organic reagent had the minimal effect on the growth of bacterial populations, according to the bacterial number, after 14 days, at 28°C.

Chemolithotroph sulphur and iron oxidizing bacteria from *Thiobacillus* genus, especially *T. ferrooxidans* have the main role in microbial leaching of ores. Through this bacterial process, metal is obtained as soluble sulphate recovering by cementation or with organic extractants. Copper extraction with solvent takes place by capturing dissolved ions in water solution in another water organic phase at acid pH. Organic extractants accomplish both the concentration and/or purification of liquids containing copper.

MATERIAL AND METHOD

In our laboratory experiments we tested the influence of the ACORGA type organic extractant M-5640 and of its components (ACORGA reagent and kerosene) on the oxidation ability of ferrous iron by the RP-1 *T. ferrooxidans*, isolated from Roşia Poieni ore.

Various concentrations of organic extractant (0.2-6%) as well as of its components – ACORGA reagent (0.09 and 0.27%) and Kerosene (2 and 6 %) – concentrations which correspond to 4.5 ml ACORGA/100 ml total solution ratio, were used.

The bacterial population was cultivated in Erlenmeyer flasks with 9K culture medium, incubated at 28°C in stationary and agitation (150 rpm) conditions for 14 days.

The quantities of ferrous and ferric iron in culture liquid and the number of bacteria/ml were determined initially at 7 and 14 days.

RESULTS AND DISCUSSIONS

The results obtained in the first experiment prove that the inhibition of the oxidative activity of *T. ferrooxidans* was directly proportional to the organic

extractant concentration added to the culture medium (Table 1). The bacterial culture grew evidently in the medium which contained up to 1% organic extractant. At the end of the first 7 days of the experiment 10^2-10^5 bacteria/ml were obtained, and after other 7 days the number evidently decreased under biological control.

Table 1

Influence of organic extract M-5640 on the growth of *Thiobacillus ferrooxidans* population by rotary shakers

extractant concentration (%)	7 days				14 days			
	Fe^{2+} (g/l)	Fe^{3+} (g/l)	pH	No. bact. /ml	Fe^{2+} (g/l)	Fe^{3+} (g/l)	pH	No. bact. /ml
0.2	0.28	6.03	2.0	95×10^2	0.11	5.58	2.0	25×10^2
0.8	0.11	5.91	2.0	45×10^4	0.11	5.36	1.8	4
1	0.11	6.14	2.0	45×10^5	0.11	6.25	2.0	95
2	0.11	5.03	1.9	25×10	0.11	4.91	1.7	0
3	0.11	5.42	2.0	95	0.11	4.90	1.8	0
4	0.11	5.03	1.8	95	0.11	5.80	1.8	0
5	0.11	5.58	2.1	95	0.11	5.36	1.8	0
6	0.11	5.45	1.9	45	0.11	6.13	1.8	0
Biological control	0.11	5.40	2.0	45×10^5	0.11	6.25	2.4	45×10^2

Initial: Fe^{2+} = 9.0g/l; Fe^{3+} = 0.29g/l; No bacteria/ml = 14×10^7

The 2-6% organic extractant concentrations had a stronger inhibitor effect, which was ascertained by pronounced reduction of the number of bacteria (45, 95 bacteria/ml) after the first 7 days and the absence of bacteria at the end of the experiment. The presence of ferric iron in a culture medium having high concentration of organic extractant is partly due to bacterial oxidation, most of it resulting, however, from the chemical reaction between ferrous iron and the organic extractant.

Of the two components of the organic extractant, ACORGA reagent had a lower effect on the bacterial culture, determining a slower growth rate. Thus, in the first 7 days the bacteria oxidized 1/3 of the ferrous iron existing initially in the medium (9 g/l), and 10^3 bacteria/ml were found in culture. At the end of the 14 days the bacterial culture had oxidized the whole quantity of ferrous iron and gave a greater number of bacteria/ml ($11\text{-}25 \times 10^5$). Comparatively, in the case of the biological control, the bacteria oxidized the whole ferrous iron quantity in the first 7 days, the resulting number being 15×10^5 bacteria/ml. As a result of the exhaustion of energetic sources, the bacterial culture decreased numerically during the following days.

The kerosene, an organic substance, had in our experiment an inhibitory action evident after several days. Thus, after 14 days the number of bacteria/ml diminished to 45 in the medium with 2% kerosene and to 2.5 in the medium with 6% kerosene.

The organic extractant had the highest inhibitory effect on the growth rate of bacterial culture, demonstrated by the total loss of bacteria in medium (Table 2).

In the recovery process of copper from solutions charged with metals, M-5640 organic extractant is used at a 1/1 ratio. A bacterial culture being in logarithmic growth phase (14×10^8 bact/ml), was put together with the organic

extractant at a 1/1 ratio. After 5 days an evident decrease in quantity of the ferric iron and the absence of bacteria were found. In the case of biological control the quantity of ferric iron was constant and the bacterial culture diminished slightly owing to the absence of the source of energy (Table 3).

Table 2

Influence of organic extractant M-5640 and its component parts on the growth of *Thiobacillus ferrooxidans* population RP-1

extractant concentration (%)	7 days				14 days			
	Fe^{2+} (g/l)	Fe^{3+} (g/l)	pH	No. bact. /ml	Fe^{2+} (g/l)	Fe^{3+} (g/l)	pH	No. bact. /ml
ACORGA 0.09	5.8	2.90	2.5	7×10^3	0.11	7.81	2.1	25×10^5
KEROSEN 2	0.11	5.64	2.0	25×10^4	0.11	5.58	1.9	45
M-5640 2	0.11	5.03	1.9	25×10	0.11	4.91	1.7	0
ACORGA 0.27	6.03	2.68	2.5	15×10^3	0.11	6.47	2.0	11×10^5
KEROSNE 6	0.11	5.70	2.0	45×10^5	0.11	5.36	1.8	2.5
M-5640 6	0.23	5.03	1.9	4.5	0.22	5.13	1.8	0
Biological control	0.11	5.70	2.0	15×10^5	0.11	6.25	2.4	45×10^2

Initial: Fe^{2+} = 9.0g/l; Fe^{3+} = 0.29g/l; No bacteria/ml = 14×10^7

Table 3

Influence of organic extractant on the *Thiobacillus ferrooxidans* population at 1/1 ratio

experimental time	Fe^{2+} (g/l)	Fe^{3+} (g/l)	No. bact./ml
initial culture (14 days old)	0.45	7.48	$> 140 \times 10^7$
4 hours	0.22	7.25	–
8 hours	0.11	7.37	$> 140 \times 10^7$
24 hours	0.11	6.81	$> 140 \times 10^7$
48 hours	0.11	5.91	$> 140 \times 10^7$
120 hours	0.11	5.58	0
Biological control			
24 hours	0.34	7.48	$> 140 \times 10^7$
48 hours	0.11	7.59	$> 140 \times 10^7$
120 hours	0.11	7.59	45×10^5

The results obtained by us are in agreement with those noted in the literature. Thus, *T. ferrooxidans* bacterium is inhibited by a high variety of organic substances, which influence iron oxidation. This inhibition appears when the concentration of the organic product approaches or surpasses that of the ferrous iron. The obtained data suggest that any organic substance having the capacity of interacting with ferrous iron, can be an apparent inhibitor of the bacterial oxidation of iron (2).

On another hand, bacterial leaching is a cyclic process. The aqueous solutions returned to the leaching process after the metal recovery. The organic compounds unfavourably affect the bacteria activity and consequently are economically prejudicial. Besides, medium pollution may increase when the solutions containing such compounds are spilled in environment. These solutions must be treated with absorbent for removing of organic substances before the solutions are returned to the leaching process (1).

An example proving the inhibiting effect is the absence of *T. ferrooxidans* bacteria in the Roşia Poieni ore through which solution, having previously been treated with M-5640 organic extractant, has been recirculated.

CONCLUSIONS

1. The organic extractant has an inhibitory effect of *Thiobacillus ferrooxidans* culture growth in a concentration over 1%.

2. Of these two components, kerosene has a higher inhibitory effect, as it kills the bacterial culture in 14 days.

3. The inhibitory effect of M-5640 organic extractant is principally due to kerosene, an inhibitory organic substance which has the higher weight in the extractant. Besides, kerosene interacting with ferrous iron removes this energetic substratum which is necessary for bacteria growth.

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Received December 28, 1995

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DETECTION AND PRELIMINARY CHARACTERIZATION OF A BACTERIOCIN PRODUCED BY A STRAIN OF *LACTOBACILLUS ACIDOPHILUS*

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A total of 8 strains of lactobacilli were tested for antimicrobial activity against different bacteria. Three out of *Lb. acidophilus* strains demonstrated inhibitory activity against other members of the same species and also against some strains of *Lb.helveticus* and *Lb. bulgaricus* . One of the active *Lb. acidophilus* strains, designated as *Lb. acidophilus* IB801 was selected and used in further experiments. The activity of the antibacterial compound produced by *Lb. acidophilus* IB801 was inhibited by protease treatment (pepsin or trypsin) but was resistant to heat. There was no reduction of antibacterial action against *L. helveticus* 102 and *Lb. bulgaricus* 509 after heating for 15 minutes at 100°C. These results demonstrate that the antibacterial compound is a heat-stable protein (bacteriocin).

Well before the existence of starter bacteria was recognized, their activities were instrumental in preserving dairy food. During growth in fermented products, dairy starters including lactobacilli and lactic streptococci produce inhibitory metabolites. Inhibitors include broad spectrum antagonists, organic acids, diacetyl and hydrogen peroxide. Some starters also produce bacteriocins or bactericidal proteins active against species that usually are closely related to producer culture (Barefoot and Nestle, 1993). The contribution of bacteriocins in fermentations is difficult to evaluate. It is suggested that they may play a role in selecting the microflora which initiates the fermentations. Bacteriocins are believed to be important in the ability of lactic acid bacteria to compete in non-fermentative ecosystems such as engastrointestinal tract (Lindgren and Dobrogosz, 1990, De Vuyst, 1994).

The aim of this study is to examine the antagonistic properties of some lactic acid bacteria.

MATERIAL AND METHODS

1. *Bacterial strains and media.* The lactobacilli used in our experiments (*Lactobacillus acidophilus, Lb. helveticus, Lb.plantarum* and *Lb.lactis*) were from the Culture Collection of the Institute for Food Research, Bucharest and of the Institute of Biology, Bucharest. As indicator strains (sensitive to inhibitory action of bacteriocin) were used *Lb. delbreuckii* subsp. *bulgaricus* 509 and *Lb. helveticus* 102. In some experiments the inhibitory activity was tested against different Gram positive bacteria (*Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *diacetylactis, Bacillus subtilis, B. cereus, Staphylococcus aureus, Clostridium sp., Listeria monocytogenes*) and Gram negative bacteria (*E.coli, Salmonella typhimurium, Pseudomonas aeruginosa, Klebsiella pneumoniae).*

REV. ROUM. BIOL. VÉGÉT., TOME 41, N° 2, P. 137–143, BUCAREST, 1996

Lactic acid bacteria were maintained in sterile skim milk supplemented with 0 .1 % yeast extract and before use they were propagated twice in MRS or RCM medium. Solid media used for lactobacilli were MRS or RCM supplemented with 0.7 % agar (toplayer) and MACA (Difco).

To cultivate the Gram positive and Gram negative bacteria we used Luria Bertani broth. *Clostridium sp.* strains were cultivated in RCM medium and *Listeria monocytogenes* strains in "Listeria enrichment broth base"(Merck) as liquid medium and McBride Listeria Agar (Bencton-Dickinson) as solid medium.

2. Detection and quantitative determination of inhibitory activity. The antagonistic activity of *Lb. acidophilus* IB8Ol was tested by agar spot test using cell-free, pH adjusted (pH 6.5-7.0) culture supernatant towards indicator strains, according to Hastings and Styles, 1991.

3. Preliminary characterization of bacteriocin produced by Lb. acidophilus IB801. For a preliminary characterization of bacteriocin produced by *Lb. acidophilus* IB801 we used culture filtrate which was treated with different proteases: trypsin, pronase and proteinase K. Before treatment the pH of the filtrate was adjusted at 6.5-7.0. All proteolytic enzymes were prepared in 3mM sodium phosphate buffer (pH 7.5), added to the samples at 0.5mg/ml final concentration and incubated for one hour at 37°C. Heat sensitivity was tested by treating the culture filtrate for 15 minutes at 100°C or for 30 minutes at 70°C. After these treatments bioactivity was checked quantitatively using critical dilution method. In this order, serial twofold dilution of samples containing bacteriocins were spotted (10 ul) onto indicator lawns of *Lb. delbreuckii* subsp. *bulgaricus* 509 or *Lb. helveticus* 102 (prepared by adding 100 ul of fresh culture suspension to 3.5ml of overlay agar). The activity was defined as the reciprocal of the highest dilution which demonstrated complete inhibition of the indicator lawn and was expressed in activity units (AU) per milliliter of culture medium.

In further experiments we concentrated the inhibitory compound by concentration with PEG 6000 (dialysis bag containing the culture filtrate was covered with solid PEG 6000 and maintained until the volume was significantly reduced) or by precipitation with ammonium sulphate (70 % final concentration). The precipitate obtained after ammonium sulphate precipitation was dissolved in 3mM phosphate buffer pH 7.5 and dialysed overnight against the same buffer. After these treatments the samples were tested quantitatively as described. In the case of bacteriocin produced by *Lb. acidophilus* IB801 the proteinaceous solution obtained after ammonium sulphate precipitation was subjected to chloroform extraction (5 volumes). The organic and the aqueous phases were examined for inhibitory activity against indicator strains.

Preliminary tests were performed in order to estimate the molecular weight. SDS-polyacrylamide gel electrophoresis was carried out to analyse protein profiles for bacteriocin activity. Preparation of stacking gel (10%) and separating gel (20%) was performed according to the method described by Giulian et al. (1983).

RESULTS AND DISCUSSION

1. *Screening of LAB for antagonistic activity*

A total of 8 strains of lactobacilli were tested for antimicrobial activity against different bacteria. At the beginning we tested the antagonism between different lactobacilli using flip-streak method.

A strong inhibitory action against lactobacilli exhibited *Lb. plantarum* (Fig. 1) but this result is rather due to the ability of this strain to generate hydrogen peroxide. It is well known that hydrogen peroxide can increase to effective antimicrobial levels because the lactic acid bacteria lack catalase activity (Lindgren and Dobrogosz, 1990).

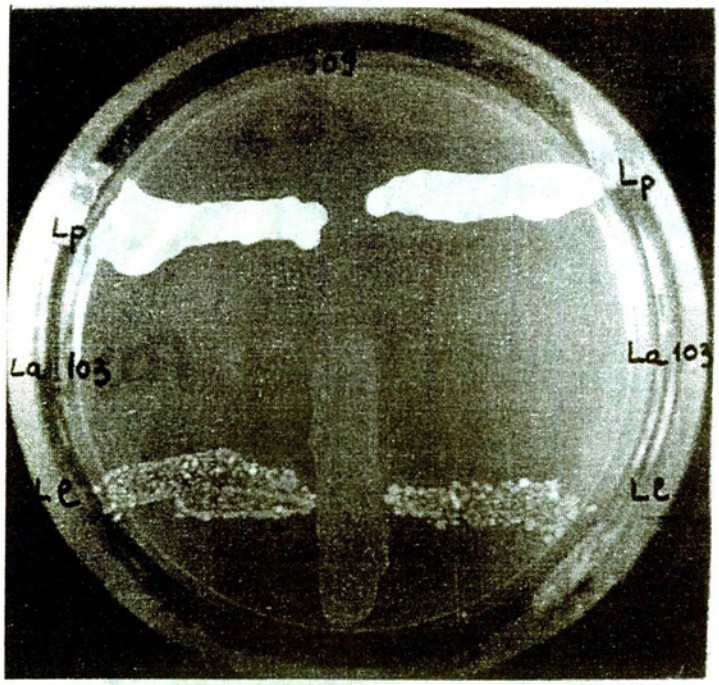

Fig.l. Antagonism between different lactobacilli.
Lp = *Lb. plantarum* 119
La103 = *Lb. acidophilus* 103
Ll = *Lb. lactis*
509 = *Lb. delbreuckii* subsp.*bulgaricus* 509

A diffusible inhibitory activity presented *Lb. acidophilus* IB801 and *Lb. acidophilus* 103 against other similar strains (*Lb. acidophilus LAP*, *Lb. delbreuckii* subsp. *bulgaricus* 509, *Lb. helveticus* 101 and 102).

No significant activity of neutralized cell-free supernatant of *Lb. acidophilus* IB801 was detected against other Gram positive and Gram negative bacteria.

2. *Preliminary characterization of inhibitory substance produced by Lb. acidophilus IB801*

To determine the nature of antibacterial compound, the effect of different factors (catalase, pH, protease or temperature) on inhibitory activity was tested.

The treatment with catalase of the supernatant from *Lb.acidophilus* IB801 has no effect on inhibitory activity. This means that hydrogen peroxide is not the inhibitory agent.

Moreover, the adjustment of pH value of the culture supernatant to 4.0, 6.5, 7.0 and 8.0 does not modify the inhibitory activity and these results show that the inhibition is not due to lactic acid.

We also tested the sensitivity of inhibitory compounds to a variety of proteolytic enzymes. Proteases used were : trypsin, pronase and proteinase K, at 0.5mg/ml final concentration for each of them. Incubation of the mixtures for 1h at 37°C completely destroyed the antimicrobial activity indicating that these substances are proteins (Fig.2).

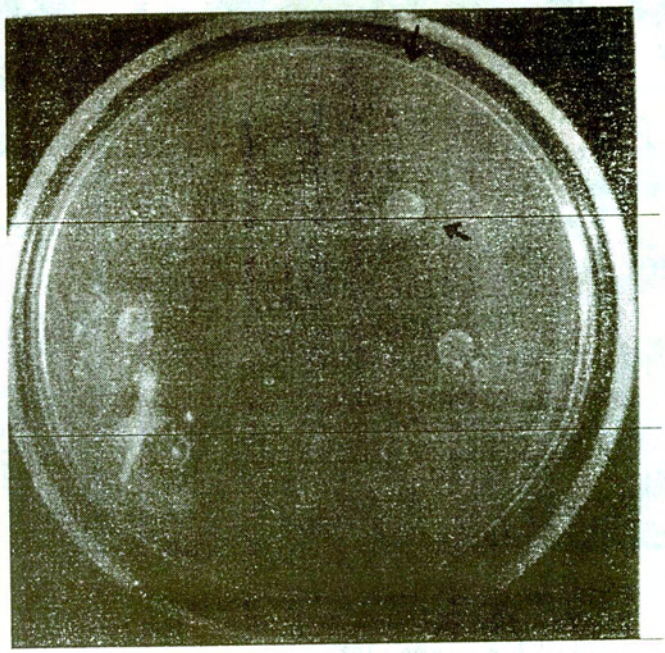

Fig.2. The effect of trypsin (0,5 mg/ml) on inhibitory activity of *Lb. acidophilus* IB801. The arrow indicates the sample treated with the proteolytic enzyme.

The culture supernatants were subjected to heat treatment: at 100°C for 15 minutes and at 70°C for 30 minutes. The inhibitory effect was not significantly altered by these treatments.

The properties of the inhibitory compound produced by *Lb. acidophilus* IB801 allow us to classify it among the bacteriocins (De Vuyst and Vandamme, 1994; Dodd and Gasson, 1994).

3. *Aspects concerning the production of bacteriocin by Lb. acidophilus IB801*

The production of the bacteriocin by *Lb.acidophilus* IB801 was examined in RCM medium. The profile of microbial growth and bacteriocin production at uncontrolled pH is presented in Fig.3. Exponential growth took place during a period of about 7-8 h and the inhibitory activity was already detectable after 3-4 h of growth. Bactericidal activity was tested against *Lb. delbreuckii* subsp. *bulgaricus* 509. pH variation during the same period of cultivation of *Lb. acidophilus* IB801 is presented in Fig. 4. The highest inhibition zone was reached after 8-10 h of cultivation (at the end of the exponential phase) and decreased after 12 h. After 24 h of cultivation the diameter of the inhibition zone was similar to that observed after 10 h.

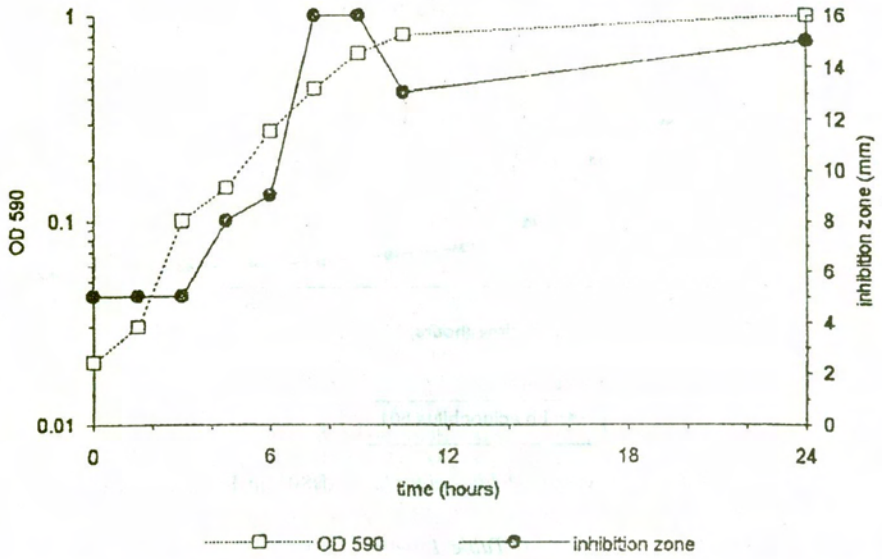

Fig.3. Production of bacteriocin by *Lb. acidophilus* IB801 in RCM liquid medium (without pH control). Antimicrobial activity was tested against *Lb.delbreuckii* subsp. *bulgaricus* 509 according to the method of Tagg and McGiven (1971). The diameter of inhibition zone includes the diameter of wall (5mm).

In order to establish the optimal conditions for higher bacteriocin production, the influence of some environmental factors was examined. One of these factors was the composition of culture medium. Using MRS liquid medium the level of inhibitory activity of *Lb. acidophilus* IB801 was reduced (100-200 AU/ml). When RCM containing Tween 80 (RCMT) was used for the cultivation of this strain, the inhibitory activity was increased (double comparative with those from MRS). In some experiments we varied the composition of RCMT: we replaced peptone with bio-Tripcase (bio-Merieux) (pancreatic hydro-lysate of casein). The level of inhibitory activity after 24h of cultivation in these conditions was significantly increased: clear inhibition zone was observed at 1/16 dilution and weakly inhibition at 1/32. Antagonistic compound produced by *Lb. acidophilus* IB801 was characterized as a

proteinaceous substance, heat-stable, active at pH 6.5-7.0, precipitable with ammonium sulphate. The bacteriocin produced by this strain was recovered as a pellicle at a saturation level of 70% ammonium sulphate. The floated precipitate as well as the pellet obtained after the centrifugation of the suspension rested were dissolved in 3mM phosphate buffer pH 7.0. The floated material was extracted with 5 volumes of chloroform and examined for inhibitory activity (Table 1). A good inhibitory activity was detected in the ammonium sulphate precipitate (both pellet and pellicle) and at the solid floated phase obtained after chloroform extraction.

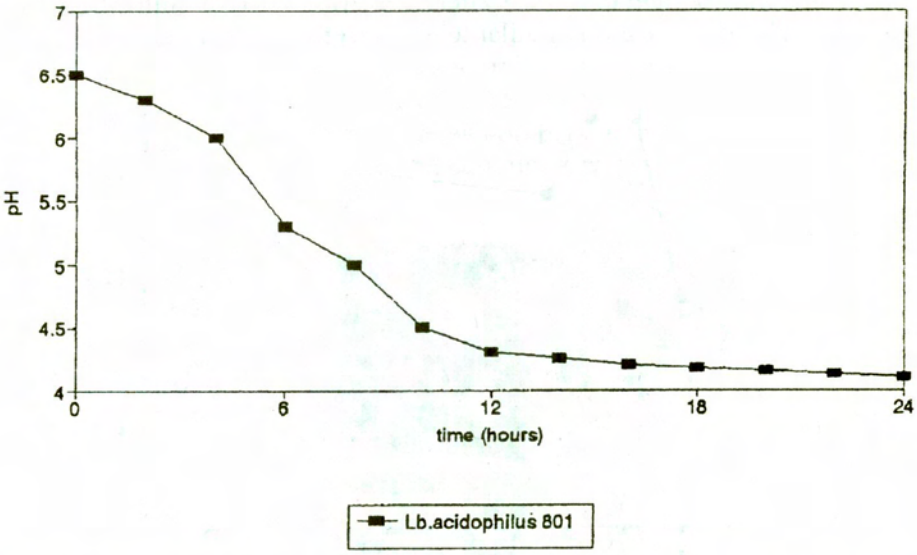

Fig.4. pH variation during cultivation of *Lb. acidophilus* IB801 in RCM liquid medium.

Table 1

Bacteriocin activity (AU-ml) of *Lb. acidophilus* IB801 in different samples

Sample	AU/ml	Activity recovery (%)*
1. culture supernatant (500ml)	400	100
2. concentrated with $(NH_4)2\ SO_4$ (70%)		
– aqueous supernatant	0	–
– pellet resuspended in 25ml of 3mM phosphate buffer pH 7.0	800	
– floated precipitate resuspended in 25ml of 3mM phosphate buffer pH 7.0	1600	60
3. organic phase		
– supernatant	100	
– sediment resuspended in 2.5ml 3mM phosphate buffer, pH 7.0	3200	4

* all the results were reported to the total activity of culture supernatant (100%)

In order to estimate the molecular weight of the bacteriocin produced by *Lb. acidophilus* IB801, partially purified sample (after ammonium sulphate precipitation) was subjected to SDS-polyacrylamide gel electrophoresis according to the method of Giulian et al (1983). The concentration of polyacrylamide in the separating gel was 20%. After 18h of electrophoresis at 40V the gel was cut: half of it, containing also some molecular weight markers (cytochrome C, lysozyme and myoglobin) was stained with Coomasie Brilliant Blue R250. The other half was extensively washed with sterile water and subjected to bioassay (with *Lb. helveticus* 102 as indicator organism). A large inhibitory area in the middle of the gel was detected after 24h of incubation corresponding to some protein bands which migrated close to the cytochrome C (16500 dal) band. These results don't allow us to estimate correctly the molecular weight of this bacteriocin. Further experiments will clarify this aspect.

ACKNOWLEDGEMENTS. This research was supported by ERBCIPACT 940160 grant (EU-Copernicus Project).

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Received April 3, 1996

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EPICOCCUM PURPURASCENS. II. "IN VITRO" RELATIONSHIPS WITH PHYTOPATHOGENIC FUNGI

TATIANA EUGENIA ŞESAN, MARIA OPREA

Investigations on the biology of fungus *Epicoccum purpurascens*, isolated from wheat kernels revealed by the method of double cultures its relationships with 22 isolates belonging to 13 plant pathogenic fungus genera, economically important for cropped plants: *Sclerotinia* (2 species, 7 isolates), *Rhizoctonia* (2 species with one isolate each), *Pythium, Fusarium, Pyrenophora* (*Helminthosporium*), *Stemphylium, Verticillium, Armillaria, Eutypa, Cytospora* (*Valsa*), *Monilinia, Botryodiplodia* (each with one species represented by one isolate).

Having in view a perspective use of the antagonistic fungus *Epicoccum purpurascens Ehrenb. ex Schlecht.* as a biocontrol agent, its developmental biological parameters have been studied (*10*), (*11*). Research continued with "in vitro" revealing of relationships between this antagonist and autochthonous isolates of some important pathogenic fungi, with a view of selecting isolates sensitive to the action of this biological agent.

MATERIAL AND METHODS

Trials used as biological material: one *E. purpurascens* isolate originating from wheat kernels (11) and 22 isolates belonging to 13 genera of plant pathogenic fungi economically significant, collected from grains, industrial and horti-viticultural plants, as well as from soil (table 1).

In order to reveal "in vitro" relationships between *E. purpurascens* and the test-plant pathogenic fungi, the method of double cultures has been used (1). Scoring was done by calculating the ratio **x** between the inner (i) and outer (e) radius of the test-fungus (A) and the antagonist *E. purpurascens* (B), with the formula: $x = iA/iB \times eB/eA$. Tests were organized in variants with 4 replications each, data being treated by the analysis of variance.

RESULTS AND DISCUSSION

When testing the antagonism of *E. purpurascens* isolate to all 22 isolates of pathogenic fungi, behaviour was different, as depending on the test-pathogen (table 2, plates I - III).

Table 1
Plant pathogenic fungi used in tests

Species/Isolates	Abbreviations	Host-plant and origin
Sclerotinia sclerotiorum (Lib.) de Bary	Scl. Scl.s. Scl.m. Scl.p. Scl.n.	Sunflower - I.C.C.P.T.Fundulea; Soybeans - I.C.C.P.T. Fundulea; Carrot - Bucharest; Parsnip - I.L.F. Braşov; Chickpeas - S.C.C.I. Valul lui Traian Constanţa;
Sclerotinia minor Jagger	S.m. S.m.1	Lettuce - Franţa (Perpignan)-P. Davet; Sunflower - Satu Mare;
Botrytis cinerea Pers.	B.c.2 B.c.4 B.c.5	Grapes - S.C.V.V. Odobeşti; Eggplants - Bucureşti; Carrot - I.L.F. Braşov;
Rhizoctonia bataticola (Taub.) Butl.= Sclerotium bataticola Taub.	Scl.bat.	Sunflower - Ialomiţa;
Rhizoctonia fragariae Husain & Mc Keen	Rh.fr.	Strawberries - Franţa - Avignon;
Pthium sp.	P	Soil - Bucharest;
Armillaria mellea (Vahl.) Karst.	Arm.	Grapevine stocks - S.C.V.V. Odobeşti;
Monilinia laxa (Aderhold & Rohl.) Hone ex Whetzel	Mon.	Apricot - S.C.P.P. Băneasa;
Eutypa lata (Pers.) Tul. & C. Tul.	Eutypa	Grapevine - S.C.V.V. Odobeşti;
Cytospora cincta Sacc. (Valsa cincta Fr.)	Cyt.	Apricot - S.C.P.P. Băneasa;
Botryodiplodia theobromae Pat.	B.th.	Magnolia grandiflora -Bucharest. Herăstrău park;
Fusarium graminearum Schwabe	F.gr.	Wheat kernels - I.C.C.P.T. Fundulea;
Pyrenophora graminea Ito & Kuribayashi = Drechslera graminea (Rabenh. ex Schlecht.) Shoem. =Helminthosporium gramineum Rabenh. ex Schlecht.	H.gr.	Barley kernels - I.C.C.P.T. Fundulea;
Stemphylium radicinum (Meier, Drechsler & Edy) Neergaard = Alternaria radicina Meier, Drechsler & Edy	St.	Carrot - Bucharest;
Verticillium dahliae Kleb.	V.dahl.	Cotton- Brânceni (Teleorman).

E. purpurascens demonstrated "in vitro" after 5 days an antagonistic capacity at various levels to 10 isolates of plant pathogenic fungi belonging to 6 genera: *Sclerotinia*, *Botrytis*, *Rhizoctonia*, *Eutypa*, *Cytospora* (*Valsa*), and *Monilinia*.

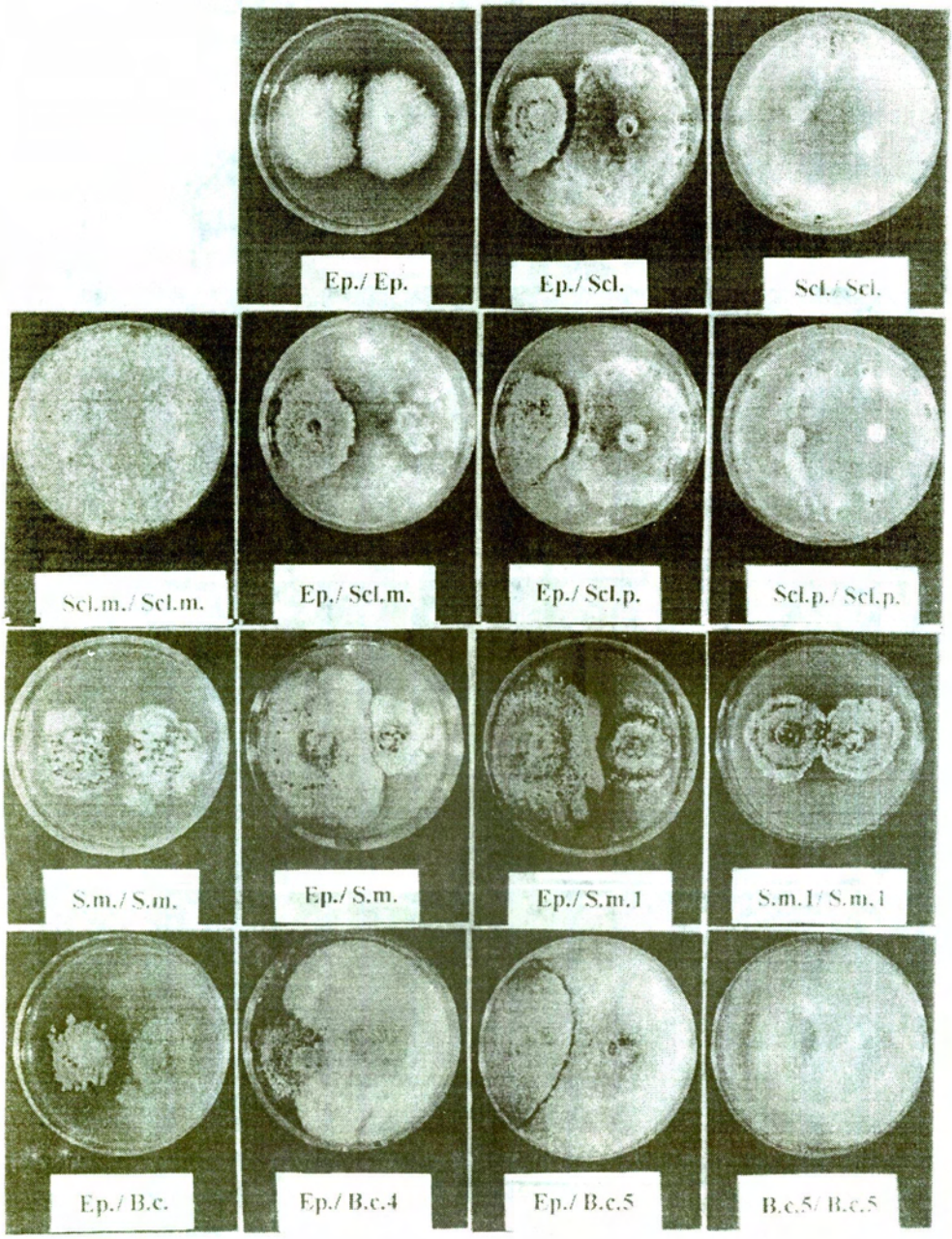

Plate I - Macroscopic aspects of *Epicoccum purpurascens* antagonism versus: *Sclerotinia sclerotiorum* (Scl., Scl.s., Scl.m., Scl.p., Scl. n.), S. *minor* (S.m., S.m.l), *Botrytis cinerea* (B.c., B.c.4, B.c. 5).

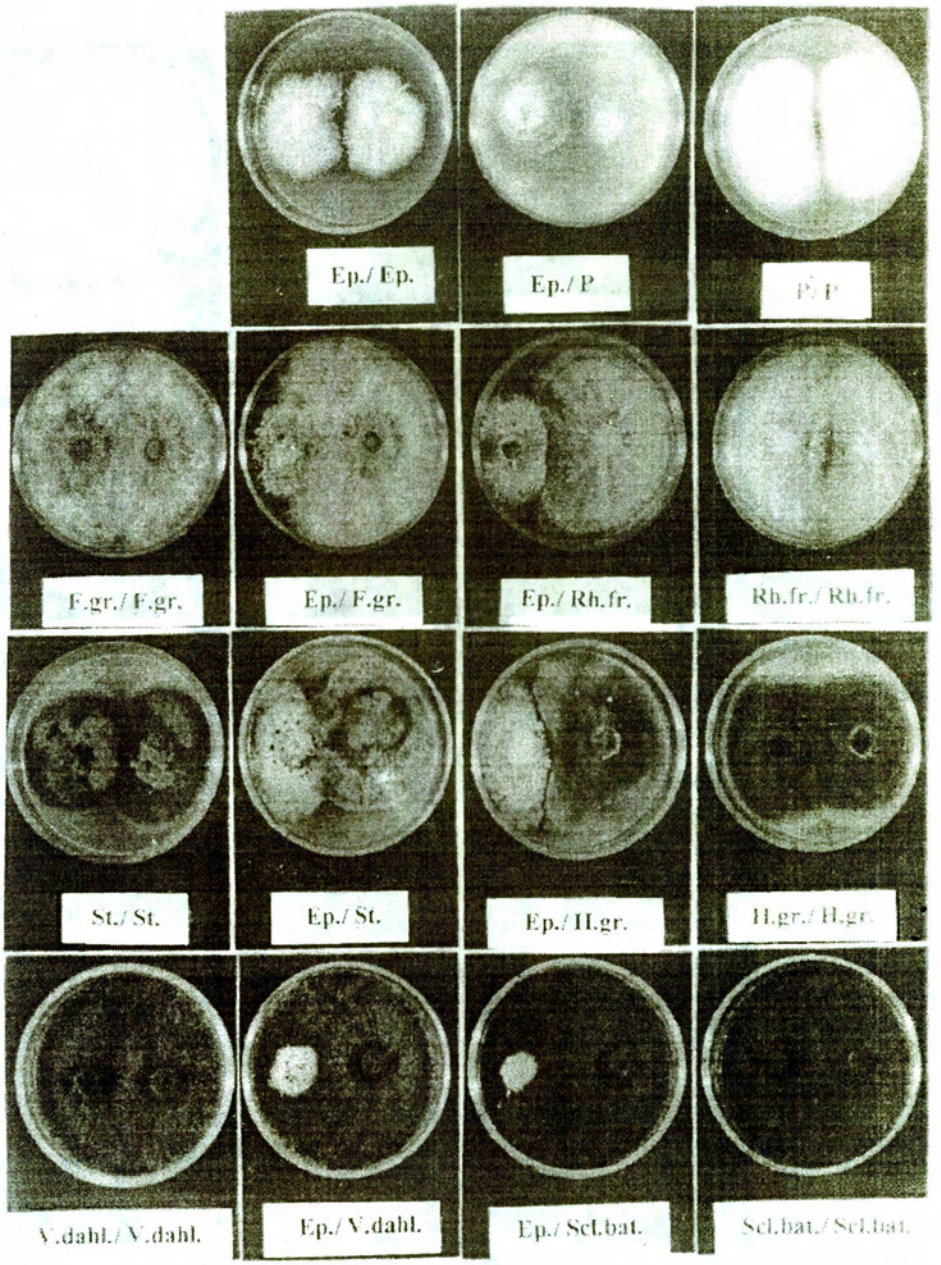

Plate II - Macroscopic aspects of *Epicoccum purpurascens* antagonism versus: *Pythium* sp. (P), *Fusarium graminearum* (F.gr.), *Rhizoctonia fragariae* (Rh.fr.), *Stemphylium radicinum* (St.), *Pyrenophora graminea*, syn. *Helminthosporium gramineum* (H.gr.), *Verticillium dahliae* (V.dahl.), *Rhizoctonia bataticola*, syn. *Sclerotium bataticola* (Scl. bat.).

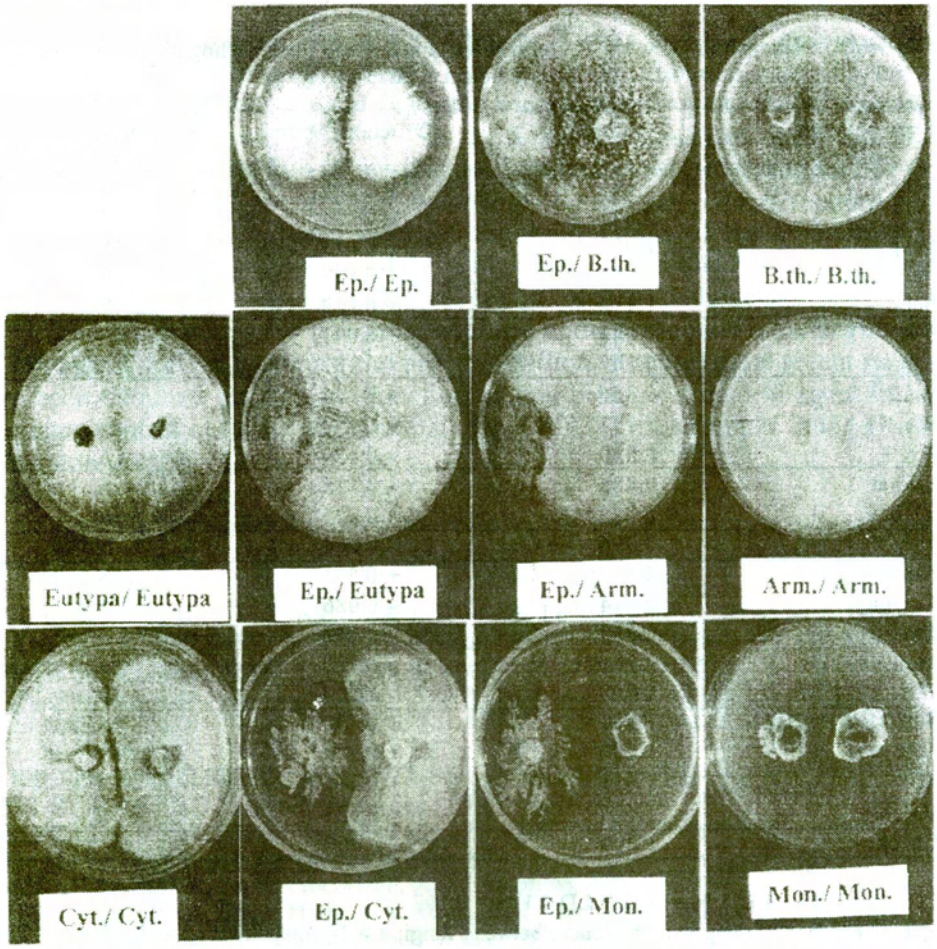

Plate III - Macroscopic aspects of *Epicoccum purpurascens* antagonism versus: *Botyodiplodia theobromae* (B.th.), *Eutypa lata* (Eutypa), *Armillaria mellea* (Arm.), *Cytospora cincta* (Cyt.), *Monilinia laxa* (Mon.).

Thus, the strongest antagonism, statistically highly and distinctly significant, was noticed against *Rhizoctonia fragariae* (**x** = 0.692) and *Monilinia laxa* (**x** = 0.771).

A slightly lower antagonistic activity of the micromyceta under study was remarked against *Sclerotinia sclerotiorum* (isolate Scl.s./ soybean) and *S. minor* (isolate S.m.l/ sunflower) (**x** = 0.847 - 0.849), against 2 isolates (B.c.2 and B.c.5) of *Botrytis cinerea* (**x** = 0.830 - 0.856), against *Cytospora cincta* (**x** = 0.858) and *Rhizoctonia (Sclerotium) bataticola* (**x** = 0.885).

The lowest antagonism was manifested against *Eutypa lata* (**x** = 0.914) and *Pythium sp.* (**x** = 0.996), practically absent.

Table 2

"In vitro" relationships between *E. purpurascens* and some plant pathogenic fungi, expressed as the coefficient **x**, after 5 days

Plant pathogenic isolate	x	Difference from check	Scoring relationships[a)]
Scl.	1.166	+ 0.166	N
Scl.s.	0.847	- 0.153	A
Scl.p.	1.073	+ 0.073	N
Scl.m.	1.157	+ 0.157	N
Scl.n.	1.152	+ 0.152	N
S.m.	1.041	+ 0.041	N
S.m.1	0.849	- 0.151	A
B.c.2	0.856	- 0.144	A
B.c.4	1.137	+ 0.137	N
B.c. 5	0.830	- 0.170	A
Scl.bat.	0.885	- 0.115	A
Rh.fr.	0.692***	- 0.305	A
P	0.996	- 0.004	N
Arm.	1.551°°°	+ 0.551	N
Eutypa	0.914	- 0.086	A
Cyt.	0.858	- 0.142	A
Mon.	0.711**	- 0.288	A
B.th.	1.000	0.000	N
F.gr	1.107	+ 0.107	N
H.gr	1.280°°	+ 0.280	N
St.	1.011	+ 0.011	N
V.dahl.	1.622°°°	+ 0.622	N
Check	1.000	0.000	–

LD 5% = 0.172; LD 1 % = 0.229; LD 0.1 % = 0.297

a) **x** =1 absence of reciprocal influences between fungi; **x** > 1- antagonism absent (N); **x** < 1 - the strongest antagonism, the lower values are (A).

E. purpurascens proved not to be antagonistic to the other 12 isolates of test-pathogenic fungi, and namely: *S. sclerotiorum* (4 isolates: Scl., Scl.p., Scl.m., Scl.n.), *S. minor* (S.m.), *Botrytis cinerea* (B.c.4). *Armillaria mellea*, *Fusarium graminearum*, *Pyrenophora graminea* (*Helminthosporium gramineum*), *Stemphylium radicinum*, *Verticillium dahliae*, *Botryodiplodia theobromae* (each with one isolate).

E. purpurascens behaviour from antagonistic standpoint differed from isolates of the same species in *S. sclerotiorum*, *S.minor* and *B.cinerea*, being however similar to both *Rhizoctonia* species tested.

After 7 days the antagonism of *E. purpurascens* isolate maintained against the pathogens: *C. cincta*, *B. cinerea* (B.c.2, B.c.5), *M. laxa*, *Pythium* sp., *R. fragariae*, but disappeared against *S. sclerotiorum* (Scl.s.), *S. minor* (S.m.1), *R. bataticola* and *E. lata*.

Our results regarding *E. purpurascens* antagonism against *C. cincta* and *M. laxa* in apricot-tree are in agreement with those in literature, on the same apricot pathogens (3), (4), (5), (8), (12), as well as on those on *B. cinerea* in grapevine (2),

(9). Data referring to inhibition of the pathogen *P. graminea* by *E. purpurascens* (7) have been not confirmed in our trials. As for the inhibition of *S. sclerotiorum* in lettuce (6), among the isolates of the same species tested by us, only the isolate from soybean (Scl.s.) was affected. Nor the *S. minor* (S.m.) isolate from lettuce was susceptible to the action of the fungus *E. purpurascens*, in our tests. For the other test-pathogens (*R. fragariae*, *R. bataticola*, *A. mellea*, *E. lata*, *B. theobromae*, *S. radicinum* and *V. dahliae*) used as target-pathogens of the antagonist *E. purpurascens*, we have not found data in literature, our results having priority in attempts to find new means for biological control of these pathogens.

The most encouraging perspective for use of the antagonist *E. purpurascens* for biological control is against the peach *Monilinia*-disease; quite recent results have been obtained through introduction of this biological control agent in the integrated control of this disease in Spain (3), (40), (5), these results being also to be extended to apricot-tree.

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Received March 20,1996

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ION T. TARNAVSCHI, GABRIELA ŞERBĂNESCU-JITARIU, NATALIA MITROIU-RĂDULESCU, *Monografia polenului florei din România* (A monograph of pollen in the flora of Romania), Editura Academiei Române, Bucureşti, vol. I (1981); II (1987); III (1990); IV (1994), 447 pp., 341 plates with 1983 figures.

The four volumes of the work we are going to speak about represent an editorial event of great importance for the Romanian botanists and came to light in the period 1981-1994. It was expected for a long time a synthesis of the researches made during the last three decades by the famous school of palynology from the University of Bucharest, a school which was initiated and guided until 1987 by the well-known phytomorphologist Ion T. Tarnavschi. This school appreciated both in the country and abroad has given several valuable contributions concerning the pollen morphology for over 2200 species of Angiosperms until 1980. It was thus absolutely necessary a synthesis of the researches so accurately made, based on the pollen analysis taken from an impressive number of taxa, especially from the spontaneous flora, on the basis of a rich literature of speciality. This synthesis comes as a direct help of the taxonomy in order to discover the ways of evolution of the vegetal kingdom and the Angiosperms especially. Lately the morphological and anatomical researches have became more and more necessary for the thorough characterisation of the **Spermatophyta**; the palynological researches have a main role in the domains used today by the taxonomy and phylogeny of the plants.

As an unanimous recognition of the value of the first volume issued in 1981 it was awarded the "Emanoil Teodorescu" prize by the Romanian Academy in 1983. We can also add the interest with which the Romanian botanists were waiting the series,which is covering a gap in the Romanian literature from this domain and in the Romanian science in general.

The four volumes, coordinated with high scientific competence by professor Gabriela Şerbănescu-Jitariu, Ph.D., comprise the morpho-palynological analysis and the determination keys of the pollen for over 2500 species (the majority belonging to the spontaneous Romanian flora) of 768 genres from 116 Dicotyledonous families (**Magnoliatae**); some of these species are cultivated in our country, only a few of them are exotic plants (tropical, subtropical and from the temperate climate).

The exposure of the morpho-palynological data and of the nomenclature was made taking into consideration the classification and the denominations comprised in the "*Flora of Romania*" (1952-1976), correlated with the"*Flora Europaea*" (1964-1980) and with the "*Syllabus der Pflanzenfamilien*" (1964). The fourth volume issued in 1994 closes the morpho-palynological analysis and presentation of the determination keys of the pollen from taxa of the last families of **Magnoliatae (Dicotyledonatae)**.

In all four volumes the description of the pollen grains morphology is extremely clear, using a very well selected terminology, widely explained in the first volume. The 1983 drawings, accomplished with an excellent accuracy and carefulness, are grouped in 341 plates together with all the necessary explanations.

Although the morpho-palynological analysis deals with such a large number of species (2479) the authors have succeeded to synthetise the information obtained as a result of multiple researches in a very economical and clear way (447 pages), without damaging the integrity of the book, the text being clear, precise and understandable, with all the necessary explanations for the drawings. All these are the proof of the wide documentation, long experience and scientific accuracy, supported by the appreciations of which all the staff of palynologists of the University of Bucharest are enjoying both in the country and abroad.

As for the utility of this remarkable work: the text presented for each species, the underline of the specific features, the used terminology, the keys that were used for the determination-by all these the monograph represents a very useful tool, for the specialists working both in the domain

of the fundamental research and in the applicative domains. More and more the interest for the palynological works has increased.

The researchers working for the analysis of the fosile and actual pollen diagrams will have at hand a synthesis work, a valuable quantity of information of this domain, which will allow them to bring in the near future, new fundamental clarifications in the study of vegetation history of our country, of Europe in general.

This work will be of a real interest for those researchers from other domains such as: geography, pedology, archeology etc.

The necessity of the pollen analysis is justified also for allergologists who are concerned with the implications of the pollen on a wide category of people. Because of the pollen, a component often presented in the breathing air, there appear pathological manifestations at the level of the eye-conjunctiva mucosa and especially, breathing problems. On this base, the detection of allergy to pollen is extremely important, being absolutely necessary the establishing of the pollen provenance, the group of plants which induce these pathological situations. More than this the importance of pollen results from the fact that it has a rich and equilibrated content of nutritive factors, being used in food (by insects and man), dieting and therapeutical purposes.

"*A monograph of pollen in the flora of Romania*" fulfills the highest scientifical exigences, representing at the same time a model for a handbook based on the shape and structure of the pollen grains, enscribing itself among the valuable contributions which are completing the monumental work "*Flora of Romania*".

As a proof of the interest with which this valuable work was received there are the book-reviews appeared for each volume, the citation and comment in different studies of speciality published both in the country and abroad, its solicitation by several specialists from different countries in the world.

As it is well known "*Flora Europaea*" represents a work of a special interest for our continent. We are convinced that this book will be followed by a morpho-palynological synthesis at the scale of the same continent but without synthesis made in different floral regions from different countries, such a work cannot be yet done. Thus it results the merit of the synthesis made by the Romanian palynologists, knowing that few countries have at present such a "palynological flora", together with such a clear, detailed and complete set of drawings.

The bibliography at the end of each volume is thoroughly selected, containing the great synthesis made until now all over the world as well as the main original contributions of the Romanian authors.

The alphabetical index of the families, genera and species with which each volume is ended, allows a slight follow of different taxa and draws the attention of the reader on some synonymies discussed where is the place and case. We shall underline the authors' considerations concerning the dependence of some genera to one family or another, the rank of one family or subfamily given to some plant groups.

Regarded and judged as a whole this monograph represents a precious synthesis of a modern domain, a valuable source of documentation, a working tool for the specialists of various domains who are directly or indirectly studying the plants, it is a model of conception, clarity, concision, accuracy and intelligibility.

But first and foremost "*A monograph of pollen in the flora of Romania*" (the four volumes are dedicated to **Magnoliatae** is a scientifical work of high class, using the original largely detailed researches of its authors, with a wide bibliographical documentation and a rich and impressive iconography.

Our justified appreciations and the satisfaction of reading these four volumes in perfect technical conditions, published by the famous Publishing House of the Romanian Academy, are accompanied by our sincere congratulations for the authors and especially for the coordinator of this work, the distinguished professor Gabriela Şerbănescu-Jitariu, Ph. D., person of special exigence and scientific probity whose efforts made possible the publication of the last three volumes with sacrifices not to be ignored. We do hope that she will prove the necessary energy to coordinate the fifth volume which should contain morpho-palynological data concerning the **Liliatae (Monocotyledonatae)** thus stimulating the eventual desire of some young people who will like to initiate themselves in this very captivating domain of biology: palynology.

C. Toma, Angela Toniuc

AVIS AUX AUTEURS

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

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

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ISSN - 0035 - 3914

REV. ROUM. BIOL. – BIOL. VÉGÉT., TOME 41, N° 2, P. 89–154 , BUCAREST, 1996

Lei 2000