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REVUE ROUMAINE DE BIOLOGIE

SÉRIE DE BIOLOGIE VÉGÉTALE

TOME 38 1993 N° 2

juillet — décembre

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GENETIC RECOMBINATION THROUGH PROTOPLAST FUSION BETWEEN *STREPTOMYCES FLAVOVIRENS* AND *S. GRISEUS*

CĂLINA PETRUȚA CORNEA[1], I. VĂTAFU[1], ANETA POP[2], AURELIA BREZEANU[1], L. SAVU[1]

Two stable hybrids obtained by protoplast fusion between *Streptomyces flavovirens* and *S. griseus* exhibit a modified pattern of endoglucanase activity. Another recombinant strain, obtained by "self-fusion" of protoplasts from *S. flavovirens* showed an enhancement of endoglucanase synthesis. Endoglucanases synthesized in the hybrids differed in their optimal conditions of activity as compared with the parental enzymes. Different aspects of protoplasts fusion are also presented on microelectronographs.

Protoplast fusion provides a potentially useful route for combining genetic characteristics from divergent strains of different species to improve commercial product yield, develop hybrid biosynthetic pathways and produce novel chemical structures (5, 12). Polyethylene glycol-induced protoplast fusion is an efficient method for producing recombinants in intraspecific crosses with a variety of Actinomycetes, particularly *Streptomyces* species (6). Aside from the advantages of high recombination frequencies, protoplast fusion overcomes the problems associated with differences between species that may limit the use of standard mating procedures. This method seems attractive to study the supposed multigenic nature of cellulose degradation systems of different cellulolytic microorganisms (1).

We report here the fusion between protoplasts isolated from two species of Streptomyces and characterization of some hybrids.

MATERIAL AND METHODS

1. MICROORGANISMS

S. flavovirens SfG3 resistant to tetracycline, having the ability to degrade cellulose and a spontaneous kanamycine resistant mutant of a streptomycin producing *S. griseus* strain were the organisms used in our experiments.

2. MEDIA AND GROWTH CONDITIONS

S. flavovirens and *S. griseus* as well as their hybrids were maintained on YEME medium (2) with the requisite antibiotics wherever needed.

For cellulase production, cultures were grown in a basal medium described by Ishaque and Kluepfel (9) supplemented with 1% CMC or 1% microcrystalline cellulose.

For protoplast isolation the bacteria were cultivated for 48 hours in YEME liquid medium with 0.8% glycine for *S. griseus* and without

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glycine for *S. flavovirens*. Hypertonic medium for protoplast formation was described elsewhere (3).

For regeneration of cell wall, two media were used : a lower layer represented by MRM (3) and an upper layer consisting of the same MRM soft agarised for *S. griseus* or MRM modified by replacing glucose with 1% CMC and agar with 0,4% agarose for *S. flavovirens* and for hybrids. To select the fusants, the upper layer was supplemented with kanamycine (20 μg/ml), tetracycline (10 μg/ml) and streptomycin (50 μg/ml).

3. PROTOPLAST ISOLATION, REGENERATION AND FUSION

Protoplast formation and regeneration in *S. flavovirens* was described in detail in a previous paper (4). Protoplasts from *S. griseus* were prepared by the same method except that the mycelium used was cultivated in the presence of 0,8% glycine to render it sensitive to lysozyme attack. Fusion was performed by mixing equal volumes of protoplast suspensions with a solution of 40% PEG 6000 in hypertonic medium according the indications of Hopwood et al. (7). Fusion frequency was estimated as the ration of the number of colonies grown on the selective medium to the number of protoplasts mixed from each strain (14).

4. ENZYME ASSAYS

The action of culture supernatants on CMC and filter paper was assayed as described by Mac Kenzie et al. (11) in 50mM phosphate buffer pH 5.8 for FP-ase or pH 7 for CMC-ase at 45°C and the reducing sugar release was determined with DNS reagent (16). One unit of enzyme activity was defined as the amount of enzyme releasing 1 μM of reducing sugar (expressed as glucose) per minute and mg of protein. Protein concentrations were estimated by the method of Lowry (10).

CMC degradation was estimated by the daily measuring of the clear zones produced by different *Streptomyces* strains (parental and hybrids) over a period of 7 days. Each strain was cultivated in 10 ml of basal medium containing 1% CMC (4 tubes for each strain). The degree of CMC digestion was evaluated by comparing the inoculated with the uninoculated tubes.

5. TRANSMISSION ELECTRON MICROSCOPY

The methods used for electron microscopy were described elsewhere (3).

RESULTS AND DISCUSSIONS

1. CELLULOLYTIC ACTIVITIES OF *S. FLAVOVIRENS* CULTURES

S. flavovirens SfG3 used in the present study was isolated and characterized in our laboratory. The strain is able to grow in minimal medium containing CMC or cellulose powder as unique source of carbon. It also

grows in basal medium containing 1% wheat bran. Growth on these types of media for 72 hours resulted in substantial level of CMC-ase and FP-ase (Table 1).

Table 1

Extracellular protein concentrations and enzyme activities of *S. flavovirens* grown on various carbon sources

Carbon source	Protein (mg/ml)	CMC-ase (mU)	FP-ase (mU)
CMC	0.2	157.4	9.5
Avicel	0.08	93	7.5
Wheat bran	0.350	52.8	1.9

The other strain used in our experiments, *S. griseus* presented a very poor growth only on basal medium containing 1% CMC but the growth was absent in the medium supplemented with cellulose powder. We did not identify any reducing sugar release in the assays for cellulase activities of *S. griseus* culture supernatants.

2. PROTOPLAST ISOLATION, REGENERATION AND FUSION

It is well known that streptomycetes have a complex cell wall so a treatment with a convenient concentration of glycine is usually necessary to convert mycelium to protoplasts (7, 13). The strain of *S. griseus* tested by us needs a pretreatment with 0.8% glycine to an efficient conversion to protoplasts. Contrarily, *S. flavovirens* SfG3 was able to form protoplasts at a good efficiency (more than 90%) without pretreatment with glycine, the bacterial cell wall being sensitive to lysozyme attack.

S. griseus protoplast suspensions usually yielded approximately 10^6 CFU/ml on MRM regeneration medium from 7×10^8 protoplasts per ml by direct count, indicating 7% regeneration. *S. flavovirens* gave a higher frequency of regeneration (19.5—23%) both on MRM regeneration medium and on the regeneration medium containing 1% CMC instead of glucose.

3. SELECTION AND CHARACTERIZATION OF HYBRIDS

Fusion between protoplasts of *S. griseus* Sm^r Km^r and *S. flavovirens* Tc^r resulted in the appearance of 1.5×10^3 colonies on selective medium (MRM with 1% CMC and antibiotics). The fusion process was also analysed by electron microscopy (Fig. 1) but at this stage we were not able to say which of the "fusion bodies" are formed by intraspecific fusion and which by interspecific fusion.

The products of interespecific fusion were selected both for the resistance to antibiotics (streptomycin, kanamycin and tetracycline) and for cellulase production. The colonies showing a large area of CMC degradation (after staining with Congo red) (15) were submitted for segregation analysis and cellulase production. Among these only 15 colonies maintained the simultaneous resistance to antibiotics. Moreover, these colonies presented a varied ability of CMC degradation. These results indicate that a large amount of the initial resistant colonies represents unstable "biparental forms" which rapidly segregate yielding parental strains sensitive to antibiotics.

We selected for subsequent studies only 5 colonies with and without resistance to all the antibiotics, designated : FG1 and FG20 ($Tc^rSm^sKm^s$), FG12 ($Tc^sSm^rKm^r$), FG2 and FG11 ($Tc^rSm^rKm^r$). These strains were tested for their ability to use CMC as a unique source of carbon and to clarify the medium as was described in Materials and Methods. The results are presented in Fig. 2. FG1 and FG20 presented an enhanced (double) ability of CMC hydrolysis but their morphology resemble *S. flavovirens* parental strain. This could be explained as a self-fusion process which was also noticed by other authors (8).

Despite of FG11 and FG2 morphological resemblance to *S. flavovirens*, their resistance to antibiotics suggested that they are hybrids resulted from interspecific fusion. However, the contribution of *S. griseus* genome in the recombination events was limited to the genes conferring resistance to streptomycin and kanamycin. A special behaviour had FG12 which was morphologically similar to *S. griseus* and also presented the same sensitivity to antibiotics. However, we presume that this strain could be a hybrid between the parental strains, taking into account its aspect and the fact that it is able to grow and hydrolyse even at a reduced extent the CMC substrate (Fig. 2).

Our results indicate that the recombination events could be achieved both in the case of intraspecific and interspecific protoplast fusion. This method (protoplast fusion) is an important tool to obtain recombinant strains with enhanced abilities of degradation of different substrates, therefore they synthesize a large amount of hydrolytic enzymes.

Received April 21, 1993

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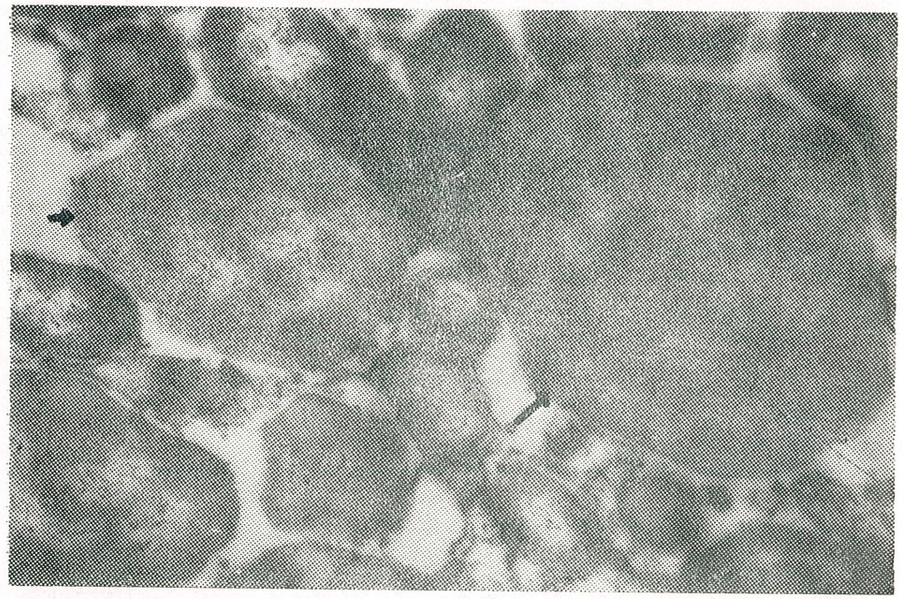

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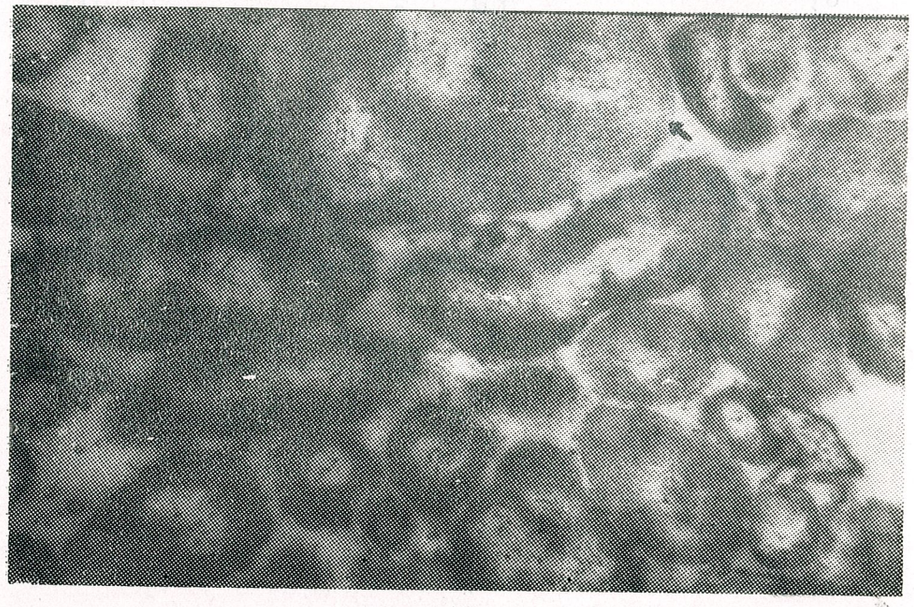

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Fig. 1. — Different stages of interspecific protoplast fusion. Arrows indicate ; a — very large cells with irregular shape ; b — "fusion bodies".

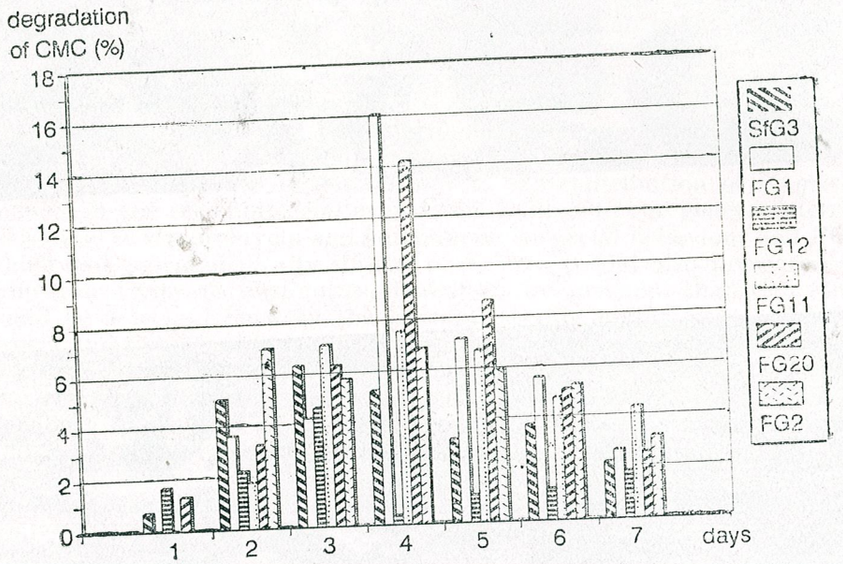

Fig. 2. — CMC degradation by different strains of *Streptomyces*.

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NECTARIES ULTRASTRUCTURE AND THEIR SECRETORY ACTIVITY IN *NIGELLA DAMASCENA* L. (Fam. *RANUNCULACEAE*)

GABRIEL C. CORNEANU*, CONSTANTIN CRĂCIUN**, VERONICA CRĂCIUN**

Unguiculate perigonal nectaries in *Nigella damascena* L. are situated on modified, reduced petals. Thoy have an internal unistratified epidermis, noctariferous parenchyma and an external epidermis. The cells from nectariferous parenchyma are in different secretory phases. In the secretory phase, the cells have a rich cytoplasm with numerous cellular organelles : mitochondria, endoplasmic reticulum, Golgi, chloroplasts with granal system and plastoglobules, ribosomes and a nucleus in an intense metabolic activity. In the synthesis of the secretion product paricipate also chloroplasts together with endoplasmie reticulum and Golgi. This is initially accumulated in subepidermal cells and then is eliminated through an exocytosis process at the cuticle surface. In the middle of the nectariferous parenchyma there are one or more cells in which are accumulated residual products. Inside these cells there are a rich endoplasmie reticulum and numerous Golgi implied in the synthesis of hydrolytic enzymes from lysosomes.

Higher plants present different types of secretory tissues represented by isolated or grouping cells dispersed through parenchymas (3). Their structural features, the cellular elements implied in the synthesis process and the ways of elimination of the secretary product, present different aspects at different species (2), (3), (4), (7), (8).

In the present study, the ultrastructural features of the nectaries at *Nigella damascena*, the cellular organelles implied in the secretary process and the exocytosis of the synthetising products were performed.

MATERIAL AND METHODS

Nigella damascena L. presents 5—8 unguicular perigonal nectaries placed on reduced, modified petals (6). The ultrastructural features of the nectaries, as well as the organelles implied in the secretory process were performed at the complete flowering. The nectaries fragments were prefixed in 3% glutaraldehyde (3 h), postfixed in 1% Millonig solution (1 1/2 h) and then included in vestopal W. The seriated asections of about 800—900 Å thickness were contrasted with uranyl acetate and lead citrate. The ultrastructural feature analysis of the cells as well as of the physiological processes (secretory activity, lysis and exocytosis process) were effectuated at the electron microscope TESLA BS-500 (Cluj University, Biology Department).

RESULTS AND DISCUSSIONS

1. NECTARIES ULTRASTRUCTURE

The internal epidermis made of cubic cells is unistratified, covered by a thin cuticle, on which surface is stored the product of secretion (nectar, Fig. 1). The spheric nucleus is placed at the cell basis. The

pellicular cytoplasm contains relatively few chloroplasts with a reduced granal system, mitochondria and profiles of endoplasmic reticulum.

Nectariferous parenchyma is formed by cells with an unregulated outline, in the different secretary process (cells from II—IV layers); inside the subepidermal layer are accumulated the secretory products (Fig. 2). Before the beginning of secretory activity, the cells have a pellicular cytoplasm inside prevailing mitochondria and fusiform chloroplasts with a granal system, plastoglobules and very rare starch granules, the endoplasmic reticulum profiles, ribosomes and rarely Golgi. The nucleus is spherical, in intense metabolic activity (Fig. 2). In the middle of the cell there is a vacuole in which a fine granular material is dispersed, or (at the cells in synthesis activity) the secretory product.

The external epidermis formed of slightly elongated cells, unistratified, presents the thickened external wall (Fig. 3). Cellular organelles are peripherally disposed a fine granular substance being in the middle of the cell. The rare chloroplasts present a reduced granal system.

2. SECRETORY PROCESSES

The investigationes effected on the nectaries from *Tropaeolum majus* evidenced that both the endoplasmic reticulum as well as Golgi participate in the secretory process (3). The similar findings were made in *Lonicera japonica*, *Vinca major*, *Musa paradisiaca*, a.o. (3), (4), (8).

The secretory parenchyma cells in *N. damascena* can be found in different stages (Fig. 2). In the synthesis stage, the cells have a rich cytoplasm with numerous cellular organelles : mitochondria, profiles of endoplasmic reticulum, numerous ribosomes, Golgi, chloroplasts with a granal system relatively well represented and with plastoglobuls, as well as a nucleus in an intense metabolic activity (Figs. 2, 4, 5). In all secretory cells, the endoplasmic reticulum is well developed, noting a swell of the canalicles and the presence of some vesicular formations on their length-ways (Figs. 4, 5). This phenomenon was raported also at other species (3), (7), (8.) Golgi present secretory cisternae on the *trans*-face with a homogeneous content (Fig. 5). This suggests their implication in the secretory process and/or in transforming the products structure syntetized in the endoplasmic reticulum at *N. damascena* nectaries. Previous researches established that "complex oligosaccharides are made during transport through the Golgi, determined by the order in which the protein encounters the enzymes localized in various Golgi stacks" (Lewin, 1990).

At the realization of the secretory product in *N. damascena* nectaries, participate also the chloroplasts from the II—IV subepidermal, cells layers, where there is synthetized a substances accumulated in a vesicular layer at the stroma periphery, near the inner membrane of the chloroplast (Fig. 4). Otherwise, the chloroplasts outside photosynthesis are implied in the synthesis of fatty acids and of some aminoacids (1). The absence of starch granules in the chloroplasts with vesicular structures, at the stroma periphery, suggests the hypothesis that starch granules represent a source for the other glucides synthesis.

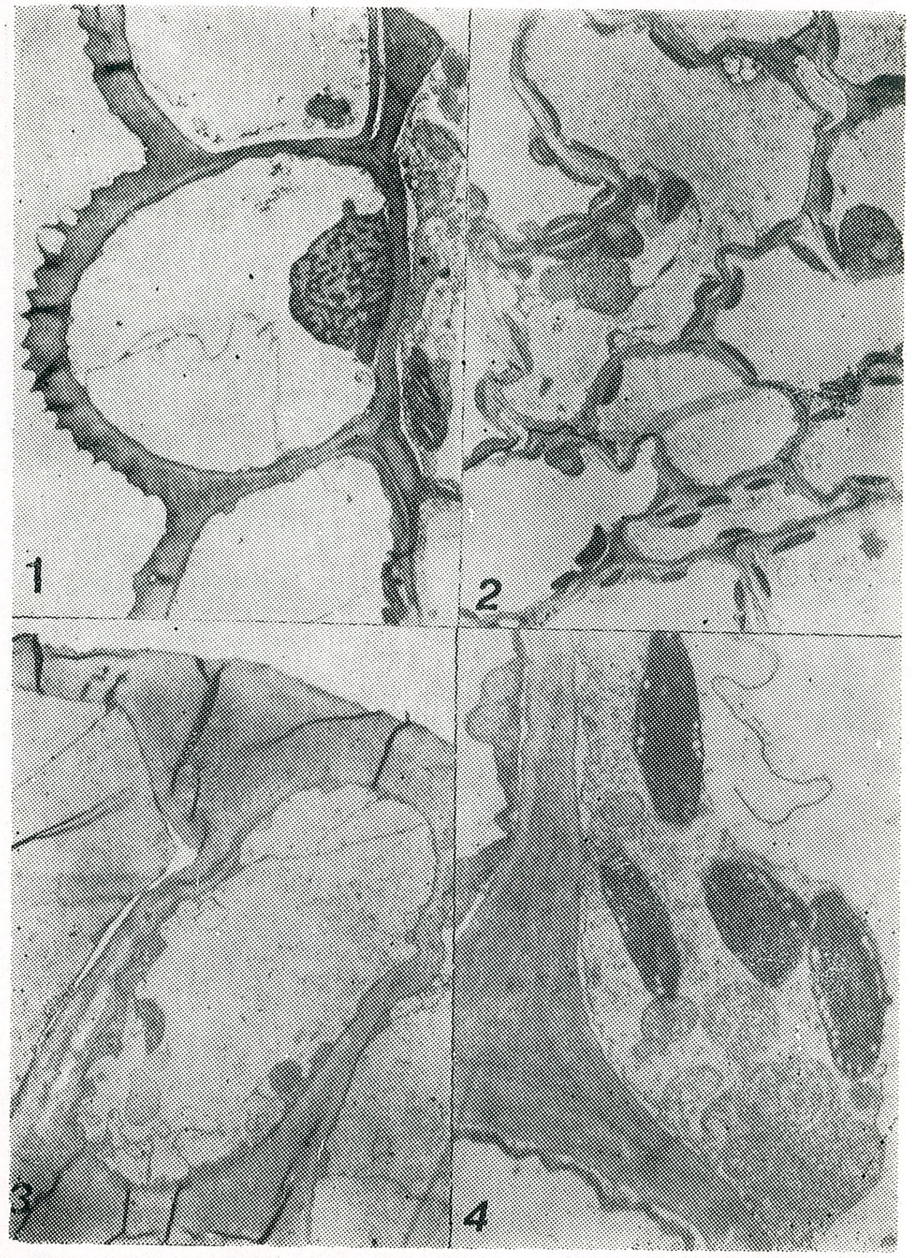

Fig. 1. — Interval epidermis of the nectaries. × 2,600.
Fig. 2. — Nectariferous parenchyma. × 1,800.
Fig. 3. — External epidermis of the nectaries. × 2,100.
Fig. 4. — The chloroplasts with secretory activity in nectariferous parenchyma. × 12,500.

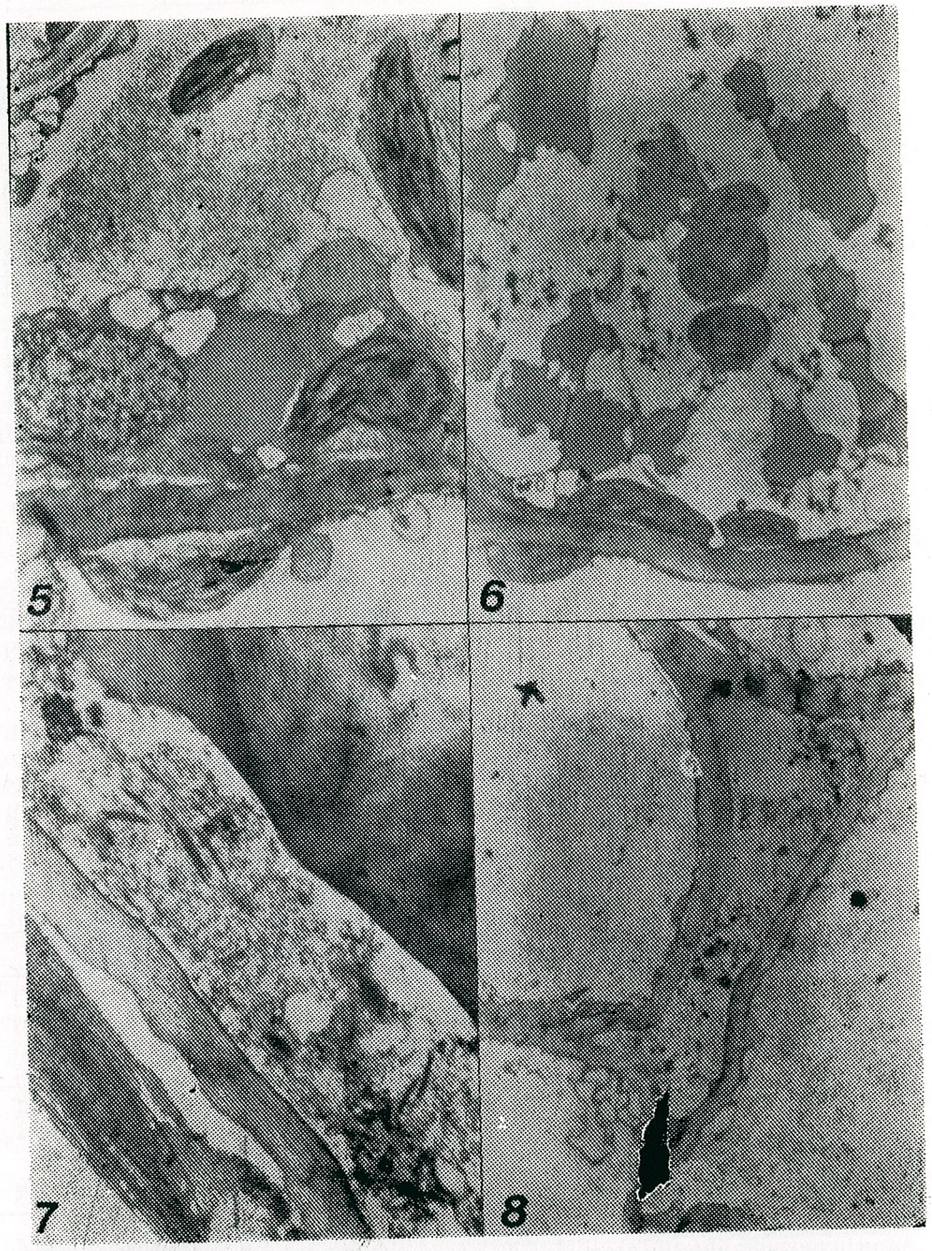

Fig. 5. — Secretory products elaborated in the vesicles. × 5,400.
Fig. 6. — Granular components of the secretory products. × 4,500.
Fig. 7. — Golgi apparatus and endoplasmic reticulum implication in the synthesis of the lysosome hydrolytic enzymes. × 15,900.
Fig. 8. — A cell with accumulated residual products. × 3,500.

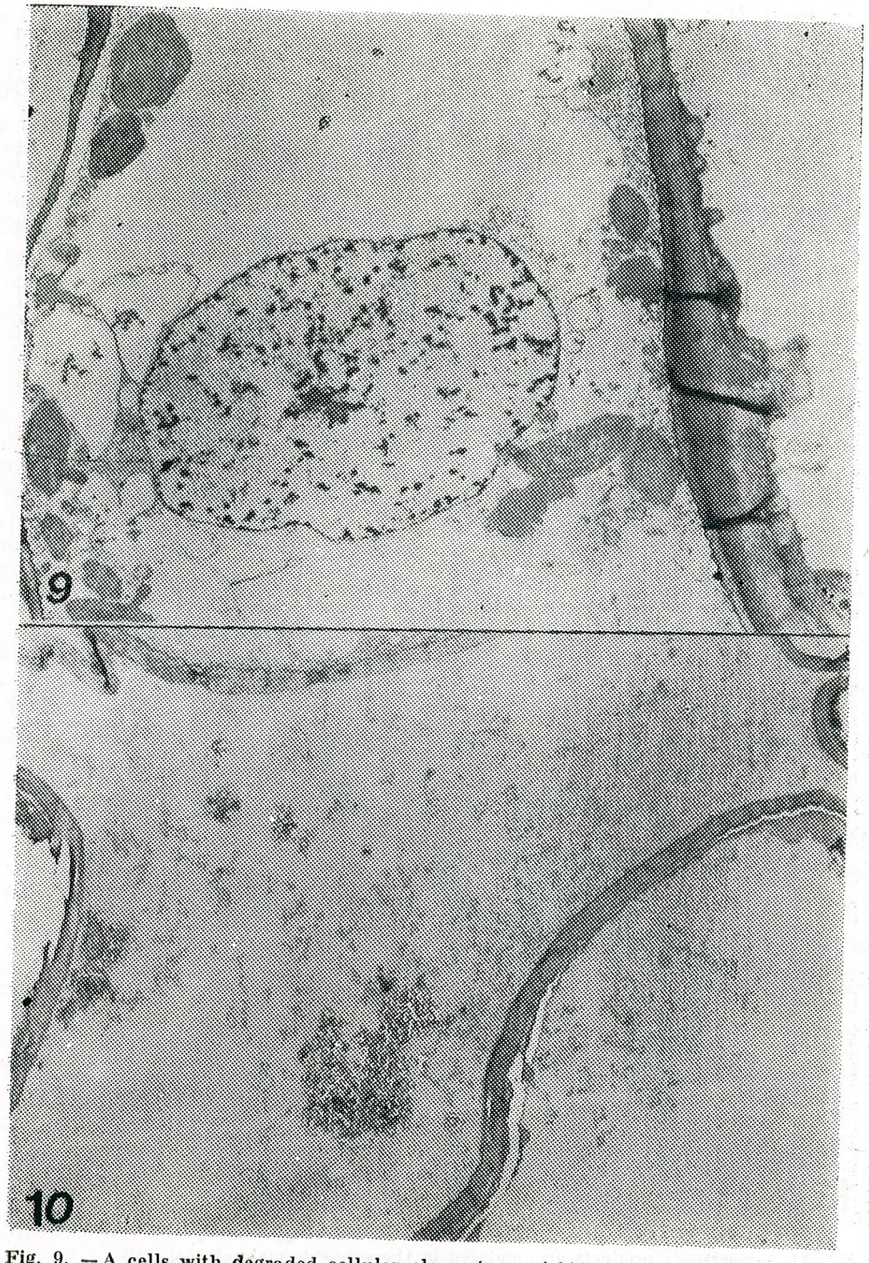

Fig. 9. — A cells with degraded cellular elements. × 4,800.
Fig. 10. — A parenchymatic cell at the lysis end of residual products. × 3,760.

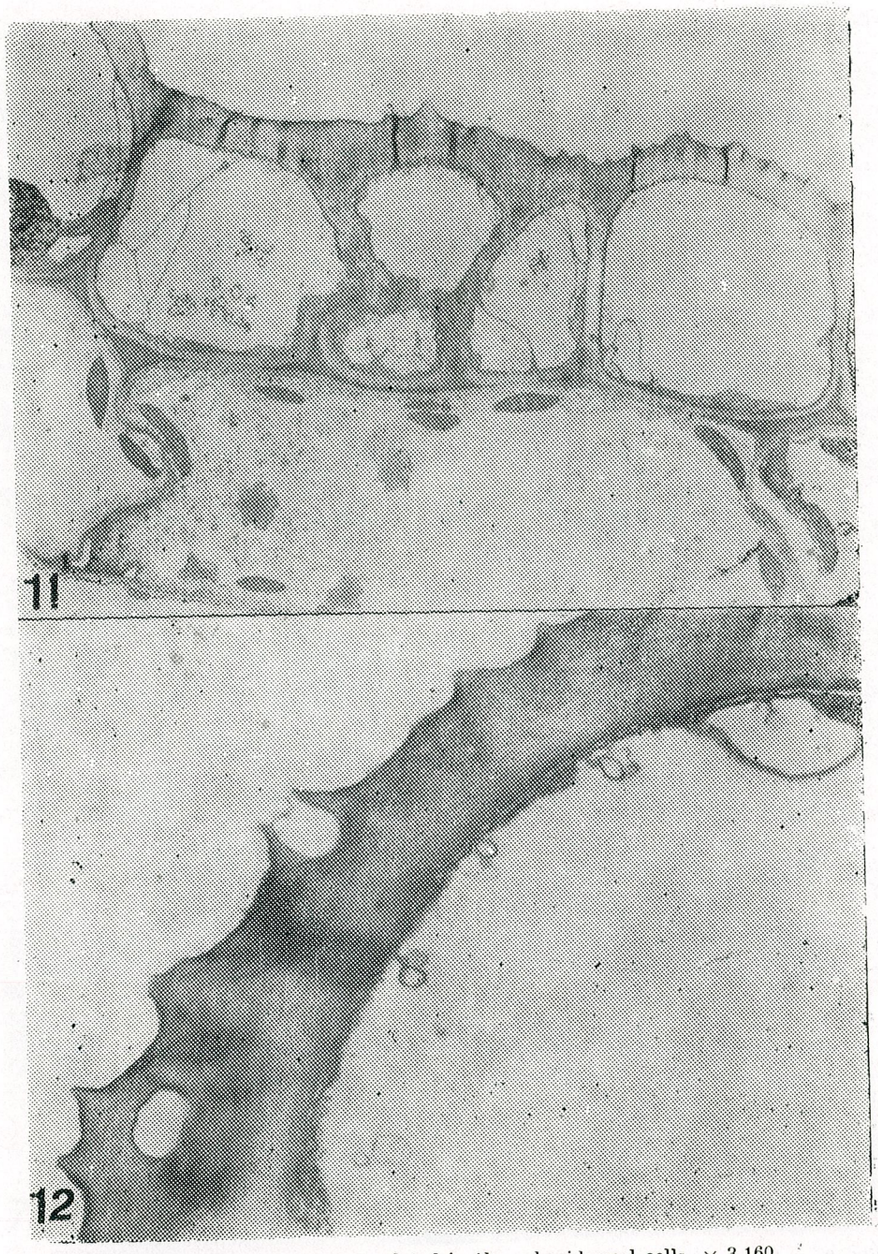

Fig. 11. — Secretory products accumulated in the subepidermal cells. × 3,160.
Fig. 12. — Exocytosis process through internal epidermis. × 9,500.

The synthesis products are accumulated as vesicles (Fig. 5), or granules of different size (Fig. 6), medium electron-dense. The last can represent also some residual products formed as a result of synthesis.

In cells there are coated vesicles as well as multivesicular and multilamelar structures, which present a transport role for the glucide solutions from a cell to the other cell, as well as a role for the cell surface enlargement (Fig. 2). They can be presented in the proximity plasma membrane in the cytoplasm, between plasma membrane and the cell wall (Figs. 1, 2), as well as at their passing through the cell wall (Fig. 10).

3. ACCUMULATION AND DEGRADATION PROCESSES OF THE RESIDUAL PRODUCTS

After the synthesis and accumulation of secretory products, the cellular organelles are gradually degraded through cytoplasm vacuolarisation, the mitochondrial crista swelling, chloroplasts and nucleus structure alteration with chromatine accentuated rarefaction (Fig. 9). In the subepidermal cells nucleus, there appear nucleolar inclusions of vesicular forms associated with nuclear envelope (Fig. 2). Similar formations were constated previously at nectariferous cells in *Vinca rosea*, *V. major* a.o. (3). Finally, the tonoplast is broken, and some secreted substances together with cell rests and residues are dispersed in the vacuole (Fig. 9). The residual products as results of secretory activities are accumulated initially in a cell placed in the middle of the parenchyma, whose content is lysed (Fig. 8). The hydrolytic enzymes from lysosomes are synthesized in the endoplasmic reticulum, pass through Golgi stack where they are transformed and activated through glycosylation, and then are incorporated in lysosomes (5). The presence of numerous Golgi stack with cisternae in a strong relation with endoplasmic reticulum and lysosomes in these cells (Fig. 7), represent an argument in favour of this process. After degradation of the residual products and cellular rests, the content of the cells is clarified (Fig. 10).

4. EXOCYTOSIS PROCESS

The researches performed by numerous authors (3), (4), (7), (8) underlined that in most cases, the nectar is eliminated from the secretory cell cytoplasm through coasted vesicles which fusion with plasma membrane. These vesicles could be active of endoplasmic reticulum, or of *trans*-cisternae Golgi, or of both structures (3), (5).

In the nectaries from *N. damascena*, secretory products are initially accumulated in the subepidermal cells (Figs. 2, 11). From here, at least the fluid component of the secondary product passes as coated vesicles in the epidermal cells (Fig. 11), from where it is removed through an exocytosis process at the cell surface (Fig. 12). Probably this process is under th control of a regulatory signal (5).

CONCLUSIONS

1. There are described the ultrastructural features of the unguiculate perigonal nectaries in *Nigella damascena*.

2. The secretory process of the nectar takes place in the parenchymatous cells (the II—IV subepidermal layers), being also implied the chloroplast together with the endoplasmic reticulum and the Golgi apparatus.

3. In the chloroplast stroma takes place the synthesis of some substances, accumulated under vesicular form near the inner membrane, probably using starch granules.

4. Synthetized products are accumulated in the subepidermal cells from where at least the fluid component of the secretory product is removed at the cell surface through an exocytosis process.

5. The residual products are accumulated in a cell (or in a few cells) from the middle of nectariferous parenchyma where subsequently are lyzed together with all cellular content. In this process are implied the endoplasmic reticulum and Golgi apparatus in which are synthetized the lysosome hydrolytic enzymes.

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Received April 15, 1993

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THE CENOTIC STRUCTURE OF SOME WOODEN ASSOCIATIONS FROM THE BUCEGI AND GÂRBOVA MOUNTAIN CHAINS

V. SANDA and A. POPESCU

The paper presents the cenotic structure of the following associations : *Symphyto cordati-Fagetum* Vida 59, *Leucanthemo waldsteinii-Piceo-Fagetum* Soó 64, *Hieracio rotundati-Piceetum* Pawl. et Br.-Bl. 39 and *Rhododendro myrtifolii-Pinetum mugi* Borza 59 em. Coldea 85 from Bucegi and Gârbova mountain chains, which are characteristic of the main vegetation levels.

In the Bucegi mountain chain the vegetation from the forest area is being distributed within the frame of two subareas as follows : the beech subarea and the spruce subarea, the last one being absent in the Gârbova mountain chain.

According to the specific composition of the forests and the altitudinal succession of the species within the frame of these subzones one can delimit the following levels of vegetation : inferior mountainous, medium mountainous and superior mountainous.

In the inferior mountainous level, situated below an 600—700 m altitude, the dominant tree mass of both mountains are those consisting only of beech or mixtures of beech and fir-tree. The superior limit of the medium mountainous level corresponds to the superior limit of the beech vegetation (1400 m). The tree-masses within this vegetation level consist predominantly of a beech and fir-tree mixture, or beech, fir-tree and spruce, but also pure beech can be found.

The superior mountainous level corresponds to the spruce subarea, characteristic only for the Bucegi mountain chain, having as superior limit the 1600—1700 m altitude.

In the inferior alpin level (2200—2350 m) of the Bucegi mountain chain the characteristic wooden phytocenoses are dominated by *Pinus mugo*.

1. *Symphyto cordati-Fagetum* Vida 59 (Table 1)

The pure or almost pure beech masses from the Gârbova mountains, situated at an altitude between 800—1250 m, generally occupy the basis and the middle of the sides with variable exposures and the slope between a medium (20—25°) and an accentuated one (40—45°).

The tree masses consistance is 0.7—0.8, with a medium height of 18—22 m, dominating exclusively *Fagus sylvatica* (Fig. 1). Isolated in this sinusy there also appear *Fraxinus excelsior*, *Acer pseudoplatanus* and sometimes *Carpinus betulus*.

Table 1

Symphyto cordati-Fagetum VIDA 59

Number of survey		1	2	3	4	5	6	7	8	9	10 K
Area (sq. m)		500	500	500	500	500	500	500	500	500	500
Vegetation height	Trees (m)	30	25	25	15	25	8	10	14	10	12
	Shrubs (m)	8	10	8	8	10	1	1	2	1	3
	Herbs (cm)	40	40	10	30	20	15	40	40	50	40
Cover area (%)	Trees	75	85	70	85	90	90	85	90	85	90
	Shrubs	10	10	10	15	5	2	2	2	10	5
	Herbs	20	10	5	10	10	5	5	5	5	5
Exposure		NV	V	SV	NV	NV	S	SE	E	E	E
Slope (degrees)		40	45	30	30	35	30	25	20	20	5

1	2	3	4	5	6	7	8	9	10	11	12
Char.ass.											
Fagus sylvatica	4	4 –5	4 –5	5	5	5	5	5	5	5	V
Symphytum cordatum	+	+	+	1	+	+	1	+	+	1	V
Symphyto-Fagion											
Dentaria glandulosa	+	+	·	+	+	·	+	+	+	+	IV
Pulmonaria rubra	·	·	·	·	·	+	+	·	+	·	II
Helleborus purpurascens	+	·	·	·	·	+	+	+	+	+	III
Fagetalia											
Euphorbia amygdaloides	·	·	·	·	·	+	+	+	+	+	II
Mercurialis perennis	+	·	·	·	·	+	·	+	+	+	III
Galeobdolon luteum	+	·	·	·	+	·	·	+	+	+	III
Carex pilosa	·	·	·	·	·	+1	+1	+1	+1	+1	III
Galium odoratum	·	·	·	·	·	+	·	+	+	+	II
Veronica urticifolia	+	+	+	+	·	+	·	+	+	+	IV
Luzula luzuloides	1	+1	+	+1	1 –2	·	·	·	·	·	III
Viola reichenbachiana	·	·	·	·	·	+	+	+	·	+	II
Epilobium montanum	+	+	+	·	·	·	·	·	+	·	II
Polygonatum verticillatum	·	·	·	·	+	+	+	+	+	+	III
Dentaria bulbifera	+	+	+	·	+	·	·	·	·	+	III
Lathyrus venetus	·	·	·	·	·	+	+	+	+	+	III
Myosotis sylvatica	+	+	·	·	+	·	·	·	·	·	II
Rubus idaeus	+	+	·	·	·	·	·	·	·	·	I
Chaerophyllum aromaticum	·	·	·	·	·	+	·	·	+	+	II
Galeopsis speciosa	·	·	·	·	·	+	·	·	+	·	I
Ajuga reptans	+	·	·	·	·	+	·	·	·	·	I
Neottia nidus-avis	·	·	·	+	·	·	·	+	·	+	I
Campanula trachelium	·	·	·	·	·	+	·	+	·	+	II
Scrophularia nodosa	·	·	·	·	·	+	·	·	·	+	I
Athyrium filix-femina	+	·	·	·	+	·	·	·	·	·	I
Polygonatum multiflorum	·	·	·	·	·	·	·	·	+	·	I

Table 1 (continued)

1	2	3	4	5	6	7	8	9	10	11	12
Anemone ranunculoides	+	·	·	·	·	·	·	+	·	·	I
Geranium robertianum	+	·	·	·	·	+	·	·	·	+	II

1 survey : *Rubus hirtus* (6), *Arum orientale* (10), *Impatiens noli-tangere* (1 : +1), *Phegopteris dryopteris* (1), *Sanicula europaea* (9), *Dryopteris filix-mas* (3), *Isopyrum thalictroides* (3), *Urtica dioica* (10), *Paris quadrifolia* (5).

Querco-Fagetea

Carpinus betulus	·	·	·	·	·	+	+	+	+	+	III
Cephalaria longifolia	·	·	·	·	·	+	+	·	·	·	I
Mycelis muralis	+	+	+	·	·	·	·	+	+	·	III
Fraxinus excelsior	·	+	·	·	·	+	·	+	·	·	II
Poa nemoralis	+	+	+	+	+	·	·	·	·	·	III
Rosa canina	·	·	·	+	·	+	·	·	·	·	I
Moehringia trinervia	+	+	+	·	+	·	·	·	·	·	II
Fragaria vesca	+	+	·	·	·	+	·	·	·	·	II
Platanthera bifolia	·	·	·	·	·	+	+	+	·	·	II
Acer campestre	·	·	·	·	·	·	+	·	·	+	I
Ranunculus auricomus	+	·	·	·	·	·	+	·	+	·	I
Hieracium murorum	+	+	+	+	+	·	·	·	·	·	III
Galium schultesii	·	+	·	·	+	+	+	·	·	·	II
Crataegus monogyna	·	·	·	·	·	+	+	·	·	·	I
Clematis vitalba	·	·	·	·	·	+	·	·	+	·	I
Chrysanthemum corymbosum	·	·	·	+	+	+	+1	+	·	+	IV
Cornus mas	+	·	·	·	+	+	+	+1	+	+	IV

1 survey : *Carex montana* (10), *Galium vernum* (6), *Populus tremula* (9), *Campanula rapunculus* 2), *Brachypodium sylvaticum* (6), *Pyrus pyraster* (4), *Corylus avellana* (9).

Carpinion

Melittis mellisophyllum	·	·	·	·	·	·	·	+	+	+	II
Lathyrus hallersteinii	·	+	·	·	·	·	·	·	+	·	I

Alno-Padion

Valeriana officinalis	+	·	·	·	+	+	·	·	·	·	II
Ranunculus repens	+	·	·	·	·	·	+	·	·	·	I

Sambuco-Salicion

Senecio fuchsii	+	+	+	·	·	·	·	·	·	·	II
1 survey : *Sambucus racemosa* (2).											

Vaccinio-Piceetea

Veronica officinalis	+	·	·	+	·	·	·	·	·	·	I
Picea excelsa (juv.)	·	+	+	·	+	·	·	·	·	·	II

1 survey : *Sorbus aucuparia* (8), *Pyrola secunda*(5), *Vaccinium myrtillus* (4 : +1).

Aceríon

Acer pseudoplatanus	+	·	·	·	+	+	+	+1	+	+	IV
Campanula latifolia	+	·	·	+	·	·	·	·	·	·	I
Polystichum lobatum	+	·	+	·	·	·	·	·	·	·	I

Table 1 (continued)

1	2	3	4	5	6	7	8	9	10	11	12	
Adenostylion												
Delphinium elatum	•	•	•	•	+	•	•	+	+	•		II
Filipendulo-Petasition												
Tussilago farfara	+	•	•	•	+	•	•	•	•	•		I
Accompanying species												
Juglans regia	•	•	•	•	•	•	+	+	•	•		I

1 survey: *Potentilla micrantha* (10), *Viola montana* (1), *Hypericum perforatum* (1), *Cerastium caespitosum* (2).

The place and data of surveys: 1—5, Izvorul Rece, Gârbova mountain, 15.VII.1979; 6—10 Posada, Gârbova mountain, 17.VII.1979.

Fig. 1. — The vernal aspect of the herbaceous stratum from the Gârbova mountain beech wood (Sinaia, Izvorul Rece).

The herbaceous layer of these beech masses is marked by an abundance of the Carpathian species *Symphytum cordatum* and of those characteristic of the *Symphyto-Fagion* alliance are to be mentioned: *Dentaria glandulosa*, *Pulmonaria rubra* and *Helleborus purpurascens*.

The tree masses of the Gârbova mountains (Posada and Izvorul Rece) present in the berbaceous layer are the following species with a more significant dominant-abundance: *Carex pilosa*, *Luzula luzuloides*, which form facies here and there.

Since these pure beech masses do not get in touch with the spruce masses, absent in the Gârbova mountains, in their floral composition, the characteristic species of the *Vaccinio-Piceetea* class are poorly represented.

2. *Leucanthemo waldstenii-Piceo-Fagetum* Soó 64 (Table 2)

The mixtures of beech, fir-tree and spruce, situated in the superior part of the medium mountainous level, placed between 1250 (1300) and 1400 (1450) m altitude, are characterized by a herbaceous flora similar to that of the beech-woods and fir-and beech woods (1, 2, 4, 9).

Table 2

Leucanthemo waldstenii-Piceo-Fagetum Soó 64

Number of survey		1	2	3	4	5	6	7	8	9	10	K
Area (sq. m)		500	500	500	500	500	500	500	500	500	500	
Vegetation height	Trees(m)	15	30	10	10	30	25	28	30	25	35	
	Shrubs(m)	2	10	6	6	3	4	3	8	2	7	
	Herbs(cm)	40	20	70	40	40	35	40	45	15	25	
Cover area (%)	Trees	80	10	75	85	20	80	70	70	75	85	
	Shrubs	10	80	—	—	90	10	5	15	10	5	
	Herbs	5	5	30	35	10	30	60	15	5	10	
Exposure		V	E	E	E	E	E	—	E	NE	S	
Slope (degrees)		30	25	15	25	40	25	—	25	20	45	

1	2	3	4	5	6	7	8	9	10	11	12	
Char. ass.												
Fagus sylvatica	4	4	4	4	4	3	3—4	2	1	3—4		V
Picea abies	1	1	+	+1	+1	2	1	3	3—4	1		V
Abies alba	+	+	•	+	•	•	•	•	+	+		III
Leucanthemum waldstenii	•	+	+	•	+	•	+	•	+	•		III
Symphyto-Fagion												
Dentaria glandulosa	+	+	•	+	•	+	•	•	•	+		III
Symphytum cordatum	+	+	+	+	+	+	+	+	•	+		V
Pulmonaria rubra	•	+	+	•	•	•	+	•	•	+		II

Table 2 (continued)

1	2	3	4	5	6	7	8	9	10	11	12
Fagetalia											
Euphorbia amygdaloides	.	.	.	.	+	+	+	.	.	.	II
Helleborus purpurascens	.	.	+	+	+	+	+	+	+	.	IV
Mercuralis perennis	.	.	+	.	+	+	3—4	1	.	1	III
Galium odoratum	.	+	+	+	+	+1	+	+	+	+1	V
Isopyrum thalictroides	.	+	+	+	+	+	+	+	+	.	V
Geranium robertianum	+	.	.	+	.	+	+	.	.	+	III
Epilobium montanum	+	.	.	.	.	+	+	.	.	+	II
Aconitum vulparia	.	.	+	.	+	.	+	.	.	.	II
Dryopteris filix-mas	.	+	.	.	.	.	.	+	+	.	II
Paris quadrifolia	.	+	.	.	.	.	.	+	+	.	II
Urtica dioica	.	+	2	.	.	.	+	+	.	+	III
Gagea lutea	.	.	.	+	.	+	.	.	.	.	I
Symphytum tuberosum	.	.	.	.	.	.	+	+	.	.	I
Galeobdolon luteum	+	+	.	+	+	+	+	.	+	+	IV
Lilium martagon	.	.	.	.	.	.	+	+	.	.	I
Dentaria bulbifera	.	.	+	.	+	.	+	.	+	+	III
Veronica urticifolia	+	+	.	.	.	+	.	.	+	+	III
Actaea spicata	+	+	.	.	.	.	.	+	+	.	II
Polygonatum verticillatum	.	.	.	.	.	.	.	+	+	+	II
Oxalis acetosella	+1	+	.	.	.	.	.	+	+1	+	II
Phegopteris dryopteris	+	.	.	.	.	.	.	.	+	.	I
Geranium phaeum	+	.	.	.	.	.	.	.	.	+	I
Aegopodium podagraria	.	.	.	+	+	.	+	+	.	.	II
Athyrium filix-femina	+	+	.	+	.	.	.	+	+	.	III
Allium ursinum	.	.	.	3	+1	.	+	+	.	.	II
Rubus idaeus	.	.	+	.	.	+	+	+	.	+	III
Cirsium erisithales	.	.	+	+	+	.	.	.	.	.	II
Myosotis sylvatica	+	.	.	+	.	+	.	.	+	+	III
Luzula luzuloides	+	.	.	.	+	2—3	+	.	.	+	III

1 survey: *Salvia glutinosa* (7), *Neottia nidus-avis* (6), *Impatiens noli-tangere* (7), *Sanicula europaea* (5), *Chaerophyllum aromaticum* (3), *Orchis purpurea* (1), *Stachys sylvatica* (9), *Ribes grossularia* (7), *Euphorbia carniolica* (10), *Lathyrus hallersteinii* (6).

Alno-Padion + Betulo-Adenostyletea

Stellaria nemorum	+	.	.	.	.	.	+	+	.	.	II
Veratrum album	.	1	+	.	.	+	+	+	.	.	III
Geranium sylvaticum	.	.	+	+	.	+	.	+	+	.	III
Doronicum columnae	+	+	.	.	.	.	.	.	.	.	I
Rumex alpinus	.	.	.	.	+	.	.	+	.	.	I

Acerion

Acer pseudoplatanus	.	.	+	+	+	+	+	+1	+	+	IV
Cystopteris fragilis	.	+	+	.	.	.	+	.	+	.	II
Polystichum lobatum	.	.	+	.	.	.	.	.	+	.	I
Ulmus glabra	.	.	.	.	.	+	.	.	+	.	I

Sambuco-Salicion

Senecio fuchsii	+	+	+	+	+	+	.	+	.	+	IV
1 survey: *Salix caprea* (1).											

Table 2 (continued)

1	2	3	4	5	6	7	8	9	10	11	12
Querco-Fagetea											
Mycelis muralis	+	+	.	.	.	.	+	.	+	+	III
Poa nemoralis	+	.	.	.	.	.	.	+	+	+	II
Hieracium murorum	+	+	.	.	.	.	.	.	.	+	II
Fragaria vesca	+	+	.	.	+	+	.	.	.	.	II
Primula officinalis	.	.	.	.	+	+	.	.	.	.	I
Stellaria holostea	.	.	.	.	.	+	+	+	.	.	II

1 survey: *Scilla bifolia* (7), *Geum urbanum* (8), *Dryopteris austriaca* (1), *Moehringia trinervia* (6), *Anemone nemorosa* (7), *Ranunculus auricomus* (7), *Glechoma hirsuta* (7), *Alliaria petiolata* (5), *Polygonatum odoratum* (6).

Vaccinio-Piceetea

Sorbus aucuparia	.	+	.	.	.	.	.	.	.	+	I
1 survey: *Pyrola secunda* (1).											

Filipendulo-Petasition

Chaerophyllum hirsutum	+	+	+	.	.	.	.	.	.	.	I
Heracleum sphondylium	.	.	.	.	.	.	+	.	.	+	I
Petasites hybridus	+	.	.	.	.	.	.	.	.	+	I
Tussilago farfara	+	+	.	+	.	.	.	.	.	.	II

1 survey: *Valeriana officinalis* (10), *Filipendula ulmaria* (3).

Accompanying species

Arabis procurrens	+	.	.	.	+	.	.	.	.	.	I

The place and date of surveys: 1—10, Poiana Stînii, 22.VII.1978

The Poiana Stînii tree masses vegetate on brown and yellowish-brown acid soils, deep, rich in humus and in total nitrogen. The arborescent edifying species for the association are in codominant relations and only at higher altitudes, where the soils are more superficial the beech becomes subdominant. Besides the species characterizing the alliance *Symphyto-Fagion* and the class *Vaccinio-Piceetea*, present in a small quantity, the preponderance is realized by a central nucleus formed by the taxons specific to the *Fagetalia* order as : *Mercurialis perennis*, *Galium odoratum*, *Isopyrum thalictroides*, *Allium ursinum*, *Luzula luzuloides*, etc.

The tree masses, in the whole Bucegi mountain chain, are characterized by a remarkable vigour and by a great capacity of natural regeneration, which emphasize the climax character of the association.

3. *Hieracio rotundati-Piceetum* Pawl. et Br.-Bl. 39 (Table 3)

In the Bucegi mountains the spruce masses occupy great surfaces in the superior basin of the Ialomiţa river and on the prahovean side of the mountain chain, where this strip is narrower due to rocky abruptnesses (Fig. 2).

Table 3

Hieracio rotundati-Piceetum Pawl. et Br.—Bl. 39

Number of survey		1	2	3	4	5	6	7	8	9	10	
Area (sq.m)		500	500	500	500	500	500	500	500	500	500	K
Vegetation height	Trees(m)	30	28	30	30	30	25	28	30	30	30	
	Shrubs(m)	—	—	1	1	3	4	4	3	4	4	
	Herbs(cm)	15	10	40	40	10	30	10	30	40	35	
Cover area	Trees	90	85	75	80	75	75	90	85	85	85	
	Shrubs	5	—	4	5	2	15	5	10	10	10	
	Herbs	20	10	50	40	30	45	20	5	5	10	
Exposure		SV	E	—	V	N	E	N	SE	SE	E	
Slope(degrees)		35	35	—	10	10	15	30	30	35	35	

1	2	3	4	5	6	7	8	9	10	11	12
Char. ass.											
Picea abies	4—5	4—5	4	4	4	4	5	4—5	4—5	4—5	V
Hieracium rotundatum	•	+	+	+	+	•	+	•	+	•	III
Vaccinio-Piceion											
Luzula slyvatica	+	•	+	+	+	•	•	+	+	•	III
Soldanella montana	•	+	+	+	+	+	•	•	+	•	II
Dryopteris dilatata	+	+	•	•	•	•	•	•	•	•	I
Vaccinio-Piceetalia											
Vaccinium myrtillus	+	+	•	+	+	•	+	•	•	•	III
Oxalis acetosella	+	1—2	1—2	3	+	+	+	+	+	+	V
Sorbus aucuparia	•	+	+	•	•	•	•	•	•	•	I
Orthilia secunda	•	•	•	•	•	•	+	•	+	•	I
Deschampsia flexuosa	•	•	•	+	•	•	+	•	•	•	I

1 survey: Moneses uniflora (5), Epipactis helleborine (7).

Symphyto-Fagion											
Pulmonaria rubra	•	+	•	•	+	•	+	•	+	•	II
Luzula luzuloides	•	•	+	+	+	+	+	+	+	+	IV
Fagus sylvatica	•	•	•	•	•	+	+	+	+	+	III
Dentaria glandulosa	•	•	+	•	+	•	•	+	•	•	II
Campanula abietina	+	+	•	+	+	+	•	•	+	+	IV
Symphytum cordatum	•	•	•	•	•	•	•	+	•	+	I

Table 3 (continued)

1	2	3	4	5	6	7	8	9	10	11	12
Fagetalia s.l.											
Geranium robertianum	•	•	•	•	+	•	+	+	•	•	II
Senecio fuchsii	•	•	•	•	•	+	+	•	+	+	II
Rubus idaeus	•	+	+	+	•	•	•	•	•	•	II
Athyrium filix-femina	•	+	+	+	•	•	•	•	•	•	II
Myosotis sylvatica	•	+	+1	•	•	•	•	•	•	+	II
Galeobdolon luteum	+	+	+	•	•	•	•	•	•	+	II
Saxifraga cuneifolia	+	+	+	+1	+1	•	+	•	•	•	III
Ajuga reptans	•	•	•	•	•	•	•	+	•	+	I
Stellaria nemorum	•	•	2—3	+	+	•	•	•	•	•	II
Helleborus purpurascens	•	•	•	•	•	+	•	+	+	+	II
Dryopteris filix-mas.	•	•	+	•	•	+	•	•	•	+	II
Geranium phaeum	•	•	•	•	•	•	•	+	•	+	I
Circaea lutetiana	•	•	+	•	+	•	•	•	•	•	I
Isopyrum thalictroides	•	•	•	•	•	•	•	+	+	+	II
Dentaria bulbifera	•	•	•	•	•	•	•	•	+	+	I
Galium odoratum	•	•	•	•	•	+1	+	+	•	+	II
Veronica urticifolia	•	•	•	•	•	+	•	•	•	+	I
Campanula persicifolia	•	•	•	•	•	•	•	•	+	+	I
Acer pseudoplatanus	•	•	•	•	•	+	•	•	•	+	I
Epilobium montanum	•	•	•	•	•	+	+	•	+	+	II
Mycelis muralis	•	•	•	•	•	+	+	+	+	+	III
Euphorbia amygdaloides	•	•	•	•	•	+	+	•	•	•	I
Bromus benekenii	•	•	•	•	•	+	+	•	•	•	I

1 survey: *Polystichum lobatum* (10), *Cardamine impatiens* (10), *Lilium martagon* (10), *Symphytum tuberosum* (10), *Chrysosplenium alternifolium* (7), *Stachys sylvatica* (6), *Festuca gigantea* (2).

Accompanying species											
Sambucus racemosa	•	•	+	+	•	•	•	•	•	•	I
Veratrum album	+	•	+	+	•	•	+	•	•	•	II
Chaerophyllum hirsutum	•	+	•	+	•	•	•	•	•	•	I
Doronicum austriacum	•	•	+	•	+	•	•	•	•	•	I
Ranunculus nemorosus	•	•	•	•	•	+	+	•	•	•	I
Potentilla ternata	+	+	•	•	•	•	•	•	•	•	I
Fragaria vesca	+	+	•	•	•	+	+	•	+	+	III
Urtica dioica	+	•	+	+	•	•	•	•	•	•	II
Cystopteris fragilis	•	+	+	•	+	•	+	•	+	+	III
Poa nemoralis	+	•	+	•	+	•	+	•	+	+	III
Moehringia trinervia	•	•	•	•	•	+	•	+	+	•	II
Platanthera bifolia	•	•	•	•	•	•	•	+	+	•	I
Campanula persicifolia	•	•	•	•	•	•	•	•	+	+	I
Arabis alpina	•	•	•	•	•	+	+	•	•	•	I

1 survey: *Calamagrostis arundinacea* (4), *Rumex acetosa* (6), *Cerastium caespitosum* (5), *Poa annua* (4), *Veronica serpyllifolia* (4), *Anthriscus nitida* (5), *Senecio sylvaticus* (6), *Ranunculus repens* (6), *Moehringia muscosa* (6), *Campanula glomerata* (6), *Asplenium trichomanes* (10).

The place and data of surveys: 1—5, Peştera-Cocora, 21.VII.1978; 6—10, Furnica, 25.VII.1978.

The podzolic brown type soils and podzolic humico-ferriiluvial moist and with a strong acid reaction determine the presence in the herbaceous synusia of a great number of acidofile species, characteristic for the alliance *Vaccinio-Piceion* and the *Vaccinio-Piceetalia* order. When these spruce masses get into contact with the beech-fir tree mixed forests, in

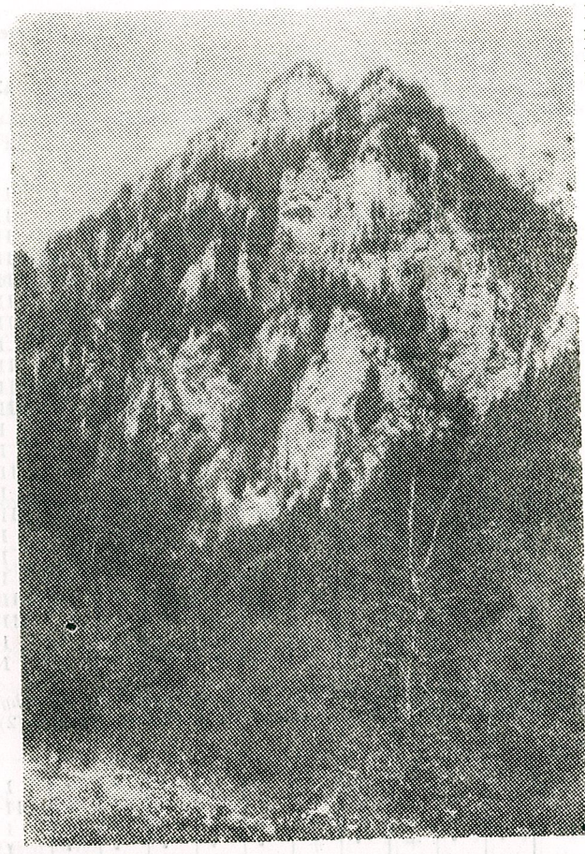

Fig. 2. — Altitude spruce masses placed on the prahovean side in the Bucegi mountain chain (Jepii Mari).

their composition constantly appear *Symphyto-Fagion* and *Fagetalia* species like : *Mysotis sylvatica*, *Geranium robertianum*, *Stellaria nemorum*, *Galium odoratum*, etc.

4. *Rhododendro myrtifolii-Pinetum mugi* Borza 59 em. Coldea 85 (Table 4)

The *Pinus mugo* bushes present a major thermodynamic role in maintaining the natural equilibrium of the subalpine level of our Carpathian mountains (5, 7). Simultaneously with the intensification of shepherd activities in high zones of the mountains, the man with a growing perseverance and incisiveness started the clearing of the potential natural vegetation, respectively of the juniper bushes (Fig. 3), in view of extending the meadows.

These destructive practices have had repercusions in course of time, especially by the dis-equilibriums caused to the regulation of the draining of waters resulted from precipitations on the mountain slopes which produced severe erosions upstream and overflowing of the rivers downstream.

Table 4

Rhododendro myrtifolii-Pinetum mugi Borza 59 em. Coldea 85

Number of survey		1	2	3	4	5	6	7	8	9	10	K
Area (sq.m)		250	200	200	200	150	400	200	500	500	200	
Vegetation height	Shrubs(m)	2	2.5	2.5	3	2.5	3.5	4	4	3	3	
	Herbs(cm)	40	40	35	40	45	40	40	45	40	45	
Cover area (%)	Shrubs	80	75	70	70	60	80	75	80	88	75	
	Herbs	35	40	30	60	40	60	60	65	50	60	
Exposure		NV	—	—	—	SE	SV	—	N	N	NE	
Slope (degrees)		5	—	—	—	10	10	—	10	10	5	

1	2	3	4	5	6	7	8	9	10	11	12
Char. ass.											
Rhododendron myrtifolium	•	•	•	•	•	+	+	+	+	+	III
Pinion mugi											
Pinus mugo	4—5	3—4	4	4	3—4	4—5	4—5	4—5	4—5	4	V
Ribes petraeum	•	+	+	•	+	•	+	+	•	+	III
Salix silesiaca	•	+	•	+	•	•	•	•	+	+	III
Junipero-Pinetalia mugi											
Soldanella hungarica	1	+	+	+	+	+	+	+	+	+	V
Campanula abietina	+	+	+	+	•	•	•	+	+	•	III
Vaccinium myrtillus	+	+	+	+	1—2	+	1—2	+	+	1	V
Homogyne alpina	+	+1	+	+	+	+	+1	+	1—2	+	V
Deschampsia flexuosa	+	+	+	•	•	+	•	•	+	+	III
Luzula sylvatica	•	•	•	+	+	•	+	•	•	+	II
Vaccinio-Piceetea											
Oxalis acetosella	1—2	+1	•	1	+1	•	+	+	•	+	IV
Dryopteris dilatata	+	+	•	+	•	•	•	+	•	•	II
Picea abies (juv.)	•	•	•	+	•	•	•	+	+	•	II
Sorbus aucuparia ssp. *glabrata*	•	+	•	+	•	+	•	+	+	+	III
Calamagrostis villosa	+	+	+	+	+	+	+	+	+	+	V
Sphagnum girgensohnii	•	•	•	+	•	+	•	•	+	•	II

1 survey : *Polytrichum alpinum* (5), *Dicranum scoparium* (7), *Hylocomium splendens* (8), *Drepanocladus uncinatus* (10).

Rumicion alpini + Betulo-Adenostyletea

Veratrum album	•	+	•	+	+	•	•	+	+	•	III
Phleum alpinums sp. *commutatum*	•	+	+	•	•	•	•	•	•	•	I

Table 4 (continued)

1	2	3	4	5	6	7	8	9	10	11	12
Rumex alpinus	.	.	+	.	+	.	.	.	.	.	I
Doronicum austriacum	+	+	.	+	.	+	+	.	+	.	III
Veronica serpyllifolia	+	.	+	+	+	.	.	.	.	.	II
Cerastium cerastioides	.	.	+	+	+	+	.	.	.	.	II
Senecio fuchsii	+	+	+	+	.	+	+	.	+	+	IV
Poa supina	.	.	+	+	+	.	.	.	.	.	II
Adenostyles alliariae	+	.	+	+	.	+	.	+	+	.	III
Taraxacum panalpinum	+	.	+	+	+	.	.	.	.	.	II
Epilobietea											
Chamaenerion angustifolium	.	.	+	.	.	.	.	.	+	.	I
Rubus idaeus	+	.	+	.	+	+	.	+	+	.	III
Stachys alpina	.	.	.	+	.	.	.	.	.	+	I
Fragaria vesca	.	+	+	.	+	.	+	.	.	+	III
Urtica dioica	.	.	+	+	+	.	.	.	.	.	II
Potentillo-Nardion+Nardetalia											
Potentilla ternata	+	+	+	+	+	.	.	+	+	.	IV
Nardus stricta	+	+	+	+	+	+	+	+	+	.	V
Alchemilla glabra	.	.	+	.	+	.	.	.	.	.	I
Ligusticum mutellina	+	+	+	.	+	.	+	+	+	+	IV
Ranunculus nemorosus	.	.	+	.	+	.	.	.	+	.	II
Caricetalia curvulae											
Festuca airoides	+	+	+	.	.	.	1	1–2	+	+1	V
Anthoxanthum alpinum	+	.	.	.	+	+	.	.	.	+	II
Accompanying species											
Poa media	+	+	+	+	+	+	.	+	+	.	IV
Sedum annuum	+	+	+	+	.	.	.	.	.	.	II
Polygala amara	+	+	.	.	.	.	.	.	.	.	I
Arenaria biflora	+	+	+	+	+	.	.	.	.	.	III
Polytrichum commune	+	+	.	.	.	+	+	.	.	+	III
Trifolium repens var. *ochranthum*	.	.	.	+	+	.	.	.	.	.	I
Gentiana punctata	.	.	.	.	.	+	+	.	.	.	I
Stellaria nemorum	+	+	+	.	+	+	.	+	+	+	IV

1 survey: *Luzula sudetica* (2), *L. luzuloides* (3), *Polygonum bistorta* (5), *Scorpidium scorpioides* (5), *Calergonella cuspidata* (7), *Pleurozium schreberi* (7), *Myurella julacea* (9), *Ditrichum flexicaule* (10), *Aulacomium palustre* (3).

The place and data of surveys: 1,2,6,7-Piatra Arsă, 19.VII.1978; 3,4,5,9,10 — Jepii Mici, 21.VII.1978.

Fig. 3. — *Pinus mugo* mushes cleared on vast surfaces (the Cocora mountain).

Fig. 4. — Phytocenoses dominated by *Pinus mugo* spread on large surfaces around the Piatra Arsă mountain shed.

The most widespread *Pinus mugo* bushes can be found presently at Piatra Arsă (Fig. 4) and Jepii Mici (Fig. 5) as well as on the Ialomiţa side of the Cocora mountains.

The phytocenoses are usually pure. Here and there in the mountain appear isolated samples of *Picea abies*, *Larix decidua*, *Pinus combra*. Among the shrubbery species one can notice the constant presence of the taxons : *Rhododendron myrtifolium*, *Vaccinium myrtillus* and *Salix silesiaca*, the last one especially in stations where the rock is present at the surface.

The herbaceous flora is characterized first of all by the presence of oligotrophic species with a great ecologic amplitude, against the light factor : *Soldanella hungarica*, *Homogyne alpina*, *Deschampsia flexuosa* and

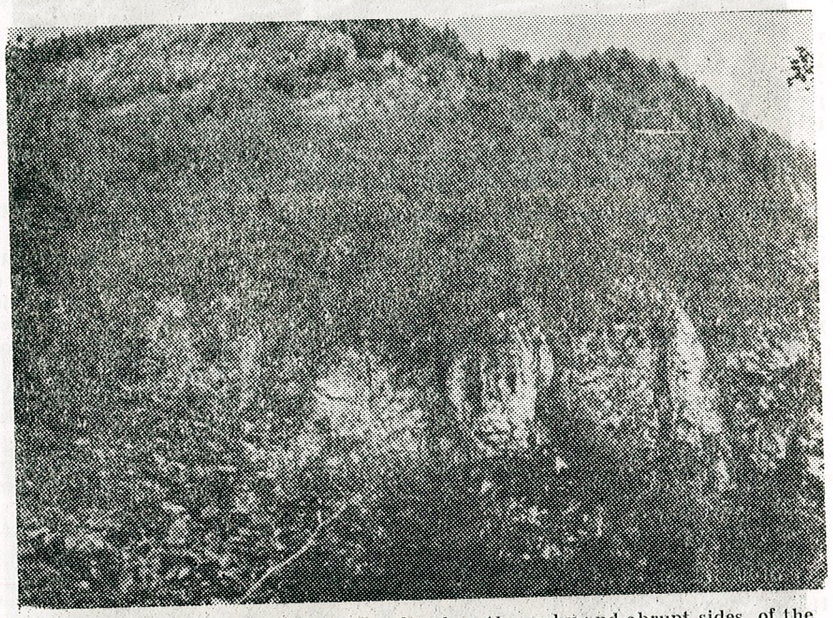

Fig. 5. — Compact *Pinus mugo* hushes placed on the rocky and abrupt sides of the Jepii Mici (Bucegi mountain chain).

Calamagrostis villosa. Besides these, there can be also added some sylvan, mountainous and subalpine elements : *Oxalis acetosella*, *Luzula sylvatica*, *Doronicum austriacum*, etc.

The moss stratum, favorized by the relative constant humidity of these bushwoods, is well represented by the species characterizing the class *Vaccinio-Piceetea* as : *Sphagnum girgensohnii*, *Polytrichum alpinum*, *Dicranum scoparium*, *Hylocomium splendens*, *Drepanocladus uncinatus* occupying an average percentage of 5—10% from the herbaceous synusia of these phytocenoses.

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Received March 17, 1993

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THE DETERMINATION OF AGE FOR INDIVIDUALS WITHIN THE POPULATIONS OF *DENTARIA BULBIFERA* L.

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Dentaria bulbifera, a perennial species with an annual cycle limited at about 4 months, maintains itself from one year to another only by rhizomes. The identified age counting criterion for this plant is the number of segments of the rhizome, because, horizontally, a single new segment is being added to the rhizome every year. The dimension of the supraterranean elements is poorly correlated with the number of segments of the rhizome and, respectively, with the age of the individual except for its weight and whole length; so these cannot help in estimating the age. The pyramids structured on age and achieved with the 2 studied natural populations in București—Băneasa and Sinaia in a oak and hornbeam mixed forest and respectively a fir and beech trees one, indicate 2 balanced populations with the dominance of mature individuals reaching the maximum age of 6 respectively 7 years old.

The reduction of biodiversity at the level of the whole biosphere but especially in the economically developed countries, imposes the demographic study of wild populations with the purpose of determining the degree of endangering the disappearance of some species.

To study the demography, a field explosively developed during the last 15 years (Harper J. L. 1977, Wilmanns O. 1985, Falinska K. 1985, Chapman D. 1987, Kelly D. 1989, Navas M. L. 1990), it is necessary to know the age of the individuals which helps the calculation of the distribution and the ratio among the young, mature and old individuals. This offers the possibility of expressing the extension character of the numerical restriction of the population.

With the herbaceous plants, the age is something more difficult to be established as compared with the woody ones, owing to the great differences of bioform and thus, to the great diversity of biological cycles.

MATERIAL AND METHODS

Two sites of research in the central area of the ecological perimeter of the species *Dentaria bulbifera* L., in the ecological perimeter of the species *Dentaria bulbifera* L., in mixed forests of oak and hornbeam (in Băneasa—București) and in those of fir and beech trees (in Sinaia), have been chosen during 1991—1992.

Within the populations of the mentioned species and from among these phytocoenoses, complete samples have been gathered by digging out on a 1 m^2 surface, in 10 repetitions spread on the whole surface; all individuals have been inventoried in 10 repetitions again. This material served as a sample for observations and biometrical measurements totalizing 263 individuals and, respectively, 406 followed by the statistical calculus of the results.

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RESULTS AND DISCUSSIONS

The analysis of the development cycle within this population shows that the species is perennial ; each year the overground part develops in March and maintains throughout for about 4 months (July) ; occasionally, according to the climatic conditions, it can last until August or September (Sinaia 1991). References about the phenology of this species were found (3) (8), but with no connection to the morphological development of the individual within the biological cycle. The overground part usually develops as a basal leaf (97.30 % from the sum of the individuals) and only 2.6 % developas astem with flowers (M. Paucă, unpublished data). The perennial part represents the rhizome usually horizontally situated. It has been observed that, although the rhizome differentiates segments in another level than the horizontal one and under different angles, there is only one single segment that differentiates horizontally (Fig. 1) ; the point from where the next segment differentiates is common with the part from where the overground part begins.

As this growth of a single horizontal segment is obvious for each year and even the colour of the last segment maintains lighter during the whole season of vegetation (a stronger degree in white) the age for each individual in the population may be determined by measuring the segments.

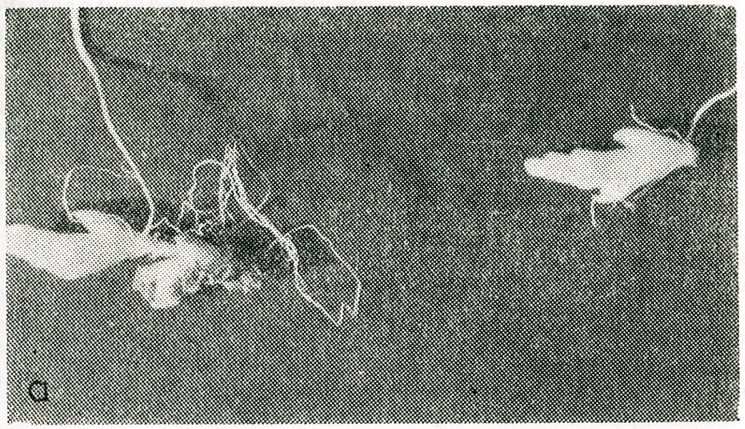
As it is rather difficult to dig out the rhizomes of these plants, different biometrical measurements have been tried to find a correlation between the morphological overground elements and the number of segments of the rhizome and thus the age of each plant (Table 1). In the case of

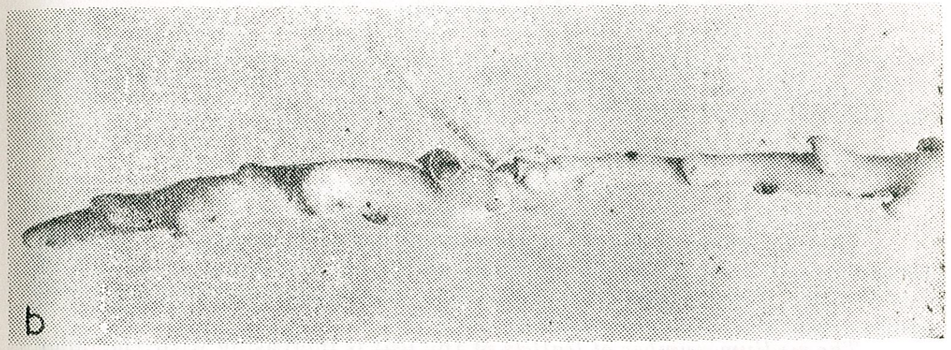
Table 1

The dynamics of morphological parameters in relation with the age of *Dentaria bulbifera* populations

Segment number of rhizome (age)	Total length of plant —cm—		Leaflet number of a leaf		Total weight of plant —g—		Segment number of rhizome different from the horizontal line	
	Sinaia	Băneasa	Sinaia	Băneasa	Sinaia	Băneasa	Sinaia	Băneasa
1	12.11	17.07	3.21	3.13	0.03	0.08	0.02	0.07
2	12.11	24.56	3.79	4.80	0.08	0.34	0.18	0.63
3	14.06	25.08	4.57	4.94	0.20	0.41	0.64	0.58
4	18.12	27.96	5.15	5.00	0.28	0.70	0.54	0.85
5	22.24	32.17	5.46	5.22	0.47	0.90	0.94	1.48
6	23.95	28.88	5.00	6.00	0.56	0.94	0.80	1.75
7	21.25	—	5.00	—	0.52	—	2.00	—

sterile individuals (that develop only one basal leaf as superterranean part), 7 age categories of plants have been identified : 1—6 years old plants which were present in the population from Băneasa and a little more longevous plants in Sinaia (7 years old plants). The age varies between 3 and 7 years with the fertile individuals. No individuals represented by rhizomes and with no overground part have been identified. Also, not all individuals of old age showed stems with flowers. No sign regar-





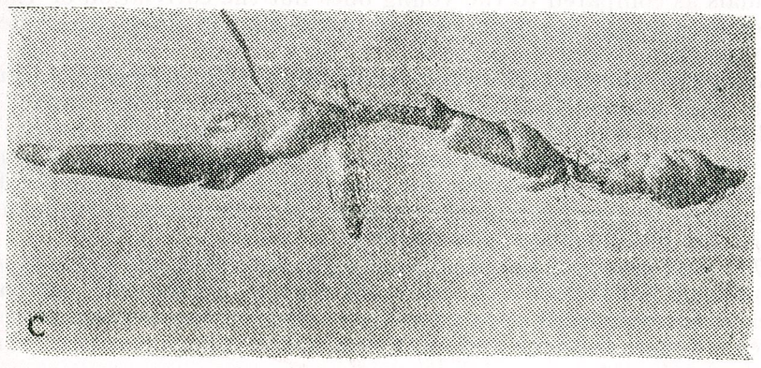


Fig. 1. — The rhizome of *Dentaria bulbifera* a) bulbil and the first segment of rhizome ; b) the rhizome in the second year of life ; c) the rhizome in the third year of life.

ding the individuals survival has been established after they passed the sexual multiplication stage, respectively, it was not noticed that the presence of stems with flower during a whole year should leave special marks on the rhizome but researches still go on.

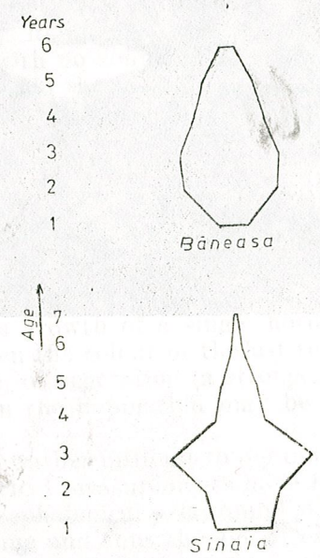


Fig. 2. — Pyramids of age at different populations of *Dentaria bulbifera*.

The total length of the plant grows together with the age : on average, there is a reduction in height with the plants of maximum age either of 7 or 6 years. The correlation is rather low (0.54—0.45) and does not represent a safe measurement criterion.

The medium number of leaflets of the leaf is increasing with the older individuals as compared to the young ones but the correlation of the two parameters is low (0.58—0.34) and we notice that it differs from one station to another.

With this morphological parameter we consider necessary to underline the high degree of intrapopulational and interspecific variety (the variation is between 3—7 leaflets for sterile individuals and for the first leaf of the fertile individual the share being most different). We emphasize this variability as many leaflets (for example, it is equal to 7 in the Flora of Romania). Our measurements showed that the greatest frequency is given by the individuals with 5 leaflets.

The total weight of the plant is also growing with the age except for the senescent individuals — 7 years old ones in Sinaia. The correlation coefficient is statistically secured (0.73—0.67), but the determination possibilities are more toilsome and more destructive than even the counting of rhizome segments.

The number of segments differently disposed as to the horizontal has a high frequency together with the age growth but their presence with a plant is not compulsory and their number is different from a plant to

another (0—3). We consider that these segments reflect an extra amount of growth energy.

The correlation among the different morphological parameters is higher when referring to the dimensions of the same organ as that between the number of segments of the rhizome and its length or weight, or between the total weight of the plant and the weight of rhizome (0.69—0.53) and much lower between the number of leaflets of the leaf and the total length of the plant (0.59—0.56).

In the end we will analyse the proportion of individuals of different ages within the two studied populations : (Fig. 2). It frames the same type of pyramid taken by Odum's model (2), characterized by a proportionally reduced participation of the young individuals, especially in Băneasa. Still, there is a balance with the large participation of the old individuals, especially in Sinaia. Under these circumstances the population is stable, with a slow rate of change both as concerns its renovation and the disappearance of some existing elements.

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LA DYNAMIQUE ANNUELLE DE LA BIOMASSE HERBEUSE DES SAPINIÈRES-HÊTRAIES DU VERSANT PRACHOVIEN DU MASSIF BUCEGI

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Le travail analyse la biomasse herbeuse des sapinières-hêtraies du versant prachovien du massif Bucegi, en mettant en évidence, par phénoaspects, la dynamique des bioaccumulations au niveau de la couche herbeuse dans la troisième surface étudiée.

Dans les écosystèmes forestiers, la synusie herbeuse représente non seulement l'une des composantes spécifiques, au rôle presque déterminant dans la classification et la définition des unités structurales, mais celle qui participe en même temps, d'une manière active, aux processus de pédogenèse par les bioaccumulations annuelles dues à la dégradation de la litière simultanément à l'implication de la couche herbeuse au ralentissement des petits écoulements de surface ou des lavages du sol sur les versants.

La couche herbeuse des sapinières-hêtraies du versant prachovien du massif Bucegi est ressemblante à celle des hêtraies pures, dominée par des éléments spécifiques à l'alliance *Symphyto-Fagion* et à l'ordre *Fagetalia*. La composition floristique des sapinières-hêtraies décrites jusqu'à présent de l'entière étendue de nos Carpates est très ressemblante et ne nous permet pas pour le moment la séparation de certaines sous-divisions.

La couche arborescente des phytocénoses des 3 surfaces analysées se compose de *Fagus sylvatica* et *Abies alba* en tant qu'espèces codominantes. Parmi celles accompagnatrices, nous rappelons, comme plus fréquentes : *Picea abies*, *Acer pseudoplatanus*, *Ulmus glabra*, *Sorbus aucuparia*. La synusie arborescente atteint une hauteur moyenne de 28—32 m, en réalisant un recouvrement de 80—85% et une constante de 0,6—0,7.

Le sous-peuplement est installé surtout dans les clairières ou au bord des peuplements. Il est représenté par : *Sambucus nigra*, *S. racemosa*, *Salix silesiaca*, *Lonicera xylosteum*, *Spiraea ulmifolia*, *Daphne mezereum*, ayant une hauteur moyenne de 4—6 m et réalisant un recouvrement de 5—15%.

En vue d'établir la structure de la couche herbeuse de l'association *Pulmonario rubro-Abieti-Fagetum* (Knapp 42) Soó 64 du versant prachovien du massif Bucegi, on a choisi 3 points représentatifs : Valea Zgarburei, valea Peleşului et Boncu (Buşteni) où l'on a effectué l'inventaire des populations spécifiques dans 100 surfaces de 0,25 m² chacune, en 3 zones de végétation de l'année 1992 et la récolte de 30 exemplaires de chaque espèce (pour les plantes très petites on a récolté 100 exemplaires). Par séchage, pésage et calcul on a déterminé la biomasse d'un exemplaire et ensuite la biomasse de l'unité de surface.

A la suite des recherches effectuées on constate que la couche herbeuse présente dans la saison vernale (mai) (tableaux 1—3) une différenciation spécifique pour les 3 surfaces analysées. Ainsi, a-t-il enregistré sur Valea Zgarburei les plus nombreuses espèces (61) présentes dans la couche

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Tableau 1

La biomasse herbeuse vernale (mai 1992) des sapinières-hêtraies de Valea Zgarburei

Espèce	Fréquence %	Densité ind./m²	Biomasse verte/m²	Biomasse sèche/m²	Contenu eau(%)
1	2	3	4	5	6
Fragaria vesca	24	2,08	0,894	0,200	77,70
Oxalis acetosella	89	172,24	43,404	4,134	90,49
Cardamine glanduligera	22	9,76	21,423	2,030	90,51
Veronica urticifolia	18	2,40	2,038	0,204	90,00
Luzula luzuloides	36	9,04	6,843	1,600	76,54
Myosotis sylvatica	35	3,80	5,100	0,441	91,35
Mycelis muralis	28	2,00	1,986	0,154	92,24
Hieracium rotundatum	15	1,96	2,936	0,470	83,95
Galeobdolon luteum	34	16,08	12,558	2,508	80,06
Adoxa moschatellina	28	14,48	3,113	0,261	91,41
Actaea spicata	11	0,80	2,042	0,256	87,46
Viola reichenbachiana	43	2,76	2,313	0,381	83,47
Geranium phaeum	13	5,24	6,236	0,655	89,49
Euphorbia amygdaloides	5	0,40	0,676	0,130	80,69
Stellaria nemorum	35	10,56	17,593	1,109	93,69
Galium odoratum	46	9,36	3,042	0,271	90,98
Campanula abietina	14	1,44	0,498	0,045	91,09
Rubus hirtus	39	2,48	12,197	4,789	60,73
Sanicula europaea	19	2,08	1,017	0,152	85,04
Cardamine impatiens	7	1,08	0,880	0,121	86,24
Phegoteris dryopteris	10	2,12	0,475	0,053	88,86
Chaerophyllum aromaticum	18	2,20	9,093	0,717	92,12
Tussilago farfara	14	1,16	4,317	0,360	91,68
Geranium robertianum	20	1,32	2,347	0,222	90,56
Anemone nemorosa	2	0,40	0,180	0,024	86,59
Galeopsis tetrahit	2	0,12	0,032	0,003	91,61
Urtied dioica	13	1,04	0,729	0,095	86,92
Stachys sylvatica	5	0,44	0,736	0,078	89,40
Polystichum lobatum	3	0,20	0,097	0,013	87,20
Lapsana communis	4	0,40	0,896	0,067	92,53
Paris quadrifolia	2	0,12	0,107	0,010	90,74
Athyrium filix-femina	9	1,44	0,907	0,091	89,96
Ranunculus repens	9	1,16	0,706	0,065	90,74
Geum urbanum	2	0,12	0,368	0,064	82,74
Impatiens noli-tangere	19	1,40	0,251	0,017	93,46
Hordelymus europaeus	5	0,68	0,518	0,097	81,39
Senecio fuchsii	13	0,80	1,858	0,131	92,95
Dentaria bulbifera	5	0,56	0,989	0,122	87,67
Chrysosplenium alternifolium	11	1,88	0,728	0,066	91,05
Petasites albus	2	0,36	4,270	0,337	92,11
Circaea lutetiana	3	0,20	0,054	0,006	89,09
Prunella vulgaris	6	0,56	0,232	0,031	86,75
Dactylis glomerata	2	0,48	0,544	0,083	84,64
Dryopteris filix-mas	3	0,44	0,883	0,136	84,63
Moehringia trinervia	6	0,40	0,096	0,010	89,93
Bellis perennis	1	0,12	0,154	0,018	88,07
Poa nemoralis	1	1,28	0,168	0,040	76,40
Calamagrostis arundinacea	11	3,84	1,475	0,530	64,01
Salvia glutinosa	3	0,20	0,210	0,021	90,09
Allium ursinum	1	0,04	0,180	0,014	92,24
Veronica officinalis	9	0,80	0,478	0,071	85,15

Tableau 1 (suite)

1	2	3	4	5	6
Orchis maculata	4	0,20	0,394	0,034	91,39
Symphytum cordatum	1	0,24	0,388	0,032	91,85
Rubus idaeus	5	0,28	0,896	0,142	73,40
Epilobium montanum	12	0,72	0,377	0,037	90,26
Pulmonaria rubra	23	2,56	7,048	0,630	91,05
Pyrola secunda	2	0,24	0,070	0,026	63,42
Total biomasse/m²			190,040	24,404	

Tableau 2

La biomasse herbeuse vernale (mai 1992) des sapinières-hêtraies de Valea Peleşului

Espèce	Fréquence %	Densité ind./m²	Biomasse verte/m²	Biomasse sèche/m²	Contenu eau (%)
1	2	3	4	5	6
Salvia glutinosa	9	1,60	12,843	1,278	90,05
Cardamine glanduligera	68	35,08	160,140	15,365	90,41
Mercurialis perennis	66	17,72	39,462	6,308	84,33
Euphorbia amygdaloides	10	1,92	10,619	2,127	79,97
Stellaria nemorum	21	2,00	2,358	0,270	88,55
Isopyrum thalictroides	69	43,32	16,115	2,816	82,53
Oxalis acetosella	62	24,48	3,354	0,392	88,47
Galium odoratum	55	6,88	4,424	0,612	86,12
Galeobdolon luteum	36	3,28	2,007	0,298	85,10
Geranium robertianum	16	1,00	2,183	0,218	90,00
Impatiens noli-tangere	19	2,16	0,646	0,037	94,36
Galeopsis tetrahit	4	0,16	0,112	0,009	91,91
Urtica dioica	17	1,60	4,682	0,595	87,29
Circaea lutetiana	14	1,16	1,414	0,199	85,87
Geum urbanum	4	0,16	0,465	0,080	82,68
Chearophyllum aromaticum	7	0,36	2,255	0,172	92,35
Mycelis muralis	6	0,32	0,469	0,040	91,53
Helleborus purpurascens	2	0,28	5,776	0,791	86,31
Stellaria holostea	1	0,08	0,035	0,005	84,78
Athyrium filix-femina	3	0,48	1,062	0,114	89,28
Senecio fuchsii	10	1,72	12,669	1,402	88,93
Rubus hirtus	11	0,52	1,006	0,292	70,98
Pulmonaria rubra	2	0,12	0,245	0,023	90,69
Dentaria bulbifera	5	0,20	0,703	0,083	88,13
Symphytum cordatum	2	0,16	0,104	0,009	91,02
Tussilago farfara	2	0,20	0,466	0,035	92,49
Chrysosplenium alternifolium	2	0,56	0,381	0,043	88,86
Corydalis marschalliana	5	0,28	0,259	0,026	89,95
Geranium phaeum	7	0,68	0,274	0,036	86,77
Epilobium montanum	1	0,04	0,015	0,002	85,14
Adoxa moschatellina	5	0,48	0,171	0,016	90,43
Anemone nemorosa	34	3,00	0,594	0,008	86,16
Polystichum setiferum	3	0,28	0,095	0,020	79,33
Dryopteris filix-mas	6	1,44	3,074	0,399	87,04
Allium ursinum	5	1,20	1,764	0,169	90,37
Total biomasse/m²			292,241	34,289	

Tableau 3

La biomasse vernale (mai 1992) des sapinières-hêtraies de Boncu (Bușteni)

Espèce	Fréquence %	Densité ind./m²	Biomasse verte/m²	Biomasse sèche/m²	Contenu eau (%)
1	2	3	4	5	6
Athyrium filix-femina	19	3,12	1,588	0,181	88,51
Geranium robertianum	24	1,36	1,179	0,167	85,82
Viola reichenbachiana	41	2,80	1,560	0,258	83,36
Oxalis acetosella	93	42,44	3,862	0,467	87,47
Mycelis muralis	15	1,20	1,104	0,106	90,42
Cardamine glanduligera	69	31,80	43,502	7,155	83,53
Euphorbia amygdaloides	30	4,24	12,427	2,442	80,33
Impatiens noli-tangere	30	4,48	1,160	0,085	92,56
Fragaria vesca	10	0,92	0,438	0,117	73,31
Galeobdolon luteum	72	14,20	6,368	1,257	80,15
Prunella vulgaris	4	0,44	0,258	0,051	80,40
Geum urbanum	1	0,04	0,065	0,011	82,41
Epilobium montanum	10	0,44	0,164	0,022	86,45
Mercurialis perennis	52	10,16	19,487	3,038	84,36
Galium odoratum	54	9,20	3,110	0,410	86,92
Stachys sylvatica	9	0,92	2,275	0,227	90,00
Circaea lutetiana	20	1,84	1,113	0,155	86,13
Paris quadrifolia	1	0,04	0,067	0,008	87,90
Anemone nemorosa	23	6,52	2,054	0,319	84,50
Urtica dioica	2	0,20	0,363	0,045	87,60
Stellaria nemorum	11	0,68	0,564	0,058	89,79
Sanicula europaea	11	0,72	1,130	0,154	86,34
Salvia glutinosa	25	3,64	9,220	1,005	89,09
Calamagrostis arundinacea	3	0,28	0,120	0,015	87,45
Isopyrum thalictroides	34	11,36	2,976	0,579	80,48
Carex sylvatica	3	0,28	0,085	0,016	80,39
Ranunculus repens	9	0,76	0,666	0,080	87,97
Adoxa moschatellina	8	1,12	0,503	0,060	87,95
Rubus hirtus	3	0,16	0,293	0,067	77,24
Galeopsis tetrahit	13	1,36	0,311	0,030	90,39
Circaea alpina	3	0,32	0,042	0,006	84,31
Glechoma hirsuta	3	0,32	0,156	0,023	85,28
Veronica officinalis	11	0,68	0,367	0,080	78,18
Actaea spicata	2	0,24	0,227	0,034	85,19
Veronica urticifolia	2	0,16	0,073	0,011	85,52
Senecio fuchsii	11	0,68	2,757	0,229	91,69
Chrysosplenium alternifolium	4	0,48	0,279	0,029	89,58
Campanula trachelium	1	0,16	0,063	0,007	88,25
Moehringia trinervia	4	0,60	0,206	0,026	87,59
Dentaria bulbifera	10	0,92	4,247	0,641	84,89
Luzula luzuloides	2	0,16	0,050	0,011	77,95
Pulmonaria rubra	2	0,36	0,727	0,069	90,54
Geranium phaeum	1	0,16	0,054	0,006	88,09
Symphytum tuberosum	1	0,04	0,030	0,020	91,47
Cardamine impatiens	2	0,12	0,155	0,020	87,20
Myosotis sylvatica	11	1,72	1,046	0,134	87,22
Poa nemoralis	1	0,20	0,027	0,006	77,94
Ajuga reptans	4	0,28	0,524	0,059	88,72
Tussilago farfara	2	0,28	0,545	0,052	90,51
Total biomasse/m²			129,587	20,048	

herbeuse, par rapport à 52 à Boncu (Bușteni) et seulement 35 sur Valea Peleșului. Cependant, la plus grande quantité de biomasse par unité de surface a été enregistrée au mois de mai à Peleș (34,289 g s.s./m²) ce qui s'explique par la taille plus haute des 6 espèces qui totalisent ensemble 85,44 % de l'entière biomasse, à savoir : *Salvia glutinosa*, *Cardamine glanduligera*, *Isopyrum thalictroides*, *Senecio fuchssii*, *Mercurialis perennis* et *Euphorbia amygdalides*.

Dans Valea Zgarburei les 6 espèces qui réalisent 66,26 % de l'entière biomasse (24,404 g s.s./m²) sont : *Oxalis acetosella*, *Cardamine glanduligera*, *Luzula luzuloides*, *Galeobdolon luteum*, *Stellaria nemorum* et *Rubus hirtus*.

Dans la surface de Boncu (Bușteni) sur les 52 espèces qui réalisent une biomasse de 20,048 g s.s./m², il y a seulement 5 espèces, à savoir : *Cardamine glanduligera*, *Euphorbia amygdaloides*, *Galeobdolon luteum*, *Mercurialis parennis* et *Salvia glutinosa* qui réalisent ensemble 72,74% de l'entière biomasse de la saison vernale.

Considérée en dynamique saisonnière, la plus grande quantité de biomasse herbeuse est obtenue dans la saison estivale (juillet) dans toutes les 3 surfaces, à savoir : 42,072 g s.s./m² à Boncu, 41,339 g s.s./m² à Peleș et 32,993 g s.s./m² à Valea Zgarburei (tableaux 4—6).

Au mois de septembre, en général, la quantité de biomasse herbeuse par unité de surface, diminue beaucoup par rapport à la saison estivale et, quelquefois, dans le cas de Peleș par exemple, même par rapport à celle vernale (tableaux 7—10), ce dernier cas pouvant être expliqué aussi par l'introduction massive des animaux dans l'entière surface.

Quoique la flore herbeuse des sapinières-hêtraies de Valea Zgarburei soit plus riche en espèces (61 pendant la saison vernale et 58 pendant celle estivale) par rapport à Boncu (52 espèces vernales et 51 estivales) comme biomasse par unité de surface, elle réalise une quantité plus réduite dans les saisons vernale et automnale).

La quantité de biomasse herbeuses plus réduite au cours de la saison vernale dans les sapinières-hêtraies de Boncu (Bușteni) s'explique par leur situation à une altitude plus grande (1050—1100 m) ce qui détermine un retard évident du développement du tapis végétal.

Quoique le noyau principal d'espèces appartienne à l'alliance *Symphyto-Fagion* de l'ordre *Fagetalia* pareillement aux hêtraies, comparativement aux peuplements de hêtre de la région (Piscul Cîinelui, le massif Gîrbova) (5), la flore des sapinières-hêtraies du versant prachovien des Bucegi est bien plus diversifiée comme nombre d'espèces, en réalisant en même temps une plus grande biomasse par unité de surface. Ce fait s'explique surtout par l'altitude plus grande à laquelle sont situées les hêtraies ce qui mène à l'appauvrissement en espèces et d'une autre part par les stations occupées par les sapinières-hêtraies, situées à la base des versants, là où les processus de dégradation de la litière sont plus actifs et, par conséquence, les bioaccumulations du sol en substances organiques, bien plus fortes.

La quantité de biomasse herbeuse accumulée par unité de surface pendant les saisons de végétation est en parfaite corrélation avec la quantité de litière (tableau 11) qui, par décomposition, influence directement les bioaccumulations respectives.

Tableau 4

La biomasse herbeuse estivale (juillet 1992) des sapinières-hêtraies de Valea Zgarburei

Espèce	Fréquence %	Densité ind./m²	Biomasse verte/m²	Biomasse sèche/m²	Contenu eau (%)
1	2	3	4	5	6
Rubus hirtus	55	5,40	25,607	7,096	72,27
Geranium robertianum	21	1,76	5,987	0,560	90,63
Calamagrostis arundinacea	15	5,48	2,603	0,553	78,60
Impatiens noli-tangere	15	1,04	1,142	0,073	93,58
Oxalis acetosella	92	127,72	17,881	2,043	88,33
Rubus idaeus	3	0,12	0,692	0,164	76,32
Athyrium filix-femina	35	6,80	10,751	1,516	85,87
Mycelis muralis	18	0,96	0,569	0,065	87,24
Urtica dioica	15	0,80	5,903	0,583	85,55
Stachys sylvatica	12	0,92	4,287	0,646	84,91
Dryopteris filix-mas	7	1,32	5,329	0,841	84,21
Stellaria nemorum	25	5,16	13,855	1,847	86,65
Galium odoratum	45	6,44	2,595	0,354	86,16
Fragaria vesca	23	1,88	1,902	0,457	75,95
Epilobium montanum	7	0,32	0,114	0,018	83,69
Adoxa moschatellina	6	0,60	0,193	0,016	91,64
Geranium phaeum	13	1,36	3,931	0,698	82,24
Viola reichenbachiana	50	3,80	4,571	0,889	80,48
Myosotis sylvatica	36	3,16	4,020	0,449	88,78
Ranunculus repens	25	1,92	4,510	0,486	89,19
Luzula luzuloides	17	4,56	6,685	2,312	65,37
Circaea lutetiana	5	0,28	0,814	0,092	88,66
Prunella vulgaris	14	1,20	1,259	0,223	82,27
Veronica officinalis	21	2,40	1,841	0,377	79,53
Tussilago farfara	18	1,88	4,958	0,382	92,27
Pyrola secunda	6	0,64	0,187	0,058	69,21
Circaea alpina	4	0,32	0,167	0,012	92,86
Hieracium rotundatum	14	2,04	3,097	0,559	81,92
Euphorbia amygdaloides	12	1,44	3,112	0,737	76,31
Carex sylvatica	23	4,20	6,565	1,630	75,13
Chaerophyllum hirsutum	32	2,36	1,121	0,123	88,96
Galeobdolon luteum	67	15,20	23,317	3,435	85,24
Cardamine glanduligera	9	0,48	0,970	0,059	93,83
Veronica urticifolia	19	1,16	1,421	0,205	85,52
Pulmonaria rubra	15	2,04	8,333	0,561	93,25
Dentaria bulbifera	3	0,28	0,114	0,010	90,73
Moehringia trinervia	6	0,88	1,359	0,255	81,21
Campanula trachelium	5	0,88	3,618	0,439	87,86
Lapsana communis	3	0,20	0,529	0,096	81,80
Campanula abietina	4	0,24	0,200	0,031	84,50
Senecio fuchsii	7	0,32	0,548	0,042	92,35
Carex remota	6	3,44	1,073	0,199	81,13
Anemone nemorosa	2	0,12	0,167	0,014	91,43
Bellis perennis	2	0,72	0,622	0,096	84,40
Chrysosplenium alternifolium	2	0,08	0,094	0,009	90,65
Actaea spicata	3	0,20	0,980	0,159	83,81
Phegopteris dryopteris	7	0,28	0,082	0,016	80,27
Hordelymus europaeus	2	1,08	0,787	0,225	71,36
Galeopsis tetrahit	1	0,04	0,023	0,002	89,94
Geum urbanum	2	0,08	0,158	0,032	79,40
Dactylis glomerata	1	0,92	3,118	0,874	71,96
Cardamine impatiens	3	0,16	0,105	0,015	85,77
Festuca drymeia	1	0,60	0,939	0,234	75,08
Polystichum lobatum	2	0,20	0,805	0,126	84,35
Total biomasse/m²			195,610	32,993	

Tableau 5

La biomasse herbeuse estivale (juillet 1992) des sapinières-hêtraies de Valea Peleşului

Espèce	Fréquence %	Densité ind./m²	Biomasse verte/m²	Biomasse sèche/m²	Contenu eau(%)
1	2	3	4	5	6
Euphorbia amygdaloides	9	1,20	4,620	0,916	80,17
Geranium robertianum	43	4,40	15,770	2,099	86,55
Galium odoratum	72	15,12	15,528	3,145	79,74
Oxalis acetosella	68	62,08	15,210	1,924	87,12
Mercurialis perennis	73	19,52	31,818	6,656	79,06
Galeobdolon luteum	42	4,28	5,414	0,937	82,50
Stachys sylvatica	23	1,40	9,782	1,754	82,06
Salvia glutinosa	28	3,16	35,566	4,797	86,48
Galeopsis tetrahit	11	0,76	1,766	0,207	88,22
Cardamine glanduligera	27	2,60	9,838	0,884	90,99
Rubus hirtus	24	1,44	6,611	2,076	68,57
Circaea lutetiana	29	3,20	7,178	0,860	83,30
Adoxa moschatellina	6	0,60	0,250	0,020	91,72
Mycelis muralis	7	0,44	1,066	0,140	86,85
Chaerophyllum hirsutum	19	1,92	16,168	2,066	87,29
Aegopodium podagraria	12	0,96	7,272	0,792	81,55
Urtica dioica	20	1,28	11,561	2,161	81,56
Impatiens noli-tangere	30	4,84	12,047	0,736	93,89
Geum urbanum	2	0,08	0,153	0,032	79,45
Tussilago farfara	7	1,04	3,180	0,324	89,78
Dryopteris filix-mas	8	1,32	6,122	1,455	76,23
Symphytum cordatum	11	1,12	2,254	0,170	92,41
Athyrium filix-femina	9	1,24	3,422	0,554	83,97
Geranium phaeum	10	1,00	0,214	0,028	86,62
Helleborus purpurascens	4	0,60	7,736	1,307	83,79
Senecio fuchsii	12	1,56	15,545	2,256	85,48
Dentaria bulbifera	3	0,44	1,469	0,153	89,75
Sanicula europaea	13	0,84	1,772	0,322	81,81
Lunaria rediviva	6	0,36	1,962	0,350	82,15
Viola reichenbachiana	8	0,44	0,303	0,064	78,83
Lamium maculatum	1	0,08	0,101	0,017	82,50
Actaea spicata	6	0,52	2,547	0,412	83,81
Polygonatum verticillatum	2	0,16	0,046	0,009	80,70
Hordelymus europaeus	6	2,08	2,286	0,499	78,11
Alliaria petiolata	1	0,12	0,510	0,094	81,56
Pulmonaria rubra	12	0,96	3,407	0,489	85,62
Ranunculus repens	7	0,68	0,910	0,122	87,65
Chrysosplenium alternifolium	4	0,52	0,390	0,025	93,40
Stellaria holostea	3	0,36	0,175	0,042	75,59
Rubus idaeus	3	0,16	0,923	0,218	76,32
Phegopteris polypodioides	1	0,56	0,163	0,032	80,27
Veronica montana	2	0,16	0,133	0,020	84,51
Myosotis sylvatica	2	0,32	0,407	0,045	88,78
Carex sylvatica	2	0,28	0,438	0,109	75,13
Moehringia trinervia	1	0,04	0,062	0,012	81,21
Anemone ranunculoides	2	0,08	0,111	0,009	91,43
Total biomasse/m²			264,206	41,339	

Tableau 6

La biomasse herbeuse estivale (juillet 1992) des sapinières-hêtraies de Boncu (Bușteni)

Espèce	Fré-quence %	Densité ind./m²	Bio-masse verte/m²	Bio-masse sèche/m²	Contenu eau(%)
1	2	3	4	5	6
Mycelis muralis	24	1,92	1,626	0,196	87,94
Viola reichenbachiana	39	2,68	1,820	0,330	81,84
Euphorbia amygdaloides	25	2,64	5,995	1,185	80,21
Oxalis acetosella	93	127,12	20,848	2,542	87,75
Galeobdolon luteum	79	21,92	75,668	6,620	91,22
Cardamine glanduligera	5	0,36	0,728	0,045	93,83
Impatiens noli-tangere	33	4,04	5,195	0,497	90,37
Galium odoratum	63	10,44	5,669	0,835	85,26
Geranium robertianum	45	3,20	11,325	1,149	89,84
Veronica urticifolia	10	0,88	1,067	0,161	84,87
Geum urbanum	3	0,20	0,383	0,079	79,40
Salvia glutinosa	35	4,76	90,683	11,614	87,18
Sanicula europaea	16	0,84	1,772	0,322	81,81
Campanula trachelium	4	0,16	0,658	0,070	87,86
Fragaria vesca	11	0,88	0,676	0,168	75,13
Rubus hirtus	6	0,40	0,783	0,235	71,48
Senecio fuchsii	26	1,92	12,651	1,066	91,56
Mercurialis perennis	58	13,88	33,131	6,010	81,76
Circaea lutetiana	37	3,68	11,080	1,737	84,30
Ajuga reptans	7	0,52	0,487	0,076	84,40
Ranunculus repens	14	0,80	1,070	0,132	87,65
Myosotis sylvatica	3	0,24	0,305	0,034	88,78
Athyrium filix-femina	19	4,44	9,271	1,483	84,00
Circaea alpina	3	2,60	1,807	0,130	92,80
Galeopsis tetrahit	14	1,28	0,727	0,073	89,94
Stachys sylvatica	18	1,36	12,935	2,007	84,47
Pulmonaria rubra	13	1,24	2,973	0,290	90,23
Phegopteris dryopteris	3	0,76	0,222	0,043	80,27
Prunella vulgaris	3	0,56	0,525	0,082	84,40
Festuca drymeia	3	0,48	1,932	0,302	84,35
Veronica officinalis	11	1,04	0,798	0,163	79,53
Stellaria nemorum	3	0,28	0,608	0,065	89,24
Cardamine impatiens	3	0,20	0,132	0,019	85,77
Tussilago farfara	8	2,00	4,482	0,414	90,74
Epilobium montanum	4	0,16	0,275	0,054	80,24
Rubus idaeus	3	0,24	1,385	0,328	76,32
Carex sylvatica	4	0,56	0,781	0,182	76,66
Luzula luzuloides	2	0,16	0,234	0,081	65,37
Actaea spicata	3	0,44	2,107	0,256	87,82
Adoxa moschatellina	11	0,94	0,400	0,034	91,40
Dentaria bulbifera	6	0,68	0,679	0,069	89,86
Hepatica transsilvanica	1	0,20	0,150	0,014	84,28
Chaerophyllum hirsutum	3	0,20	1,695	0,215	87,29
Moehringia trinervia	4	0,96	0,537	0,070	86,91
Chrysosplenium alternifolium	2	0,20	0,235	0,022	90,65
Urtica dioica	2	0,16	1,120	0,198	82,33
Polystichum lobatum	2	0,40	1,610	0,252	84,35
Anemone nemorosa	3	0,24	0,333	0,028	91,43
Dryopteris filix-mas	1	0,12	0,484	0,076	84,21
Symphytum cordatum	4	0,32	0,236	0,019	91,82
Total biomasse/m²			319,359	42,072	

Tableau 7

La biomasse automnale (septembre 1992) des sapinières-hêtraies de Valea Zgarburei

Espèce	Fré-quence %	Densité ind./m²	Bio-masse verte/m²	Bio-masse sèche/m²	Contenu eau(%)
1	2	3	4	5	6
Oxalis acetosella	82	66,16	8,071	1,058	86,66
Galium odoratum	36	5,28	2,841	0,496	82,48
Galeobdolon luteum	44	7,52	4,076	0,880	78,32
Fragaria vesca	12	1,20	0,943	0,221	76,55
Sanicula europaea	16	3,20	6,493	1,264	80,55
Luzula luzuloides	7	4,08	5,010	1,379	72,49
Carex sylvatica	22	5,12	5,376	1,382	74,25
Viola reichenbachiana	20	1,44	0,962	0,212	77,92
Veronica officinalis	8	0,40	0,216	0,047	78,18
Euphorbia amygdaloides	6	1,44	6,160	1,362	77,88
Rubus hirtus	26	2,72	12,221	4,232	65,36
Pulmonaria rubra	36	2,80	7,174	0,876	87,78
Poa nemoralis	6	1,92	0,251	0,059	76,40
Carex remota	14	6,72	1,882	0,443	76,32
Stellaria nemorum	52	9,84	28,634	3,139	89,04
Urtica dioica	8	0,80	2,941	0,534	81,82
Impatiens noli-tangere	12	0,96	1,475	0,140	90,52
Hordelymus europaeus	6	3,84	3,529	0,998	71,70
Prunella vulgaris	24	3,28	2,985	0,738	75,27
Ranunculus repens	16	3,28	1,997	0,184	90,74
Tussilago farfara	10	1,36	2,074	0,198	90,39
Moehringia trinervia	10	1,20	0,709	0,096	86,51
Chaerophyllum hirsutum	14	0,72	6,820	0,724	89,38
Athyrium filix-femina	26	5,60	13,961	2,274	83,72
Geranium robertianum	28	2,40	4,562	0,566	87,60
Mycelis muralis	10	0,48	0,290	0,052	81,94
Dryopteris filix-mas	2	0,40	2,647	0,483	81,75
Veronica urticifolia	12	0,72	0,214	0,122	81,66
Hieracium rotundatum	6	0,48	0,715	0,115	83,95
Festuca drymeia	4	0,56	0,449	0,109	75,80
Galeopsis tetrahit	6	0,32	0,410	0,058	85,79
Epilobium montanum	2	0,64	0,644	0,168	73,95
Myosotis sylvatica	6	0,56	0,751	0,065	91,35
Total biomasse/m²			137,483	24,674	

Tableau 8

La biomasse herbeuse automnale (septembre 1992) des sapinières-hêtraies de Valea Peleșului

Espèce	Fré-quence %	Densité ind./m²	Biomasse verte/m²	Biomasse sèche/m²	Contenu eau(%)
1	2	3	4	5	6
Mercurialis perennis	54	8,69	10,463	2,172	79,25
Geranium robertianum	35	2,40	6,348	0,953	84,98
Oxalis acetosella	73	17,31	1,731	0,242	85,89
Rubus hirtus	19	0,84	2,935	1,041	64,54
Anthriscus nitidus	6	0,38	1,953	0,223	88,61

Tableau 8 (suite)

1	2	3	4	5	6
Galeopsis tetrahit	4	0,23	0,702	0,092	86,82
Galeobdolon luteum	44	3,15	4,229	0,855	79,81
Galium odoratum	60	7,08	4,347	0,856	80,26
Salvia glutinosa	29	2,85	18,351	2,987	83,73
Athyrium filix-femina	10	1,31	5,370	1,136	82,31
Actaea spicata	4	0,15	0,453	0,079	82,46
Mycelis muralis	6	1,08	1,218	0,241	80,16
Hordelymus europaeus	2	0,61	0,565	0,160	71,70
Urtica dioica	17	1,15	10,764	2,153	79,99
Circaea lutetiana	8	1,00	1,338	0,261	80,45
Euphorbia amygdaloides	12	1,23	4,138	0,946	77,13
Stellaria nemorum	21	2,31	6,276	0,824	86,89
Lunaria rediviva	2	0,31	7,087	1,358	80,82
Stachys sylvatica	6	0,38	2,561	0,412	83,91
Dryopteris filix-mas	10	1,31	4,845	0,859	82,26
Helleborus purpurascens	4	0,61	4,332	0,651	85,09
Senecio fuchsii	6	0,46	3,186	0,474	85,11
Viola reichenbachiana	10	0,54	0,249	0,062	75,06
Sanicula europaea	8	1,38	1,540	0,329	78,59
Pulmonaria rubra	2	0,15	0,522	0,066	87,28
Moehringia trinervia	6	1,08	0,636	0,086	86,51
Dentaria bulbifera	2	0,54	0,220	0,020	90,73
Fragaria vesca	2	0,38	0,302	0,071	76,55
Total biomasse/m²			106,661	19,609	

Tableau 9

La biomasse herbeuse automnale (septembre 1992) des sapinières-hêtraies de Boncu (Bușteni)

Espèce	Fréquence %	Densité ind./m²	Biomasse verte/m²	Biomasse sèche/m²	Contenu eau(%)
1	2	3	4	5	6
Galeobdolon luteum	67	10,69	8,243	1,529	81,44
Oxalis acetosella	100	229,00	19,694	2,290	87,77
Viola reichenbachiana	41	3,31	1,409	0,321	77,24
Salvia glutinosa	51	6,31	39,467	6,730	82,95
Athyrium filix-femina	37	4,46	7,754	1,089	85,98
Mercurialis perennis	40	4,85	9,891	1,982	79,95
Euphorbia amygdaloides	26	3,15	7,109	1,602	77,44
Sanicula europaea	24	5,61	5,896	1,190	79,84
Stellaria nemorum	6	0,61	1,048	0,161	84,69
Carex sylvatica	6	0,92	0,916	0,257	71,98
Hordelymus europaeus	2	0,15	0,141	0,040	71,70
Epilobium montanum	8	1,23	1,287	0,348	72,79
Galium odoratum	43	6,85	3,211	0,794	82,20
Geranium robertianum	46	3,31	4,677	0,724	84,50
Luzula luzuloides	6	1,00	1,228	0,338	72,49
Ajuga reptans	13	1,23	1,444	0,265	81,64
Myosotis sylvatica	27	1,61	0,982	0,126	87,22
Circaea alpina	40	6,46	2,358	0,226	90,35
Pulmonaria rubra	4	0,23	0,699	0,102	85,41

Tableau 9 (suite)

1	2	3	4	5	6
Rubus idaeus	6	0,31	0,858	0,417	51,38
Circaea lutetiana	15	1,61	2,891	0,515	82,20
Mycelis muralis	17	1,08	0,650	0,117	81,94
Fragaria vesca	13	1,31	1,421	0,469	66,96
Senecio fuchsii	27	1,54	14,491	2,711	81,30
Veronica officinalis	4	0,31	0,166	0,036	78,18
Stachys sylvatica	6	0,54	3,586	0,577	83,91
Impatiens noli-tangere	13	2,15	0,793	0,069	91,18
Tussilago farfara	15	2,92	8,141	0,891	89,06
Veronica urticifolia	8	0,46	0,285	0,042	85,25
Geum urbanum	2	0,54	0,767	0,149	80,63
Phegopteris dryopteris	10	0,85	0,665	0,139	79,18
Urtica dioica	2	0,15	1,435	0,287	79,99
Prunella vulgaris	2	0,15	0,140	0,035	75,27
Moehringia trinervia	2	0,46	0,273	0,037	86,51
Festuca drymeia	2	1,08	0,883	0,209	75,80
Ranunculus repens	2	0,38	0,337	0,040	87,97
Rubus hirtus	2	0,23	1,382	0,479	65,36
Polygonatum verticillatum	2	0,92	0,873	0,118	86,43
Total biomasse/m²			157,491	27,451	

Tableau 10

La biomasse herbeuse (g s.s./m²) des 3 surfaces analysées

Les surfaces analysées	Mois					
	mai		juillet		septembre	
	biomasse verte	biomasse sèche	biomasse verte	biomasse sèche	biomasse verte	biomasse sèche
Zgarbura	190,040	24,404	195,610	32,993	137,483	24,674
Peleș	292,241	34,289	264,206	41,339	106,661	19,609
Boncu (Bușteni)	129,587	20,048	319,359	42,072	157,491	27,451

Tableau 11

La quantité de litière (g s.s./m²) des sapinières-hêtraies du massif Bucegi

Les surfaces analysées	Litière ancienne	Litière nouvelle	Total
Zgarbura	220,896	43,131	264,027
Peleș	189,267	118,933	308,200
Boncu (Bușteni)	310,466	234,200	544,666

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Reçu le 25 janvier 1993

Institut de sciences biologiques Bucarest
Splaiul Independenței 296

METABOLICAL ADAPTATION TO INDUSTRIAL POLLUTION IN BEECH (*FAGUS SYLVATICA* L.) AND HORNBEAM (*CARPINUS BETULUS* L.) LEAVES

II. EVOLUTION OF CARBOHYDRATES AND LIPIDS

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The authors have investigated the effect of polution with SO_2, nitrogen oxides and fallouts consisting of heavy metal compounds (Pb. Cd, Zn, Cu) upon season dynamics of carbohydrates and lipids in beech (*Fagus sylvatica* L.) and hornbeam (*Carpinus betulus* L.) leaves. The trees from which sample leaves were collected belonged to four distinct regions : a) unpolluted landscape located on the Arieș Valley ; b) highly polluted landscape lying close to the polluting source (Works for Processing Unferrous Metals in Zlatna, Alba county, Romania) ; c) less severely polluted landscape, about 15 km upstream from the polluting source ; d) rather severely polluted landscape, due to the main course of air currents along the Ampoi Valley, about 25 km from polluting source. The resulting data have revealed that under the influence of polluting elements, i. e. toxic gases (SO_2, nitrogen oxides) and fallouts consisting of heavy metal compounds (Pb, Cu, Cd, Zn), the amounts of soluble carbohydtates and lipids presents in beech and hornbeam leaves had a wider range than insoluble carbohydrates. These variations were wider in the trees of the highly polluted landscape, while they were closer to control values in the trees located about 15 km from the polluting source (less affected by air currents loaded with polluting substances). The researches carried out have proved that of all the vegetal metabolities analysed, those which revealed best the adapting reactions to pollution caused stress were the variations in the amounts of soluble carbohydrates.

Our previous paper (4) dealt with the evolution of dry substanc (net primary production) and total proteins in beech (*Fagus sylvatica* L and hornbeam (*Carpinus betulus* L.) leaves sampled from trees pollut with SO_2, nitrogen oxides and fallouts of heavy metal compounds (P Cd, Zn, Cu) and also from trees located in an unpolluted area. The pr sent paper investigates the evolution of carbohydrates and lipids in beech and hornbeam leaves subjected to the influence of a mixture of polluting elements discharged by the "Works for Processing Unferrous Metals" in Zlatna, Alba county, Romania.

MATERIAL AND METHOD

Our previous paper (4) thoroughly presented the stationary conditions (ecotops) and the periods when the vegetal material was sampled for analyses.

In order to estimate the amounts of carbohydrates and lipids present in beech and hornbeam leaves, the discs cut off from these leaves were dried at 65 °C and then minced into a fine powder. Carbohydrates were dosed spectrophotometrically according to the method established by Dubois at al. (10), treating with phenol-sulphuric acid first the soluble fractions in the presence of heat, and then hydrolyzing with HCl 1N the

resulting residue. The contents in total extractible lipids was estimated according to the gravimetrical method established by Folch et al. (13). In order to extract lipids, a mixture of chloroform-ethanol in a ratio of 2 : 1 was used.

The resulting data were expressed in mg of carbohydrates and lipids per cm² of foliar surface.

RESULTS AND DISCUSSION

A. *Evolution of the amount of carbohydrates.* Ever since the investigations carried out by Hugo von Mohl (1845, 1855) and mainly by Julius Sachs (1862, 1864) in the 19th e. (21), and later by Malvin Calvin, John A. Bassham and their collaborators (2), (3), (7) in the second half of our century it has been known that the primary products of photosynthesis are carbohydrates (40%) aminoacids and proteins (30%) and other 30% lipids (11). The first carbohydrates occurring during photosynthesis are trioses and certain acids bearing 4 carbon atoms (6), then hexoses (fructose 1,6-diphosphate and fructose 6-phosphate), which accumulate rapidly both in leaves and the vegetal organism either as soluble monosaccharides (fructose and glucose) and disaccharides (saccharose) or as insoluble polysaccharides of the starch type. A somewhat larger amount of carbohydrates such as pentoses (ribose 5-phosphate, ribulose 5-phosphate, ribulose 1,5-diphosphate, xylulose 5-phosphate), heptoses, tetroses and trioses are rapidly produced during CO_2 fixation in Calvin's cycle (7), and another significant amount of organic acids (malic acid, glycolic acid and succinic acid) occur during CO_2 fixation in Hatch and Slack's cycle (11), (18), a cycle typical for tropical, subtropical and succulent plants. In beech and hornbeam, CO_2 fixation takes place in Calvin's cycle, this fact accounting for the larger amount of pentoses than hexoses present in these leaves. It is known (24) that Calvin's cycle is accomplished, under normal photosynthesis conditions, by 13 molecules of various carbohydrates according to the following proportion : 0.8% trioses, 7.6% tetroses, 30.8% pentoses, 15.4% hexoses and 15.4% heptoses. The larger amount of soluble pentoses recorded in beech and hornbeam leaves is also due to the share taken by these substances as Desoxy-D-ribose and D-ribose in the composition of DNA and RNA, as ribic alcohol in the composition of certain vitamins (vitamin B_{12}) and the coenzymes of some dehydrogenases (diphosphopyridin-nucleotide and triphosphopyridin-nucleotide). Eventually, mention should be made of xylose and arabinose the former contributing to the formation of wooden parts in plants, and the latter to the genesis of gums and mucilages in the vegetal organism (24).

1. *Evolution of the amount of soluble carbohydrates in control beech and hornbeam leaves.* Soluble carbohydrates (pentoses and hexoses) were present in much lower amounts in beech and hornbeam leaves sampled from the control area. During vegetation season they ranged between 0.02—0.12 mg . cm^{-2} in beech leaves, i.e. 0.1—0.2% of the dry substance, and between 0.008—0.05 mg . cm^{-2} in hornbeam leaves, i.e. 0.04—0.09% of the dry substance.

Figure 1 (A and B) presents the evolution of soluble pentoses and hexoses in beech leaves (Fig. 1A) and hornbeam leaves (Fig. 1 B) during

the vegetation period of 1991. The leaves were sampled from an unpolluted landscape (Baia de Arieș, Alba county). The investigations carried out have revealed that the increase in soluble carbohydrates in the leaves of the two species followed different patterns.

In beech leaves, noteworthy was (Fig. 1A) the significant presence of carbohydrates when buds burst into leaves resulting mainly from the sap

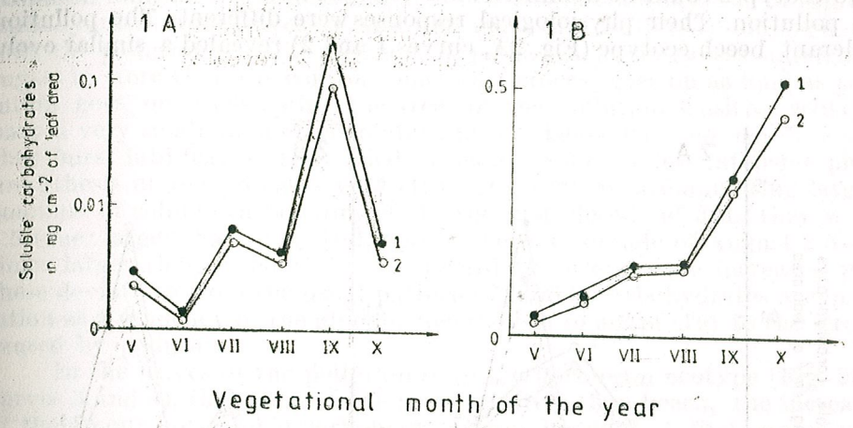

Fig. 1. — Evolution of the amount of soluble carbohydrates in control trees during the vegetation period of 1991. A — carbohydrates in beech leaves (*Fagus sylvatica* L.) 1 — pentoses, 2 — hexoses; B — carbohydrates in hornbeam leaves (*Carpinus betulus* L.) 1 — pentoses, 2 — hexoses.

(i.e. from the previous-year store of glucids fixed in the bark and roots). When hornbeam trees leafed out (Fig. 1B) this surplus in glucids did not occur. In fact, the amount of soluble glucids recorded in beech leaves in the first decade of May was about 2.2—2.5 times larger than the one in hornbeam leaves, and this difference remained between the two species all along the vegetation period.

In the first decade of June, when the growth period of foliar surfaces came to an end, the amount of soluble carbohydrates was seen to decrease in beech leaves and to increase in hornbeam leaves.

In the first decade of July, a relatively significant increase in glucids was recorded ; it was more obvious in beech (Fig. 1A) than in hornbeam (Fig. 1B). The first decade of August was characterized by stability in soluble carbohydrates synthesis. This may have been due to ecological factors such as diminished rainfall and high temperatures, or the physiological response to such stress-causing factors, i.e. more intense respiration and lower photosynthesis brought about by the closing of stomata during part of the day in order to control transpiration, or by scarce water supply in soil, leaves and atmosphere. This process was more obvious in beech and less obvious in hornbeam. The maximum accumulation of soluble carbohydrates was reached in the first decade of September (in beech) and the first decade of October (in hornbeam). The beginning of leaf senescence processes led to the translocation of soluble glucids in the bark

and roots. This process was clearly visible in beech, but it had not started yet in hornbeam when leaves were sampled.

2. The effect of pollution upon the evolution of the amount of soluble carbohydrates in beech and hornbeam leaves. The effects of pollution upon the amount of soluble carbohydrates were analysed according to the landscape the leaves had been collected from.

In the highly polluted landscape lying close to the polluting source, two ecotypes could be delimited : one tolerant and the other one sensitive to pollution. Their physiological responses were different. The pollution-tolerant beech ecotype (Fig. 2A, curves 1 and 2) revealed a similar evolu-

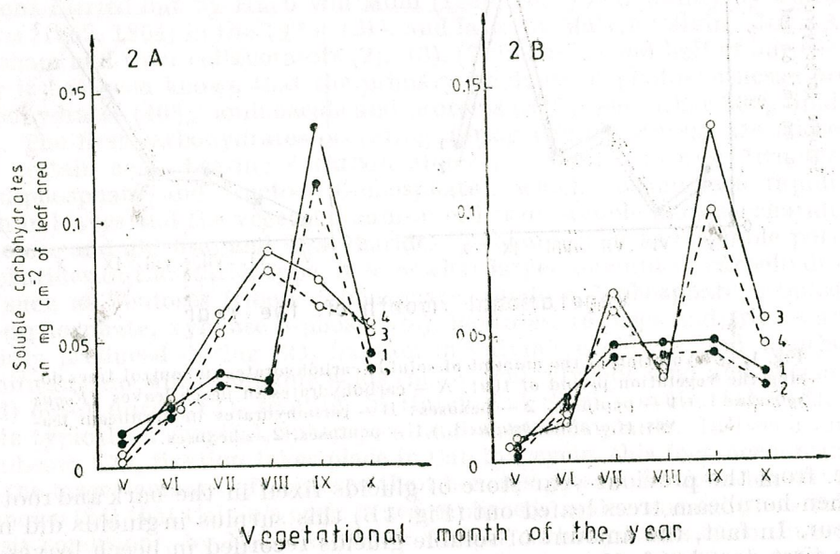

Fig. 2. — Evolution of the amount of soluble carbohydrates in the highly polluted landscape during the vegetation period of 1991. A — carbohydrates in beech leaves (*Fagus sylvatica* L.) 1 — pentoses in the tolerant ecotype ; 2 — hexoses in the tolerant ecotype ; 3 — pentoses in the sensitive ecotype ; 4 — hexoses in the sensitive ecotype. B — carbohydrates in hornbeam leaves (*Carpinus betulus* L.) 1 — pentoses in the tolerant ecotype ; 2 — hexoses in the tolerant ecotype ; 3 — pentoses in the sensitive ecotype ; 4 — hexoses in the sensitive ecotype.

tion of soluble carbohydrates to that in control, but its higher values were reached only in the first decade of September. The pollution-tolerant hornbeam ecotype (Fig. 2B curves 1 and 2) differed in the evolution of soluble carbohydrates from beech leaves. The respective growth curves (Fig. 2B, curves 1 and 2) were of a logistic nature, indicating a saturation in soluble pentoses and hexoses starting with the first decade of August. Towards the end of the vegetation period, the amount of soluble carbohydrates in hornbeam leaves dropped because of translocation, just like in beech. The presence of this process unrecorded in the leaves of control trees shows that air pollutants brought about early senescence.

Pollution-sensitive beech and hornbeam ecotypes have revealed a different evolution of the amount of glucids in leaves during the vegetation period of 1991. In the pollution-sensitive beech ecotype (Fig. 2A, curves 3 and 4) the growth curves for the amount of soluble carbohydrates followed a polynomic exponential pattern (1), (14), (20), (29), with a maximum accumulation in the first decade of August. The decrease in soluble carbohydrates recorded in September and October was mainly due to pollution-induced senescence and less to autumn translocation of soluble carbohydrates from leaves to bark and roots (22), (25), (33), (35). Therefore, the pollution-sensitive beech ecotype has each winter less starch and sugars in store than the control. Since this process goes on as long as pollution goes on, each spring the trees of the pollution-sensitive ecotype have a very small amount of soluble glucids (below 0.01 mg. cm^{-2}) when they burst into leaves. They tried to make up for the less intensive photosynthesis of the previous year (19), (26), (32) by accumulating larger amounts of soluble carbohydrates. In the first decade of July they were 1.5 times larger than in control, and in the first decade of August 2.5 —3 times larger than in control. We regard these excessive increases and these deviations from the usual pattern of soluble carbohydrates accumulation as a tendency of the glucidic metabolism to adapt (19) to the stress caused by pollution.

In the leaves of the pollution-sensitive hornbeam ecotype (Fig. 2B, curves 3 and 4), that seems to be more sensitive than beech, the increase of the amount of soluble carbohydrates was bimodal. A first maximum peak (0.06 —0.07 mg . cm^{-2} was reached in the first decade of July, while a second, almost a double peak in glucid accumulation (0.1 mg . cm^{-2} for hexoses and 0.13 mg . cm^{-2} for pentoses) was reached in the first decade of September. It is interesting to note that the pattern of glucid accumulation in hornbeam leaves during the whole vegetation period of 1991 was similar to that in control beech leaves. This adapting behaviour suggests that this wooden species is a little more sensitive to pollution than beech, under the conditions characterizing the Ampoi Valley. Several authors (5), (8), (15), (22), (23), (26) consider that beech and hornbeam are not resistant species to SO_2, but rather species with variable sensitivity. Our researches have confirmed this opinion, providing aditional information on the fact that within the populations of these two species there are tolerant ecotypes (more resistant to pollution) and sensitive ones to the presence of SO_2 and other polluting agents.

The evolution of the amount of soluble carbohydrates in beech and hornbeam leaves of trees upstream and downstream from the polluting source during the vegetation period of 1991 is given in Figures 3A and B and 4A and 4B. These data suggest that under the mentioned conditions beech proved to be more resistant to pollution than hornbeam, because in the latter species downstream from the polluting source the amount of soluble carbohydrates accumulated in the first decade of September was still 4 times larger than in control trees (compare Fig. 1B with Fig. 4B). In beech (compare Fig. 1A with Fig. 4B) this increase in soluble carbohydrates was much lower (about 1.5 times).

The fact that beech proved to be tolerant to pollution than hornbeam is probably due to several internal factors such as : leaf consistency,

thickness of the epidermal cuticle, stomata distribution and their physiology (12), (27), (36).

3. The effect of pollution upon the evolution of the amount of insoluble carbohydrates in beech and hornbeam leaves. Insoluble carbohydrates (mainly polyoses or polysaccharides such as starch, amylopectin, polyfructosan, xylans, mannans and others) all storage substances (6), (9),

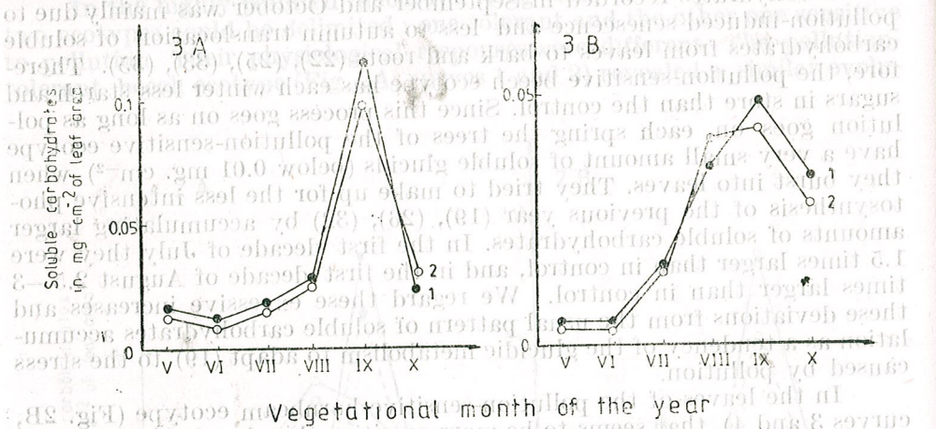

Fig. 3. — Evolution of the amount of soluble carbohydrates in the landscape upstream from the polluting source during the vegetation period of 1991. A — carbohydrates in beech leaves (*Fagus sylvatica* L.) 1 — pentoses. 2 — hexoses; B — carbohydrates in hornbeam leaves (*Carpinus betulus* L.) 1 — pentoses, 2 — hexoses.

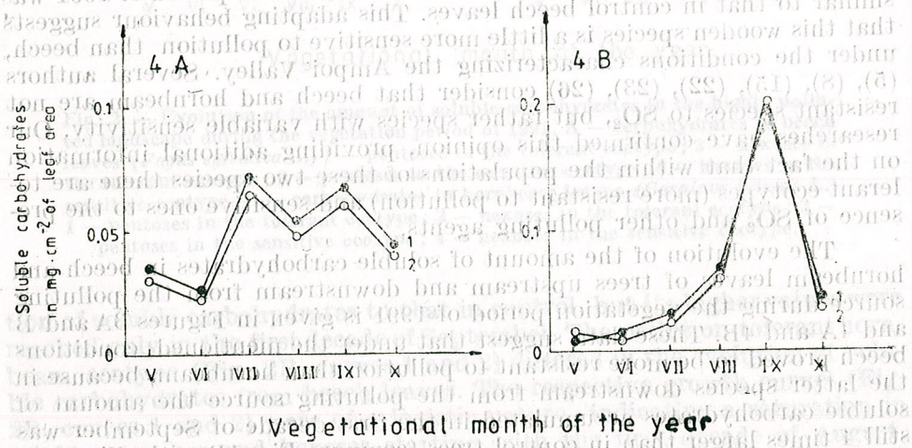

Fig. 4. — Evolution of the amount of soluble carbohydrates in the landscape downstream from the polluting source during the vegetation period of 1991. A — carbohydrates in beech leaves (*Fagus sylvatica* L.) 1 — pentoses. 2 — hexoses; B — carbohydrates in hornbeam leaves (*Carpinus betulus* L.) 1 — pentoses, 2 — hexoses.

(16), (30) have been recorded in large amounts in beech and hornbeam, 1.5 times larger in the former and 4.4 times larger in the latter. Consequently, the interconnected dynamics of soluble and insoluble carbohydrates in the processes of physiological or ecophysiological adaptation to stress-inducing factors could be more variable, and thus more malleable in beech than in hornbeam. The much lower proportion of soluble carbohydrates in hornbeam, when stress-inducing factors change rapidly, may bring about a slowing down of adapting reactions as the insoluble carbohydrates present in larger amounts need time for hydrolyzation in order to obtain soluble glucids able to be rapidly metabolized.

Figure 5A and B presents the evolution of insoluble carbohydrates in beech and hornbeam leaves sampled from control trees in the unpol-

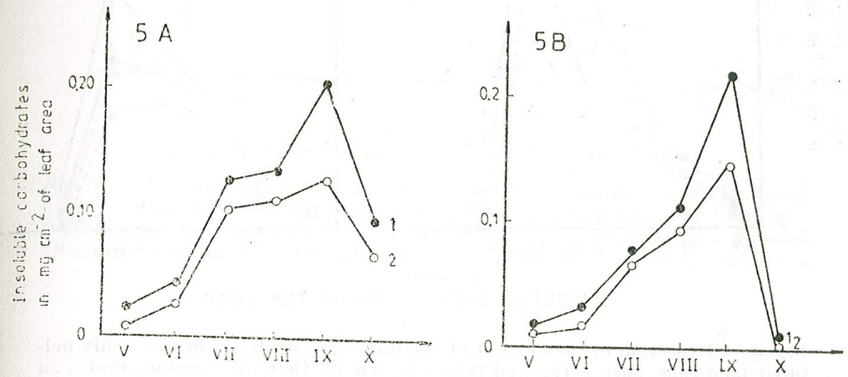

Fig. 5. — Evolution of the amount of insoluble carbohydrates in control tress during the vegetation period of 1991. A — carbohydrates in beech leaves (*Fagus sylvatica* L.) 1 — pentoses, 2 — hexoses; carbohydrates in hornbeam leaves (*Carpinus betulus* L.) 1 — pentoses, 2 — hexoses.

luted landscape on the Aries Valley. It can be seen that the evolution of this glucid fraction, with insignificant variations from one species to the other, was similar in both species : the amount of insoluble carbohydrates per cm² of foliar surface increased almost exponentially until the first decade of September, after which it dropped (more pronounced in hornbeam than in beech) to values close to these recorded in young leaves in spring. The diagrams in Fig. 5A and B show that the insoluble carbohydrates in hornbeam leaves were almost totally solubilized and translocated in bark and roots until the end of the vegetation period. In beech leaves, it seems that this transfer occurs only in October, a stage uninvestigated by us. This difference in behaviour between beech and hornbeam is first of all accounted for by their ecological preferences within their own phytogeographic area (31). According to Walter (34) hornbeam is a species that finds its ecological optimum in moderately dry sites also characterized by a slight shortage in soil water, while beech requires moderately humid sites with scarce periods of water shortage. If the month of September is

poor in rainfall it can bring about earlier senescence in hornbeam leaves, as compared to beech leaves, and this means translocation of glucids in bark and roots.

In the highly polluted landscape (Fig. 6A and B) the curves showing the evolution of the amounts of insoluble carbohydrates both in the tole-

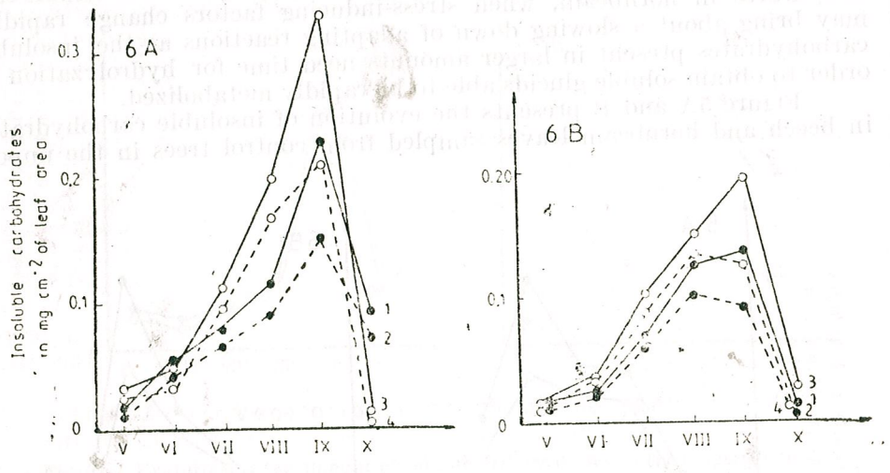

Fig. 6. — Evolution of the amount of insoluble carbohydrates in the highly polluted landscape during the vegetation period of 1991. A — carbohydrates in beech leaves (*Fagus sylvatica* L.) 1 — pentoses in the tolerant ecotype ; 2 — hexoses in the tolerant ecotype ; 3 — pentoses in the sensitive ecotype ; 4 — hexoses in the sensitive ecotype. B — carbohydrates in beech leaves (*Fagus sylvatica* L.) 1 — pentoses in the tolerant ecotype ; 2 — hexoses in the tolerant ecotype ; 3 — pentoses in the sensitive ecotype ; 4 — hexoses in the sensitive ecotype.

rant beech ecotype and in the pollution-sensitive beech ecotype are similar. In beech leaves, both of the tolerant and the sensitive ecotypes, the amounts of insoluble carbohydrates accumulated are much larger than in control in the first decade of September (about 1.3 —2.7 times in the sensitive ecotype and 1.1 —1.2 times in the tolerant ecotype). This increase, according to Kreeb (23), is a proof that beech grows resistant against SO_2 aggression. But in hornbeam leaves, the amount of insoluble carbohydrates accumulated in the first decade of September was smaller than in the control. Krebs (23) thinks that this shows that hornbeam is more sensitive to air pollution than beech, fact also confirmed by other researchers (6), (8), (15), (26).

The changes recorded in the amounts of insoluble carbohydrates in beech and hornbeam leaves from trees upstream and downstream from the polluted source suggest that pollution is heavier downstream. Resistance and physiological adaptation to pollution-induced stress both in beech and hornbeam is well reflected by the accumulation of insoluble carbohydrates in leaves in higher amounts than those in control (down-

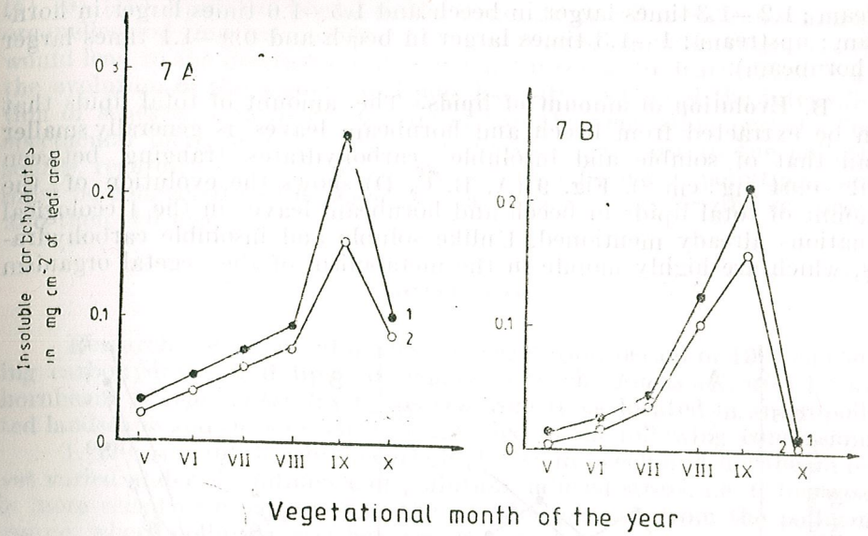

Fig. 7. — Evolution of the amount of insoluble carbohydrates in the landscape upstream from the polluting source during the vegetation period of 1991. A — carbohydrates in beech leaves (*Fagus sylvatica* L.) 1 — pentosea, 2 — hexoses. B — carbohydrates in hornbeam leaves (*Carpinus betulus* L.) 1 — pentoses, 2 — hexoses.

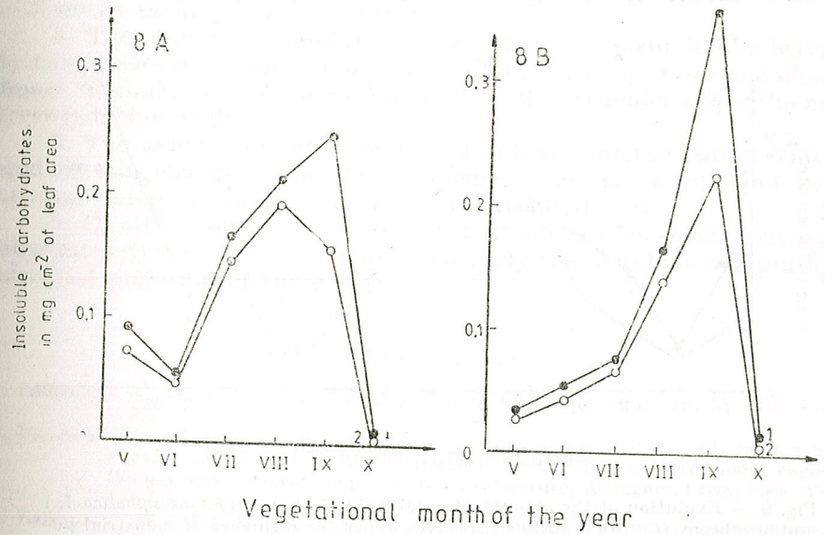

Fig. 8. — Evolution of the amount of insoluble carbohydrates in the landscape downstream from the polluting source during the vegetation period of 1991. A — carbohydrates in beech leaves (*Fagus sylvatica* L.) 1 — pentoses, 2 — hexoses. B — carbohydrates in hornbeam leaves (*Carpinus betulus* L.) 1 — pentoses, 2 — hexoses.

stream : 1.2 —1.3 times larger in beech and 1.5 —1.6 times larger in hornbeam ; upstream : 1 —1.3 times larger in beech and 0.9 —1.1 times larger in hornbeam).

B. **Evolution of amount of lipids.** The amount of total lipids that can be extracted from beech and hornbeam leaves is generally smaller than that of soluble and insoluble carbohydrates (ranging between 0.01 —0.04 mg · cm^{-2}). Fig. 9 (A, B, C, D) shows the evolution of the amount of total lipids in beech and hornbeam leaves in the 4 ecological situations already mentioned. Unlike soluble and insoluble carbohydrates, which are highly mobile in the metabolism of the vegetal organism

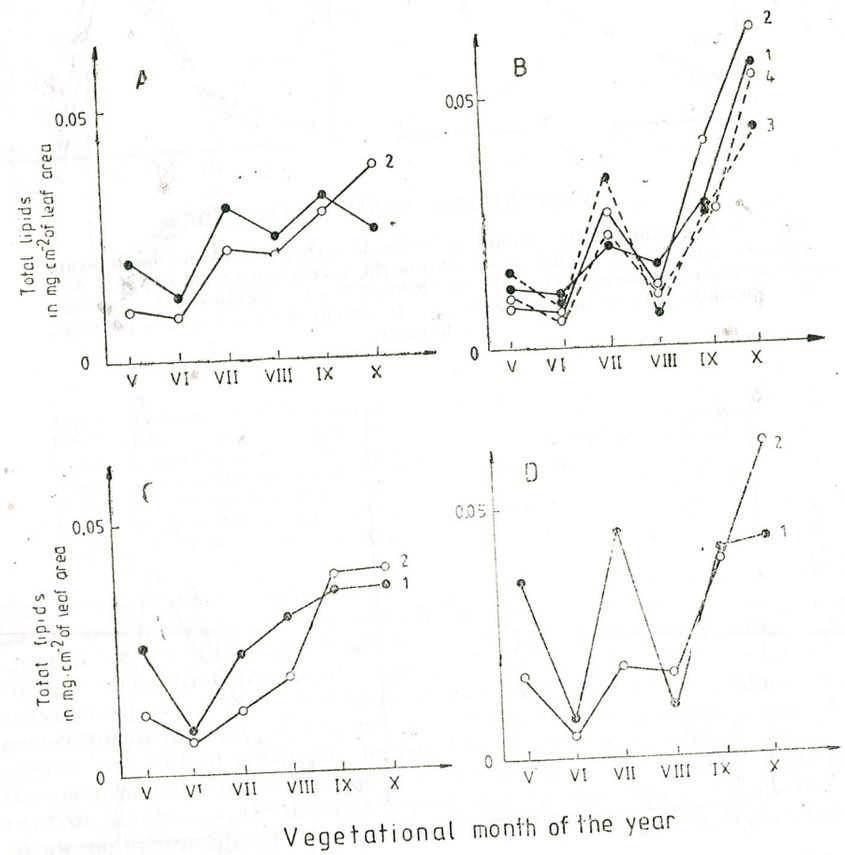

Fig. 9. — Evolution of the amount of total lipids in beech (*Fagus sylvatica* L.) and hornbeam (*Carpinus betulus* L.) leaves under the influence of industrial pollution during the vegetation period of 1991. A — total lipids in control trees : 1 — beech, 2 — hornbeam. B — total lipids in trees of the highly polluted landscape : 1 — tolerant beech ecotype, 2 — tolerant hornbeam ecotype, 3 — sensitive beech ecotype, 4 — sensitive hornbeam ecotype. C — total lipids in trees upstream from the polluting source : 1 — beech, 2 — hornbeam. D — total lipids in trees downstream from the polluting source : 1 — beech, 2 — hornbeam.

(6), (9), (16), lipids are bound to the structure of cell membranes and cell organelles and thus less mobile. If they were used similarly to glucids this would lead to the destruction of cell structures and to death. That is why the evolution of the amount of lipids has often reflected the intensification of senescence in leaves by the end of the vegetation period. Their spectacular increase in the leaves of trees located close to the polluting source, regardless of ecotype, or downstream from the polluting source must be connected to lipophanerosis, a process characterizing senescence and then cell death.

CONCLUSIONS

Researches carried out during the vegetation period of 1991 concerning carbohydrates and lipids dynamics in beech *(Fagus sylvatica* L.) and hornbeam *(Carpinus betulus* L.) leaves from trees located in an unpolluted landscape and in polluted ones have led to the following conclusions :

1. The amount of soluble carbohydrates in beech and hornbeam leaves varied under the influence of pollution-induced stress, i.e. it increased in more sensitive ecotypes or in the site downstream from the polluting source, where pollution was heavier than upstream.

2. In pollution-tolerant beech and hornbeam ecotypes the change in the amount of soluble carbohydrates was insignificant as compared to control.

3. In sensitive beech and hornbeam ecotypes and in the site downstream from the polluting source, autumn senescence affects leaves at least 2 —3 weeks earlier than in control.

4. The amount of insoluble carbohydrates present in the leaves of the two species was larger than that in soluble carbohydrates and changed almost similarly to that of soluble carbohydrates under the influence of stress-inducing pollution.

5. The amount of total lipids in the leaves of the two species suffered certain changes under the influence of air pollutants, but not to the same extent as soluble and insoluble carbohydrates.

6. Of all the metabolites present in beech and hornbeam leaves, carbohydrates dynamics reveals most accurately the metabolic adaptation to industrial pollution of these trees.

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Received March 21, 1993

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INFLUENCE OF INOCULUM ON EXOPOLYSACCHARIDE PRODUCTION BY *SCHIZOPHYLLUM COMMUNE*

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The production of exopolysaccharides by *Schizophyllum commune* depends on the type, amount and age of inoculum. The best inoculum was 10 ml from the first seed obtained from hyphae and the best media was potato-dextrose. The large spherical pellets of 3—5 mm size produce the highest amounts of polysaccharides.
1,3-β-glucanase and 1,6-β-glucanase which could degrade polysaccharides are inhibited by glucose present in a culture liquid.

β-glucans are produced by different fungi : *Auricularia auricullajudae*, *Claviceps fusiformis*, *Lentinus edodes*, *Monilia fructigena*, *Piricularia oryzae*, *Porio cocos*, *Pythium acanthiacum*, *Sclerotium glucanicum* (3, 5, 10, 11, 13), *Acremonium diospyri* (11) *Acremonium persicinum* (12), *Cephalosporium* (12).

The chemical composition and rheological properties of liquids had determined the possibility to use them in the food (emulsions, gelling agents), in the oil industry (drilling, enhanced oil recovery) paper finishing as biosurfactants, antitumor or antiviral agents.

Schizophyllan, a water soluble β-1,3-D glucan with single β-1,6-linked glucopyranose residues is produced, extracellular by white rot fungus *Schizophyllum commune*.

The present study was undertaken to establish the best inoculum for the biosynthesis of schizophyllan (type, quantitaty, age) and the possibility of the fungus to degrade it.

MATERIAL AND METHODS

The investigations were carried out on *Schizophyllum commune* 109 isolated by wood, and there is in Fungal Collection of Institute of Biology (Bucharest, Romania). In was maintained on potato-dextrose agar at 4°C.

We used as inoculum both basidiospores and mycelium. In the first case, *S. commune* was grown 4 days on potato-dextrose agar in Petri dishes at 25°C in darkness. Squares of mycelium (10 mm) behind the colony border were cut and thrown out. Before the exposure to light colonies were aerated for a few seconds by lifting the lids and Petri dishes were placed upside down. After 2 —4 days incubation in continuous light fruit bodies were obtained. Basidiospores accumulated on the lids after fructification of the mycelium were harvested immediately or after 2 weeks in distilled water and counted at the haemocytometer.

Spore suspensions were inoculated into liquid medium (potato-dextrose = medium 1 or medium 2 : KH_2PO_4-lg, $MgSO_4 \cdot 7\,H_2O$ — 0.5 g, yeast extract 3 g, glucose 35 g, distilled water 1000 ml) in Erlenmeyer flasks (with 100 ml medium) to a density of approximative $1.6 \cdot 10^7$ spo-

res/ml and 2 . 10^9 spores/ml, in a rotary shaker. Volumes of 5 ml, 10 ml, 15 ml, 20 ml from 2 and 4 days cultures were added as an inoculum to 750 ml Erlenmeyer flasks containing the same media. The flasks were incubated as described above.

In the second case the colonies were grown in 50 mm Petri dishes at 25°C as surface culture on cellophane membranes overlying malt agar medium. The mycelium was scraped, homogenized and floated on liquid media (medium 1 and medium 2) in a 750 ml Erlenmeyer flask in a rotatory shaker. After 2 days, volumes of 5 ml, 10 ml, 15 ml, 20 ml of this culture were added as inoculum to 750 ml Erlenmeyer flasks containing 100 ml of the same media. The flasks were incubated as described above.

Germination of spores was observed at light microscope.

Growth measurement. Biomass was appraaised by determining the mycelial dry weight. Mycelium was separated from polysaccharide by centrifugation at 17.500 g. The pellets were washed three times with distilled water and dried to a constant weight.

Isolation of polysaccharide. Both culture supernatant and washing water of the pellets were precipitated by adding 2 volumes of ethanol and were dried.

1,3-β-Glucanase and 1,6-β-Glucanase activities were assayed by incubating 1 ml laminarin or pustulan (0.5%) in 0.07 M-sodium phosphate buffer at pH 4.0 with 1 ml culture liquid at 55°C for 5 min (10).

Polysaccharide content was measured by the difference between total carbohydrate contents (phenol-sulphuric acid procedure (4) and reducing sugars (copper reductometric method (8)).

RESULTS AND DISCUSSION

INFLUENCE OF INOCULUM PROVIDED BY BASIDIOSPORES ON POLYSACCHARIDE PRODUCTION

Influence of 1.6 . 10^7 spores/ml amount on polysaccharide production and growth is shown in Tables 1 and 2. We supposed that age of inoculum could influence production of polysaccharide and we used for it 1 ml, 5 ml, 10 ml, 15 ml, and 20 ml from the first seed by 2 days (Table 1) and 4 days old (Table 2). Although different volumes of inoculum

Table 1

Production of polysaccharide and biomass by different volumes of inoculum from 2 days old cultures obtained from 1.6 · 10^7 spores/ml

Volume of inoculum (ml)	Polysaccharide content (g/l) on		Biomass (g%) on	
	medium 1	medium 2	medium 1	medium 2
1	3.0	4.1	0.02	0.10
5	6.2	7.6	0.08	0.20
10	8.3	10.7	0.29	0.40
15	7.0	8.3	0.10	0.36
20	7.0	7.8	0.09	0.31

Table 2

Production of polysaccharide and biomass by different volumes of inoculum from 4 days old cultures obtained from 1.6 · 10^7 spores/ml

Volume of inoculum(ml)	Polysaccharide content (g/l) on		Biomass (g%) on	
	medium 1	medium 2	medium 1	medium 2
1	5.2	8.9	0.08	0.33
5	7.1	10.6	0.09	0.46
10	10.8	13.1	0.40	0.67
15	8.7	12.3	0.36	0.52
20	6.3	9.1	0.10	0.49

were used, the best was 10 ml both for 2 days and 4 days old cultures (10.8 g/l, respectively 13.1 g/l). Production of polysaccharide was lower when there were used 5 ml, 15 ml and 20 ml of inoculum and the lowest when 1 ml of inoculum was used. Medium 2 was the best both for polysaccharide production and biomass. Using 4 days old inoculum we could cut time for polysaccharide production with 2 days.

Influence of 2 . 10^9 spores/ml amount on polysaccharide production and growth is shown in Table 3.

Table 3

Production of polysaccharides and biomass by different volumes of inoculum-4 days old obtained from 2 · 10^2 spores/ml

Volume of inoculum (ml)	Polysaccharide content (g/l) on		Biomass (g%) on	
	medium 1	medium 2	medium 1	medium 2
1	2.4	3.0	0.02	0.03
5	3.6	3.8	0.02	0.03
10	5.1	5.7	0.04	0.05
15	5.9	6.2	0.05	0.06
20	6.2	6.7	0.05	0.06

The best inoculum was 15 ml and the best medium was medium 2. If we compare the level of polysaccharides produced by both amounts of inoculum we can see that a lower inoculum gives better results.

Microscopical studies indicated that in a medium with a high concentration of spores only some of them can germinate. We noticed ungerminated spores attached on the surface of the germinated tubes. Sometimes they were attached on the tip of germinate tubes and growth became impossible. Small amounts of extracellular polysaccharide could determine adhesion of spores on the tip of germinate tubes or their aggregation. These phenomena are lower when the amount of spores was lower.

Although approximately 75% were germinated and the size of pellets was 1.0—1.8 mm. The size of pellets growing from the first concentration of inoculum was 1.5—2.5 mm. The smallest pellets provided by breaking of hyphae in shake conditions look like flocks.

In all these experiments we used spores harvested after 2 weeks of accumulating on the lids of Petri dishes and germination started after 6 hours. When spores were used in the same concentration but harvested immediatly after they appeared on the lids, germination started after 8—10 hours. The delay in germination can be attributed to the presence of auto-inhibitors which had been identified to other fungi (1, 7).

We agree with Nguyen's Hypothesis (9) : intrinsic inhibitors are formed during sporulation and metabolically block the germination process. The regulatory role of auto-inhibitors would have to be temporally modulated and in time its effect is abolished and germination takes place.

INFLUENCE OF INOCULUM PROVIDED BY HYPHAE

Influence of different volumes of inoculum obtained by growing of hyphae 2 and 4 days in shake conditions on polysaccharide production and growth is shown in Tables 4 and 5. The best volume of inoculum was 10 ml both from 2 days old culture and 4 days old culture. The highest

Table 4

Production of polysaccharide and biomass by different volumes of inoculum 2 days old cultures from hyphae

Volume of inoculum (ml)	Polysaccharide content (g/l)on		Biomass (g%) on	
	medium 1	medium 2	medium 1	medium 2
1	5.3	6.2	0.07	0.09
5	8.7	12.3	0.35	0.50
10	10.1	15.2	0.39	0.80
15	9.3	14.6	0.38	0.76
20	9.0	13.1	0.38	0.66

Table 5

Production of polysaccharide and biomass by different volumes of inoculum 4 days old cultures from hyphae

Volume of inoculum (ml)	Polysaccharide content (g/l) on		Biomass (g%) on	
	medium 1	medium 2	medium 1	medium 2
1	7.8	10.3	0.55	0.60
5	13.0	15.4	[0.63]	0.82
10	16.1	19.2	0.71	1.0
15	15.6	18.0	[0.69]	0.86
20	14.3	16.2	0.67	0.80

polysaccharide content was on medium 2. The size of pellets in the last case was 3—5 mm. This size of pellets offered bigger surface with fungal tip.

In filamentous fungi growth takes place on apical area (2, 6, 14).

Steady-state theory (15) assumes the continuous secretion at the apex of an expansible mixture of wall polymers that is continously removed at the base of the extension zone as a rigid complex.

Wessels (15) suggests a continuous flow of wall polymers from the inside to the outside of the wall in the area where secreted proteins are released in the wall. The special structure of cell wall at the growing hyphal tip suggests that the wall may be more porous to allow for secretion of proteins through the wall. There are two possibilities for polysaccharide to reach in culture : to pass through the porous wall or to assemble in the proximity of the cell wall. Biochemical and electromicroscopical studies will confirm or infirm these suppositions.

During the growth *S. commune* uses glucose (35 g/l initial) and after 10 days glucose concentration in culture liquid was 8.03 mg ml^{-1}. We found low 1,3-β-glucanase and 1,6-β-glucanase activities (0.001 and 0.002 U ml^{-1}). Rapp (11), working with *Sclerotium glucanicum*, obtained the same results when he added an excess of glucose. It is clear that glucose inhibits 1,3-β-glucanase and 1,6-β-glucanase.

CONCLUSIONS

L : The best inoculum for production of exopolysaccharide by *Schizophyllum commune* was from hyphae and the best medium was potato-dextrose.

2. The large spherical pellets of size 3—5 mm determined the highest amounts of polysaccharide.

3. Glucose present in culture liquid inhibits 1,3-β-glucanase and 1,6-β-glucanase which could degrade polysaccharide.

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Received January 16, 1993

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NEW ASPECTS OF THE BIOLOGY OF THE HYPERPARASITIC FUNGUS *CONIOTHYRIUM MINITANS* Campbell

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This paper presents the results on the influence of some culture media, of some initial pH values of the PDA medium, and of some fungicides from various chemical groups on the growth and sporulation of the hyperparasitic fungus *Coniothyrium minitans*, new aspects having priority in plant protection literature in general, and in that on mycological and of biological control of plant pathogens, in particular.

Among the 17 culture media tested, *C. minitans* grew and sporulated best on the medium Sabouraud and Leonian, well on media Hansen and agarized soybean meal, followed in decreasing order by the media : Conn-agar-variant B, malt-agar, malt extract-carrot, carrot-agar, Czapek, Hotson ; the most unfavourable to fungus growth and sporulation were the media Bilai and the agarized water.

C. minitans proved the best growth and sporulation on the PDA acid medium (pH 4.0—6.0) up to neutral (pH 7.0) and the poorest on a strongly alkaline medium (pH 9.0—13.0).

Out of 20 fungicides from various groups, 14 proved to be highly toxic, i.e. unselective to *C. minitans* (Cuzin 15 SC, Tiuram 75 PU, Dithane M—45, Metozir, Metoben 70 PU, Fundazol 50 WP, Tecto 450 Fl, Tilt 250 EC, Baycor 25, Anvil 5 SC, Fademorf 200, Falimorf, IAMN-SN—210, Quinolate 400), whereas 6 fungicides were slightly selective (Turdacupral 50 PU, Captadin 50 PU, Sumilex 50 WP, Bayleton 5 WP, Ridomil plus 48 and Sandofan C).

Recently an increased interest in the study of biological agents of controlling the plant diseases has been noticed, among which a particular place has *Coniothyrium minitans* Campbell, a hyperparasitic fungus specific to sclerotial parasites, namely : *Sclerotinia*, *Sclerotium*, *Botrytis*, etc. (1), (7), (9), (10).

In this paper we bring some new aspects to our previous results (8), still not studied till now, of the "in vitro" biology of the fungus *Coniothyrium minitans*, as follows :

a) growth and sporulation on some culture media, not tested as yet ;

b) growth and sporulation on the PDA medium with various initial pH values ;

c) influence of some pesticides on the growth and sporulation of the fungus, and establishing selective and unselective compounds to this biologic control agent.

MATERIAL AND METHODS

The biological material used was a *C. minitans* isolate, previously obtained by us (8).

A number of 17 culture media were tested (Table 1), and among these the PDA medium with 10 various initial pH values, ranging between 4.0 and 13.0 (Table 2), aiming a determining the culture parameters of this fungus for its mass rearing, as well as the effect of 20 fungicides from

REV. ROUM. BIOL. — BIOL. VÉGÉT., TOME 38, Nº 2, P. 131—137, BUCAREST, 1993

Table 1

Growth of *Coniothyrium minitans* on different culture media

Culture medium	Diameter (cm) after :			Sporulation
	2 days	11 days	25 days	
1 (check) *	0.7	0.8	1.4	—
2*	0.7	1.9	2.8	—
3	0.8	2.0	3.3	+++
4	0.8	2.1	3.0	++++
5*	0.7	2.5	3.4	—
6	0.7	2.4	2.5	—
7	0.7	2.2	3.4	+++
8*	0.8	2.8	3.9	—
9	0.7	2.7	3.7	+++
10*	0.8	2.3	3.6	++++
11*	0.8	3.0	4.5	++++
12	0.9	2.4	3.8	++++
13	0.9	2.4	3.7	++++
14	0.8	2.4	4.4	++++
15	0.7	2.0	3.7	++++
16	0.7	2.2	2.8	+++
17	0.8	3.3	4.1	++++

++++ = heavy sporulation
+++ = high sporulation
++ = moderate sporulation
+ = low sporulation
— = no sporulation

Media : 1. water. agar (check) ; 2. Bilai ; 3. potato-dextrose-agar (PDA) ; 4. potato-carrot-agar ; 5. Conn-agar-variant B ; 6. Czapek ; 7. wheat (meal)-agar ; 8. Hansen ; 9. soybean (meal)-agar ; 10. Hotson ; 11. Leonian-agar ; 12. malt-agar ; 13. malt extract carrot ; 14. carrot-agar ; 15. oat(meal)-agar ; 16. corn(meal)-agar ; 17. Sabouraud

× = media unexperimented as yet

Table 2

Influence of pH values of PDA medium on *Coniothyrium minitans* growth

pH	Diameter (cm) after :			Sporulation
	2 days	11 days	25 days	
4.0	1.0	4.0	7.0	++++
5.0	1.0	3.7	7.0	++++
6.0	0.9	3.1	6.5	+++
7.0	0.9	3.3	6.5	+++
8.0	0.9	3.2	6.0	+++
9.0	0.9	3.0	5.0	++
10.0	1.0	3.0	5.0	++
11.0	0.9	3.2	5.0	++
12.0	0.9	3.1	5.0	++
13.0	0.9	3.1	5.0	++

++++ = heavy sporulation
+++ = high sporulation
++ = moderate sporulation
+ = low sporulation
— = no sporulation

various chemical groups (Table 3), which could interfere with the possible utilizations of *C. minitans*, with a view to setting up their selectivity.

The methods previously described (5), (8) were used to study the influence of culture media and of their initial pH values on the growth and sporulation of the test-fungus, while for revealing the selectivity of fungicides the method of their inclusion in the PDA nutritive medium was used, in Petri dishes, on which disks 0.7 cm in diameter and 12 days old of the test-fungus were placed, these being further kept in an incubator at 20—22°C (2), (3), (4).

The influence of culture media and of the initial pH values of the PDA medium were scored by the diameter of the colony of test-fungus, measured at various time elapses, until the whole medium surface was covered by the fungus growth, and also by the microscopical analysis of sporulation (8).

The action of fungicides on the test-fungus was established by measuring the diameter of the fungus colonies at the time when in check Petri dishes (without fungicide) the culture had covered the whole surface, and it was estimated by calculation of the inhibition percentage of its growth, and also by calculating the regression line ; interpretation was made by using the scale previously developed (3).

For some fungicides additional determinations have been made (table 4), with various doses, to set the EC 50 (the efficient concentration) and EC 95, calculated with a IBM—PC—1 computer. Interpretation of results was made through the previously developed methodology (2), (4).

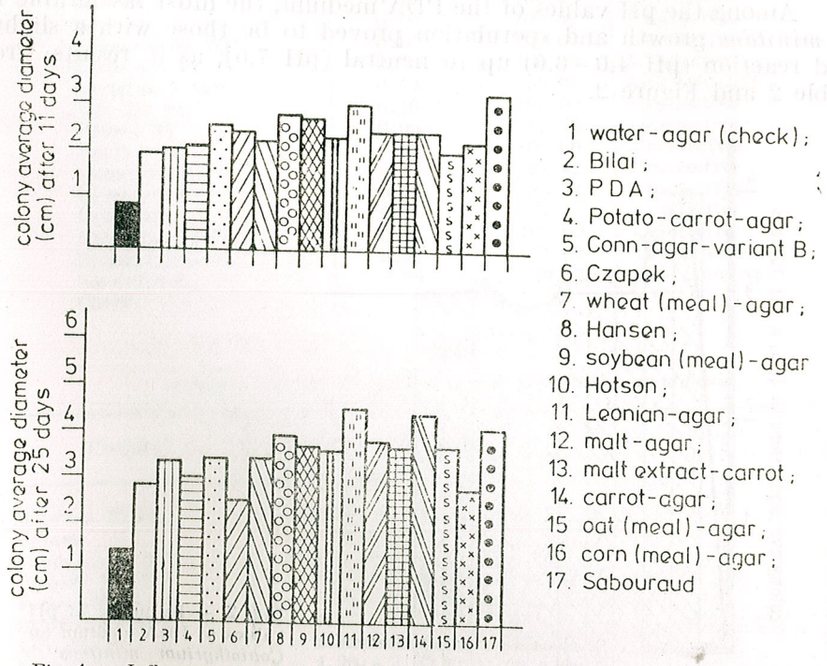

Fig. 1. — Influence of solid culture media on *Coniothyrium minitans growth*.

RESULTS AND DISCUSSION

a) GROWTH AND SPORULATION OF *C. MINITANS* ON VARIOUS CULTURE MEDIA

In tables 1 and 2 it could be seen that *C. minitans* grew very well on Sabouraud and Leonian media, well on Hansen and soybean meal media, followed by the media Conn-agar-variant B, malt-agar, malt extract-carrot, carrot-agar, Czapek and Hotson. The poorest growth was noted on Bilai medium and agarized water.

These results confirm those obtained for the media previously used : Sabouraud, PDA, malt-agar, malt extract-carrot and carrot-agar (8).

For the 6 newly-introduced media, their influence on the growth and sporulation of the hyperparasite fungus was established; thus, the most favourable were : Leonian, followed by Hansen, Conn-agar-variant B, Hotson, whereas more unfavourable proved to be Bilai and the agarized water, on which both fungus growth and sporulation were poor.

These results agree with those obtained by Iakubova and Chaban (6), when culturing on liquid Leonian, PDA and potato extract media, as well as those reviewed by Whipps and Gerlagh (10).

b) GROWTH AND SPORULATION OF *C. MINITANS* ON PDA MEDIUM WITH VARIOUS pH INITIAL VALUES

Among the pH values of the PDA medium, the most favourable for *C. minitans* growth and sporulation proved to be those with a slightly acid reaction (pH 4.0—6.0) up to neutral (pH 7.0), as it results from Table 2 and Figure 2.

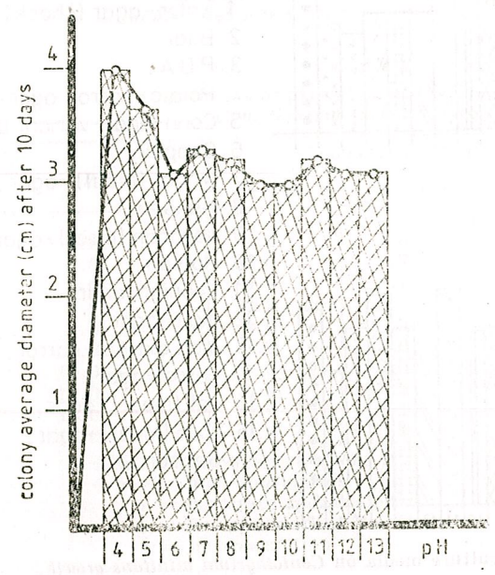

Fig. 2. — Influence of pH values of PDA medium on *Coniothyrium minitans* growth.

As the pH effect on the growth and sporulation of this fungus has not been studied as yet (10), these results represent the first contributions on a world-wide scale.

c) INFLUENCE OF SOME PESTICIDES ON THE GROWTH AND SPORULATION OF *C. MINITANS*

Tables 3 and 5 show a high sensitivity of the hyperparasite fungus *C. minitans* to the fungicides tested, at the usual concentration.

According to the selectivity scale, all products inducing an inhibition superior to 80% are considered highly toxic and, consequently, unse-

Table 3

Toxicity of some fungicides against *Coniothyrium minitans*

Product	Concentration %	% of inhibition	Selectivity
Turdacupral 50 PU	0.50	69.71	Low selective
Cuzin 15 SC	1.00	100.00	Unselective
Captadin 50 PU	0.25	77.95	Low selective
Sumilex 50 WP	0.05	76.48	Low selective
Tiuram 75 PU	0.30	100.00	Unselective
Dithane M—45	0.20	100.00	Unselective
Metozir	0.40	100.00	Unselective
Metoben 70 PU	0.10	100.00	Unselective
Fundazol 50 WP	0.10	100.00	Unselective
Tecto 450 Fl	0.10	100.00	Unselective
Bayleton 5 WP	0.10	69.86	Low selective
Tilt 250 EC	0.10	100.00	Unselective
Baycor 25	0.05	82.65	Unselective
Anvil 5 SC	0.05	100.00	Unselective
Quinolate 400	0.175	92.91	Unselective
Fademorf 200	0.15	80.74	Unselective
Falimorf	0.30	87.80	Unselective
IAMN-SN—210	0.25	100.00	Unselective
Ridomil plus 48	0.25	71.62	Low selective
Sandofan C	0.25	69.12	Low selective
Check	—	0	

Table 4

Toxicity of some fungicides against *Sclerotinia sclerotiorum*

Product	Equation of regression	EC 50 mg/l a.i.	EC 90 mg/l a.i.	Correlation coefficient
Metoben 70 PU	$y = 6.36 + 2.38\ x$	0.26	1.29	0.99
Metozir	$y = 4.89 + 1.95\ x$	1.12	7.76	0.98
Fundazol 50 WP	$y = 6.21 + 2.40\ x$	0.31	1.50	0.97
Dithane M—45	$y = 3.24 + 2.11\ x$	6.75	40.26	0.98
Tecto 450 Fl	$y = 7.20 + 2.69\ x$	0.15	0.61	0.97
IAMN-SN—210	$x = 4.57 + 2.21\ x$	1.55	8.56	0.99
Tilt 250 EC	$y = 7.35 + 2.27\ x$	0.09	0.48	0,99
Falimorf	$y = 4.96 + 2.33\ x$	1.03	5.21	0.99

Table 5

Toxicity of some fungicides against *Coniothyrium minitans*

Product	Equation of regression	EC 50 mg/l a.i.	EC 90 mg/l a.i.	Correlation coefficient
Metoben 70 PU	$y = 1.85 + 2.30\ x$	23.09	118.84	0.99
Metozir	$y = 1.34 + 2.46\ x$	30.60	141.92	0.99
Fundazol 50 WP	$y = 2.32 + 2.20\ x$	16.34	90.50	0.99
Cuzin 15 SC	$y = 1.47 + 2.23\ x$	37.52	202.89	0.97
Tecto 450 Fl	$y = 2.56 + 2.28\ x$	11.57	60.31	0.99
Tilt 250 EC	$y = 3.37 + 2.16\ x$	5.62	32.07	0.98
IAMN-SN—210	$y = 1.69 + 2.28\ x$	27.80	144.69	0.99
Anvil 5 SC	$y = 6.23 + 2.45\ x$	0.31	1.46	0.97

lective. In this category are enclosed : Cuzin 15 SC, Tiuram 75 PU, Dithane M—45, Metozir, Metoben 70 PU, Fundazol 50 WP, Tecto 450 Fl, Tilt 250 EC, Baycor 25, Anvil 4 SC, Fademorf 200, Falimorf, IAMN-SN-210 and Quinolate 400.

In the category of toxic compounds (slightly selective), the compounds inducing a 63—80% inhibition are included. Here are comprised all other products : Turdacupral 50 PU, Captadin 50 PU, Sumilex 50 WP, Bayleton 5 WP, Ridomil plus 48 and Sandofan C.

Table 4 presents the data on toxicity of products to *Sclerotinia sclerotiorum* (Lib.) de Bary. The most toxic products to this fungus were : Tilt 250 EC, Tecto 450 Fl, Fundazol 50 WP and Metoben 70 PU. Otherwise, the other products too, having an EC 50 of about 1 mg/l, can be considered as very active.

The fungus *C. minitans*, which can be used as a biological control means against *S. sclerotiorum*, is affected mainly by the products Anvil 5 SC and Tilt 250 EC ; however, *C. minitans* is generally more resistant to Anvil 5 SC, comparatively to the pathogen *S. sclerotiorum*.

Table 6

Selectivity index (SI) of some fungicides and the ratio of using concentration (UC) and efficient concentration (EC) 95 for *Sclerotinia sclerotiorum* (S.s.) and *Coniothyrium minitans* (C.m.)

Product	EC 50 mg a.i./l S.s.	EC 50 mg a.i./l C.m.	SI	UC mg a.i./l	EC 95 mg a.i./l S.s.	EC 95 mg a.i./l C.m.	Ratio UC/EC 95 S.s.	Ratio UC/EC 95 C.m.
Metoben 70 PU	0.26	23.09	88.80	700	1.29	118.84	542.0	5.89
Metozir	1.12	30.60	27.32	1600	7.76	141.92	206.0	11.27
Fundazol 50 WP	0.31	16.34	52.37	500	1.50	90.50	333.0	5.52
Tecto 450 Fl	0.15	11.57	77.13	450	0.61	60.30	737.7	7.46
Tilt 250 EC	0.09	5.62	62.44	250	0.48	32.07	520.8	2.79
IAMN-SN—210	1.55	27.80	17.93	1250	8.56	144.69	146.0	8.64

The selectivity index for some fungicides is rather high, *S. sclerotiorum* being much more sensitive to Anvil 5 SC. If the ratio between the usual rate and the EC 95 is calculated, this has values between 5.52 and 11.27 for *C. minitans*. That means that a field application with these fungicides to control *S. sclerotiorum* will considerably affect the mycelium and spores of *C. minitans*.

When examining data on fungicide selectivity, one can assess that none of the compounds tested can be recommended to be applied together with the hyperparasitic fungus. Therefore, fungicide spraying should be performed before or after the hyperparasite application.

An alternative solution could be seed dressing with Metoben 70 PU, Fundazol 50 WP, Tiuram 75 PU, Quinolate 400, Tecto 450 Fl, Sumilex 50 WP, which act locally, at the seed surface and in the soil, around the seed (spermosphere), while the potential *C. minitans* treatments have to be directed to destroy sclerotia in the soil, or by sprayings onto the plants.

When consulting and commenting the updated literature on *C. minitans* biology and its potential for biological control of sclerotial fungi (10), one can conclude that this paper brings contributions representing world-wide priorities, particularly those referring to the new culture media tested, to the pH values, and establishing the selectivity of some fungicides to this beneficial fungus for plant protection.

As a result, we made specifications regarding the most favourable culture media (Sabouraud, Leonian, Hansen, soybean meal-agar) and the most favourable pH values (4.0—7.0), these conditions being able to ensure mass culture of *C. minitans*, with a view of developing a new bioproduct whose action spectrum includes the sclerotial plant pathogenic fungi.

As a consequence of the lack of selectivity of the fungicides tested against *C. minitans*, one reaches the conclusion of a practical alternative for seed dressing with some fungicides specific to control the white rot, and the possible application of the biological treatment with *C. minitans* to destroy sclerotia occurring in the soil biological supply, or for season applications.

Likewise, it is considered necessary to extend research, having as an objective to find unpolluting alternatives within the technologies for integrated control of agricultural crops.

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Received March 17, 1993

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NEW OR RARE HOST-PLANTS FOR ROMANIAN USTILAGINALES

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A number of 7 smuts species and 67 "matrix nova" are presented for Romania.

The investigations on Ustilaginales have not been our special target. Notwithstanding, during 30 years of fieldwork throughout Romania, there were gathered numerous herbarium-samples from this important group of fungi. On the ground of most recent bibliographical synthesis on fungi from Romania (1) and of our files from the Mycologic Herbarium of the Institute of Biology in Bucharest, we choose for publication the following records.

The list comprises 7 species new for Romania and 67 "matrix nova". The number of host-plants is 95 and that of the combinations fungus-host is 104. There are also listed some combinations, rarely reported from Romania. From among the records we mention the species *Melanotaenium adoxae* (Bref.) S. Ito, an extremely rare fungus, found by us near the site from which it was first reported by C. Petrescu (2). Of the host-plants we stress especially the genus *Polyschemone*, endemic in Romania (Rodna Mountains), which has been established for the first time as a host for a smuts-fungus. Many hosts are endemites with a quite restricted area of distribution like *Dianthus barbatus compactis*, *D. henteri*, *D. nardiformis*, *Hieracium pojoritense*, *Silene nutans dubia* etc.

The list presents the hosts in alphabetical order. The counties are abbreviated as usual, they being given from N to S, in a counter clockwise manner.

The entire material is inventoried and deposited in the mycologic collection of the Institute of Biological Sciences of Bucharest (B U C M).

Acknowledgement. We express our gratitude to the biologists St. Roman, N. Roman and E. Docea who have kindly put at our disposal plants infested by smuts, and to Mr. O. Constantinescu who has identified some fungus samples.

Achillea millefolium L. subsp. *millefolium* : *ENTYLOMA ACHILLEAE* Magnus — *HR* : Cristurul Secuiesc, Fântâna Sărată, 46°15′48″N, 25°02′48″E, alt. 480 m, 18 X 1991, GN (BUCM 123.242).

Adoxa moschatellina L. : *MELANOTAENIUM ADOXAE* (Bref.) S. Ito — *BT* : Rediu, in silva Rediu, 47°32′09″N, 27°14′05″E, alt. 130 m, 25 V 1989, GN (BUCM 115.337), id. 47°31′47″N, 27°14′22″E, alt. 125 m, 1 VI 1990, GN (BUCM 118.232). Published in Romania at first by C. Petrescu in 1923 (2) also at same zone ("Valea Ioanei"). Herbarium material to be wanted. In our material the sori to be found in stolons, very rare in the rhizomes.

Agropyron brandzae Panțu & Solacolu subsp. *ciliatum* (G. Grinț.) Dihoru & Negrean : *Tilletia controversa* Kühn — All the 14 specimens

from BUCM belong to this subspecies, also T. Săvulescu's '*Agropyron brandzae*'.

Agrostis stolonifera L.: *TILLETIA SPHAEROCOCCA* (Wallr.) Fisch. v. Waldh.-*SM :* Scărişoara Nouă, 47°35'...N, 22°14'...E, alt. 135 m, 23 VI 1977, GN (BUCM 53.268).

Alopecurus arundinaceus Poiret: *USTILAGO STRIIFORMIS* (Westend.) Niessl — *IS :* Cârlig, Valea Cacaina,47°13'13''N, 27°33'03''E, alt. 60 m, 24 V 1969, GN (BUCM).

Anemone ranunculoides L.: *UROCYSTIS ANEMONES* (Pers.: Pers.) Winter — On this host the smuth is much more rare than in *A. nemorosa*. *GR :* Malul Spart, 28 IV 1974, GN (BUCM 22017); Călugăreni, 44°11'05''N, 25°59'15''E, alt. 50 m, 8 V 1982, GN (BUCM 70164). *PH :* Brazi, in silva Bădârlan, 44°50'38''N, 26°02'06''E, alt. 120 m, 28 IV 1985, GN (BUCM 87384). *NT :* Viişoara, in vallis Agârcia, 46°55'03''N, 26°17'47''E, alt. 365 m, 5 V 1986, GN (BUCM 97703).

Arabis sagittata (Bertol.) DC.: *USTILAGO THLASPEOS* (G. Beck) Lagerh. — *HD :* Petroşeni, Montes Piatra Leşului, 45°27'48''N, 23°23'00''E alt. 1100 m, 14 VII 1983, GN (BUCM 77405). *BZ :* Pâclele, 45°19'32''N, 26°42'23''E, alt. 250 m, 9 VII 1987, GN (BUCM 104056). "Matrix nova" for Romania.

Arenaria biflora L.: *MICROBOTRYUM VIOLACEUM* (Pers.: Pers.) Deml & Oberwinkler — *BV :* Montes Făgăraş, Gălăşescu, 45°36'55''N, 24°46'55''E, alt. 2000 m, 24 VII 1987, GN (BUCM). "Matrix nova".

Arenaria serpyllifolia L.: *USTILAGO DURIAEANA* Maire — *MH :* Copăcioasa, 10 VI 1960, leg. S. & N. Roman, det. GN (BUCM 81.699).

Arnica montana L.: *ENTYLOMA ARNICALE* Ell. & Ev. — *SV :* Montes Căliman, Vatra-Dornei, Valea Negreştilor, 47°20'00''N, 25°20'43''E alt. 920 m, 7 VI 1987, GN (BUCM 103.580).

Arum maculatum L.: *MELANOTAENIUM ARI* (Cooke) Lagerh. — *CS :* Băile Herculane, Cheile Pecinişca, 10 V 1976, GN (BUCM 56.122). In the absence of rhizomes and of spots on leaves, it is difficult to separate *A. maculatum* from *A. orientale*. Much frequent is the smuth on *A. orientale* (55 specimens in BUCM).

Bilderdykia aubertii (Louis Henry) Moldenke (cult.): *USTILAGO ANOMALA* J. Kunze ex Winter — Bucureşti, Parcul Carol I, 44°24'35''N, 26°05'50''E, alt. 82 m, 26 X 1990 and 5 X 1991, GN (BUCM). "Matrix nova" for Romania.

Bromus inermis Leysser: *USTILAGO STRIIFORMIS* (Westend.) Niessl — *SV :* Zamostea Pârâul Putred, 47°51'20''N, 26°12'33''E, alt. 311 m, GN (BUCM 109395).

Bromus riparius Rehm: *UROCYSTIS BROMI* (Lavrov) Zundel — *CT :* Basarabi, 17 V 1974, leg. GN, det. O. Constantinescu (BUCM 41456). "Matrix nova" for Romania.

Bromus squarrosus L.: *USTILAGO BULLATA* Berk. — *CT :* Hagieni, 7 VI 1967, leg. GN, det. O. Constantinescu (BUCM 40741).

Carex brevicollis DC.: *SCHIZONELLA MELANOGRAMMA* (DC.) Schröter — Not to be recorded from Moldavia. *GL :* Gârboavele, 11 VI 1978, GN (BUCM 51893).

Carex depressa Link subsp. *transsilvanica* (Schur) Egorova — *ANTHRACOIDEA CARYOPHYLLEAE* Kukk. — *SM :* Ţara Oaşului, Montes Buiana, Poiana Fătăciune, 47°53'04''N, 23°33'50''E, alt. 690 m, 3 VI 1983, GN (BUCM 83056); Negreşti-Oaş, Valea Albă, Culmea Brebu, 47°52'28''N, 23°32'27''E, alt. 640 m, 3 VI 1983, GN (BUCM 76.720). "Matrix nova".

Carex fuliginosa Schkuhr subsp. *fuliginosa : ANTHRACOIDEA MISANDRAE* Kukk. — *MM :* Montes Rodnei, in cacumine Puzdrele, 47°34'44''N, 24°43'36''E, alt. 2100 m, 4 IX 1974, GN (BUCM 41.618). New for Romania.

Carex halleriana Asso: *ANTHRACOIDEA CARICIS* (Pers.) Bref. — *CT :* Pădurea Hagieni, 28 V 1981, GN (BUCM 59.279), id. 44°47'44''N, 28°27'47''E, alt. 25 m, 24 V 1987, GN (BUCM).? "Matrix nova". *SCHIZONELLA COCCONII* (Morini) Liro — *MH :* Sviniţa, 44°30'30''N, 22°06'00''E, 18 V 1979, GN (BUCM 67997); Porţile de Fier, Viaductul Scarpiei, 10 VI 1981, GN (BUCM 59418). *TL :* Babadag, Valea Caugagia, 44°48'58''N, 28°40'01''E, alt. 65 m, 31 V 1984, GN (BUCM 82910). *CT :* Medgidia, 10 VII 1974, GN (BUCM 41613); Basarabi, 17 V 1974, GN, det. O. Constantinescu (BUCM 42388); Canaraua Fetii, 20 V 1976, GN (BUCM 53301); Dumbrăveni, 27 V 1981, GN (BUCM 59265); In silva Hagieni, 44°47'44''N, 28°27'47''E, alt. 25 m, 24 V 1987, GN (BUCM 103344), id. Cazanul Mare, 28 V 1981, GN (BUCM 59276). *TOLYPOSPORIUM ATERRIMUM* (Tul.) Dietel — *MH :* Sviniţa, 18 V 1979, GN (BUCM 67946). "Matrix nova" for Romania.

Carex hirta L.: *ANTHRACOIDEA SUBINCLUSA* (Körn.) Bref. — *TR :* in silva Brânceni, 24 VI 1980, GN (BUCM).

Carex ligerica Gay: *ANTHRACOIDEA ARENARIA* (H. Syd.) Nannf. — *TL :* Sfiştofca, 8 VI 1978, GN (BUCM 52461); C. A. Rosetti, 45°30'...N, 29°32'...E, alt. 5 m, 14 VIII 1978, GN (BUCM 67940). "Matrix nova" for Romania.

Carex liparocarpos Gaudin subsp. *liparocarpos : ANTHRACOIDEA CARICIS* (Pers.) Bref. s.l. — *BZ :* Vulcanii Noroioşi, "Pâclele Mici", 45°21'26''N, 26°41'40''E, alt. 410 m, 10 VI 1982, GN (BUCM 71066).

Carex michelii Host: while I gathered *Schizonella melanogramma* from 16 localities, the *TOLYPOSPORIUM ATERRIMUM* (Tul.) Dietel I gathered only from *IS :* Valea Lupului, Reservatio Valea lui David, 47°11'41''N, 27°28'12''E, alt. 110 m, 23 V 1992, GN (BUCM 124.362). "Matrix nova" for Romania.

Carex montana L.: *SCHIZONELLA MELANOGRAMMA* (DC.) Schröter — *BV :* Hărman, Dealul Lempeş. 28 V 1978, GN (BUCM). *PH :* Stânca Tohanilor, 4 V and 18 VI 1979, GN (BUCM 53571 et 53873). *GR :* in silva Comana, 1 VI 1980, GN (BUCM). "Matrix nova" for Romania.

Carex pilosa L.: *ANTHRACOIDEA PILOSAE* Vánky — *SV :* Solca, Valea Trei Iazuri, 47°43'37''N, 25°51'14''E, alt. 462 m, 4 VII 1980, GN (BUCM 67987). New for Romania.

Carex praecox Schreber: *ANTHRACOIDEA ARENARIA* (H. Syd.) Nannfeldt — *MH :* Gura Motrului, 44°32'51''N, 23°25'20''E, alt. 270 m, 17 V 1983, GN (BUCM 78204); Butoieşti, 16 V 1983, GN (BUCM 78207). *TL :* Greci, 'Montis' Ţuţuiatu, 45°11'52''N, 28°15'55''E, alt.

300 m, 7 VI 1989, GN (BUCM 112.242). *GR :* Vadul Lat, 5 VI 1977, GN (BUCM 50599). "Matrix nova" for Romania. *SCHIZONELLA MELANOGRAMMA* — Mentioned only from Ostrov, I also add *TR :* Drăgănești-Vlașca, 10 VII 1980, GN (BUCM 56406). *CT :* Canaraua Fetii, 30 V 1981, GN (BUCM 59324).

Carex rostrata Stokes : *ANTHRACOIDEA INCLUSA* Bref. — *SV :* Dorna Depresion, Roșu, 47°21′04″N, 25°18′30″E, alt. 810 m, 22 VIII 1980, GN (BUCM). New for Romania.

Carex rupestris All. : *ANTHRACOIDEA RUPESTRIS* Kukk. — *VL :* Montes Cozia, 27 VI 1975, GN (BUCM 55.377). New for Romania.

Carex vesicaria L. : *ANTHRACOIDEA SUBINCLUSA — SM :* Urziceni, 47°44′34″N, 22°23′01″E, alt. 117 m, 31 V 1983, GN (BUCM 76625).

Cichorium intybus L. : *ENTYLOMA CICHORII* Wróbl. — It was not mentioned in the southern Romania — *IF :* Balta Neagră, 44°41′05″N, 26°18′40″E, alt. 75 m, 21 V 1982, GN (BUCM 70328). *BZ :* Buzău, in silva Frasin, 45°26′10″N, 26°48′40″E, alt. 85 m, 10 VII 1987, GN (BUCM 104.087).

Colchicum biebersteinii Rouy : *UROCYSTIS COLCHICI* (Schlecht.) Rabenh. — to the only known locality, I add *CT :* Dealul Allah-Bair, 16 V 1974, GN (BUCM 41289) and silva Hagieni, 7 IV 1977, GN (BUCM 48.676).

CUCUBALUS BACCIFER L. : *MICROBOTRYUM VIOLACEUM* (Pers. : Pers.) G. Deml & Oberw. — *MS :* Gara Sovata, 46°34′55″N, 25°03′06″E, alt. 440 m, 6 VIII 1979, GN (BUCM 54361). "Matrix nova" for Romania.

Dianthus armeria L. subsp. *armeriastrum* (Wolfner) Velen. : *SOROSPORIUM SAPONARIAE* Rud. — *VL :* Tomșani, 26 VII 1961, leg. N. Roman, det. GN (BUCM 58705) ; Bunești, 20 VI 1961, leg. N. Roman, det. GN (BUCM 58706). "Matrix nova".

Dianthus barbatus L. subsp. *compactus* (Kit.) Heuffel : *MICROBOTRYUM VIOLACEUM — SV :* Lucina, 47°38′47″N, 25°11′28″E, alt. 1205 m, 14 VII 1986, GN (BUCM 99323) ; Vatra-Dornei, Montes Runc, 47°21′35″N, 25°20′06″E, alt. 1060 m, 14 VIII 1992, GN (BUCM 124721). "Matrix nova".

Dianthus deltoides L. : *SOROSPORIUM SAPONARIAE* Rud. — *SV :* Marginea, in vallis Iaslovăț, 47°46′52″N, 25°50′46″E, alt. 465 m, 27 VI 1989, GN (BUCM 113592). *MICROBOTRYUM VIOLACEUM — SV :* Marginea, in the same place with *Sorosporium* (BUCM 113.593) ; Zamostea, 47°48′52″N, 26°11′30″E, alt. 475 m, 15 VI 1988, GN (BUCM).

Dianthus henteri Heuffel ex Griseb. & Schenk : *SOROSPORIUM SAPONARIAE* Rud. — *GJ :* Piatra Cloșani, 45°06′00″N, 22°46′31″E, alt. 1160 m, 10 VI 1983, GN (BUCM 76868) and 11 VII 1984, GN (BUCM 84072). *VL :* Defileul Oltului, inter vallis Puturoasa et vallis Lotrișorul, 29 VI 1975, GN (BUCM 42737). "Matrix nova". *MICROBOTRYUM VIOLACEUM — VL :* Montes Cozia, Șirul de Pietre, 45°20′26″N, 24° 22′04″E, alt. 1500 m, 2 VIII 1987, GN (BUCM). "Matrix nova".

Dianthus membranaceus Borbás : *SOROSPORIUM SAPONARIAE* Rud. — *IS :* Voinești, in vallis Slavnicul, 47°04′16″N, 27°23′51″E, alt. 235 m, 27 V 1989, GN (BUCM 113.186). "Matrix nova".

Dianthus nardiformis Janka : *MICROBOTRYUM VIOLACEUM — TL :* Măcin, 'Montes' Cheia, 45°16′01″N, 28°10′38″E, alt. 200 m, 18 IX 1989, GN (BUCM). „Matrix nova".

Dianthus patraeus Waldst. & Kit. subsp. *petraeus : MICROBOTRYUM VIOLACEUM — CS :* Muntele Arjana, 45°00′55″N, 22°27′15″E, alt. 1400 m, 28 VII 1984, GN (BUCM 84394) ; Băile Herculane, Montes Domogled, 44°52′36″N, 22°26′20″E, alt. 1080 m, 30 VII 1984, GN (BUCM 84515). *GJ :* Montes Piatra Mică Cloșani, 45°05′36″N, 22°45′45″E, alt. 1100 m, 11 VII 1984, GN (BUCM 84063) ; Montes Piatra Mare Cloșani, 45°06′07″N, 22°46′16″E, alt. 1200 m, 11 VII 1984, GN (BUCM 84075), id. alt. 1420 m, 13 VII 1984, GN (BUCM 84128).

Dianthus pontederae Kerner subsp. *pontederae : MICROBOTRYUM VIOLACEUM — SM :* Urziceni, Grădina Cailor, 47°43′08″N, 22°21′22″E, alt. 130 m, 13 X 1983, GN (BUCM 80277). "Matrix nova" for Romania.

Dianthus pontederae subsp. *giganteiformis* (Borbás) Soó : *MICROBOTRYM VIOLACEUM — VL :* Montes Cozia, Surdoiu, Priboiasa, 45°20′58″N, 24°23′34″E, alt. 780 m, 3 VIII 1987, GN (BUCM 104575). "Matrix nova".

Dianthus spiculifolius Schur : *MICROBOTRYUM VIOLACEUM — BV :* Montes Postăvarul, Muchia Cheii, 45°33′58″N, 25°33′41″E, alt. 1700 m, 16 VIII 1989, GN (BUCM 114884). "Matrix nova".

Dianthus superbus L. subsp. *superbus : MICROBOTRYUM VIOLACEUM* — known only from two localities, I also add : *BN :* Tiha-Bârgăului, Dealul Strâmbei, 47°13′26″N, 24°47′31″E, alt. 600 m, 1 X 1987, GN (BUCM 106307).

Dianthus superbus subsp. *speciosus* (Reichenb.) Pawl. : *MICROBOTRYUM VIOLACEUM — BV :* Montes Făgăraș, Piatra Caprei. 45°37′31″N, 24°38′39″E, alt. 2160 m, 23 VII 1987, GN (BUCM 104328), "Matrix nova".

Dianthus tenuifolius Schur : *SOROSPORIUM SAPONARIAE — AG :* Rucăr, Cheile Dâmboviței, 4 VII 1981, GN (BUCM 59573). *MICROBOTRYUM VIOLACEUM — SV :* Benia, Dealul Glodului, Răchitișul Mare, 47°38′44″N, 25°15′25″E, alt. 1020 m, 15 VII 1986, GN (BUCM 99385). *HR* Lacul-Roșu, Montes Suhardul Mic, 46°47′54″N, 25°47′41″E, alt. 1160 m, 24 VI 1986, GN (BUCM 98807). *GJ :* Cloșani, montes Piatra Mică Cloșani, 45°05′36″N, 22°45′45″E, alt. 1100 m, 11 VII 1984, GN (BUCM 84064) ; Montes Piatra Mare Cloșani, 45°06′07″N, 22°46′16″E, alt. 1200 m, 11 VII 1984 and 18 VII 1985, GN (BUCH 84146 & 89420).

Digitaria ischaemum (Schreber) Muhl. : *USTILAGO SYNTHERISMAE* (Schwein.) Peck — *TL :* Montes Pricopan, 45°14′36″N, 28°12′14″E, alt. 325 m, 22 IX 1989 GN (BUCM 114366).

Echinochloa oryzoides (Ard.) Fritsch : *USTILAGO TRICHOPHORA* (Link) Körn. — *CL :* Căscioarele, 44°06′44″N, 26°27′01″E, alt. 14 m, 7 X 1983, GN (BUCM 80734). "Matrix nova".

Elymus farctus (Viv.) Runemark ex Melderis : *USTILAGO HYPODITES* (Schlecht.) Fr. — *CT :* Cetatea Histria, VIII 1987, leg. N. Roman, det. GN (BUCM 105093). "Matrix nova".

Filaginella uliginosa (L.) Opiz : *ENTYLOMA MAGNUSII* (Ule) Woronin — *SV :* Clit, 47°44′55″N, 25°51′35″E, alt. 445 m, 26 VIII 1982,

GN (BUCM 72820), id. 1987, 1988, 1991; Bogdănești, Dealul Moișa, 16 VII 1948, leg. C. Burduja, det. & comm. GN (BUCM 73457).

Gagea minima (L.) Ker. — Gawl.: *USTILAGO ORNITHOGALI* (Schmidt & Kunze) Magnus — *B :* in silva Dudu, 2 V 1976, leg. GN, det. O. Constantinescu (BUCM 55384), "Matrix nova".

Gagea arvensis (Pers.) Dumort.: *USTILAGO ORNITHOGALI* — *B :* in silva Băneasa, 44°31′01″N, 26°05′54″E, alt. 91 m, 27 III 1988, GN (BUCM 108267).

Glyceria nemoralis (Uechtr.) Uechtr. & Körnicke: *USTILAGO FILIFORMIS* (Schrank) Rostrup — *AG :* Podul Dâmboviței, in vallis Cheia, 21 X 1981, GN (BUCM 68047). *PH :* Montes Ciucaș, in vallis Berii, 15 VII 1982, GN (BUCM).

Hieracium argillaceum Jordan: *ENTYLOMA HIERACII* H. & P. Syd. ex Cif. — *SV :* Montes Căliman, Dornișoara, 7 IX 1982, GN (BUCM). *PH :* Cheile Doftanei, 14 VI 1980, GN (BUCM 56225). "Matrix nova".

Hieracium murorum L.: *ENTYLOMA HIERACII* — *SV :* Poieni-Solca, 4 VII 1980, GN (BUCM). *HR :* Băile Tușnad, 10 VI 1985, GN (BUCM).

Hieracium pojoritense Woloszczak: *ENTYLOMA HIERACII* — *NT :* Bicaz-Chei, Cheile Șugăului, 46°49′36″N, 25°51′21″E, alt. 750 m, 25 VI 1986, GN (BUCM 98860). "Matrix nova".

Hieracium rotundatum Kit. ex Schultes: *ENTYLOMA HIERACII* — *SV :* Vatra-Dornei, Dealul Negru, 11 VIII 1980, GN (BUCM 56622).

Juncus minutulus Albert & Jahandiez: *ENTORRHIZA ASCHERSONIANA* (Magnus) Lagerh. — *MM :* Borșa, 3 IX 1974, GN (BUCM 57874). *SV :* Clit, 'Pe Tolocuță', 2 IX 1980, GN (BUCM 57233); Solca, La Trei Iazuri, 4 VII 1980, GN (BUCM 56294); Montes Căliman, Dornișoara, Canton Strunior, 23 VIII 1980, GN (BUCM 56899). "Matrix nova".

Leymus racemosus (Lam.) Tzvelev subsp. *sabulosus* (Bieb.) Tzvelev: *USTILAGO HYPODITES* — mentioned only in a seaside place; the material is missing, I also found it in *TL :* Caraorman, 45°03′26″N, 29°22′55″E, alt. 6 m, 3 X 1980 and 20 V 1988, GN (BUCM 57437 & 108382).

Luzula alpinopilosa (Chaix) Breistr. subsp. *obscura* Fröhner: *USTILAGO SPADICEA* (Liro) Vánky — *HD :* Montes Retezat, Tăul Negru, 45°21′32″N, 22°49′55″E, alt. 2000 m, 26 VIII 1985, GN (BUCM). New for Romania.

Luzula pilosa (L.) Willd.: *USTILAGO LUZULAE* Sacc. — mentioned only in one place, I gathered it from 15 localities, in the mountains: Obcina Mare, Suhard, Bârgău, Căliman, Gurghiu and Stânișoarei (BUCM).

Minuartia recurva (All.) Schinz & Thell.: *MICROBOTRYUM VIOLACEUM* — *PH :* Montes Bucegi, Jepii Mici, 4 VII 1906, leg. I. Prodan, det. & comm. GN (BUCM 93872). It is also mentioned in the Bucegi Mountains, but the herbarium material is missing.

Moehringia muscosa L.: *SOROSPORIUM SAPONARIAE* — *GJ :* Motru Sec, Vallis Lupșa, 45°03′30″N, 22°47′46″E, alt. 400 m, 17 VII 1985, GN (BUCM 89381), vallis Motru Sec, 18 VI 1987, GN (BUCM 103679); Cloșani, Vallis Calu, 45°04′04″N, 22° 48′58″E, alt. 500 m, 30 VII 1986, GN

(BUCM 100348); Cheile Motrului, 45°06′50″N, 22°48′14″E, alt. 425 m, 12 VI 1983, GN (BUCM 76957). "Matrix nova".

Myosotis sylvatica Hoffm. subsp. *sylvatica : ENTYLOMA FERGUSSONII* (Berk. & Broome) Plowr. — *VL :* Montes Buila, inter Schitul Pahomie et Stâna 'La Oale', 25 IX 1980, GN (BUCM). "Matrix nova" for Romania.

Myosoton aquaticum (L.) Moench: *MICROBOTRYUM VIOLACEUM* — *MS :* Sovata-Băi, Lacus Ursu, 6 VIII 1979, GN (BUCM 54392). *DJ :* Târmurești, 20 V 1977, GN (BUCM 55386). *TR :* in silva Nanov, 39°59′...N, 26°19′...E, alt. 70 m, 1 V 1979, GN (BUCM 53537). *VL :* Montes Buila, Schitul Pahomie, 25 IX 1980, GN (BUCM 57360). *IF :* in silva Bufteianca, 44°56′04″N, 25°58′32″E, alt. 106 m, 3 X 1982, GN (BUCM 73587) and 4 VI 1989, GN (BUCM 113.234). *GR :* Grădiștea, 44°13′10″N, 26° 09′55″E, alt. 50 m, 30 V 1982, GN (BUCM 72193). "Matrix nova" for Romania.

Oenanthe banatica Heuffel: *ENTYLOMA OENANTHES* R. Maire. — *SM :* highway Livada — Orașul Nou, 47°50″42″N, 23°14′44″E, alt. 148 m, 1 VI 1983, GN (BUCM 76697). *GR :* Iepurești, Lunca Neajlovului, 22 VI 1980, leg. GN, det. O. Constantinescu (BUCM 56275). "Matrix nova".

Poa trivialis L. subsp. *silvicola* (Guss.) H. Lindb. fil.: *UROCYSTIS POAE* (Liro) Padw. & Khan — *TR :* Nanov, 39°59′...N, 25°19′...E, alt. 70 m, 1 V 1979, GN (BUCM 57858). *GR :* in silva Malu-Spart, 28 IV 1974, GN (BUCM). "Matrix nova".

Polygonatum hirtum (Bosc ex Poiret) Pursh (= *P. latifolium* (Jacq.) Desf.): *UROCYSTIS POLYGONATI* Moesz & Ulbrich — *CT :* Negureni, in silva Mezarlic, 44°04′55″N, 27°44′48″E, alt. 130 m, 12 V 1992, GN (BUCM 124.204). New for Romania and "matrix nova".

Polygonatum multiflorum (L.) All.: *UROCYSTIS POLYGONATI* — *CS :* Montes Aninei, in vallis Beușnița 8 V 1976, GN, lecturer O. Constantinescu (BUCM 46890); Cârșia Beușniței, 6 VI 1976, GN (BUCM 55383); Montes Locva, Moldova Nouă, Cornetul Dracului, 44°45′01″N, 21°43′00″E, alt. 600 m, 11 V 1989, GN (BUCM 111971). "Matrix nova" for Romania.

Polygonatum odoratum (Miller) Druce: *UROCYSTIS POLYGONATI* — *CT :* in silva Seid-Orman, 10 V 1978, GN (BUCM 51644). "Matrix nova" for Romania.

Polyschemone nivalis (Kit.) Schott: *MICROBOTRYUM VIOLACEUM* — *MM :* Montes Rodnei, alt. 2000 m, sine datum, leg. A. P. Alexi (ante 1900), comm. & det. GN (BUCM 94700), in cacumine Pietrosul, 47°35′43″N, 24°38′17″E, alt. 2260 m, 22 VIII 1987, GN (BUCM 104856), Curmătura Pietrosului, 23 VIII 1987, GN (BUCM 104.882). "Matrix nova" (genus!).

Ranunculus acris L. subsp. *acris : ENTYLOMA FICARIAE* A. Fischer v. Waldheim — *BN :* Tiha-Bârgăului 47°13′48″N, 24°46′37″E, alt. 450 m, 28 IX 1987, GN (BUCM 106029). *HR :* Băile Tușnad, ad cacumine Cetății, 46°08′53″N, 25°53′01″ E, alt 1030 m, 8 VI 1985, GN (BUCM 88666). *MS :* Sighișoara, Dealul Strâmb 6°11′55″N, 24°45′39″E, alt. 500 m, 19 X 1991, GN (BUCM 123401). *GJ :* Cloșani, 45°04′03″N, 22°48′03″E, alt. 385 m, 11 V 1984, GN (BUCM 32265). *DB :* Montes

Bucegi, Cheile Urşilor, 45°23′56″N, 25°26′30″E, alt. 1600 m, 1 X 1982, GN (BUCM 72130). *NT :* Leghin, 16 VIII 1981, GN (BUCM 63280). *SV :* Montes Suhard, Runc, 47°21′09″N, 25°21′06″E, alt. 880 m, 6 IX 1983, GN (BUCM 78634) ; Poieni-Solca, 47°41′43″N, 25°51′30″E, alt. 500 m, 10 IX 1982, GN (BUCM 73173). "Matrix nova" for Romania. *ENTYLOMA MICROSPORUM* (Unger) Schröter — *SV :* Clit, 47°44′48″N, 25°51′25″E, alt. 455 m, 29 VIII 1982, GN (BUCM 72837), Dealul Ederii, 26 VIII 1983, GN (BUCM 78291). *UROCYSTIS RANUNCULI* (Lib.) Moesz — *HR :* Băile Tuşnad, ad Cacumine Cetăţii, 46°08′53″N, 25°53′01″E, alt. 1030 m, 8 VI 1985, GN (BUCM 88667). *HD :* Petroşeni, 45°25′25″N, 23°22′10″E, alt. 610 m, 10 VIII 1988, GN (BUCM 109753).

Ranunculus acris subsp. *strigulosus* (Schur) Hyl. (= *R. stevenii* auct. rom.) : *ENTYLOMA FICARIAE — SM :* Carei, in fossa castellii, 47°41′01″N, 22°18′05″ E, alt. 129 m, 31 V 1983, GN (BUCM 76598) et 2 X 1978, GN (BUCM 75017). *PH :* Buşteni, 45°24′44″N, 25°32′06″E, alt. 900 m, 7 X 1984, GN (BUCM 86074) ; Cocorăştii Mislii, 11 IX 1979, GN (BUCM 58788). *SV :* Fălticeni, Dumbrava Minunată, 13 VIII 1981, GN (BUCM 63147) ; Clit, in silva Cărbunărie, 47°44′28″N, 25°50′36″E, alt. 440 m, 26 VIII 1982, GN (BUCM 72815). *ENTYLOMA MICROSPORUM — SV :* Fălticeni, in silva 'În Poduri', 47°26′16″N, 26°21′04″E, alt. 390 m, 14 VIII 1981, GN (BUCM 83057) ; Solca, 47°41′00″N, 25°50′40″E, alt. 550 m, 10 IX 1982, GN (BUCM 73142) ; Clit, 47°45′05″N, 25°51′33″E, alt. 435 m, 24 VIII 1983, GN (BUCM 78235).

Ranunculus binatus Kit. : *ENTYLOMA EICARIAE — SV :* Clit, rivulum Maha, 47°44′53″N, 25°51′34″E, alt. 433 m, 9 IX 1991, GN (BUCM). "Matrix nova".

Ranunculus bulbosus L. s. l. : *ENTYLOMA MICROSPORUM — AB :* Zlatna, Valea lui Lal, 16 V 1947, leg. T. Bunea, det. GN (BUCM 69056).

Ranunculus constantinopolitanus (DC.) D'Urv. : *ENTYLOMA FICARIAE — GR :* Crânguri, 44°11′30″N, 25°59′08″E, alt. 55 m, 8 V 1982, GN (BUCM). "Matrix nova".

Ranunculus ficaria L. subsp. *bulbifer* Lawalrée : *UROCYSTIS FICARIAE* (Liro) Moesz — *NT :* Piatra-Neamţ, Dealul Cernegura, 46°55′14″N, 26°21′38″E, alt. 400 m, 1 V 1986, GN (BUCM). *CT :* Dobromir, in silva Sâtma, 44°02′09″N, 27°48′50″ E, alt. 110 m, 12 V 1992, GN (BUCM 124207.) "Matrix nova".

Ranunculus flammula L. subsp. *flammula : UROCYSTIS RANUNCULI — SV :* Montes Suhard, Runcu, 47°21′09″N, 25°21′06″E, alt. 880 m, 6 IX 1983, GN (BUCM 78638). "Matrix nova".

Ranunculus nemorosus DC. subsp. *nemorosus : ENTYLOMA MICROSPORUM — PH :* Montes Ciucaş, Creasta Zăganu, 14 VII 1982, GN (BUCM 71506). *DB :* Montes Bucegi, Cheile Urşilor, 1 X 1982, GN (BUCM 72140). *HD :* Montes Piule — Piatra Iorgovan, Poiana Funduri, 12 VIII 1982, GN (BUCM 72404).

Ranunculus pseudomontanus Schur : *UROCYSTIS RANUNCULI — HD :* Montes Piule — Piatra Iorgovan, 31 VII 1974, GN (BUCM 57847). "Matrix nova".

Sesleria rigida Heuffel ex Reichenb. subsp. *rigida : TILLETIA SESLERIAE* Juel — *AG :* Dragoslavele, Montes Vârtoapele (Piatra

Dragoslavelor), 45°21′40″N, 25°12′30″E, alt. 1400 m, 5 VII 1981, GN (BUCM 67254). New for Romania.

Silene borysthenica (Gruner) Walters : *USTILAGO MAJOR* Schröter — *CT :* Mamaia, 44°16′28″N, 28°37′18″E, alt. 2 m, 15 VIII 1991, GN (BUCM 121325). *TL :* Delta Dunării, C. A. Rosetti, 14 VIII 1978, GN (BUCM 67949). "Matrix nova" for Romania.

Silene bupleuroides L. subsp. *bupleuroides : MICROBOTRYUM VIOLACEUM — CJ :* Turda, 28 VI 1915, leg. A Borza, det. GN (BUCM 94662). *CT :* Istria, 44°33′20″N, 28°41′55″E, alt. 18 m, 21 IX 1982, GN (BUCM 83061).

Silene heuffelii Soó : *MICROBOTRYUM VIOLACEUM — HR :* Băile-Tuşnad, Ciucaş Lake, 46°08′45″N, 25°51′36″ E, alt. 632 m, 18 IX 1985, GN (BUCM 92208). "Matrix nova".

Silene pseudotites Besser ex Reichenb. : *USTILAGO MAJOR — MH :* Porţile de Fier, Viaductul Scarpiei, 10 VI 1981, GN (BUCM 59420). *TL :* Danube Delta, in silva Caraorman, 23 VI 1982, GN (BUCM 71179). "Matrix nova".

Silene nutans L. subsp. *dubia* (Herbich) Zapal. : *MICROBOTRYUM VIOLACEUM — PH :* Izvoare, 28 VI 1951, T. Săvulescu (sub *Silene nutans*), rev. GN (BUCM 41029). "Matrix nova".

Silene pusilla Waldst. & Kit. : *MICROBOTRYUM VIOLACEUM — MM :* Montes Rodnei, Fundul Divezilor, 21 VIII 1987, GN (BUCM). "Matrix nova" for Romania.

Silene saxifraga L. : *SOROSPORIUM SAPONARIAE — GJ,* Montes Mehedinţi, in Vallis Ţesna, ad 'Gaura Fetei', 44°58′00″N, 22°30′44″E, alt. 680 m, 27 VII 1984, GN (BUCM). *MH :* Băile Herculane, Montes Domogled, Crucea Albă, 44°53′11″N, 22°25′43″E, alt. 550 m, 30 VII 1984, GN (BUCM 84493), 6 XI 1986, GN (BUCM 102135) ; Cheile Pecinişca, 44°51′47″N, 22°25′32″E, alt. 480 m, 20 VII 1984, GN (BUCM 84510). "Matrix nova". *MICROBOTRYUM VIOLACEUM — CS :* Montes Arjana, 45°00′42″N, 22°27′25″E, alt. 1360 m, 28 VII 1984, GN (BUCM). *VL :* Montes Cozia, Bulzu, 45°10′00″N, 24°20′50″E, alt. 1520 m, 31 VII 1987, GN (BUCM 104461). "Matrix nova".

Stellaria holostea L. : *SOROSPORIUM SAPONARIAE — CS :* Cheile Nerei, 5 VI 1976, matrix leg. & det GN, fungus comm. GN, det. O. Constantinescu (BUCM 46839). *OT :* in silva Saru, 26 IV 1972, matrix leg. & det. GN, fungus comm. GN, det. O. Constantinescu (BUCM 40355). *GR :* Roata de Jos, in silva Cartojani, 44°26′35″N, 25°33′30″E alt. 125 m, 5 VI 1982, GN (BUCM 70994). "Matrix nova" for Romania.

Stipa capillata L. : *USTILAGO WILLIAMSII* (Griffiths) Lavrov 1— *CT :* Dunăreni, 44°12′27″N, 28°45′00″E, alt. 50 m, 22 V 1985, GN (BUCM 88196). "Matrix nova".

Stipa tirsa Steven subsp. *tirsa : USTILAGO HYPODITES* (Schecht.) Fr. — *BZ :* Pâclele, 45°19′25″N, 26°42′31″E, alt. 277 m, 10 VII 1987, GN (BUCM 104059). "Matrix nova".

Symphytum cordatum Waldst. & Kit. : *ENTYLOMA SEROTINUM* Schröter — *MM :* Montes Rodnei, vallis Măgurii, 47°35′02″N, 24°33′53″E, alt. 1270 m, 25 VIII 1987, GN (BUCM 104936). *BV :* Predeal, 2 VII 1954, leg. E. Docea, det. GN (BUCM 83379). "Matrix nova".

Symphytum tuberosum L. subsp. *nodosum* (Schur) Soó: *ENTYLOMA SEROTINUM — HD :* Almaşul Mic de Munte, 5 V 1946, leg. T. Bunea, det. GN (BUCM 69807). *MS :* Sovata-Băi, 28 VII 1979, GN (BUCM 57899). *MH :* Gara Gura Motrului, 44°34′27″N, 23°25′55″E, alt. 106 m, 17 V 1983, GN (BUCM 76549). All indications with *S. tuberosum*, are referring, for Romania, to the subspecies *nodosum*.

Thalictrum minus L. subsp. *pubescens* (Schleicher ex DC.) Rouy & Fouc.: *UROCYSTIS SOROSPOROIDES* Körn. ex Fischer v. Waldh. — *TL :* Delta Danubii, in silva Letea, 45°19′28″N, 29°31′00″E, alt. 2,5 m, 19 VI 1982, GN (BUCM 71067); Haşmacul Mare, 29 V 1979, GN (BUCM 53826). "Matrix nova".

Tragus racemosus (L.) All.: *USTILAGO TRAGI-RACEMOSI* Zogg (= *U. tragica* Vánky; *Sphacelotheca tragi* Săvul.

The smuts are known only from the 'locus classicus' (Murfatlar). I find to *CT :* Medgidia, 15 X 1974, GN (BUCM 41740); Topalu, Valea Mare, 44°33′27″N, 28°02′50″E, alt. 50 m, 3 VII 1985, GN (BUCM 89242).

Viola ambigua Waldst. & Kit.: *UROCYSTIS VIOLAE* (Sow.) Fischer v. Waldh. — *IS :* Valea Lupului, Reservatio Valea lui David, 47°11′52″N, 27°28′05″E, alt. 120 m, 24 V 1992, GN (BUCM 124420). "Matrix nova".

Viola kitaibeliana Schultes: *UROCYSTIS KMETIANA* Magnus — *DJ :* Ciupercenii Vechi, Arceru, 14 VI 1979, GN (BUCM 53966). *CT :* Albeşti, in silva Hagieni, 43°48′12″N, 28°28′20″E, alt. 14 m, 14 V 1992, GN (BUCM 124254). "Matrix nova" for Romania.

Viola hymettia Boiss. & Heldr.: *UROCYSTIS KMETIANA — DJ :* Cernele, 18 V 1977, GN (BUCM). "Matrix nova".

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