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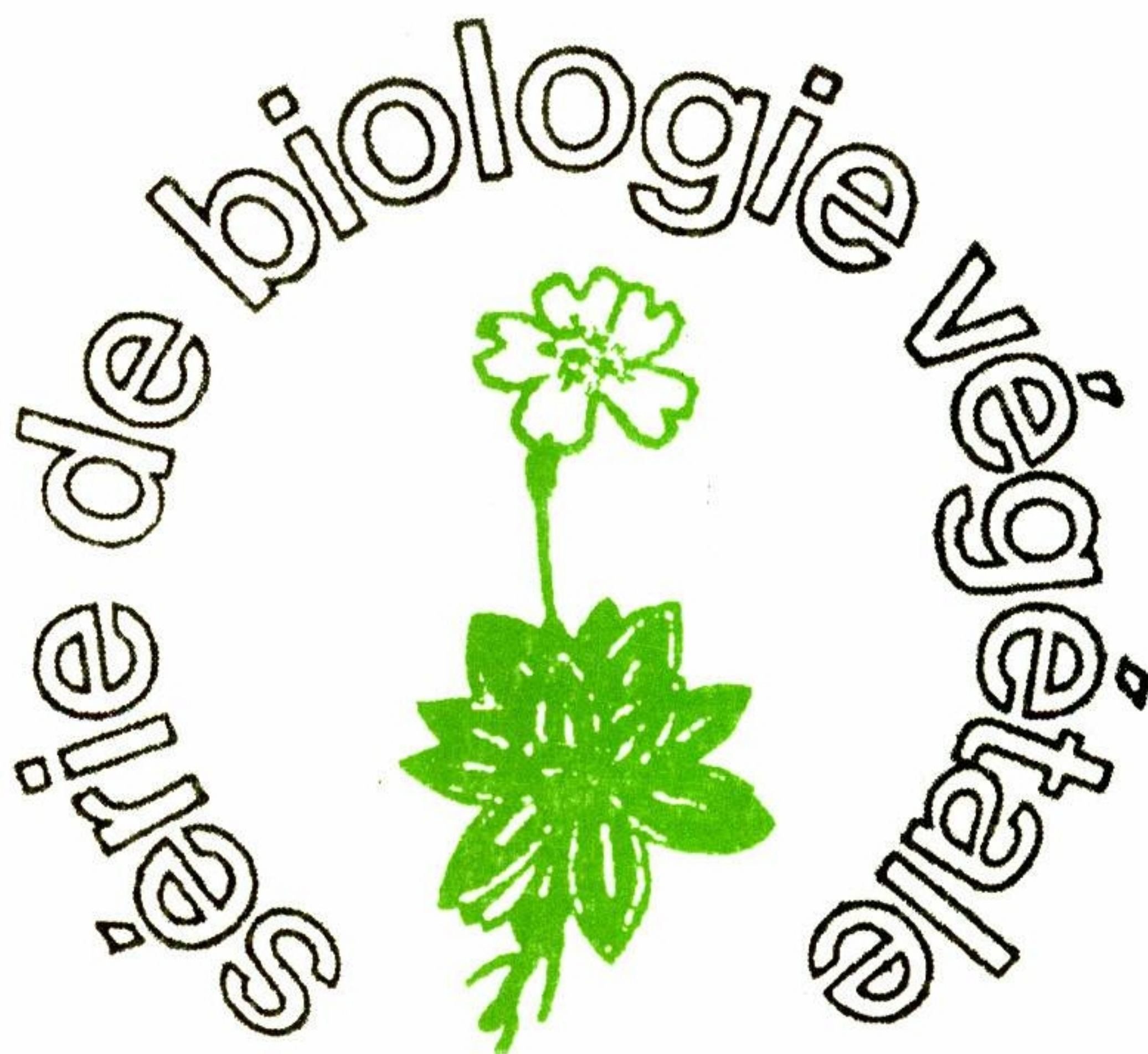
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THE RELATIONSHIP BETWEEN POLLEN GRAIN AND OVULE IN SOME PINACEAE

MARIN ANDREI*, PAULINA ANASTASIU**

Embryological studies in some Pinaceae (*Abies alba*, *Larix decidua* subsp. *polonica*, *Pinus mugo*, *Pinus nigra* subsp. *banatica*, *Pinus sylvestris*) show the penetration of pollen grains into pollen chamber induces alterations of integumental cells and generates a protoplasmic micropylar obturator. This closes the micropyle, but as we observed in some sections, the obturator can function as an optimal nutritive medium for the pollen grains germination. Also we suggest the replacement of the term "pollination drop" with the term "germination drop" that seems to be more adequate and realistical.

Key words: Pinaceae, pollen grain, integumental cells, micropylar obturator, pollination drop, germination drop.

INTRODUCTION

The male gametophyte of Gymnosperms, extremely reduced (2-cellular at full maturity), has to fulfil a series of specialized functions.

The first part of the male gametophyte existence, after meiosis, is spent within the sporophytic tissues of the pollen sac (microsporangium), where microgametogenesis takes place.

After the pollen grains were shed from the pollen sacs, they start an independent existence as free unicellular haploid organisms. Arrived in the pollen chamber with the aid of air currents, the grain begins the second stage of its life. If it is compatible, the pollen grain germinates producing the pollen tube, into which the vegetative and the generative cells migrate. The latter divides in the pollen tube resulting in two sperm cells (spermata).

In opposition to Angiosperms, where the interaction takes place between the male gametophyte and components of the ovary (stigma and style), in the studied Pinaceae and generally in Gymnosperms, interaction occurs between the male gametophyte and the tissues of the ovule (integument and nucellus) of sporophytic origin.

McWilliam (1958) and Ziegler (1959), cited by KNOX (1984), have shown, that, when receptive, the megaspores secrete a pollination drop; the pollen grains are transported and germinate in this fluid. In the pollination drop of *Pinus* sp. and *Taxus baccata* there were identified fructose, glucose and sucrose, and in *Ephedra*

and *Taxus baccata* free aminoacids. According to these authors and others (Colangeli and Owens 1990; Aderkas and Leary 1999), the pollination drop may be a product of the pollen chamber, respectively the nucellus or the megagametophyte.

Our observations do not agree with the literature cited, as will prove the microphotographs taken.

MATERIAL AND METHOD

The following species were analyzed: *Abies alba*, *Larix decidua* subsp. *polonica*, *Pinus mugo*, *Pinus nigra* subsp. *banatica*, *Pinus sylvestris*.

The collected material (ovules in various stages of development) was processed according to the rules of paraffin wax imbedding (Andrei and Rădulescu 1972). Fixation was carried out in the mixture by Navashin-Bruun, the staining with Ehrlich's hematoxylin. The sections were included in Canada balsam.

After being analyzed under the light microscope, the sections were photographed at the Docuval microscope and commented.

The results obtained were compared with the data of the consulted literature.

RESULTS AND DISCUSSIONS

Whereas in the case of the Angiosperms there is a close interaction between the pollen tube and the tissues of the gynoecium, in Gymnosperms the pollen grains come directly into contact with the outer envelope of the ovule at the level of the micropyle, which serves as receptive or stigmatic surface, always holocrine secretory in character.

In Angiosperms one suggested the top of the growing pollen tube, separated from the rest of the tube by a callose plug, might represent a mobile cell. It could be interpreted as a special case of cell migration in plants.

Referring to Gymnosperms there are no data published, neither did we observe pollen tubes interrupted by callose plugs.

In the taxon *Larix decidua* subsp. *polonica* the entering of the pollen grains into the pollen chamber starts drastic alterations of the cells of the inner layers belonging to the integument on either side of the micropyle. The cells walls are disorganized partially or wholly, and their content forms an obturator or plug which closes the micropyle, preventing the penetration of other pollen grains, of microorganisms or dust, etc. (Fig. 1). On the analyzed material we observed pollen grains germinating in that same zone of the micropyle. From this ensues that the

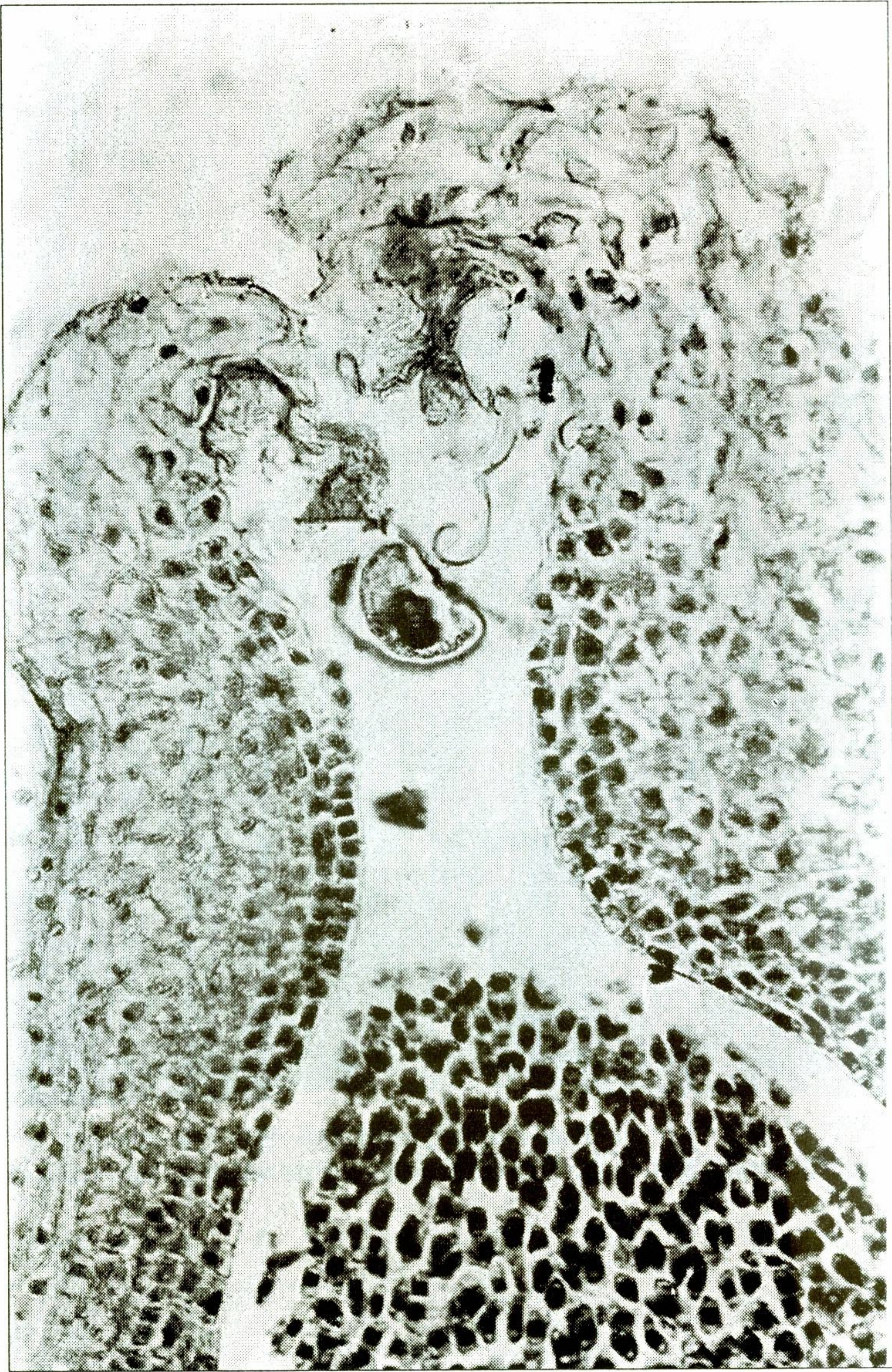


Fig. 1 – Longitudinal section through an ovule of *Larix decidua* subsp. *polonica*.
In the apical part of the micropyle are shown disorganized cells, belonging to the integument;
beneath a pollen grain (Oc. 12.5×; Ob. 10; Amplif. 12.5; Orig.).



Fig. 2 – Longitudinal section through an ovule of *Pinus mugo*.
The mucilage resulting from the lysis of the walls can be seen (Oc. 12.5×; Ob. 25; Orig.).

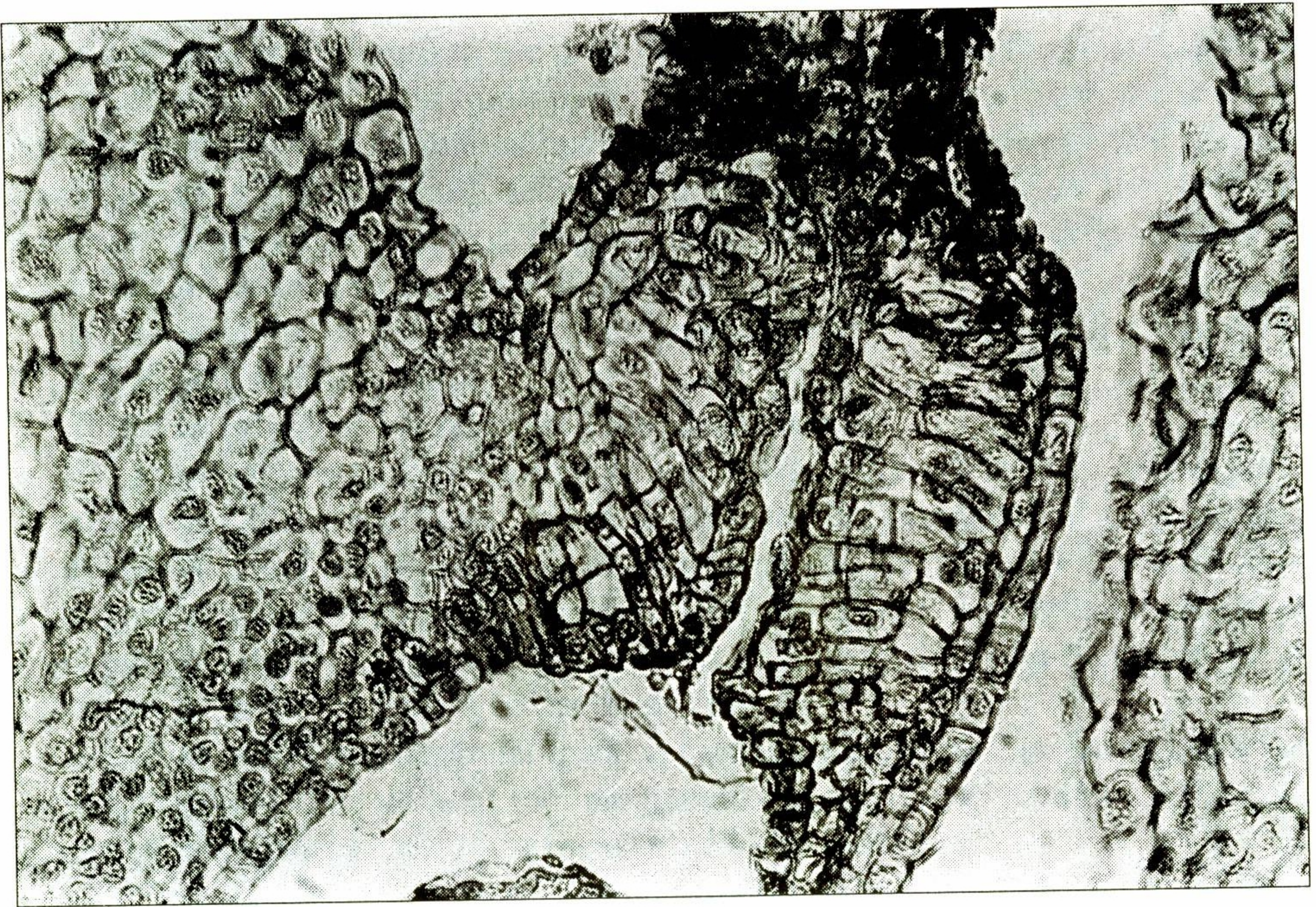


Fig. 3 – Longitudinal section through an ovule of *Pinus nigra* subsp. *banatica*, showing the apical micropylar mucilage and the integumental secretory cells (Oc. 12.5×; Ob. 10; Amplif. 16; Orig.).

protoplasmic micropylar obturator functions at the same time as an optimal nutritive medium for the germination of pollen grains – equivalent of the stigma in Angiosperms.

In *Pinus mugo* the cells of the inner integumental epidermis are transversally elongated, and in the external part of the micropyle they are being disorganized, together with almost all other integumental cells, inclusive those of the outer epidermis.

The content of the respective cells forms mucilage, which obturates the micropylar opening (Fig. 2).

Additional with regard to the preceding species we stress that the cells of the inner epidermis, coating the micropylar canal are much greater than those of the outer epidermis, have voluminous nuclei and play a secretory role, so that it can be considered as a differentiated secretory epidermis. This reminds the secretory epidermis of the stylar canal of Angiosperms. Thus, the pollen drop might be a product of these cells too, not only of nucellus.

In view of the shape of the integumental cells, all these will change into mucilage during a further stage.

In *Pinus sylvestris* we registered the same situation.

In *Pinus nigra* subsp. *banatica* the integument represents obviously a prolongation of the ovule body. In the upper part the integumental cells between both epidermes, incl. these are being disorganized and transformed into a mucilage which obturates the micropyle. Judging after the shape and orientation of the integumental cells, we appreciate that all will be transformed into mucilage during a late stage in the development of the ovule (Fig. 3).

In *Abies alba* the micropylar canal is coated by elongated secretory cells, rich in cellular content. The ends of the integument are, by the orientation of the cells, ready to be disorganized and to be transformed into mucilage.

CONCLUSIONS

The pollen grain of conifers is a specialized, independent cell, which interacts with a tissue complex, namely the ovule. In the present paper we demonstrate that consequent to pollination some extragametophytic tissues of the ovule get transformed into an obturator of mucilage, originating from the protoplasts of integumental cells.

In the species analyzed by us (*Larix decidua* subsp. *polonica*, *Pinus mugo*, *Pinus nigra* subsp. *banatica*, *Pinus sylvestris* and *Abies alba*) the cells forming the extremity of the integument are being disorganized and generate a plug of mucilage, which obturates the micropyle. Thus the pollen grains can no more penetrate into the pollen chamber.

For the first time we cannot state precisely the relation between the pollination drop of nucellar origin and the mucilage obturator of integumental origin. Further research will certainly clarify this.

Probably the signal for the formation of the mucilage obturator stems from the germination of the pollen and the formation of pollen tubes in the pollen chamber.

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STUDY ON EARLY ANDROGENESIS IN WHEAT ANTHERS CULTURE UNDER NORMAL AND STRESS CONDITIONS

MARCELA ELENA BADEA, IRINA HOLOBIUC

Our results regarding the early androgenesis process in three Romanian wheat varieties showed that, unlike Fundulea 4 and Dropia, in Lovrin 41 a greater number of asymmetric divisions of spores took place, but almost always the vegetative cells act as the embryogenic precursor. In Dropia variety, presence of salt in the culture medium had different effects on the early androgenesis, depending on the concentration (0.25%, 1%).

After Al^{3+} treatment with nonselective doses, an activator effect of the embryogenic program in the microspores was observed.

Key words: wheat early androgenesis, microspore sporophytic development, Al^{3+} and NaCl supplemented medium.

INTRODUCTION

When microspores or young pollen grains are cultivated *in vitro*, gametophytic development stops and a new developmental pathway begins to form a haploid sporophyte, either directly by embryogenesis, or indirectly via callus. Generally, the factors that determine the success of regeneration in cereals anther culture are: genotype, physiological state of the donor plants, pollen microspore stage, culture conditions, medium composition (Bajaj, 1990).

The stimulatory effect of some stress factors – thermal shocks or starvation – on androgenesis in anthers and isolated pollen, is also well-documented (Touraev *et al.*, 1997).

The goals of our study were to compare the early androgenesis in three Romanian wheat varieties and to establish the effect of salt and Al^{3+} on the sporophytic development in one variety -Dropia.

MATERIAL AND METHODS

Three Romanian winter wheat cultivars – Lovrin 41, Fundulea 4 and Dropia – were chosen as experimental material. In preliminary experiments, these genotypes showed androgenetic ability in anther culture.

The plants were grown in the experimental fields of the Fundulea Research Institute for Cereals and Industrial Crops. Spikes with anthers containing microspores in the mid- or -late uninuclear stage were collected and were maintained at 4 °C for 2–7 days.

Anthers were taken aseptically and were inoculated on 15 ml solidified N6 medium (Chu, 1978) in 60 mm Petri dishes.

The medium was supplemented with sucrose (90 g/l), 2,4- D (1.5 mg/l) and glutamine (200 mg/l). Anthers cultures were incubated at 26 °C, in the dark.

In order to study the effect of salt on early androgenesis, before anther's inoculation, NaCl (250 and 1000 mg/l) was added into the N6 medium.

The effect of Al^{3+} on early androgenesis was studied on anthers after pretreatment for three days on liquid modified Schenk-Hildebrandt [Kamp-Glass *et al.*, 1993] medium added with 50 and 150 μM Al^{3+} . Then the anthers were transferred on solidified N6 medium and incubated at 26 °C, in the dark.

For the cytological study of the early stages of androgenesis, anthers were removed from culture after 6, 9, 11 and 13 days respectively and squashed in 1% aceto-carmin.

Only dividing microspores and bi-or multicellular pollen grains were registered for each variety, the number of induced spores was counted for 50 anthers.

The pollen grains were divided in several categories according to the type and number of cells/several nuclei – vegetative, generative. Every microscopical sample was considered a repetition. The experience was made after randomized blocks method and the results was interpreted after variance analysis and F test.

RESULTS AND DISCUSSION

FIRST HAPLOID MITOSIS AND ORIGIN OF THE MULTICELLULAR STRUCTURES IN NORMAL CULTURE CONDITIONS

The results concerning the cytological differentiation of the pollen derived structures showed that in all three winter wheat varieties, microspores in culture were divided both asymmetrically and symmetrically to form typical or abnormal pollen.

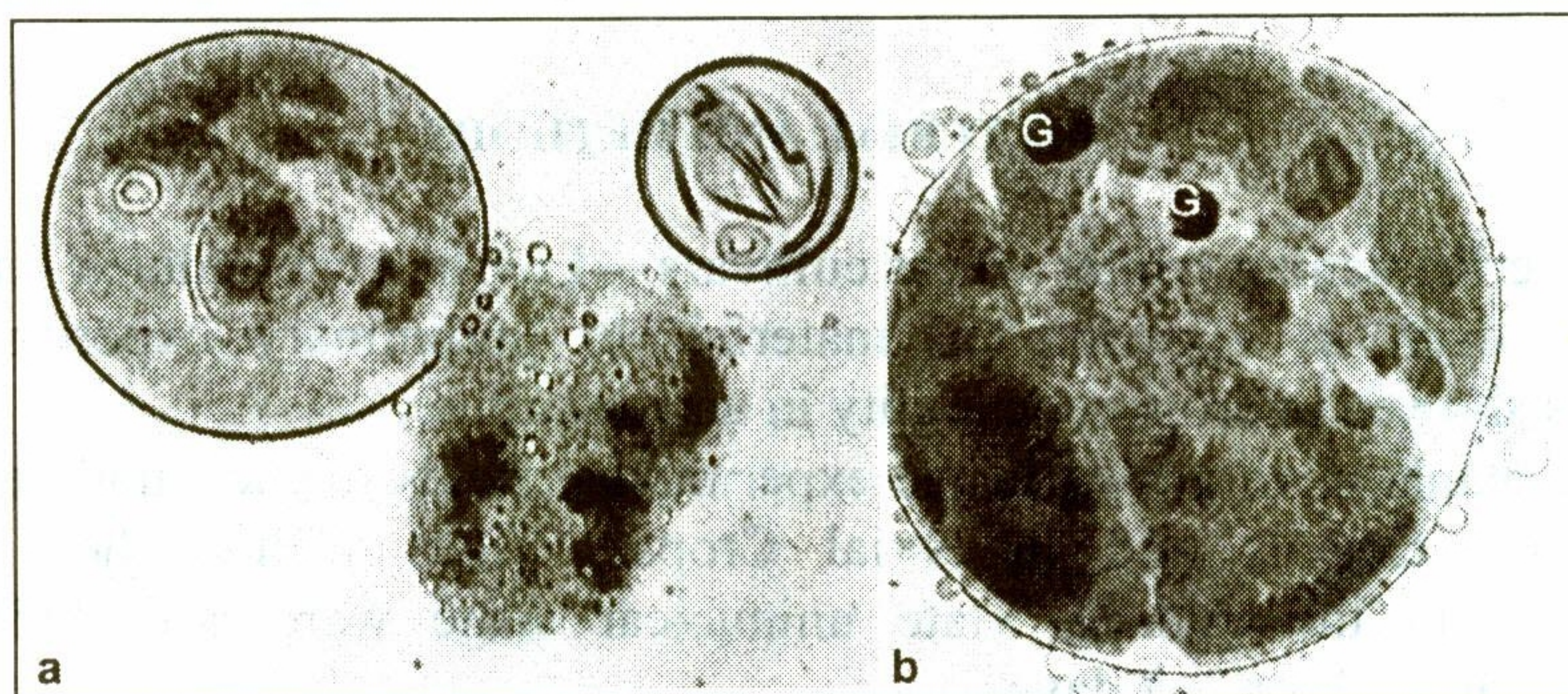


Fig. 1A–B – Light microscopy of wheat anthers culture after either 11 or 13 days of culture on N6 medium.
A. A multicellular pollen grain after 11 days of culture, showing only vegetative cell derivatives.
B. A multicellular pollen grain after 13 days of culture, showing two degenerated generative cells and many vegetative derivatives.

Then the vegetative cell type can initiate sporophytic divisions, whereas the generative cells degenerate or undergo only one or two divisions (Table 1).

Table 1

The cell types recorded in pollen grains, which were reprogrammed for sporophytic development

Number of bi-and multicellular pollen grains with	Variety	Fundulea 4	Lovrin 41	Dropia	Mean frequency of the experience
1	2	3	4	5	6
	Total number of embryogenic microspores/ 50 anthers	48	67	66	—
vegetative cells	Number/ cellular type	28	31	40	—
	Frequency	58	46	60	54
	% of mean	107	85	111	100
	Difference from the mean and significance	4	-8°	6	0
one vegetative cell and one generative cell	Number/ cellular type	17	34	17	—
	Frequency	35	51	26	37
	% of mean	94	138	70	100
	Difference from the mean and significance	-2	14*	-11	0
2-3 vegetative cells or 1-2 generative cells	Number/ cellular type	1	0	6	—
	Frequency	2	0	9	4
	% of mean	50	0	225	100
	Difference from the mean and significance	-2	-4°	5*	0
2 generative cells or 2-3 generative cells and a vegetative cell	Number/ cellular type	2	2	3	—
	Frequency	4	3	5	4
	% of mean	100	75	125	100
	Difference from the mean and significance	0	-1	1	0

Significant for $P < 0.05$; $P < 0.01$, and $P < 0.001$

Unlike Dropia and Fundulea 4, in Lovrin 41, the early androgenesis is characterized by a greater number of asymmetric divisions of the spores, but almost always the vegetative cells act as the embryogenic precursor (Fig. 1A) because we observed multicellular structures with no more than two generative cells (Fig. 1B).

THE EFFECT OF SALINE STRESS ON EARLY ANDROGENESIS

Presence of salt in the culture medium had different effects on the early androgenesis, depending on the concentration (0.25% or 1%). On the medium added with 1% NaCl after six days of culture, most of the spores were in various stages of degeneration (Fig. 2A) or showed no signs of subsequent development.

On the contrary, on 0.25% NaCl containing medium, many spores underwent both an asymmetric and a symmetric first mitotic division, appearing bicellular pollen grains. Multicellular pollen grains with 3–4 vegetative cells or 4 vegetative cells and one generative cell (Fig. 2B) could be observed, after seven days of anther culture. After two weeks proembryos appeared. On 1% NaCl, structures with no more than four nuclei were formed (Fig. 2C).

Cytological evidence indicates that concentration of 1% NaCl is lethal, whereas 0.25% could be selective (Table 2).

Table 2

The effect of some abiotic stress factors on androgenetic induction in Dropia wheat variety

Number of bi-and multicellular pollen grains with	Experimental variant	Control	1 % NaCl	0.25 % NaCl	150µM Al ³⁺	50µM Al ³⁺
1	2	3	4	5	6	7
	Total number of embryonic microspores/50 anthers	80	18	86	136	129–
vegetative cells	Number/cellular type	40	8	36	28	30
	Frequency	54	44	42	20	23
	% of Control	100	81	77	37	42
	Difference from the mean and significance	0	10	–12	–34°	–31°
one vegetative cell and one generative cell	Number/cellular type	17	2	21	28	25
	Frequency	23	11	25	20	19
	% of control	100	48	108	87	82

Table 2 (continued)

1	2	3	4	5	6	7
	Difference from the control and significance	0	-12 ^{ooo}	2	-3	-4
2-3 vegetative cells or 1-2 generative cells	Number/cellular type	17	8	27	71	65
	Frequency	23	44	32	52	50
	% of mean	100	191	139	226	217
	Difference from the mean and significance	0	21*	9	29**	27**
2 generative cells or 2-3 generative cells and a vegetative cell	Number/cellular type	6	0	2	9	9
	Frequency	12	0	1	8	7
	% of mean	100	0	8	67	58
	Difference from the mean and significance	0	-12*	-11*	-4	-5

Significant for P < 0.05; P < 0.01, and P < 0.001

No more than 18 spores were induced in 50 anthers collected from 1% NaCl supplemented medium, while 86 spores underwent sporophytic induction on 0.25% NaCl supplemented medium.

In spores maintained for two weeks on medium added with NaCl we also observed some types of aberrations micronuclei of different size (Fig. 2D) – a source of chromosome loss –, altered, uncoiling and sticky chromosomes (Fig. 2E–H), dihaploid metaphase (Fig. 2I).

All these abnormalities could be explained by the effect of salt added in the culture medium. While micronuclei can also be seen in spores cultivated in normal conditions (with lower frequency), dihaploid nuclei were recorded only in correlation with saline stress which could act as a trigger of mechanisms involved in the appearance of dihaploid and polyhaploid plants (Henry, 1998). Microspectrophotometry studies showed that wheat microspores suitable for the anther culture have a 2 C DNA content, indicating the G 2 phase of the cell cycle (Raquin *et al.*, 1982). The fact that diploid metaphases are observed in microspores indicates that doubling may occur before the first mitosis. The absence of dihaploid nuclei in the spores maintained in normal conditions suggests that osmotic stress could induce an extra round of DNA synthesis. Cytological evidence indicates that salt could also induce nuclear restitution recognized by the overcontraction of chromosomes, their enhanced stickiness, and the failure of the ordered arrangement due to the malfunction of the nuclear spindle (Nagl, 1978) (Fig. 3A, B).

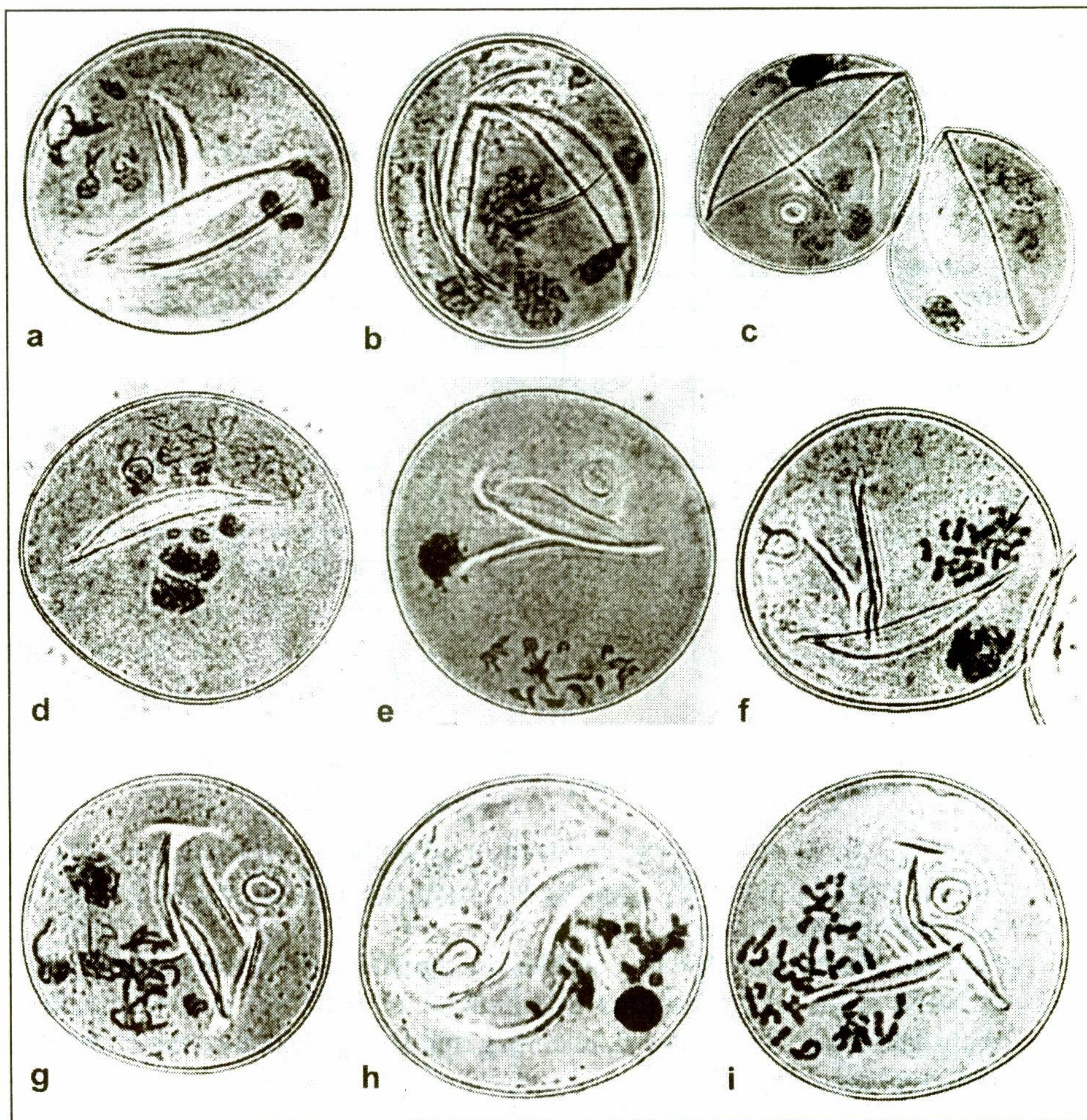


Fig. 2A-I – Light microscopy of *Drobia* wheat anthers after 6 or 9 days of culture in N6 medium added with 0.25% or 1% NaCl.

- A. A pollen grain 1 % NaCl after 6 days of culture on 1% degraded chromosomes.
- B. A multicellular pollen grain with five nuclei after 7 days of culture on 0.25% NaCl added medium.
- C. Two pollen grains, one with three and other with four nuclei, after 9 days of culture on medium added with 1% NaCl.
- D. A pollen grain after 6 days of culture on 0.25% NaCl supplemented medium, with two generative nuclei, three micronuclei and one dividing nucleus.
- E. Pollen grains after 6 days of culture on 0.25% NaCl added medium, showing altered chromosomes.
- F–G. Pollen grains after 9 days of culture on 1% NaCl added medium, showing uncoiling chromosomes.
- I. A microspore after 6 days of culture on 1% NaCl added medium showing a dihaploid metaphase.
- H. A pollen grain after 6 days of culture on 1% NaCl supplemented medium with sticky chromosomes.

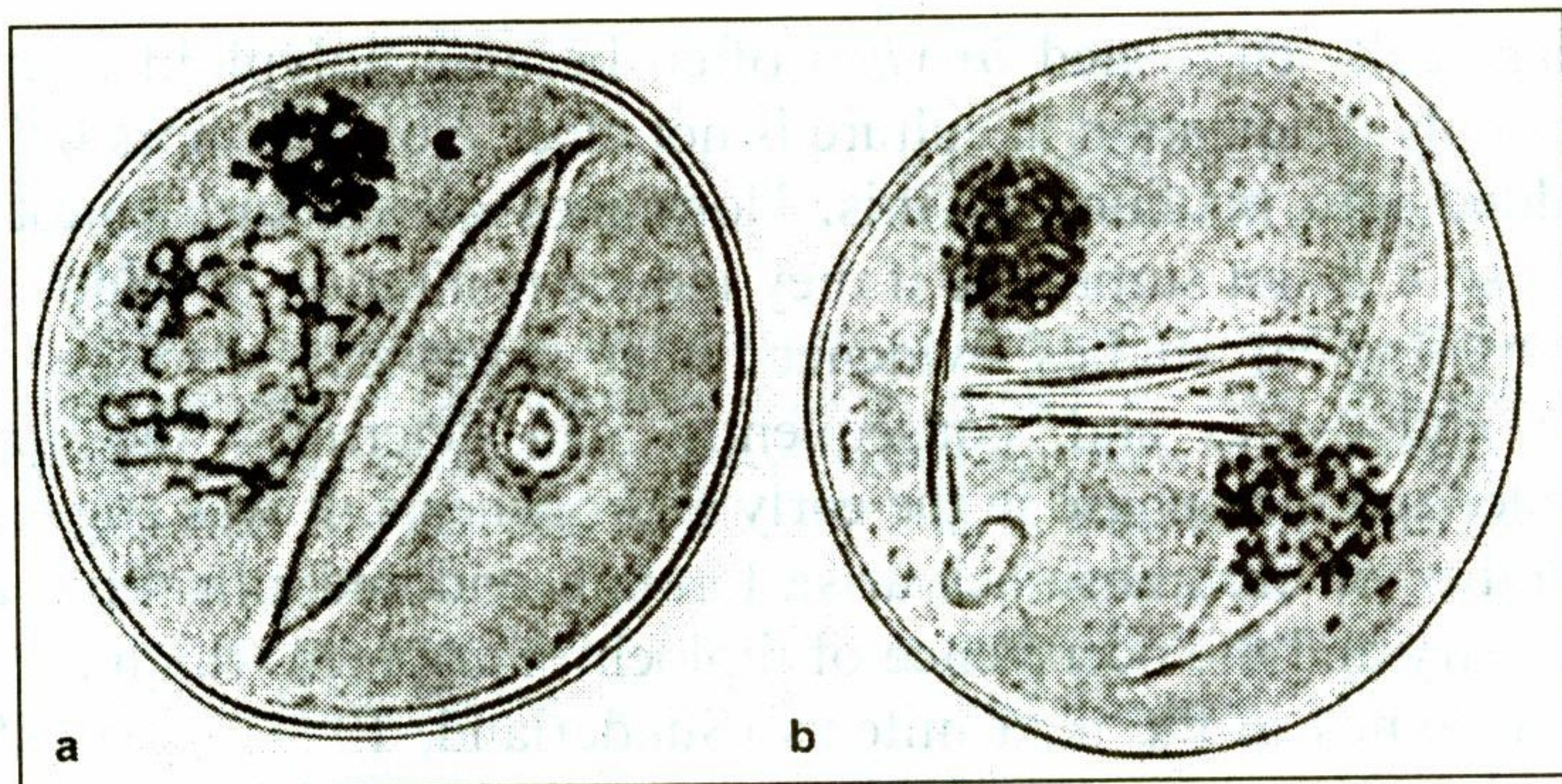


Fig. 3 A–B – Light microscopy of Dropia wheat anthers cultivated in stress conditions.
 A. A pollen grain after 6 days of culture in salt stress conditions showing a network of thin threads and a restitution nucleus.
 B. A pollen grain after 7 days of culture in saline stress conditions, showing an interphasic vegetative nucleus and a restitution one.

THE EFFECT OF Al^{3+} ON EARLY ANDROGENESIS

As it can be seen in Table 2, the maintenance of anthers of Dropia wheat cultivar, for three days on medium added with Al^{3+} , had a stimulatory effect on the induction process after placing them on N6 medium; more microspores underwent sporophytic induction than those from the anthers cultivated on the salted medium.

However, the great majority of spores underwent an asymmetric mitosis. Consequently, after one week in culture, many structures with one or two generative cells appeared (Fig. 4A). The occurrence of dihaploid spores and divisions (Fig. 4B) suggests that Al^{3+} , like salt, induced either restitution or endocycles (Fig. 4C).

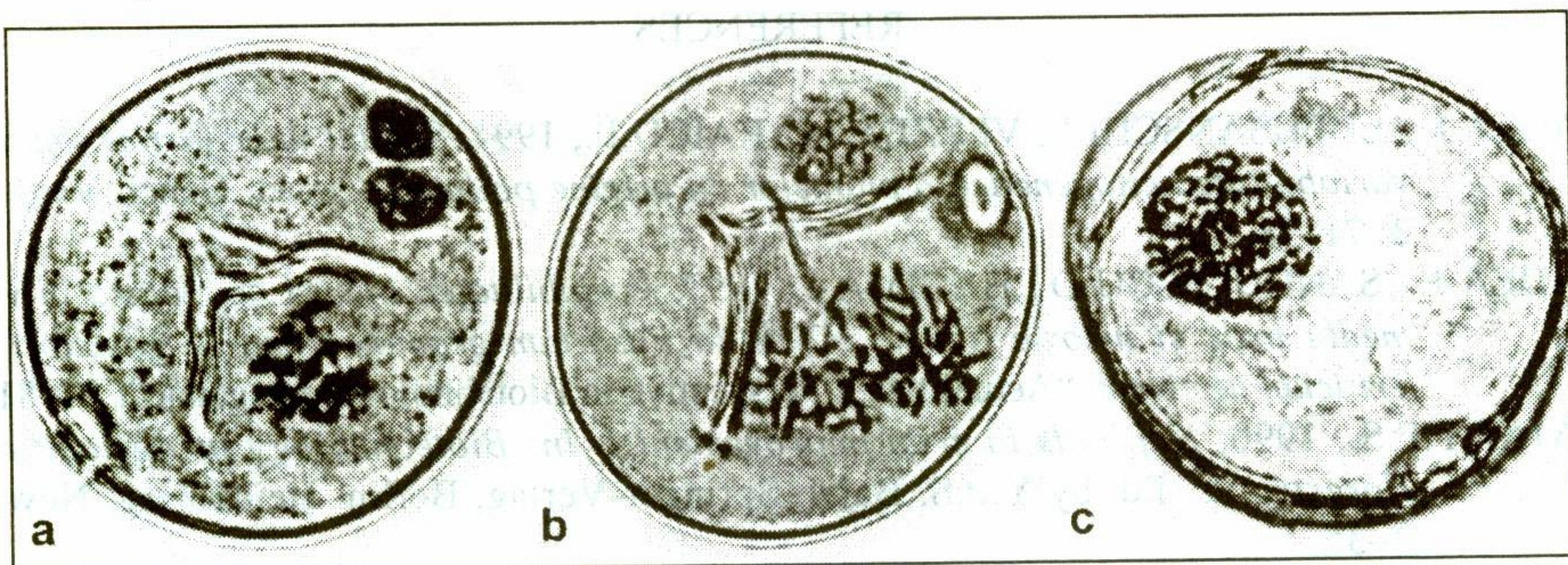


Fig. 4 A–C – Light microscopy of wheat anthers after pretreatment for three days on $150 \mu M Al^{3+}$ and 6 days of culture in N6 medium.

- A. A pollen grain after 6 days of culture, with two generative nuclei and one dividing (probably) vegetative nucleus.
- B. A pollen grain after 6 days of culture showing one dihaploid dividing nucleus.
- C. Microspore after 6 days of culture, showing an endoreduplication within nucleus.

The plant cells cultivated *in vitro* often became polyploid (Bayliss, 1980). What induces polyploidization in culture is not clear. Some authors believe that the hormones added into medium do this. Hormones can disturb mitotic cycles in various ways, so it is not surprising if they induce restitution of endo-cycles (Nagl, 1978). Our cytological studies evidenced that stress can also be a trigger of restitution or endocycles and, consequently, can determine the appearance of dihaploid nuclei and metaphase in the early androgenesis. It was suggested that the dihaploid number of chromosomes arise through endoreduplication, as indicated both by cytometry and the occurrence of diplochromosomes which separate in two normal chromosomes in the next mitosis (Sunderland, 1974). The extremely low frequency (under 1%) of the dihaploid spores could be explained by the hexaploid origin of the wheat. Generally, the frequency of spontaneous dihaploids and polyploids in plant population derived by androgenesis is correlated with the number of genomes in microspores: the greater the number of the genomes, the lower the frequency of polyploids (Badea *et al.*, 1994).

In many species, stress applied in non-selective doses to the cells *in vitro* cultivated triggers the embryogenic program (Badea *et al.* 1994, 1997).

Dudits *et al.*, (1991), considered that the cellular functions related to stress response can also be involved in the activation of the embryogenic program. Our results evidenced that Al^{3+} can act as an activator of the embryogenic program in the microspores, when it has been used in non-selective doses. In order to select spontaneous mutants, which have Al^{3+} tolerance, it would be necessary to use concentrations higher than 150 μ M. Regarding salt, 0.25% being a selective concentration, no stimulatory effect on androgenesis could be observed. To this effect 0.1 or 0.15% NaCl could be used.

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THE INFLUENCE OF THE MAGNETIC FLUIDS ON GROWTH AND PHOTOSYNTHESIS IN *CHLORELLA*

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The reactions of *Chlorella vulgaris* Beij. cells treated with magnetic fluid were investigated. For one culture cycle, the effects on cell numbers, growth and photosynthesis were recorded. Magnetic fluid was added in Arnon nutritive medium of algal suspension in increasing concentrations from 0.06 to 0.5 ml/l.

Irrespective of the concentration of the magnetic fluid and sampling day it was possible to obtain a good estimation of the cell number/ml using a relationship based on absorbance of algal culture (adjusted with the absorbance of the magnetic fluid).

For all used concentrations, in the first week of the experiment, values relatively close to control were noticed.

The growth of *Chlorella vulgaris* was stimulated by lower concentrations of magnetic fluid (0.06%) and the process was affected by higher concentrations (0.25 and 0.5‰).

A stimulation effect on photosynthesis was noticed for 0.06 and 0.12‰ concentrations and an inhibitory influence of the magnetic fluid on the same process was observed in case of 0.25 and 0.5‰ treatments.

Key words: *Chlorella vulgaris*, magnetic fluids, growth, photosynthesis.

INTRODUCTION

The unicellular algae represent a useful experimental material for the study of the magnetic fluids impact on plant physiology. Because of the great number of cells from the volume unit of the algal suspension, the effects of the individual variations are eliminated.

Also, the algal cultures are the subject of a special theoretical and practical interest for the definition of optimal culture conditions, able to ensure an intense growth and implicitly a good productivity.

A special interest is presented by the plant behavior in biocompatible magnetic fluids.

Previous researches in this field showed that plant metabolism is influenced by treatments with magnetic fluids, which are able to induce phenotypic and genotypic effects able to stimulate the productivity.

Magnetic fluids are colloidal suspensions of metallic magnetic particles obtained by thermal decomposition of metallic carbonyls: $\text{Ca}(\text{CO})_8$; $\text{Fe}(\text{CO})_5$; $\text{Fe}(\text{CO})_9$ in the presence of a polymeric or anionic stabilizing agent.

The properties of the ferro-fluids are influenced by the solvent, used stabilizing agent and carbonyl nature.

The researches on the influence of magnetic field in plants are mainly focused on the following aspects:

- influence of direction and intensity of magnetic fields on the development and stress resistance plant;
- clearing up of the role of terrestrial magnetic field (if its intensity is enough to influence the biological processes in plants; if there is any correlation between the growth direction of roots and field direction; and also if there is any correlation between the direction and intensity of the field and the vegetal production).

For the explanation of these problems, the biocompatible magnetic fluids are bringing some suggestions.

A new research direction in the general subject of plant – magnetic field interactions was opened by the researches on the influence of magnetic fluids on plants in different development stages, researches started in Timișoara several years ago.

One may point out that through treatments of seeds, seedlings and mature plants with magnetic fluids, magnetic particles penetrate into different plant structures where they develop a strong local magnetic field in a small volume with a ray of several tens of Å.

Many researchers like Tenforde (7); Haque and Kreuzberg (4), Asashima *et al.* (1) showed that through treatments with biocompatible magnetic fluids the plant metabolism is changed and it is possible to induce some phenotypic and genotypic effects able to stimulate the plant productivity.

Van Dyke and Halpern (8) pointed out that plants respond to the magnitude and direction of the magnetic field. It was observed that root degeneration and growth inhibition occurred in the presence of a strong magnetic field. This is suggesting that there is a threshold above which the stimulator effect of the magnetic field disappears leading to growth inhibition.

In a magnetic field with a little greater than magnetic field of the Earth, Savostin (6) observed that germination of wheat caryopsis is quicker than in normal conditions.

Audus (2) studied the root development in several plants like *Lepidium sativum*, *Phleum pratense*, *Poa nemoralis*, in magnetic fields up to $B = 0.4\text{T}$, with a gradient of 0.5T/cm .

Roots were oriented to form 90° angle with the surface of Earth and the magnetic poles were parallel with this surface. The roots, initially horizontally oriented, changed their direction towards the zone without magnetic field so as to escape from the zone with high magnetic flux.

The unicellular green alga *Chlorella vulgaris* was considered by Warburg as a standard species for the research of the physiological processes. That is why we choose this alga for our experimental study. Most of the papers which refer to the physiological processes of algae used prevalingly this alga.

The reactions of *Chlorella vulgaris* Beij. cells treated with different concentrations of magnetic fluids were investigated and the main results (the effects on cell numbers, growth and photosynthesis) are presented in this paper.

MATERIAL AND METHODS

Chlorella vulgaris alga was cultivated on Arnon nutritive medium (3). The experiments were carried out in a chamber with artificial illumination of 8 000 lux. To avoid mutual shadowing, the algal suspensions, in cylindrical glass recipients of 1 000 ml, were bubbled with steady stream of air produced by vibrator devices. The culture medium was inoculated with an amount of algal biomass producing a 100 000 cells/ml suspension in all experimental variants. The ambient temperature varied between 21–25 °C. The results represent average values of two repetitions for each variant.

The initial culture of *Chlorella vulgaris* Beij. was obtained from the Plant Physiology Laboratory of the Institute of Biology in Bucharest.

The magnetic fluid was introduced in the culture medium before inoculation. Four concentrations of the magnetic fluid solutions 0.06, 0.12, 0.25 and 0.5 ml/l were used.

The growth of alga was observed at 13 sampling days within the culture cycle (established at 16 days) with a “Cecil 1020” spectrophotometer at $\lambda = 676$ nm. The absorbance of the blank sample with the same concentration was subtracted from the sample absorbance in order to obtain the absorbance of the algal suspension.

The number of algal cells was counted with a Thoma haemocytometric mount.

The photosynthesis rate was investigated with the Warburg method using the treated algal suspension and the Warburg buffer solution number 6 (25 ml Na_2CO_3 0.1M + 25 ml NaHCO_3 0.1M) (5).

RESULTS AND DISCUSSION

A significant correlation was found between absorbance and number of algal cells per volume unit ($R^2 = 0.95^{***}$). This relationship may be used to convert the absorbance into number of cells/ml (Fig. 1), but for the discussion of the growth, it was preferred to express the values as absorbance.

The obtained values for growth suggest a slow growth in the first 5 days of the experiment, but after this period an intensifying of growth was noticed (Fig. 2).

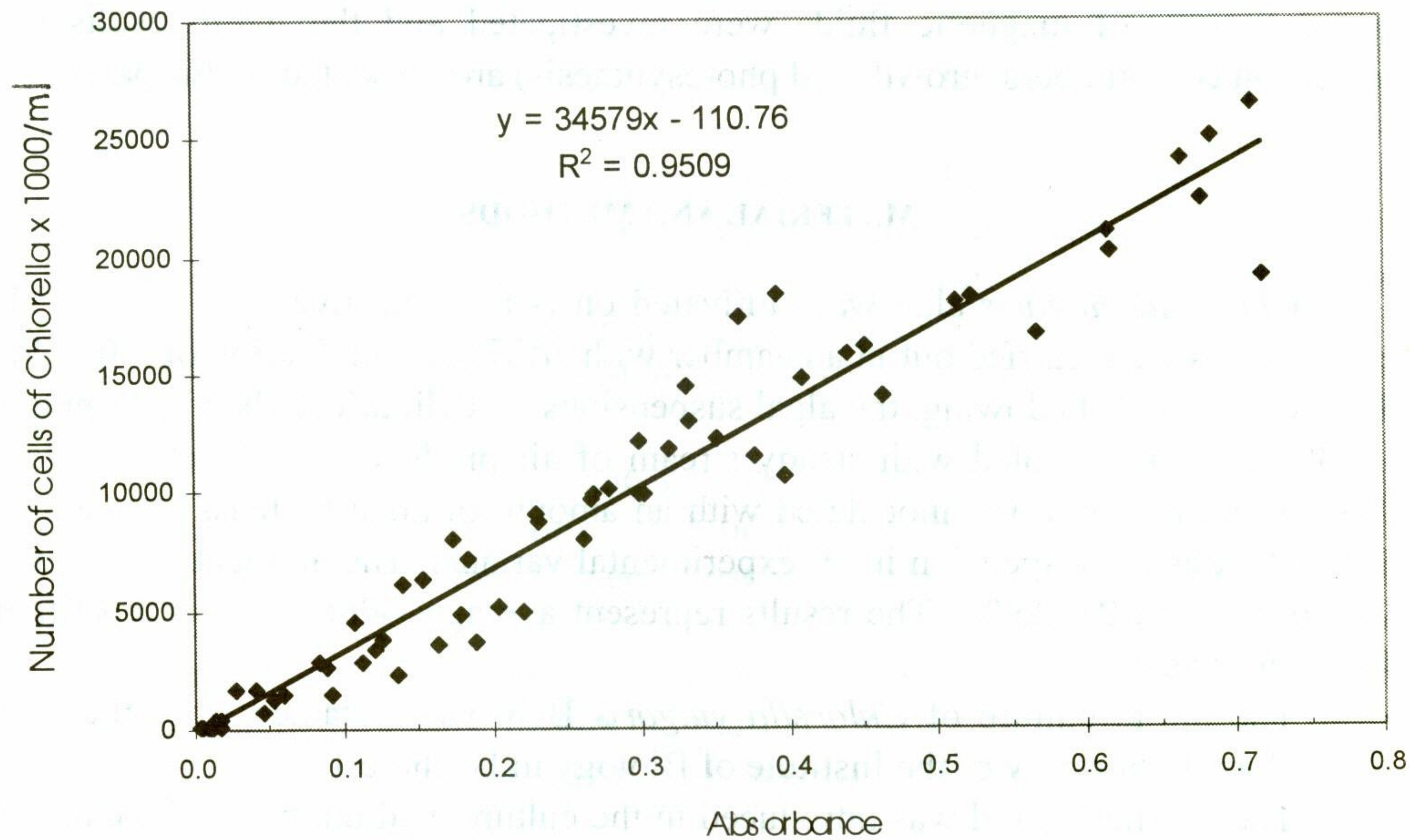
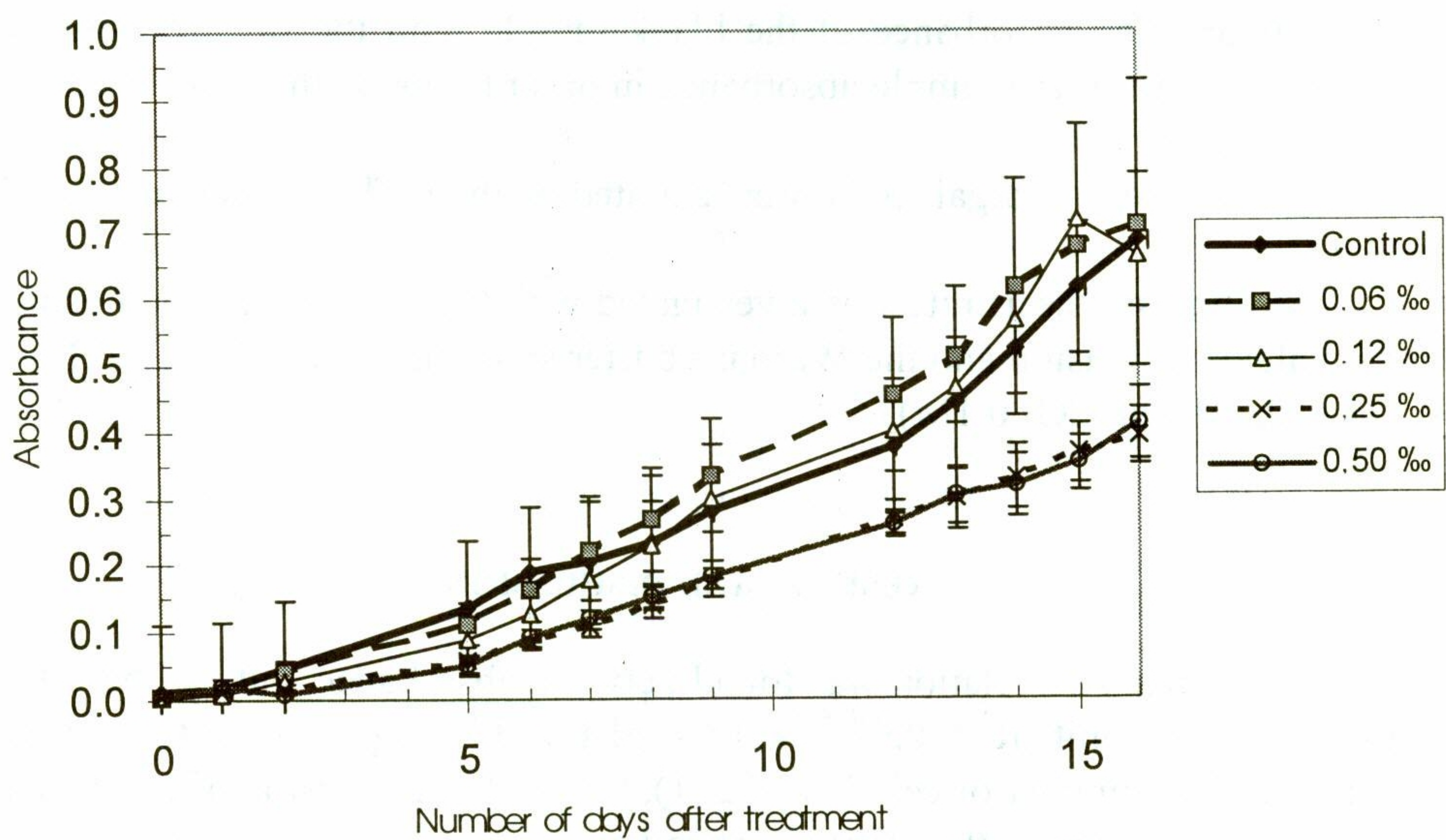


Fig. 1 – The relationship between the absorbance and cells number from the culture of *Chlorella vulgaris*.



(The vertical bars are equal to two standard deviations)

Fig. 2 – The influence of magnetic fluids on absorbance of culture of *Chlorella vulgaris*.

In the first week of the experiment, the obtained values of all treatments were relatively close to control.

In the second week from the addition of the magnetic fluid, for the 0.06 ‰ concentration a relative constant stimulating effect on growth was observed, but the differences from control were not statistically significant. For the concentration of 0.12 ‰ a similar trend was noticed, but with smaller differences from control and to a less extent.

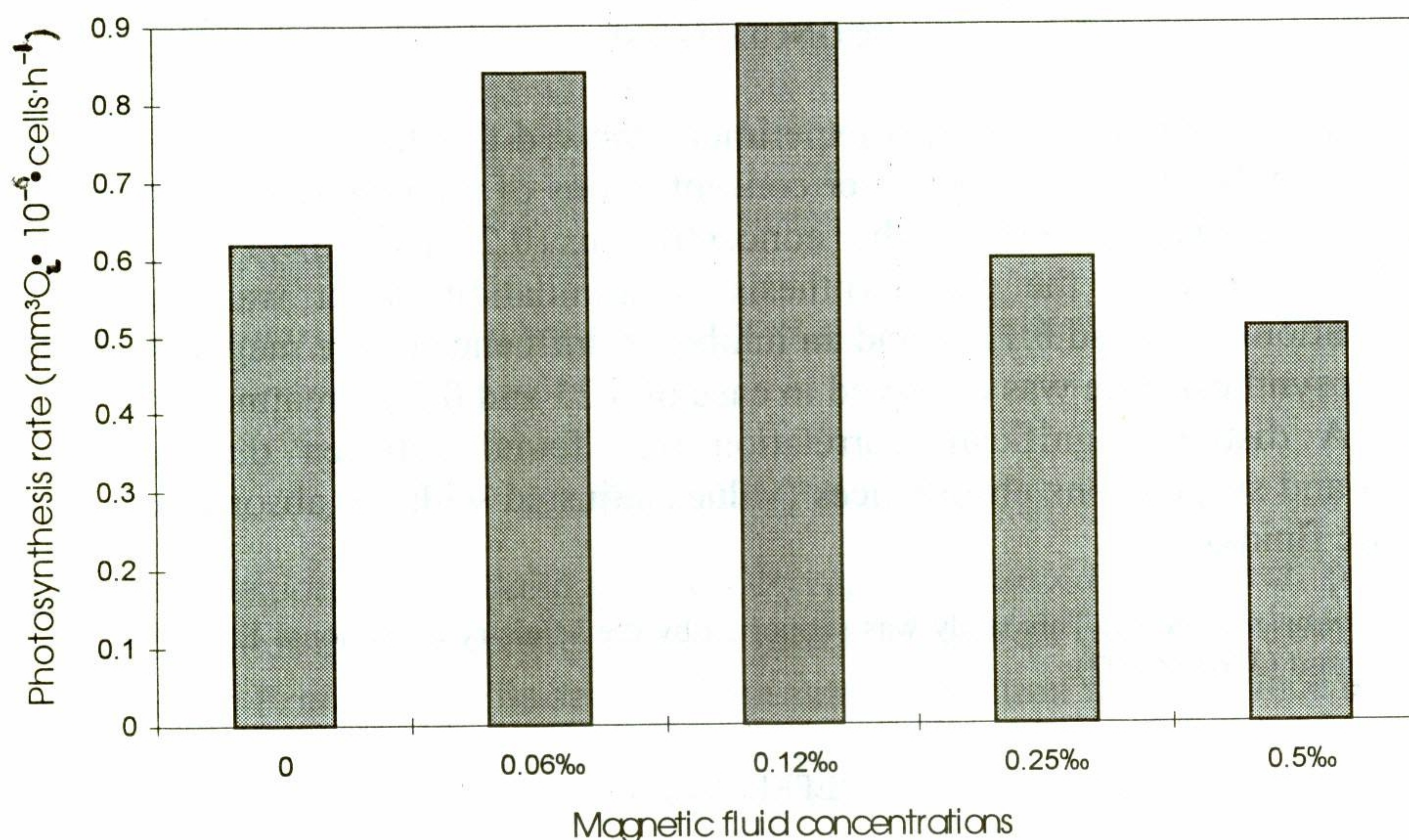


Fig. 3 – The influence of magnetic fluids on photosynthesis rate of *Chlorella vulgaris*.

After the second week, in case of the algal suspensions cultivated with magnetic fluid in concentrations higher than 0.25 ‰, a significant inhibitor effect on growth was noticed.

The raising of the growing rates occurred later in the cultures with higher concentrations of magnetic fluid and sooner in case of control followed by the treatments with lower concentrations.

Since there are no significant differences between the effect of the 0.25–0.50 ‰ concentrations, the existence of a maximal threshold for the effect on growth may be supposed between the concentrations of magnetic fluids of 0.12 ‰–0.25 ‰, but further investigations are required.

In case of photosynthesis intensity (established after the amount of oxygen released), for the concentrations of 0.06 and 0.12 ‰, on the 7th day of the experiment, a relative stimulating effect was noticed. The concentrations of the magnetic fluid higher than 0.25 ‰ obviously affected the photosynthesis in *Chlorella vulgaris*.

The different concentrations of the magnetic fluid affected in the same way both growth and photosynthesis intensity of the algal cultures. For the concentrations of 0.06 and 0.12‰, a relatively stimulating effect was noticed, but the concentrations of 0.25 and 0.5‰ obviously affected both processes in *Chlorella vulgaris*. But in the case of photosynthesis intensity the largest stimulation effect was recorded for the 0.12‰ concentration.

CONCLUSIONS

1. The data obtained in this experiment showed that the growth of *Chlorella vulgaris* may be stimulated by lower concentrations of the magnetic fluid (0.06‰) and the process is affected by higher concentrations (0.25 and 0.5‰).
2. In regard of the photosynthesis, a stimulation effect was noticed for concentrations 0.06 and 0.12‰ and an inhibitory influence of the magnetic fluid on the photosynthesis rate was observed in case of 0.25 and 0.5‰ treatments.
3. A distinct significant correlation was found between the number of cells/ml and suspensions absorbances (values adjusted with the absorbances of the magnetic fluids).

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L'INFLUENCE DU MILIEU DE CULTURE ET DU pH SUR LA CROISSANCE ET LA SPORULATION DU *CERCOSPORA BETICOLA* SACC.

CRĂIȚA-MARIA ROȘU*, CĂTĂLIN TĂNASE**, MIHAI MITITIUC**

The effects of some natural culture media and of different pH values on mycelium growth on spore production of *Cercospora beticola* Sacc. were tested in the laboratory. The vegetative growth and sporulation of the fungus were influenced by these factors in different ways. Thus, the sugar beet molasses-agar and potato-dextrose-agar media favored the radial growth of the colonies, while the sporulation of the fungus was strongly stimulated by the cultivation on the media of sugar beet molasses-agar and carrot leaf infusion-agar. The optimum pH for the vegetative growth of the fungus was 5.5, while the optimum pH for the sporulation varied as a function of the tested culture media.

Key words: *Cercospora beticola*, vegetative growth, sporulation.

INTRODUCTION

La cercosporose, produite par la *Cercospora beticola* Sacc., est, parmi les maladies foliaires de la betterave, une des plus nuisibles qu'on puisse trouver sur le territoire de la Roumanie. Elle se manifeste par l'apparition sur la surface du limbe foliaire de nombreuses petites taches grises, entourées par un anneau brun.

Les attaques sont, dans des conditions favorables, fortes et conduisent, à la fin, au dessèchement complet des feuilles attaquées. La maladie est fréquemment présente dans toutes les zones cultivées avec de la betterave mais elle produit des pertes importantes en particulier dans les parties d'ouest et de sud du pays, ainsi que dans les zones irriguées et pendant les années avec des précipitations abondantes.

La fréquence de l'attaque a augmenté de 2,17% par an et au niveau de la production de sucre les pertes sont de 120 kg/ha par an (16).

C'est la raison pour laquelle la connaissance de la biologie du champignon *Cercospora beticola* Sacc. et, en particulier, des aspects liés à la physiologie du champignon représentent un facteur essentiel dans l'étude de l'interaction plante hôte-parasite. Dans le cas du patosystème *Beta vulgaris* L.–*Cercospora beticola* Sacc. le but de l'étude est de sélectionner des variétés avec une résistance accrue à l'attaque du champignon, dans quelques situations même à un certain isolé (race physiologique). Des recherches sur la biologie du champignon ont été réalisées au niveau du comportement *in vitro* (2, 3, 4, 6, 9, 13).

Parmi les aspects étudiés, l'induction de la sporulation du champignon dans des cultures pures a posé les plus grands problèmes à cause du fait qu'il y a une série des facteurs impliqués qui interfèrent. Quelques-uns de ces facteurs sont: la nature du milieu de culture (les espèces du genre *Cercospora* ne sporulent pas en général dans des milieux artificiels) et l'interaction entre la lumière et la température. Ces problèmes ont été élucidés, partiellement (4, 12, 14). Les auteurs ont réussi l'obtention de cultures sporulées de *Cercospora* par le transfert direct des conidies trouvées sur des plantes infectées dans des milieux de culture naturels. Des difficultés dans l'induction de la sporulation *in vitro* ont été rencontrées dans le cas d'autres espèces appartenant au genre *Cercospora*, tels que: *Cercospora zea-maydis* (1), *Cercospora asparagi* (7), *Cercospora citrullina* (10) et *Cercospora kikuckii* (11).

Notre ouvrage apporte des informations nouvelles concernant l'influence du pH et de certains milieux de culture naturels sur la croissance et la sporulation du champignon dans des conditions de laboratoire. L'obtention d'une grande quantité de biomasse et aussi d'une bonne production de spores est nécessaire pour assurer un inoculé adéquat dans les études quantitatives impliquant la relation hôte-parasite.

MATÉRIEL ET MÉTHODE

L'isolé de *Cercospora beticola* Sacc. utilisé dans les expérimentations a été obtenu sur des plantes de betterave naturellement infectées. La source de ces plantes se trouve dans les champs expérimentaux de la Station de Recherche et Protection pour la betterave de Roman (département de Neamț).

Pour isoler et purifier le champignon nous avons utilisé les milieux pomme de terre-dextrose-agar (PDA). Le champignon a été conservé à 4°C, dans des éprouvettes contenant les mêmes milieux de culture.

En vue de maintenir un niveau élevé de la vigueur du champignon celui-ci a été passé périodiquement sur la plante hôte et, après, réisolée. Ainsi, des plantes saines de betterave, âgées de 6 semaines, élevées en laboratoire, ont été infectées avec une suspension de spores (5 000 spores/ml + Tween 20–0,5%) et recouvertes avec une feuille en plastique pour assurer une humidité des plus élevées (RH – 90%). La température a été de 28 °C et la photopériode de 24 h, pendant trois jours. Après cette période la feuille a été enlevée et les plantes passées à 24°C et 16 h de lumière par jour, conditions assurées par des lampes avec halides métalliques, 400 W (Luxten Company, Roumanie).

Pour le réisolement du champignon, après 3 semaines, les feuilles malades ont été récoltées et passées dans des chambres humides pendant 3 jours, pour sporuler. A l'aide de l'anse et de la loupe binoculaire nous avons recueilli les

spores des surfaces des lésions. Pour réisoler et purifier le champignon on a utilisé le même milieu (PDA).

Dans tous les cas on a travaillé avec un isolé monosporal, qui a été au troisième passage sur le milieu de culture.

MILIEUX DE CULTURE UTILISÉS, INOCULATION ET INCUBATION

L'influence du milieu de culture sur la croissance du champignon a été suivie dans les milieux: pomme de terre-dextrose-agar (PDA), pomme de terre-carotte-agar (PCA), mélasse de betterave-agar (MBA), décocté de feuilles de betterave-agar (DBA) et malt-agar (MA) en variante agarisée et non-agarisée.

La sporulation du champignon a été testée dans les milieux: mélasse de betterave-agar (MBA), décocté de feuilles de betterave-agar (DBA), décocté de feuilles de carotte-agar (DCA) et pomme de terre-dextrose-agar (PDA).

Les milieux de culture ont été préparés (8) conformément à Dhingra D.O. et Sinclair J.B. (1985) et répartis sur des plaques Pétri de diamètre de 80 mm (la variante agarisée) et dans des flacons Erlemeyer de 100 ml, chacun contenant 20 ml (la variante liquide). L'ajustement du pH aux valeurs désirées (4,00–11,00) a été fait à l'aide de solutions de KOH 1N et acide lactique 25%, en utilisant un pH-mètre de type ORION–model 210A. Après la stérilisation des milieux, dans chaque flacon on a ajouté du sulfate de streptomycine, en concentration de 40 U/ml, pour prévenir la contamination bactérienne.

Tableau 1

Le développement du *Cercospora beticola* Sacc. dans les milieux de culture agarisée et non-agarisée, aux pH 5,5 (* – $\bar{x} \pm SE$)

Milieu de culture	La durée de l'incubation (jours)		La production du mycélium (g/flacon)
	7	14	
	Diamètre de la colonie (mm)		
1. PDA (control)	*28,6 ± 0,02	55,4 ± 0,02	0,30692 ± 0,02
2. MBA	30,2 ± 0,03	60,1 ± 0,04	0,50990 ± 0,04
3. DBA	25,0 ± 0,04	41,2 ± 0,04	0,05200 ± 0,04
4. PCA	24,6 ± 0,02	50,2 ± 0,03	0,03540 ± 0,05
5. MA	23,6 ± 0,02	43,8 ± 0,03	0,09459 ± 0,03

Les milieux de culture sélectionnés ont été inoculés ainsi: ceux destinés à l'observation de la croissance radiale de la culture ont étéensemencés par le placement au milieu de la plaque, de disques avec un diamètre de 8 mm, découpés du bord d'une culture de *Cercospora beticola* Sacc., âgée de 10 jours, cultivée sur

un milieu PDA. Les milieux liquides ont été ensemencés avec des disques mycéliens avec un diamètre de 4 mm provenant de la même culture. Après inoculation les milieux ont reçu une température constante de 25°C, et une photopériode de 18 h par jour.

L'inoculation des milieux de culture destinés à la sporulation du champignon a été faite par le placement, sur chaque plaque, à l'aide de la pipette automatique (5), de 4 gouttes de suspension des spores avec une concentration de 3.000 spores/ml (40 µl/goutte). Les cultures ont été incubées à 20°C, dans les mêmes conditions de lumière assurées par des tubes fluorescents (daylight-40 W/Tungsram), placés à 30 cm en dessus des cultures et qui ont assuré un flux lumineux de 100 lux.

La croissance radiale du champignon dans les 5 milieux testés a été évaluée à 7 et 14 jours d'incubation, suivant le mesurage du diamètre des colonies (5 répliques). L'intensité de la croissance a été appréciée à 7 jours d'incubation par la détermination de la biomasse sèche (15), les valeurs présentées étant la moyenne des 5 répliques.

La sporulation a été estimée après 5 jours d'incubation, à l'aide d'un hemocytomètre. Pour déterminer la moyenne des nombres de spores sur une plaque, on a ajouté 10 ml d'eau distillée sur chaque plaque (3 répliques), opération suivie par une légère dislocation des spores à l'aide d'une brosse. Avant le dénombrement la suspension de spores a été agitée et filtrée.

On a utilisé le test t-Student pour l'analyse statistique des données.

RÉSULTATS ET DISCUSSION

A. L'INFLUENCE DU MILIEU DE CULTURE SUR LA CROISSANCE DU CHAMPIGNON

Les milieux de culture utilisés ont influencé d'une manière différente la croissance du champignon *Cercospora beticola* Sacc.

Ainsi, dans le cas des variantes agarisés, les plus favorables milieux de culture ont été les milieux MBA et PDA, qui ont assuré une croissance de diamètre des colonies supérieure à ceux des autres milieux testés. Plus précisément, le diamètre a été de 30,2 mm après 7 jours et de 60,1 mm après 14 jours dans le milieu MBA et de 28,6 mm après 7 jours et de 55,4 mm après 14 jours dans le milieu PDA. Le mycélium aérien formé dans ces milieux est aussi bien développé et dense. Si on considérait le milieu PDA comme celui de contrôle, la croissance dans les milieux MBA est accrue après 7 jours ($p < 0,01$) mais aussi après 14 jours ($p < 0,001$).

Dans les milieux de culture DBA, PCA et MA le développement du champignon a été plus lent en comparaison avec celui du champignon dans les

milieux PDA. La différence est visible du point de vue de la croissance du diamètre de la colonie, diamètre qui est bien plus réduit par rapport au contrôle ($p < 0,001$) et aussi de celui de l'aspect du mycélium aérien qui est dans les trois cas moins abondant, ceci étant le plus visible dans le cas du milieu DBA. Parmi les trois milieux, le PCA est le plus favorable pour le développement du champignon, le diamètre de la colonie étant après 14 jours de 50,2 mm. Dans le cas du milieu DBA, même si dans la première période l'extension en espace des hyphes a conduit à la formation d'une colonie avec un diamètre de 25,0 mm, à la fin de la période d'incubation (14 jours) le diamètre des colonies mesuré dans ce milieu a été le plus petit (41,2 mm).

Parce que le mycélium du champignon peut se développer différemment en fonction du substrat utilisé, on a aussi mesuré la biomasse sèche obtenue dans les mêmes milieux mais sous formes liquides. Les résultats obtenus confirment, en général, ceux obtenus dans la variante agarisée.

Ainsi, on a remarqué d'importantes différences selon l'utilisation du substrat, les plus favorables milieux étant toujours le MBA (0,50990 g/flacon – $p < 0,001$) et PDA (0,30692 g/flacon – contrôle). Dans le cas des autres milieux testés les biomasses obtenues ont été dans une moindre quantité, plus précisément de 0,09459 g/flacon (MA), de 0,05200 g/flacon (DBA) et de 0,03540 g/flacon (PCA) toutes les valeurs étant négatives par rapport au contrôle ($p < 0,001$).

B. L'INFLUENCE DU pH SUR LA CROISSANCE DU CHAMPIGNON

Les champignons phytopathogènes peuvent se développer dans des limites très larges des pH, grâce au pouvoir du champignon de tamponner les milieux, soit par absorption d'un anion ou cation, soit par production d'acides organiques ou ammoniac.

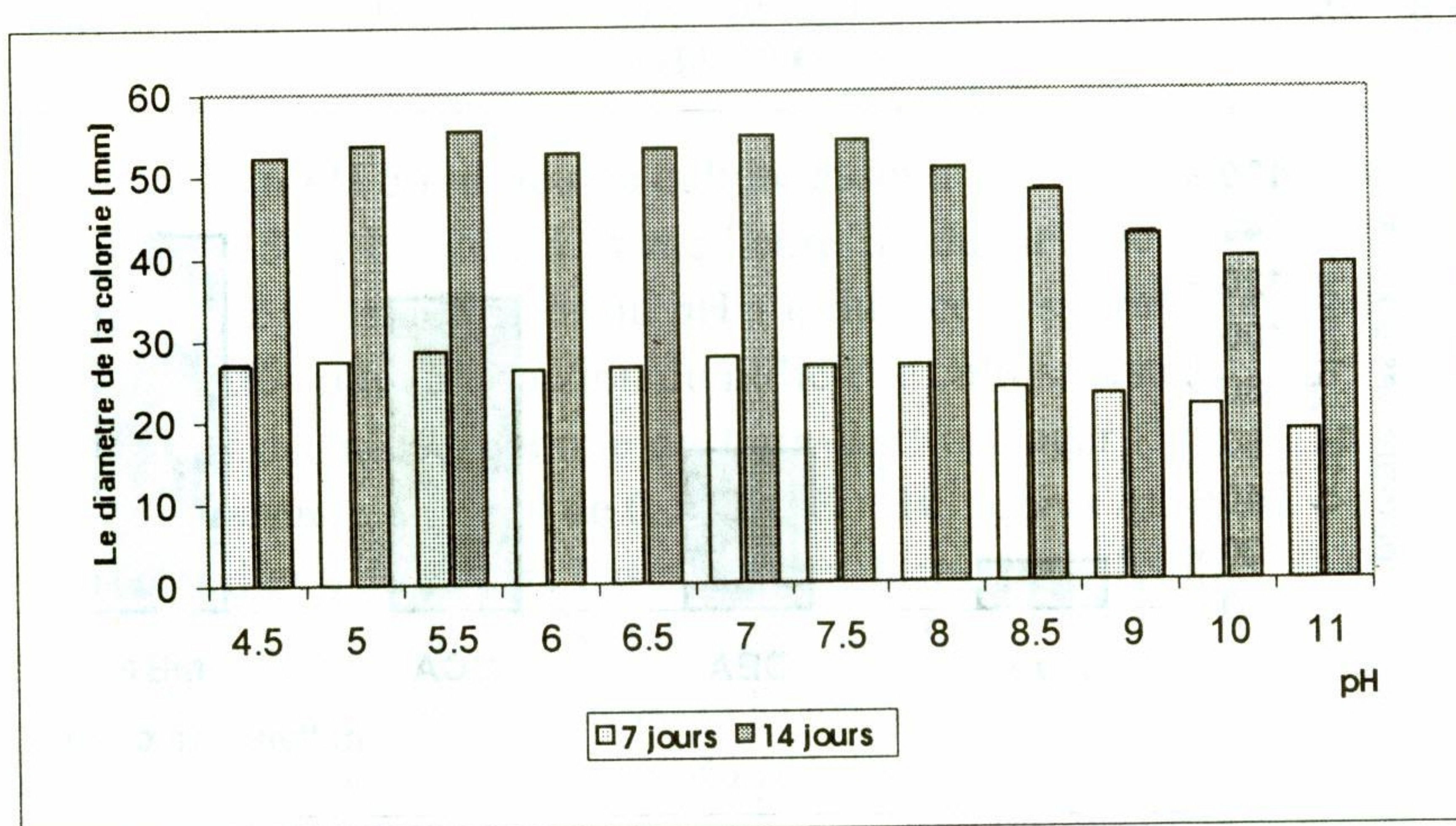


Fig. 1 – La croissance radiale des colonies de *Cercospora beticola* Sacc. dans les milieux PDA, en fonction de la valeur initiale du pH (pH = 6,0 – contrôle).

Dans le cas du *Cercospora beticola* Sacc. cultivé sur les milieux PDA, on a remarqué la même disponibilité de se développer dans différentes valeurs de pH, avec quand même des préférences (Fig. 1). Ainsi, les valeurs des pH compris entre 5,0 et 8,0 sont favorables à la croissance radiale de la colonie. La valeur optimale du pH est de 5,5 parce que pour cette valeur du pH on a mesuré le diamètre maximal de la colonie (55,4 mm après 14 jours de culture).

La préférence du champignon de se développer dans un milieu légèrement acide vers neutre est donnée par les valeurs mesurées après 7 et 14 jours de culture, la croissance du champignon étant fortement ralentie aux valeurs supérieures à 8,0.

Si on considérait la valeur du pH naturel du milieu (pH = 6,0) comme contrôle, la croissance du pH à 5,5 produit une importante croissance du diamètre de la colonie ($p < 0,001$). Les autres valeurs testées du pH ont influencé d'une manière négative le développement des colonies ($p \leq 0,05$), à l'exception des valeurs comprises entre 6,5 et 8,0, pour lesquelles la modification du diamètre des colonies a été très petite ($p > 0,05$).

C. L'INFLUENCE DU MILIEU DE CULTURE SUR LA SPORULATION DU CHAMPIGNON

Parmi les milieux de culture testés par nous et connus comme étant favorables à la sporulation du *Cercospora beticola* Sacc., les MBA et DCA sont optimaux. Nos expériences ont démontré que la production de spores obtenue dans ces milieux a été 4,96 fois (MBA) et 3,88 fois (DCA) plus grande que celle obtenue dans le milieu PDA. Dans le milieu DBA la sporulation a été moins abondante, la croissance étant seulement 1,09 fois plus grande que dans le PDA (Fig. 2).

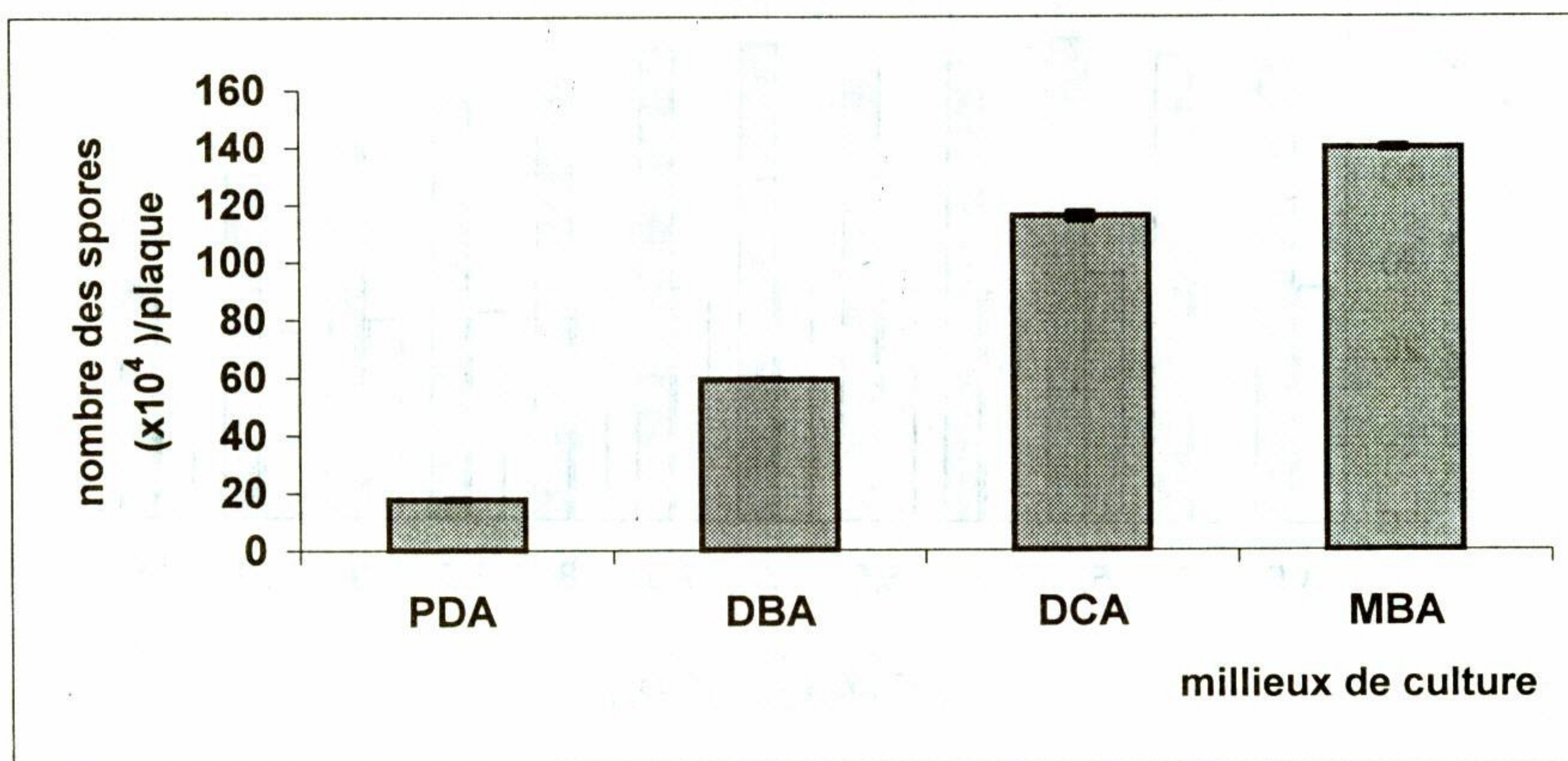


Fig. 2 – L'influence du milieu de culture sur la sporulation du *Cercospora beticola* Sacc. (PDA – contrôle; $p < 0,001$).

Des observations microscopiques on a constaté que seulement dans les milieux DCA et DBA les spores ont été typiques du point de vue de la dimension, semblables à ceux isolés des lésions.

Les spores obtenues dans le milieu MBA sont 2-3 fois plus grandes, de même que celles obtenues dans le milieu PDA. Des résultats analogues ont été obtenus dans le cas des spores de *Cercospora carotae*, obtenues dans différents milieux de culture (5).

Un autre aspect est donné par le fait que les milieux PDA et MBA assurent une très bonne croissance du mycélium, au contraire des milieux obtenus à base du décocté de feuilles (ce qu'on a remarqué aussi dans le cas de l'expérience antérieure), ce qui, du point de vue théorique, agrandit la surface de sporulation; mais, entre les deux milieux il existe une grande différence en ce qui concerne l'induction de la sporulation du champignon. Cette différence peut être expliquée par la composition des deux milieux de culture. Le PDA étant très riche en amidon, il favorise plus la croissance abondante du mycélium et non la sporulation, tandis que la mélasse de betterave, qui est composante du MBA, a un riche contenu en aminoacides libres (en spécial l'acide δ -aminobutyrique) et amides (glutamine et asparagine) qui peuvent influencer la sporulation. Les recherches démontrent que l'acide δ -aminobutyrique favorise la sporulation du *Cercospora beticola* Sacc. (3). Evidemment, il peut y avoir des facteurs nutritifs parmi ceux qui influencent la sporulation.

D. L'INFLUENCE DU pH DU MILIEU DE CULTURE SUR LA SPORULATION DU CHAMPIGNON

En général, un pH du milieu de culture assurant un domaine légèrement acide vers neutre (5,5-6,5) a conduit à la meilleure production de spores pour tous les milieux testés. Une certaine valeur du pH a influencé la sporulation du champignon d'une manière différente, en fonction du milieu de culture testé.

Dans le cas du milieu pomme du terre-dextrose-agar (Fig. 3), la sporulation maximale a été mesurée à un pH de 6,5 ($23,48 \times 10^4$ spores /plaque).

Dans les milieux de culture basés sur des décoctés de feuilles, la sporulation a été optimale pour un pH naturel: 6,0 dans les cas DBA ($59,2 \times 10^4$ spores/plaque – Fig. 4) et 5,5 dans le cas du DCA ($115,76 \times 10^4$ spores/plaque – Fig. 5). On remarque également, pour des valeurs du pH supérieur à 7,0 que la sporulation est favorisée, mais la production de spores n'atteint pas dans ces cas les valeurs maximales enregistrées.

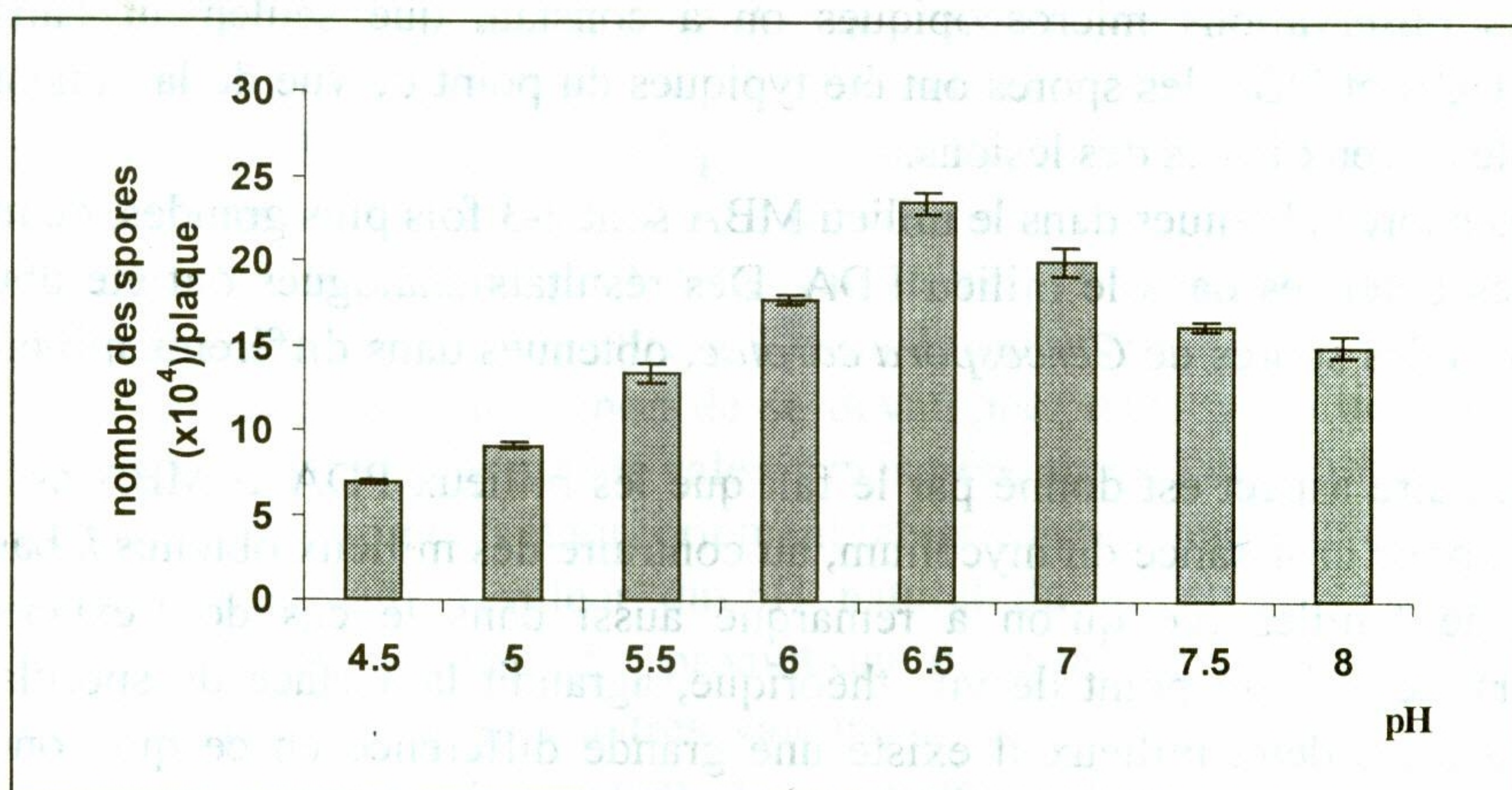


Fig. 3 – L'influence du pH sur la sporulation du *Cercospora beticola* Sacc. dans les milieux PDA ($pH = 6,0$ – contrôle; $p \leq 0,05$).

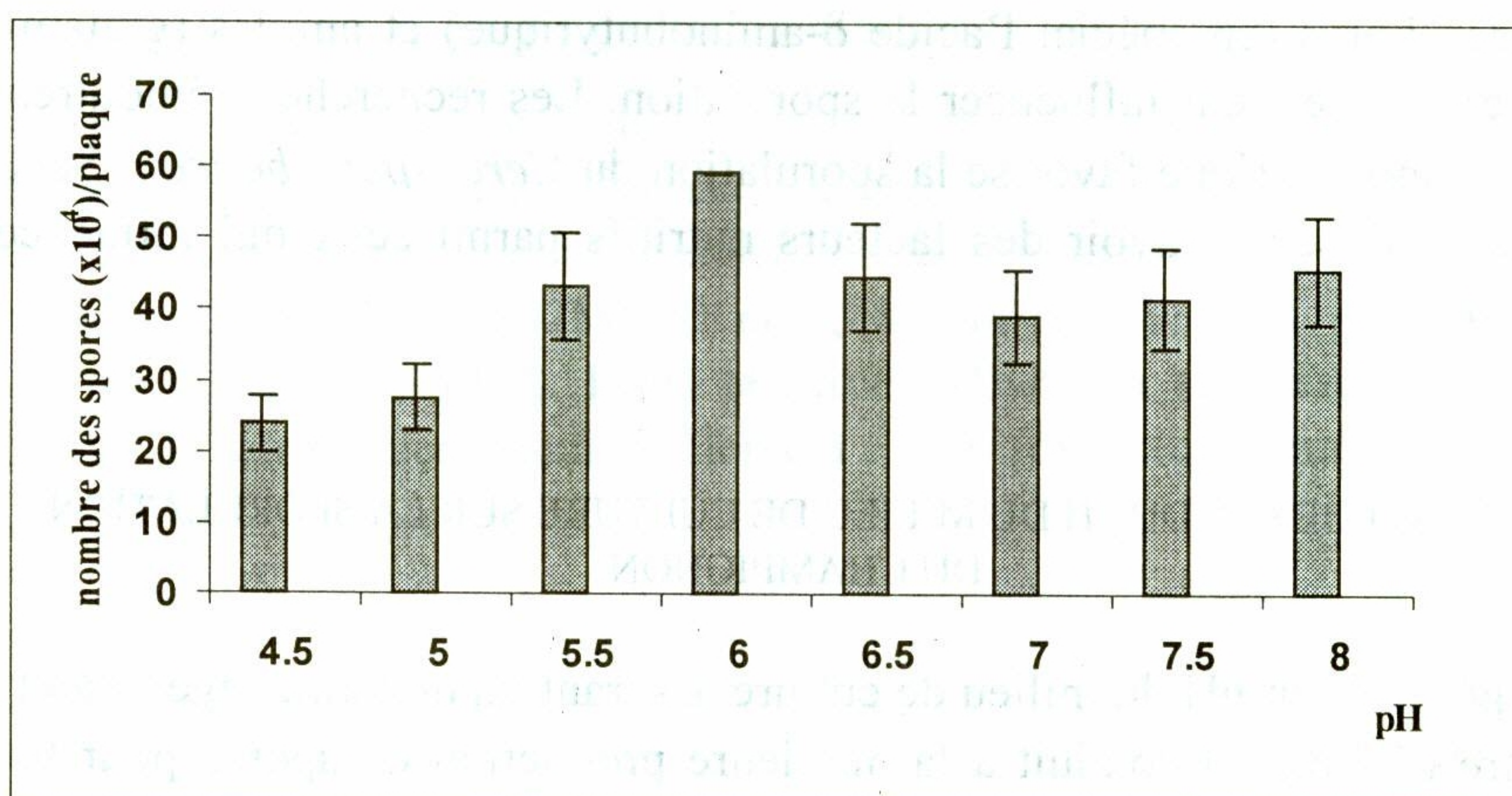


Fig. 4 – L'influence du pH sur la sporulation du *Cercospora beticola* Sacc. dans les milieux DBA ($pH = 6,0$ – contrôle; $p \leq 0,001$).

Le milieu MBA assure une très bonne sporulation pour presque toutes les valeurs du pH testé (pH supérieur à 5,0), avec un nombre maximal de spores (139,66 spores ($\times 10^4$)/plaque) pour un pH égal à 6,5 (Fig. 6).

Dans les cas des milieux MBA et PDA on a remarqué l'augmentation de l'intensité de la sporulation au fur et à mesure que le pH augmente vers la valeur optimale, suivie d'une diminution de la production au fur et à mesure que le pH augmente vers des valeurs alcalines.

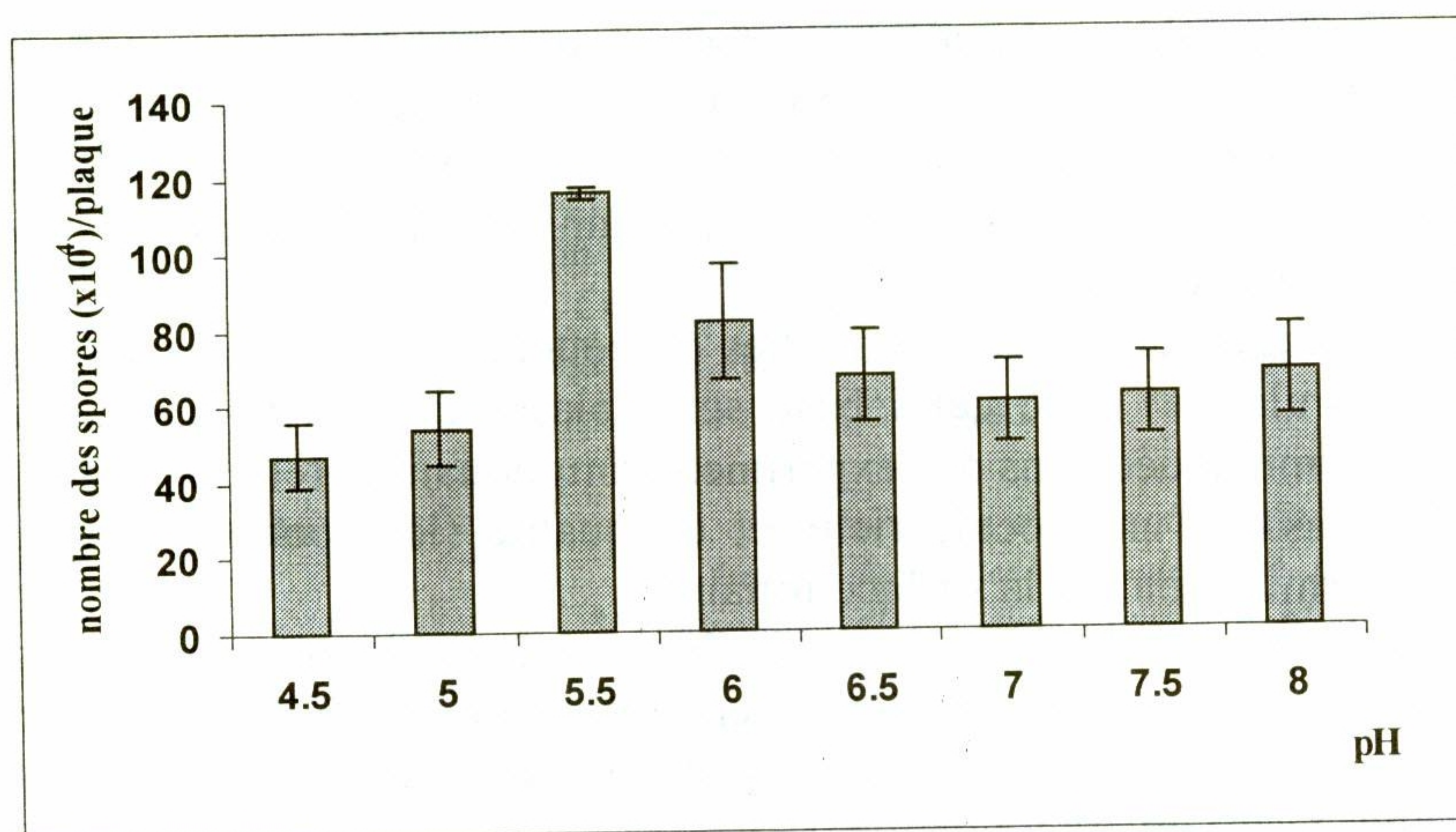


Fig. 5 – L'influence du pH sur la sporulation du *Cercospora beticola* Sacc. dans les milieux DCA (pH = 5,5 – contrôle; $p \leq 0,001$).

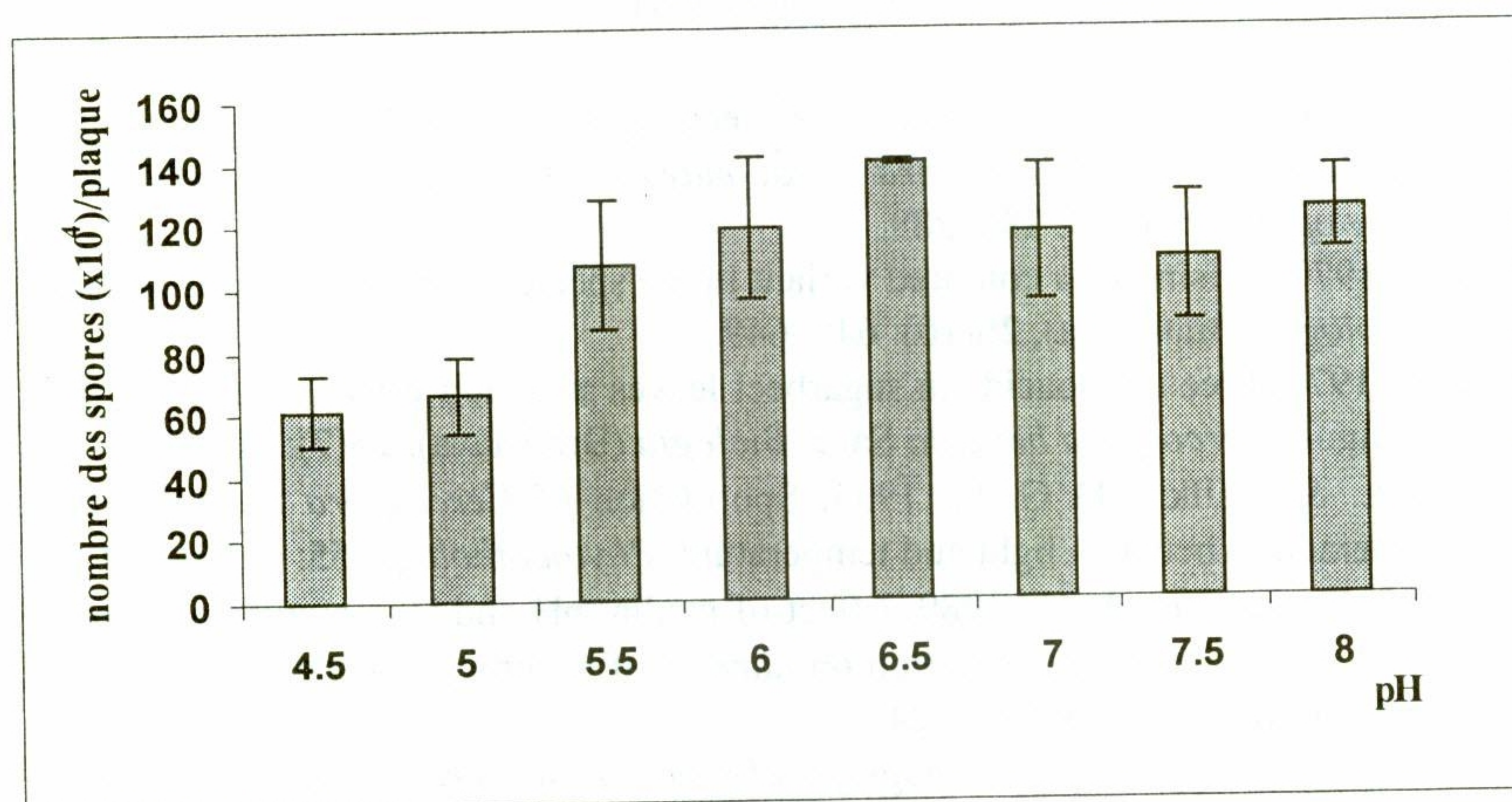


Fig. 6 – L'influence du pH sur la sporulation du *Cercospora beticola* Sacc. dans le milieu MBA (pH = 6,5 – contrôle; $p \leq 0,05$).

Dans tous les milieux testés, la diminution du pH en dessous de la valeur optimale inhibe graduellement la sporulation.

CONCLUSIONS

Nos expériences ont démontré que l'utilisation d'un milieu de culture basé sur mélasse de betterave est très favorable pour la croissance et la sporulation du *Cercospora beticola* Sacc. en comparaison avec les autres variantes des milieux

testés. Toutes les valeurs du pH testé (supérieures à 5,0) ont eu une influence positive sur la sporulation dans ce milieu, des différences significatives dans la production de spores n'étant pas signalées (pour les valeurs entre 6,0–7,0). Des résultats presque semblables ont été obtenus dans le cas d'un milieu basé sur le décocté de feuilles de carotte.

La substitution de décoct de feuilles de betterave (utilisé fréquemment pour induire la sporulation) avec celui basé sur mélasse de betterave ou feuilles de carotte est recommandée dans des expériences impliquant la relation hôte–parasite, parce qu'elle assure un inoculé riche et de qualité (les spores ont une grande capacité de germination sur la surface foliaire).

La légère acidité du milieu pomme de terre-dextrose-agar (pour un pH égal à 5,5) assure une plus rapide croissance en surface du mycélium, la connaissance de cet aspect étant importante, aussi, pour divers tests avec *C. beticola* *in vitro* (par exemple, pour tester divers fongicides).

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JURINEA TZAR-FERDINANDII NEW FOR THE ROMANIAN FLORA

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Jurinea tzar-ferdinandii Davidov is recorded for the first time in Romania (Cotul Văii, Constanța County). Morphological and ecological characteristics of this plant, as compared with those of *Jurinea stoechadifolia* and *J. linearifolia*, are presented and discussed.

Key words: *Jurinea tzar-ferdinandii*, Romania, morphology, taxonomy, chorology, ecology.

INTRODUCTION

Among the *Stenocephalae* Bentham et Hooker section, *Linearifoliae* Iljin series, *Jurinea stoechadifolia* and *Jurinea linearifolia* have been recorded for the Romanian flora till now.

During botanical investigations in South Dobrogea we observed many rare and very rare plant species. Among these, *Jurinea tzar-ferdinandii* has not been reported for the Romanian flora.

In the present preliminary account the morphological and ecological features, and also the chorology are described and briefly discussed.

Further information on chromosomes and anatomical specific features will be presented in a forthcoming contribution.

MATERIAL AND METHODS

Morphological, ecological and chorological data were obtained from live plants observed "in situ" and collected:

- Cotul Văii S, sinistra vallis Valea Mare, in saxosis calcareis (sarmatian), 43°48'31"N, 28°20'12"E, alt. 73 m, 22 VII 2001, G. Negrean [BUC].
- Cotul Văii S, sinistra vallis Valea Mare, "La Ic", in saxosis calcareis, 43°48'26"N, 28°20'17"E, alt. 65 m, 21 X 2001, G. Negrean & Paulina Anastasiu [BUC].
- Cotul Văii ESE, dextra vallis Valea Mare, in saxosis calcareis, 43°48'55"N, 28°20'53"E, alt. 74 m, 3 VI 2001, G. Negrean [BUC].

Informations for *J. stoechadifolia* and *J. linearifolia* were obtained from the literature (1, 3, 5, 6, 7, 8, 9, 12) and from herbarium specimens preserved in BUC:

- *Jurinea linearifolia*: Basarabia, distr. Cahul. In foenatis steppaceis septentrionalis expositionis propre pagum Ciumai, alt. cca 20–50 m s.m., solo argill. (pH = 7), 22 Jun. 1939, legit G. Bujorean, A. Grimalschi, C. Zahariadi, determinavit C. Zahariadi. [FRE 2068] (1).

- *Jurinea stoechadifolia*: In campis aridis ad marginem silvae Babadag, prope pag. Caugagia, 22 Jul. 1910, legit & determinavit I. Prodan. [FRE 1581] (1).

The nomenclature is according to Flora României (10) and Flora Europaea (11, 12).

Herbarium abbreviations are those used by Holmgren *et al.* (1990).

RESULTS AND DISCUSSIONS

Morphological data: Perennial plant with numerous rosettes of leaves, sometimes non-flowering.

Stems 15–30 cm, woody at base, angular, tomentose, with sessile glands, as whole plant.

Numerous basal leaves, 4–15 × 1.2 cm, with entire, revolute margin, green, setose and thin tomentose above, arachnoid-tomentose beneath. The length of cauline leaves decreases as far as capitula. Very rarely some leaves are pinnatifid.

Capitula 4–8, 15–20 × 4.5–7 mm, obconical. Involucral bracts narrowly linear-lanceolate, long-acuminate, straight and appressed, scabrid on margin, arachnoid-tomentose, white distally.

Flowers pale pinkish, smelling chocolate with milk.

Achenes 3–5.5 mm, tetragonal, whitish, apical with brown spots, finely ribbed, glabrous; pappus hairs in several rows, 3–4 times as long as achene, with 3–4 of the inner hairs twice (8–9 mm) as long as the outer, shortly plumose.

Ecology: The plant grows on stony, rocky slopes, on sarmatian limestone, together with the following species: *Achillea clypeolata*, *Adonis vernalis*, *Asperula tenella*, *Astragalus cornutus*, *Astragalus spruneri*, *Bromus riparius*, *Chamaecytisus jankae*, *Crepis pulchra*, *Echinops ritro ruthenicus*, *Elymus hispidus barbulatus*, *Euphorbia dobrogensis*, *Ferulago confusa*, *Filipendula vulgaris*, *Genista sessilifolia romanica*, *Gypsophila pallasii*, *Haplophyllum ciliatum*, *Hyacinthella leucophaea*, *Inula oculus-christi*, *Khoeleria nitidula*, *Linum tauricum*, *Minuartia bilykiana*, *Nepeta parviflora*, *Ononis pusilla*, *Poa versicolor*, *Polygala major*, *Prunus tenella*, *Salvia austriaca*, *Salvia nutans*, *Salvia ringens*, *Satureja coerulea*, *Scabiosa argentea*, *Scorzonera mollis*, *Scutellaria orientalis*, *Serratula radiata*, *Stipa lessingiana*, *Tanacetum millefolium*, *Teucrium chamaedrys*, *Teucrium polium capitatum*, *Thymus zygioides*, *Vicia peregrina*, etc.

Chorology: In Romania, *Jurinea tzar-ferdinandii* is distributed in Dobrogea (Cotul Văii, Constanța County).

For Europe this species is endemic. Kožuharov (1976) indicates it only from Bulgaria. We collected the plant in N-E Bulgaria, at E and W of Balcic town (where is parasited by *Puccinia calcitrapae* DC.) [BUCM 136.752; SOM].

Jurinea tzar-ferdinandii is a dobrogean element.

In Romania other two species belonging to those with cylindrical capitula were indicated. We present a key for the three species from *Linearifolia* series below:

- 1a Capitula cylindrical, 27–30 mm; involucre 17 mm $\times$ 5–7 mm; leaves 5–7 $\times$ 0.2–0.3 cm *J. stoechadifolia*
- 1b Capitula cylindrical or obconical, 7–20 mm 2
- 2a Basal leaves 3–8 (–10) $\times$ 0.2–0.6 (–0.9) cm. Capitula 7–15 (–18) $\times$ 4–6 mm, cylindrical. Involucral bracts shortly acuminate, glabrous or slightly tomentose, pink or reddish-purple distally; steppic plant *J. linearifolia*
- 2b Basal leaves 4–15 $\times$ 0.12 cm. Capitula 15–20 $\times$ 4.5–7 mm, obconical. Involucral bracts narrow linear-lanceolate, long acuminate, with dense arachnoid indumentum, white distally; on calcareous slopes *J. tzar-ferdinandii*

Because all these species are rare in Romania, we present also their chorology:

J. stoechadifolia (Bieb.) DC. – “între marginea pădurii Babadag, satul Căugăgia” (3). This is LOCUS CLASSICUS for *Centaurea jankae*; “Locuri aride de pe colina dela Găugăgia aproape de marginea pădurii Babadag (Br. Db. 217); în locul susindicat pe marginea semănăturilor [Prodan Flora Romaniae Exsiccata cent. VI și XVI]” (PRODAN 1939: 53); “In campis aridis ad marginem silvae Babadag, prope pag. Găugăgia. – 22 Iul. 1910, leg. & det. I. Prodan [FRE 1581] (1); “Căugăgia aproape de marginea pădurii Babadag și la marginea semănăturilor” (8).

In a map regarding the distribution of southern and south-eastern European plants in Romanian Flora, *J. stoechadifolia* is noticed at the point (no. 32) situated on Middle Bârlad Valley (2).

General distribution: from S-E Europe to Caucasus (Bulgaria, Romania, S-E Russia, Ukraine, Crimea). It is a dobrogean-pontic-tauric-caucasian element.

J. linearifolia DC. – Galați County, near Brateș lake, C. Zahariadi [HERB. C. Zahariadi]; Tulcea County, Casimcea upland, Nistorești (5); Medgidia, with var. *bessarabica* Prodan (9).

General distribution: from S-E Romania to W China (Romania, Ukraine, S Russia, Caucasus, Siberia, Mongolia, China). It is a continental element. This species has not been recorded in Bulgaria. According to Iljin (1962) the correct name of the plant is *J. multiflora* (L.) B. Fedtsch. (= *J. linearifolia* DC.).

We mention that the place where we discovered *Jurinea tzar-ferdinandii* is very rich in rare species, about 30 taxa of them being included in the red lists. Among these we underline: *Salvia ringens*, indicated till now only from Hagieni (Constanța County), *Genista sessilifolia romanica*, *Ononis pusilla*, *Astragalus spruneri*, *Linum tauricum*, *Scorzonera mollis*. For this reason we are thinking to propose the slopes between Cotul Văii and Vâlcele as a Natural Reserve.

CONCLUSIONS

Based on morphological features one can establish that the species observed and collected in Constanța County is:

***Jurinea tzar-ferdinandii* Davidov,**
Sborn. Bălg. Akad. Nauk. 15: 28–44 (1909).

This has not been recorded till now in our country. Its presence in Romania is very interesting completing its area to North.

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THE CALCICOLOUS GROUPS SYNTAXONOMY OF *PAPAVERO-THYMION PULCHERRIMI* POP 1968 FROM THE ROMANIAN CARPATHIANS

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The Carpathic phytocoenoses of calcarous debris or of stones from *Papavero-Thymion pulcherrimi* Pop 1968 are better delimited than another from Central Europe due to their cenotic structure, with numerous oligothermous (hekistothermic, subcryophilous and microthermophilous) and endemic species.

Their place on a substrate in various mobility degrees causes the beginning of the colonization and the fastening of the debris and it also permits the subsequent installation of the other characteristic elements of *Elyno-Seslerietea*, *Salicetea herbaceae*, etc, with an important role in ulterior dynamics of the vegetal succession in these ecotopes.

Key words: syntaxonomy, debris vegetation, *Thlaspietea rotundifolii*.

INTRODUCTION

The phytocoenoses of calcarous debris or of stones placed in an alpine belt where the processes of degradation and erosion are more intense, are an initial link in the natural vegetation installation.

This installation is conditioned by various factors: the particles dimensions of the fragments of the substrate, their nature, their stability degree, the exposition and the slope, the altitude, etc.

On these debris the phytocoenoses remain as long as it is a permanent disaggregate recent material rolled on.

As the calm down phases of the debris are going on (this phenomenon is more active in the mountain belt) it is produced a gradual lying fallow of these habitats due to a colluvial process.

The *Papavero-Thymion pulcherrimi* Pop 1968 alliance was created to characterise opened, heliophile vegetation of calcarous debris or of stones placed in the alpine belt of the Romanian Carpathians.(10)

From the characteristic species, most of them Carpathic endemic species, we noticed: *Cerastium arvense* ssp. *lerchenfeldianum*, *C. transsilvanicum*, *Papaver corona-santi-stephani*, *Thymus pulcherrimus*, *Acinos alpinus* ssp. *majoranifolia*, *Doronicum carpaticum*, *Alyssum repens* and *Poa molinerii*.

MATERIAL AND METHODS

On the basis of literature data (the synthesis of 168 surveys) and of our observations, we reproduced in Table 1 the coenotic structure of eight associations

from *Papavero-Thymion pulcherrimi* Pop 1968. In the critical examination of each coenotaxon we reproduced the principal synonyms under which the groups of debris have been described.

Table 1
The associations of *Papavero-Thymion plcherrimi* Pop 1968

Number of association	1	2	3	4	5	6	7	8
Number of surveys	23	18	35	27	10	46	5	18
Characteristic of associations								
<i>Cerastium lerchenfeldianum</i>	IV		I	I		II		
<i>Papaver corona-santi-stephani</i>	V	V						
<i>Cardaminopsis neglecta</i>		II						
<i>Acinos alpinus</i>	III		V			I		
<i>Galium anisophyllum</i>	III	II	V	I	I	III	III	II
<i>Doronicum columnae</i>	I			II				
<i>Rumex scutatus</i>				V		II		
<i>Cerastium arvense</i> ssp. <i>calcicolum</i>			II		IV	II		IV
<i>Saxifraga moschata</i>	II	III	III	I	V	IV		
<i>Saxifraga aizoides</i>	II	III	II	II	I	V		
<i>Cerastium transsilvanicum</i>							V	
<i>Galium lucidum</i>			I				V	
<i>Poa alpina</i>	I	II	II	I		II		V
<i>Alyssum repens</i>	IV		I	I		I		IV
<i>Papavero-Thymion pulcherrimi</i>								
<i>Thymus pulcherrimus</i>	IV	II	V	I		III		III
<i>Doronicum carpaticum</i>		III		I	I	I		
<i>Poa molinerii</i>	III		III	I				
<i>Rhodiola rosea</i>		II	I		III			
<i>Viola alpina</i>	II					I		
<i>Thymus comosus</i>			I				I	

I: *Thymus balcanus* (3), II: *Saxifraga hieraciifolia* (5), III: *Phleum alpinum* (8), *Festuca nitida* ssp. *flaccida* (2).

<i>Androsacetalia alpinae</i> s.l.								
<i>Poa laxa</i> (<i>P. tremula</i>)		II	II	II	II			II
<i>Saxifraga adscendens</i>	I	I	II		II	II		
<i>Oxyria dygina</i>		IV		I	I	I		
<i>Luzula spicata</i>		I			II	I		II
<i>Saxifraga bryoides</i>		II			I	I		
<i>Veronica baumgartenii</i>		II			V	I		
<i>Silene acaulis</i>	I	III	II					
<i>Saxifraga androsacea</i>					II	I		
<i>Dianthus glacialis</i>		II				I		

I: *Epilobium anagallidifolium* (6), *Leucanthemopsis alpinus* (6), *Poa granitica* (3), *Arenaria rotundifolia* (3), *Saxifraga pedemontana* ssp. *cymosa* (3), II: *Erigeron uniflorus* (8), *Soldanella pusilla* (6), *Poa cenisia* ssp. *contracta* (3, 6), *Taraxacum nigricans* (4), III: *Anthemis pyrethroides* (8).

Table 1 (continued)

<i>Thlaspietea rotundifolii</i>								
<i>Arabis alpina</i>	V	III	I	III	V	III		
<i>Senecio rupestris</i>	V		II	IV		I		
<i>Cardaminopsis arenosa</i>			I	I	III			
<i>Polystichum lonchitis</i>			I	I		I		
<i>Hutchinsia alpina</i> ssp. <i>brevicaulis</i>	II				I	II		
<i>Saxifraga carpatica</i>					I	I		

I: *Senecio squalidus* (3), III: *Cardaminopsis halleri* ssp. *ovirensis* (6), *Androsace obtusifolia* (1), *Galium album* (4).

<i>Seslerietalia s.l.</i>								
<i>Achillea schurii</i>	I	III	III	I	I	II		II
<i>Polygonum viviparum</i>		II	I		II I	II		V
<i>Biscutella laevigata</i>	II						V	II
<i>Campanula kladiana</i>		II	I		I	I		
<i>Sedum atratum</i>	III		II		I	I		
<i>Saxifraga oppositifolia</i>		II			II	I		
<i>Artemisia eriantha</i>	I	II			I	I		
<i>Ranunculus oreophilus</i>	IV	I	II	I		I	III	I
<i>Myosotis alpestris</i>	IV	I	III	II			II	V
<i>Festuca versicolor</i>	I	II			I		II	I
<i>Scabiosa lucida</i>			II	I				II
<i>Festuca amethystina</i>			I	I				
<i>Cerastium alpinum</i> var. <i>lanatum</i>		III			II			
<i>Sesleria rigida</i> var. <i>haynaldiana</i>							V	I
<i>Carduus kernerii</i>			I	I				
<i>Sesleria bielzii</i>			I			I		
<i>Polygala alpestris</i>			II				II	
<i>Carex sempervirens</i>						I		IV
<i>Gentiana verna</i>	II		I					II

I: *Helianthemum tomerntosum* (8), *Onobrychis transsilvanica* (8), *Minuartia verna* (8), *Helianthemum alpestre* (8), *Lloydia serotina* (6), *Dryas octopetala* (1), *Ranunculus thora* (3), *Silene zawadzkii* (3), *Bartschia alpina* (3).

II: *Primula elatior* (8), *Thymus parviflorus* (8), *Oxytropis halleri* (8), *O. carpatica* (8), *Veronica aphylla* (6), *Linum extraaxilare* (8), *Dianthus callizonus* (7), III: *Potentilla crantzii* (8), *Festuca pachyphylla* (1), *Dianthus spiculifolius* (7), IV: *Armeria alpina* (8), *Trifolium repens* ssp. *ochranthum* (8).

<i>Potentilletalia caulescentis</i> s.l.								
<i>Saxifraga paniculata</i>	I	II	II	I	V	II		
<i>Asplenium viride</i>			II	I		I		
<i>Draba lasiocarpa</i>	III					I		
<i>Cystopteris fragilis</i>			II			I		
I: <i>Silene dubia</i> (3)								
<i>Salicion herbaceae</i>								
<i>Luzula alpinopilosa</i>		I			IV			
<i>Primula minima</i>					V	II		

Table 1 (continued)

<i>Geum montanum</i>			II			I		III
<i>Sedum alpestre</i>						I		IV
<i>Pedicularis oederi</i>	I					I		
I: <i>Poa supina</i> (3)								
<i>Accompanied</i>								
<i>Cystopteris regia</i>	II			III				
<i>Pedicularis verticillata</i>	I	II		I		I	I	IV
<i>Taraxacum alpinum</i>	II		II	I		I		III
<i>Silene pusilla</i>	II	I		I		II		
<i>Alchemilla fissa</i>				I	I	II		
<i>Viola biflora</i>				I	III	I		
<i>Parnassia palustris</i>			I	II		I		
<i>Huperzia sellago</i>					III	I		
<i>Potentilla ternata</i>						I		IV
<i>Agrostis rupestris</i>						I		III
<i>Festuca airoides</i>			I			I		IV
<i>Carlina acaulis</i>						I		I
<i>Draba lasiocarpa</i>			I			I		
<i>Alchemilla xanthochlora</i>			I					I

I: *Kernera saxatilis* (3), *Seseli libanotis* (3), *Petasites albus* (3), *Hornungia petraea* (6), *Veronica alpina* (6), *Cortusa matthioli* (6), *Pulsatilla alpina* (8), *Gentianella lutescens* (6), *Ranunculus crenatus* (6), *Saxifraga stellaris* (6), *Veronica chamaedrys* (8), *Saxifraga tridactylis* (6), *Trifolium badium* (8), *Saxifraga heucherifolia* (6), *Nigritella rubra* (8), *Centaurea nervosa* (8), *Campanula abietina* (8), *C. serrata* (8), *Achillea sudetica* (8), *Scrophularia scopolii* (3), *Epilobium nutans* (3), II: *Ligusticum mutellina* (8), *Poa violacea* (8), *Alchemilla glabra* (8), *Trifolium pratense* (8), *Viola declinata* (8), *Cruciata glabra* (8), *Gnaphalium supinum* (6), *Doronicum clusii* (5), *Veronica bellidioides* (3), *Plantago atrata* (3), *Epilobium nutans* (4).
III: *Luzula campestris* (8), *Poa media* (8), *Alchemilla flabellata* (8), *Deschampsia caespitosa* var. *alpina* (8), *Achillea stricta* (8), *Festuca rupicola* ssp. *saxatilis* (7), *Oreochloa disticha* (5), *Campanula alpina* (5), IV: *Ranunculus pseudomontanus* (8), *Libanotis pyrenaica* (7), *Aconitum tauricum* (7).

Providence of surveys:

Ass. 1 *Cerastio lerchenfeldiani-Papaveretum*, 2 surveys by Csűrös et al. 1956, Retezat Mountains, 10 surveys by Boșcaiu et al. 1977, Retezat Mountains, 5 surveys by Evd. Pușcaru-Soroceanu et al. 1981, Făgăraș Mountains, 5 surveys by Ileana D. Stancu, 2002 Polița lui Vodă și Râiosu Mountain.
Ass. 2 *Cardaminopsio neglectae-Papaveretum*, 8 surveys by Beldie, 1967, Bucegi Mountains, 10 surveys by Coldea et Pânzaru, 1986, Rodnei Mountains.
Ass. 3 *Acino-Galietum anisophylli*, 10 surveys by Beldie, 1967, Bucegi Mountains, 5 surveys by Boșcaiu, 1971, Țarcu, Godeanu Mountains, 7 surveys by Coldea, 1990, Rodnei Mountains, 5 surveys by Nechita et Mititelu, 1996, Hășmaș and Cheile Bicazului Mountains, 8 surveys by Ileana D. Stancu, 2002, Buda and Râiosu Mountains.
Ass. 4 *Doronico columnae- Rumicetum scutati*, 7 surveys by Coldea, 1990, Rodnei Mountains, 7 surveys by Voik et Schneider, 1978, Făgăraș Mountains, 6 surveys by Boșcaiu, 1971, Țarcu, Godeanu Mountains, 10 surveys by Boșcaiu et al. 1977, Retezat Mountains.
Ass. 5 *Cerastio calcicolae-Saxifragetum moschatae*, 10 surveys by Coldea, 1990, Rodnei Mountains.
Ass. 6 *Saxifragetum moschatae-aizoidis* (incl. *Saxifrageto aizoidis-Rumicetum scutati*), 9 surveys by Voik, 1976, Făgăraș Mountains, 9 surveys by Voik et Schneider, 1978, Făgăraș Mountains, 21

surveys by Boşcaiu, 1971, Țarcu, Godeanu Mountains, 7 surveys by Coldea, 1990, Rodnei Mountains.

Ass. 7 *Cerastio transsylvanici-Galietum lucidi*, 5 surveys by Monica Boşcaiu, 1998, Piatra Craiului Mică.

Ass. 8. *Poo alpinae-Alysetum repentis*, 18 surveys by Beldie, 1967, Bucegi Mountains.

RESULTS AND DISCUSSIONS

The species from the main nucleus of phytocoenoses that we have analysed are generally microthermophilous elements, subcryophilic or even cryophilic; in their structure many elements from *Seslerietalia* and *Asplenietea* have penetrated.

1. *Cerastio lerchenfeldiani-Papaveretum* Boşcaiu *et al.* 1977 (Table 1).

(Syn. *Papavereto-Cystopteridetum* Csűrös *et al.* 1956, *Papaver pyrenaicum-Linaria alpina* ass. Puşcaru *et al.* 1956, *Papaver pyrenaicum-Viola alpina* Evd. Puşcaru *et al.* 1981).

The phytocoenoses enlightened by *Papaver corona santi-stepheni* have been described and observed in Bucegi, Făgăraş and Retezat Massifs (1, 3, 4, 8). These present in their coenotic structure as a characteristic and co-enlightened species the carpathic endemic *Cerastium arvense ssp. lerchenfeldianum*.

On semi-fixed and sunny calcarous rocks the following species could be frequent: *Acinos alpinus*, *Thymus pulcherrimus*, *Poa molinerii*, *Alyssum repens*, *Arabis alpina*, *Senecio rupestris*, *Ranunculus oreophilus*, *Myosotis alpestris* in these coenoses structure.

2. *Cardaminopsio neglectae-Papaveretum* Coldea et Pânzaru 1986 (Syn. *Papaver pyrenaicum-Festuca violacea* ass. Beldie 1967).

They are sciaphilous and chionophilous phytocoenoses on the bottom of glacial hollow and on high grassy paths from rocky walls of the Rodna and Bucegi Mountains (1, 5, 6).

The endemic species *Doronicum carpaticum* and *Cardaminopsis neglecta*, characteristic for this association, are accompanied by numerous oligothermous elements as: *Oxyria dygina*, *Silene acaulis*, *Saxifraga aizoides*, *S. moschata*, *Arabis alpina*, *Achillea schurii*, *Cerastium alpinum ssp. lanatum*. All these species emphasize the better-marked microthermous character than the previous community.

3. *Acino-Galietum anisophylli* Beldie 1967

It populates small debris from the Rodnei, Hăşmaş, Bucegi, Țarcu and Godeanu Mountains (1, 3, 4, 5, 6, 9), and it grows with priority on sunny sides, edges of glacial hollows, etc. The species of *Seslerietalia* as: *Achillea schurii*, *Sedum atratum*, *Ranunculus oreophilus*, *Myosotis alpestris*, *Scabiosa lucida*, *Polygala alpestris* are better represented. Besides them, there could be found many

elements of *Thlaspietalia* and *Asplenietea* that characterise more than once these biotopes.

4. *Doronico columnae-Rumicetum scutati* Boșcaiu *et al.* 1977 (Syn. *Rumicetum scutati* auct. roman.)

The coenoses enlightened by *Rumex scutatus* and *Doronicum columnae* present an optimum growth on mobile, small and wet debris from the Oriental Carpathians (Rodnei Massif) (5, 6) and the Meridional Carpathians (Făgăraș, Retezat, Țarcu, Godeanu) (2, 3, 14).

From frequently accompanied species we notice: *Arabis alpina*, *Senecio rupestris*, *Cystopteris regia*, *Myosotis alpestris*.

In consequence of organic material colluviation, favorable conditions are created for the colony of *Seslerietalia* species that contribute to the strengthening of the substrate.

5. *Cerastio calcicolae-Saxifragetum moschatae* Coldea 1990

They are phytocoenoses from the alpine belt of Rodnei Massif (5, 6) and they grow on jurassic calcareous fragments, amphibolite and sericitoase schists. In phytocoenosis, there could be found both basophilous species characteristic for *Thlaspietalia rotundifolii* order and acidophilous species characteristic for *Androsacetalia alpina*.

The most frequently accompanied of these two codominated *Cerastium arvense* ssp. *calcicolum* and *Saxifraga moschata* are *Rhodiola rosea*, *Poa laxa*, *Saxifraga ascendens*, *Veronica baumgartenii*, *Arabis alpina*, *Cardaminopsis arenosa*, *Polygonum viviparum*, *Saxifraga oppositifolia*.

6. *Saxifragetum moschatae-aizoidis* Boșcaiu 1971 (incl. *Saxifrageto aizoidis-Rumicetum scutati* Boșcaiu 1971)

It is established on low mobility and high humidity debris due to snow thawed accumulation. Consequently, in these phytocoenoses are a large number of hygrophilous elements such as *Silene pusila*, *Saxifraga stellaris*, *Parnassia palustris*, *Viola biflora*, etc. even chionophytes: *Soldanella pusilla* and *Gnaphalium supinum*.

The subassociation *dianthetosum glacialis* Voik et Schneider-Binder 1978 is established in Făgăraș Massif (15, 16), on amphibolic schists and on crystalline calcareous ones. In these phytocoenoses the species *Saxifraga moschata* has a lower presence, it is not found in the majority of the surveys (from four surveys it is found in only one, with +)

7. *Cerastio transsylvanici-Galietum lucidi* Monica Boșcaiu *et al.* 1998

Those five surveys from Piatra Craiului Mică (4) debris are described on the south and sloping sides (45°). The phytocoenoses from 1660–1680 m altitude present a medium cover by 20–25% and they have as accompanied species: *Galium anisophyllum*, *Thymus comosus*, *Biscutella laevigata*, *Bupleurum cernuum*,

Dianthus callizonus, *D. spiculifolius*, *Sesleria rigida* ssp. *haynaldiana* and *Libanotis pyrenaica*.

8. *Poo alpine-Alysetum repentis* Beldie 1967

They are established at the bottom and on the gentle sloping sides of glacial valleys, on skeleton soil formed on calcareous, strong colluvinate debris (11).

This community is remarkable due to the large number of component species that realize a large cover of elements with great constancy, from which we notice: *Poa alpina*, *Arenaria alpina*, *Alyssum repens*, *Festuca airoides*. The presence of many oligothermous and eutrophic characteristic species of these habitats that tend to evaluate to installation of characteristic *Seslerietalia* order meadow.

CONCLUSIONS

The coenotic nucleus of characteristic species of *Papavero-Thymion pulcherrimi* Pop 1968 alliance is composed of many endemic Carpathic species, oligothermes (microthermes, hekistothermes and subcryophiles) that grow on calcareous debris in an incipient phase of colonisation. As the processes of humus accumulation and of colluviation advance there are created conditions for the installation of characteristic *Elyno-Seslerietea* class vegetation, with the most important role in debris fixing and generally in unroll of successive phases of vegetation installation.

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CONTRIBUTIONS À L'ÉTUDE DE LA VÉGÉTATION FORESTIÈRE DU BASSIN DE LA MOLDOVIȚA (DÉPARTEMENT DE SUCEAVA)

CIPRIAN MÂNZU, TOADER CHIFU

In this paper are presented three wooden vegetation associations, frequently met in forests (*Leucanthemo waldsteinii* – *Fagetum* (Soó 1964) Taüber 1987, *Hieracio rotundati* – *Piceetum* Pawl. et Br.-Bl. 1939) and water courses (*Telekio speciosae* – *Alnetum incanae* Coldea (1986) 1990) from the Moldovița river's basin (Suceava county).

Key words: phytocoenology, wooden vegetation.

Le bassin de la Moldovița se superpose sur la Dépression de Moldovița, largement développée entre Obcina Feredeului, à l'ouest, Obcina Mare, à l'est, Obcinele Brodinei, au nord et Culmea Ascuțită, du système orographique du Voroneț au sud. La Moldovița, qui draine longitudinalement cette dépression, prend sa source dans le mont Vejul (Obcina Feredeului) et après un cours de 51,6 km, se joint à la Moldova à Vama (555 m alt.). La surface de son bassin est de 653 km² et l'altitude moyenne du relief est de 814 m. Du point de vue de la distribution régionale géomorphologique des Obcinele Bucovinei, le bassin de la Moldovița fait partie des «Obcinele du flysch».

Etant donné la situation vers l'extrémité nord-est de la province centrale-européenne, le climat général est de type tempéré-moderé-continentale, avec certaines influences du climat continental de l'est et celui sous-baltique (boréal) du nord. Par sa position, le bassin de la Moldovița appartient au sous-district du nord-ouest des Obcinele Bucovinei (ayant des températures et des amplitudes thermiques plus réduites, des précipitations plus riches), mais, tout de même, le long de son couloir (comme pour le couloir de la Moldova), on retrouve les caractéristiques climatiques du sous-district de sud-est des Obcinele Bucovinei (caractérisées par des températures et des amplitudes thermiques plus élevées, des précipitations plus réduites)[1].

Des recherches sur la végétation des Obcinele Bucovinei, dans le périmètre desquelles se trouve aussi le bassin de la Moldovița, ont débuté et se sont concentrées surtout pendant les années 1950–1965 [7, 12, 13, 16] et 1990–2000 [2, 3, 4, 15].

Comparées aux recherches sur la flore vasculaire [10, 11, 14] des Obcinele Bucovinei, les recherches sur la végétation sont relativement réduites. C'est pourquoi notre attention s'est concentrée sur cette zone.

On peut mentionner aussi que le bassin de la rivière Moldovița a constitué le thème d'une thèse de doctorat [9], mais les recherches portent seulement sur la végétation des prairies.

Suite aux recherches de terrain effectuées entre 2000–2001, on présente dans l'ouvrage trois associations végétales forestières qui ont une vaste distribution dans la zone recherchée.

1. *Leucanthemo waldsteinii* – *Fagetum* (Soó 1964) Taüber 1987

(Cl. *Querco-Fagetea*, Ord. *Fagetalia*, Al. *Symphyto-Fagion*, Subal. *Symphyto-Fagenion*) (Tableau 1)

C'est une association dans laquelle les phytocoenoses sont constituées constamment et d'une manière dominante par *Picea abies* et *Fagus sylvatica*, mais dans certaines phytocoenoses l'espèce *Abies alba* devient codominante soit avec *Fagus sylvatica*, soit avec *Picea abies*. Cette composition mixte de la strate arborescente a été signalée dans Obcina Mare [4], et elle représente une caractéristique des forêts d'épicéa avec hêtre de Bucovine. Les trois espèces, fréquemment co-dominantes, réalisent un recouvrement élevé, de 80-90%, rarement de 70-75% et *Acer pseudoplatanus* et *Sorbus aucuparia* sont présentes seulement dans quelques phytocoenoses. L'espèce caractéristique, *Leucanthemum waldsteinii*, avec les espèces caractéristiques à la classe *Querco-Fagetea*, sont majoritaires par rapport aux espèces caractéristiques à la classe *Vaccinio-Piceetea*. Une teinte régionale spécifique est donnée par la fréquence d'un noyau important d'espèces caractéristiques à l'alliance *Alnion incanae*, exprimant une affinité des espèces pour l'humidité du sol, ce qui est significatif, aussi dans l'écogramme de l'association (Fig. 1a). L'analyse phytogéographique indique la prédominance des éléments euroasiatiques, européens et circonpolaires (Fig. 1b).

2. *Hieracio rotundati* – *Piceetum* Pawl. et Br.-Bl. 1939

(Cl. *Vaccinio-Piceetea*, Ord. *Piceetalia excelsae*, Al. *Piceion excelsae*) (Tableau 2)

Les phytocenoses de l'association sont dominées par *Picea abies*, espèce qui leur imprime une physionomie uniforme, monotone. Cette monotonie est rarement interrompue par des espèces feuillues telles *Betula pendula*, *Sorbus aucuparia*, *Fagus sylvatica*, *Acer pseudoplatanus*, etc.

Dans la composition floristique, un important cortège d'espèces caractéristique à l'alliance *Piceion excelsae* et l'ordre *Vaccinio-Piceetalia* s'impose mais, vu la répartition de l'association à la limite inférieure de l'étage des épicéas, à l'altitude de 750-1000 m, ces phytocoenoses se caractérisent par la présence massive des espèces caractéristiques à la classe *Querco-Fagetea*. L'analyse des éléments phytogéographiques relève la nette prédominance des espèces euroasiatiques, auxquelles s'ajoutent les éléments circonpolaires et européens (Fig. 2b) et ceux modérément-faiblement acidophiles (Fig. 2a).

Tableau 1 (suite)

Moehringio muscosae-Acerenion													
<i>Acer pseudoplatanus</i> <i>Acer pseudoplatanus</i> (juv.)	-	-	-	-	-	-	-	-	-	-	-	-	I I
	-	-	-	-	-	-	-	-	-	-	-	-	I
Calamagrostio-Fagenion													
<i>Hieracium rotundatum</i> <i>Luzula luzuloides</i>	+	+	+	-	-	+	+	+	+	+	+	+	IV IV
Lathyro-Carpinion													
<i>Glechoma hirsuta</i> <i>Galium schultesii</i> <i>Stellaria holostea</i>	-	-	-	+	-	-	-	-	-	-	+	+	I I I
<i>Alnion incanae</i>													
<i>Senecio nemorensis</i> ssp. <i>fuchsii</i> <i>Circaea intermedia</i> <i>Carex remota</i> <i>Veronica montana</i> <i>Myosotis sylvatica</i> <i>Galeopsis speciosa</i> <i>Impatiens noli-tangere</i> <i>Carex pendula</i> <i>Glechoma hederacea</i> <i>Chrysosplenium alternifolium</i> <i>Festuca gigantea</i> <i>Circaea lutetiana</i> <i>Stellaria nemorum</i>	+	+	+	-	-	+	-	-	-	-	-	-	II I II I IV I II I II I I I I

Tableau 1 (suite)

Tableau 1 (suite)

Quercus-Fagetea							
Lonicera xylosteum	-	-	-	-	-	-	I
Dryopteris filix-mas	+	-	+	+	+	+	V
Athyrium filix-femina	+	+	+	-	+	+	IV
Viola reichenbachiana	-	+	+	-	-	+	II
Fragaria vesca	+	+	-	+	+	-	II
Anemone nemorosa	-	+	-	-	-	-	I
Geum urbanum	-	-	+	-	+	-	I
Poa nemoralis	-	-	-	-	+	+	II
Brachypodium sylvaticum	-	-	-	-	+	+	I
Calamintha clinopodium	-	-	-	-	-	-	I
Vaccinio-Piceetea s. l.							
Abies alba	2	+	2	2	1	3	V
Picea abies	2	1	2	2	3	1	V
Abies alba (juv.)	-	-	-	-	+	-	I
Picea abies (juv.)	-	-	-	-	-	-	I
Streptopus amplexifolius	+	-	-	-	-	-	I
Lonicera caerulea	+	+	-	-	-	-	I
Circaea alpina	+	+	+	+	-	-	III
Lycopodium annotinum	-	+	-	-	-	-	I
Luzula sylvatica	-	2	+	-	+	+	IV
Lonicera nigra	-	-	-	+	-	-	I
Dryopteris dilatata	-	-	-	+	-	+	II
Sorbus aucuparia	-	-	+	+	-	-	II
Sorbus aucuparia (juv.)	-	+	-	-	-	-	I
Homogyne alpina	-	-	-	-	-	-	I
Campanula abietina	-	-	-	-	-	+	I

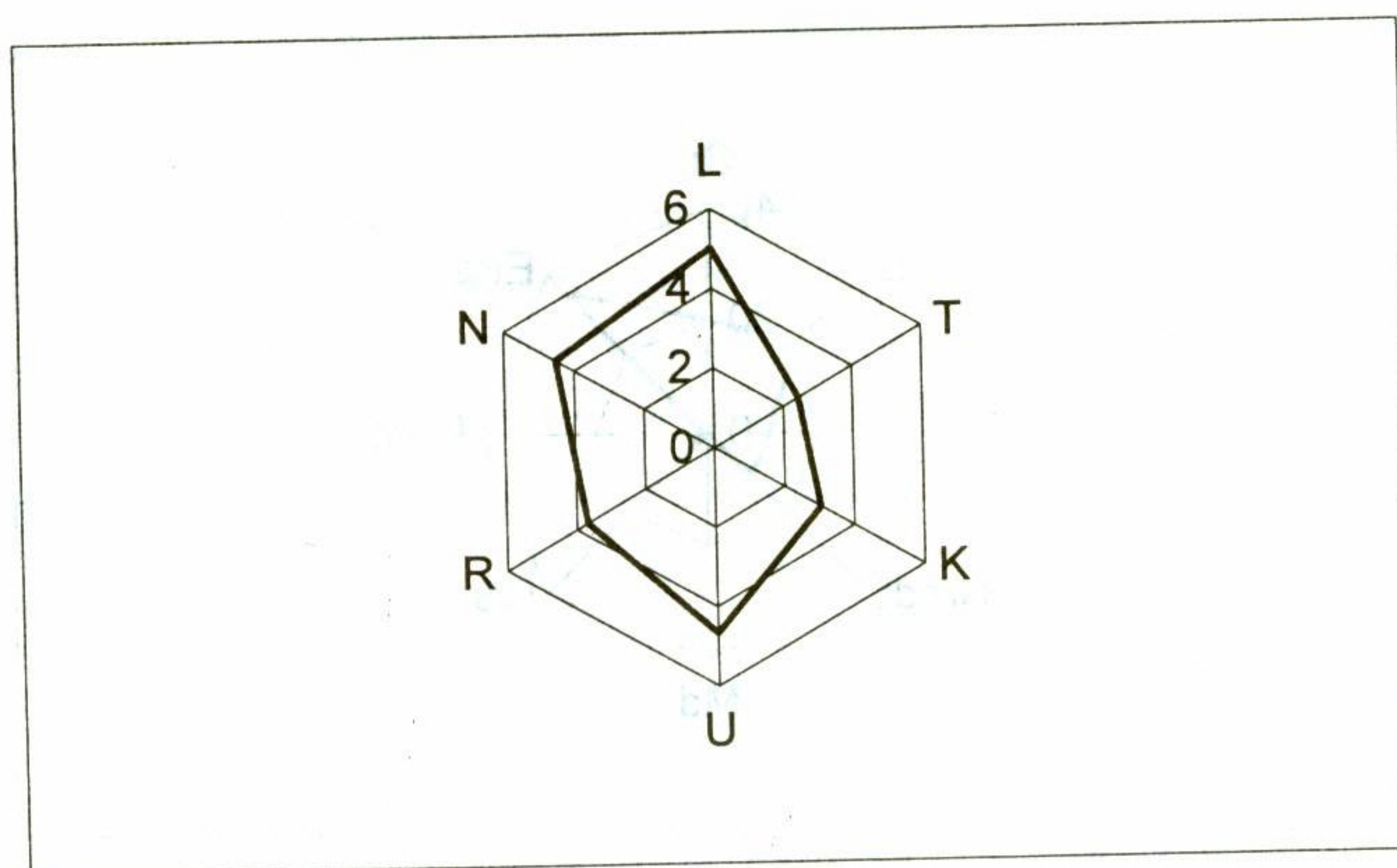
Tableau 2

Cl. Vaccinio-Piceetea Br.-Bl. in Br.-Bl. et al. 1939
Ord. Piceetalia excelsae Pawlowski in Pawlowski et al. 1928
Al. *Piceion excelsae* Pawlowski in Pawlowski et al.1928
As. *Hieracio rotundati-Piceetum* Pawl. et Br.-Bl. 1939

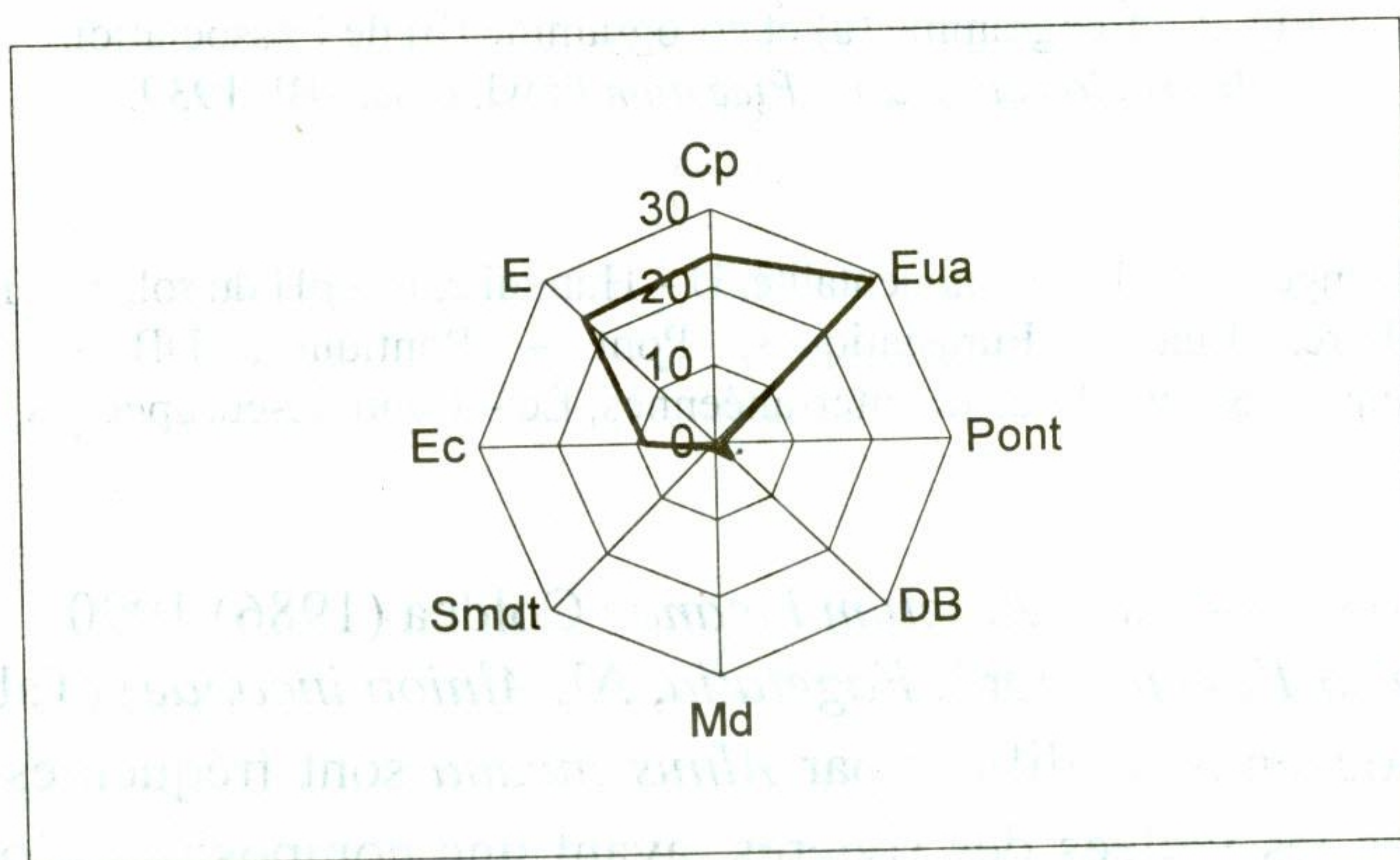
Numéro du relevé	1	2	3	4	5	6	7	8	9	
Altitude (m.s.m.)	1000	1000	1000	1000	750	750	800	800	800	
Surface (m²)	400	400	400	400	400	400	400	400	400	
Exposition	E	N-V	S	V	N	V	N	-	S-E	
Pente (en degrés)	30	15	15	15	15	30-35	5	-	2-3	
Recouvrement de la strate arborescente (%)	85	70	80	80	80	90	95	80	65	K
Recouvrement de la strate arbustive (%) et juv. (%)	-	60	40	10	3-5	5	-	-	30	
Recouvrement de la strate herbacée (%)	10	70	30	50	60	8	3	50	20	
Diamètre des arbres (cm)	30-50	30-35	30-35	40	10-50	10-30	15-30	20-60	60-70	
Hauteur des arbres (m)	30	20	20	22-25	26	20	20-22	27-28	35-37	
Caractéristiques de l'association										
<i>Hieracium rotundatum</i>	+	-	-	+	+	+	-	+	-	III
<i>Piceion excelsae</i>										
<i>Picea abies</i>	4	5	4	5	5	5	5	4	4	V
<i>Picea abies</i> (juv.)	-	3	-	-	-	-	-	-	2	II
<i>Abies alba</i>	+	-	-	-	+	-	-	-	+	II
<i>Abies alba</i> (juv.)	-	-	-	+	-	-	-	-	-	I
<i>Homogyne alpina</i>	-	+	-	-	-	-	-	-	-	I
<i>Lonicera nigra</i>	-	-	-	-	-	+	-	-	-	I
<i>Circaea alpina</i>	+	-	-	-	-	-	+	-	-	II
<i>Luzula sylvatica</i>	+	-	-	-	-	-	-	+	-	II
<i>Calamagrostis villosa</i>	+	-	-	-	-	-	-	-	-	I
<i>Oxalis acetosella</i>	1	-	-	-	+	1	+	2	2	IV
<i>Dryopteris dilatata</i>	-	-	-	-	-	-	-	-	+	I
<i>Lonicera caerulea</i>	+	-	-	-	-	-	-	-	-	I
<i>Luzula luzuloides</i>	+	+	-	+	+	+	+	-	+	IV

Tableau 2 (suite)

Dicrano-Pinion												
<i>Calluna vulgaris</i>	-	+	-	-	-	-	-	-	-	-	-	I
<i>Betula pendula</i>	-	+	-	-	-	1	+	-	-	-	-	II
<i>Calamagrostis varia</i>	-	1	+	+	-	-	-	-	-	-	-	II
<i>Trientalis europaea</i>	-	+	+	+	-	-	-	-	-	-	-	II
Piceetalia excelsae et Vaccinio-Piceetea												
<i>Vaccinium vitis-idaea</i>	-	+	1	+	-	+	-	-	-	-	-	II
<i>Sorbus aucuparia</i>	+	+	-	+	-	-	+	+	-	+	-	IV
<i>Sorbus aucuparia</i> (juv.)	-	3	-	2	+	4	-	-	-	+	+	II
<i>Vaccinium myrtillus</i>	-	+	-	+	-	-	-	-	-	-	-	III
<i>Deschampsia flexuosa</i>	-	+	-	+	-	-	+	+	-	+	-	II
<i>Maianthemum bifolium</i>	+	-	-	-	-	-	-	-	-	-	-	III
<i>Lycopodium annotinum</i>	-	-	-	-	-	-	-	-	-	1	-	I
<i>Athyrium filix-femina</i>	+	-	-	-	-	-	-	-	-	-	+	II
<i>Gentiana asclepiadea</i>	-	-	-	-	-	-	-	-	-	-	+	I
Mulgedio-Aconietea s. l.												
<i>Valeriana montana</i>	-	-	-	-	-	-	-	-	-	-	-	I
<i>Athyrium distentifolium</i>	-	-	-	-	-	-	-	-	-	-	-	I
<i>Rosa pendulina</i>	+	-	-	-	-	-	-	-	-	+	-	II
<i>Polygonatum verticillatum</i>	+	-	-	-	-	-	-	-	-	+	-	II
<i>Stellaria nemorum</i>	-	-	-	-	-	-	-	-	-	+	+	I
<i>Salix silesiaca</i>	-	-	-	-	-	-	-	-	-	-	-	I
<i>Veronica urticifolia</i>	+	-	-	-	-	-	-	-	-	-	-	I
<i>Urtica dioica</i>	-	-	-	-	-	-	-	-	-	+	-	II
Fagetalia sylvaticae s. l.												
<i>Fagus sylvatica</i>	-	-	-	-	-	-	+	-	-	-	-	I
<i>Fagus sylvatica</i> (juv.)	+	-	-	-	-	-	-	-	+	+	+	II
<i>Corylus avellana</i>	+	-	-	-	-	-	+	-	-	-	-	III
<i>Galium schultesii</i>	-	-	-	-	-	-	-	-	-	-	-	I
<i>Acer pseudoplatanus</i>	+	-	-	-	-	-	-	-	-	-	+	I
<i>Acer pseudoplatanus</i> (juv.)	-	+	-	-	-	-	-	-	-	-	-	II
<i>Pteridium aquilinum</i>	-	-	+	+	-	+	-	-	-	-	-	II

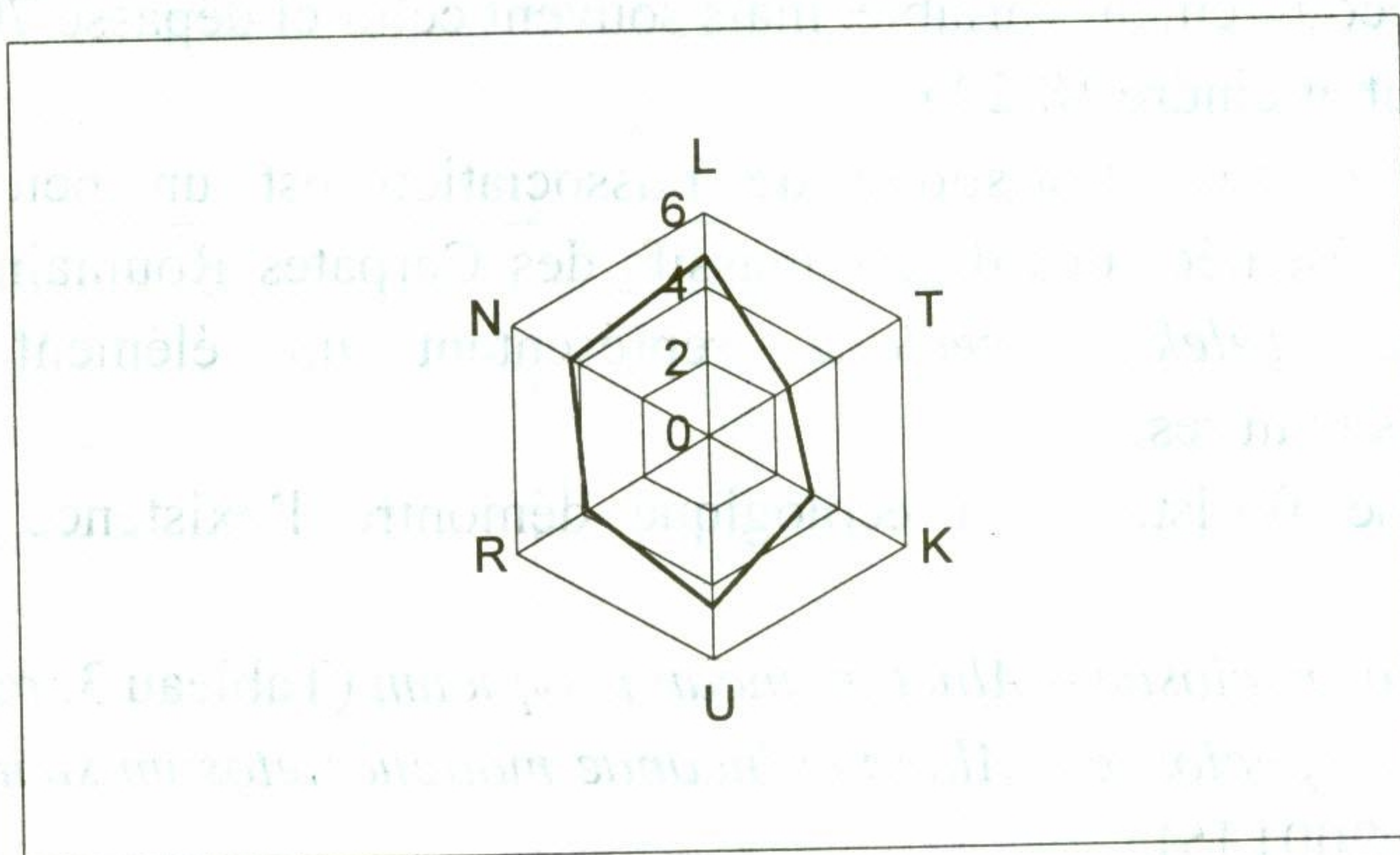


1a

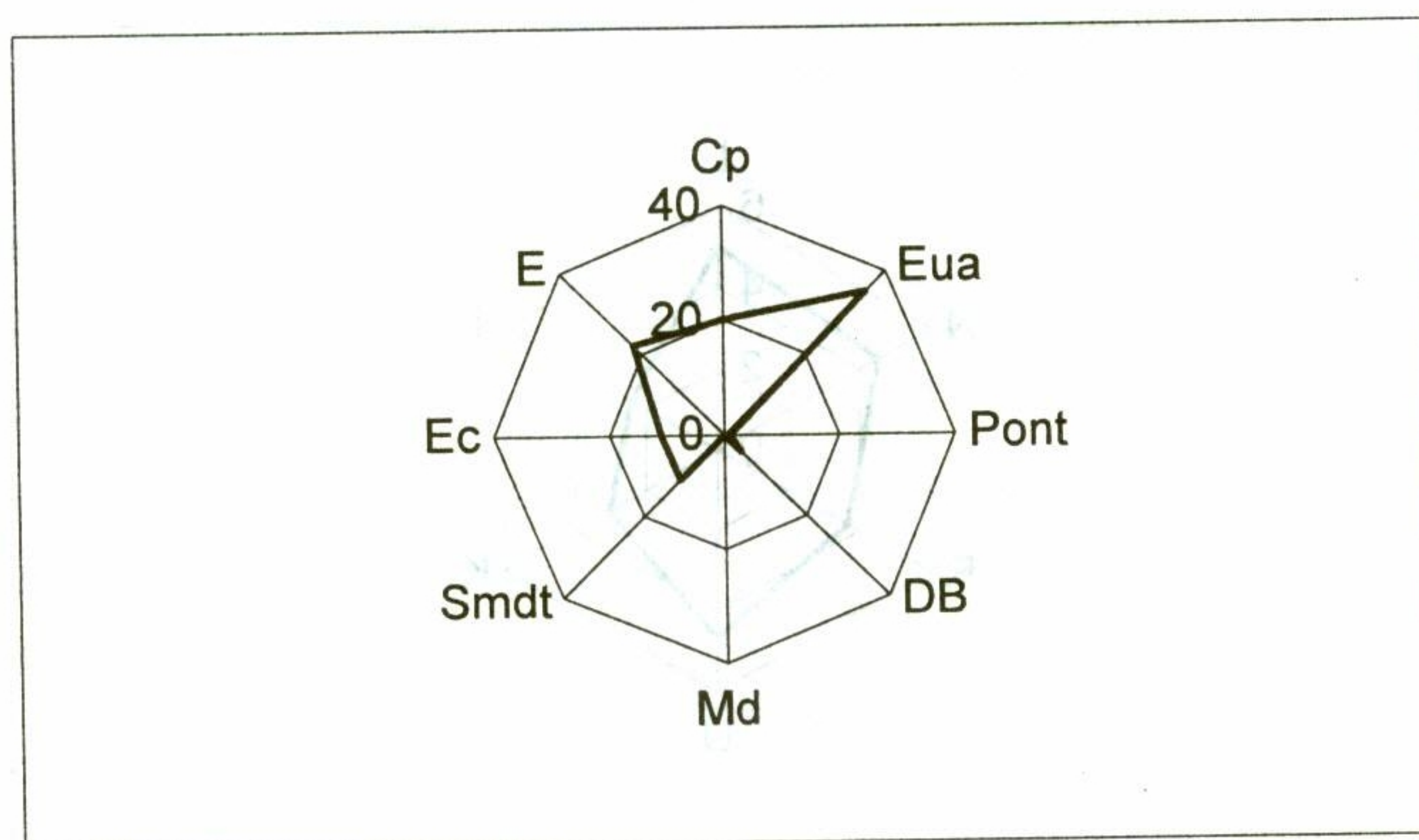


1b

Fig. 1 – Écogramme (a) et corogramme (b) de l'association *Leucanthemo waldsteinii* – *Fagetum* (Soó 1964) Taüber 1987.



2a



2b

Fig. 2 – Écogramme (a) et corogramme (b) de l'association *Hieracio rotundati* – *Piceetum* Pawl. et Br.-Bl. 1939.

Légende:

L – Lumière, T – Température, K – Continentalité, U – Humidité, R – pH du sol, N – Trophicité du sol
 Cp – Circonpolaire, Eua – Eurasiatiques, Pont – Pontiques, DB – Daco-Balkaniques,
 Md – Méditerranéennes, Smdt – Sous méditerranéennes, Ec – Centrale-européennes, E – Européennes

3. *Telekio speciosae* – *Alnetum incanae* Coldea (1986) 1990
 (Cl. *Quercio-Fagetea*, Ord. *Fagetalia*, Al. *Alnion incanae*) (Tableau 3)

Les phytocoenoses édifiées par *Alnus incana* sont fréquentes dans Obcinele Bucovinei, dans les vallées des rivières, ayant une composition floristique variée, mais dans laquelle les espèces caractéristiques à l'alliance *Alnion incanae* prennent une place importante, ayant un caractère fortement mésohygrophile, mis en évidence aussi dans l'écogramme de l'association (Fig. 3a). La strate arborescente présente un recouvrement variable, mais souvent celui-ci dépasse 70% et la hauteur des arbres peut atteindre 18-20 m.

La composition floristique de l'association est un peu différente des descriptions présentées des divers massifs des Carpates Roumaines [6], l'espèce caractéristique, *Telekia speciosa*, représentant un élément constant des phytocoenoses matures.

L'analyse floristique et écologique démontre l'existence de deux sous-associations:

- *Telekio speciosae* – *Alnetum incanae typicum* (Tableau 3, rel. 1)
- *Telekio speciosae* – *Alnetum incanae matteuccietosum struthiopteris* Chifu et Zamfirescu 2001 [5].

Tableau 3

Cl. Querco-Fagetea Br.-Bl. et Vlieg. in Vlieger 1937

Ord. Fagetalia sylvaticae Pawlowski in Pawlowski et al. 1928

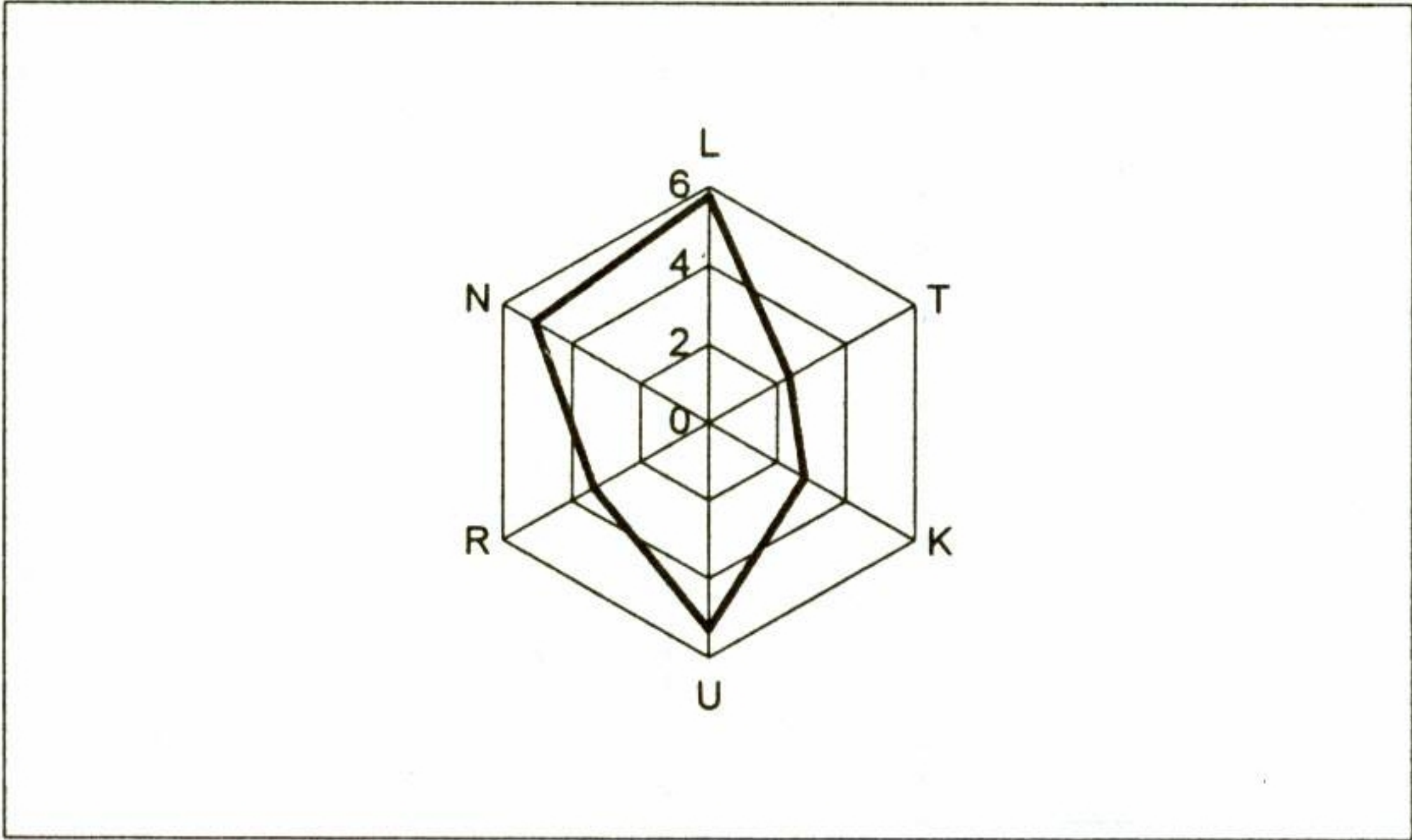
Al. *Alnion incanae* Pawlowski in Pawlowski et Wallisch 1928Subal. *Alnenion glutinoso-incanae* Oberd. 1953As. *Telekio speciosae-Alnetum incanae* Coldea (1986) 1990

Numéro du relevé	1	2	3	4	5	
Altitude (m.s.m.)	700	750	700	750	700	
Surface (m ²)	100	200	200	200	100	
Exposition	-	-	-	-	-	
Pente (en degrés)	-	-	-	-	-	K
Recouvrement de la strate arborescente (%)	95	70	50	70	70	
Recouvrement de la strate arbustive (%)	-	15	-	15	1	
Recouvrement de la strate herbacée (%)	30	30	45	30	70	
Diamètre des arbres (cm)	10	10-35	25	10-35	5-20	
Hauteur des arbres (m)	8	15-20	10-12	15-20	8	
Caractéristiques de l'association						
<i>Telekia speciosa</i>	-	+	+	+	-	III
<i>Alnion incanae</i>						
<i>Alnus incana</i>	5	4	3	4	4	V
<i>Impatiens noli-tangere</i>	-	+	-	+	+	III
<i>Stachys sylvatica</i>	-	1	-	1	-	II
<i>Thalictrum aquilegifolium</i>	-	+	-	+	+	III
<i>Matteuccia struthiopteris</i>	-	2	3	2	2	IV
<i>Geranium phaeum</i>	-	+	-	+	+	III
<i>Stellaria nemorum</i>	-	-	-	-	+	I
<i>Scirpus sylvaticus</i>	-	-	-	-	+	I
<i>Chaerophyllum hirsutum</i>	-	+	+	+	-	III
<i>Cirsium oleraceum</i>	-	+	-	+	+	III
<i>Senecio nemorensis</i> ssp. <i>fuchsii</i>	+	+	-	+	+	III
<i>Glechoma hederacea</i>	-	+	+	+	+	V
<i>Galeopsis speciosa</i>	-	+	-	+	-	II
<i>Equisetum sylvaticum</i>	-	+	-	+	-	II
<i>Aegopodium podagraria</i>	-	+	+	+	+	IV
<i>Angelica sylvestris</i>	-	-	+	-	-	I
<i>Sambucus nigra</i>	+	-	-	-	-	I
<i>Rubus caesius</i>	-	-	-	-	+	I
<i>Symphyto-Fagion</i>						
<i>Aconitum moldavicum</i>	-	+	-	+	-	II
<i>Luzula luzuloides</i>	-	-	+	-	-	I
<i>Fagetalia sylvaticae</i>						
<i>Geranium robertianum</i>	-	+	-	+	-	II
<i>Hordelymus europaeus</i>	-	+	-	+	-	II
<i>Euphorbia amygdaloides</i>	-	+	-	+	+	III
<i>Lamium maculatum</i>	-	-	+	-	+	II
<i>Pulmonaria officinalis</i>	-	-	-	-	+	I
<i>Pulmonaria rubra</i>	-	-	-	-	+	I
<i>Carex sylvatica</i>	-	-	-	-	+	I
<i>Daphne mezereum</i>	-	-	-	-	+	I
<i>Lapsana communis</i>	-	+	-	+	-	II
<i>Ribes uva-crispa</i>	-	+	-	+	+	III
<i>Stellaria holostea</i>	-	-	+	-	-	I
<i>Rubus hirtus</i>	-	-	-	-	+	I

Tableau 3 (suite)

Querco-Fagetea						
Anemone nemorosa	-	+	-	+	+	III
Dryopteris filix mas	-	+	-	+	-	II
Geum urbanum	+	+	-	+	+	IV
Corylus avellana	+	-	-	-	+	II
Vaccinio-Piceetea						
Sorbus aucuparia	-	+	-	+	-	II
Compagnes						
Rubus idaeus	-	2	+	2	+	IV
Mentha longifolia	1	+	-	+	-	III
Salix purpurea	+	-	-	-	-	I
Lysimachia nummularia	+	-	-	-	-	I
Ranunculus repens	1	+	+	+	+	V
Caltha palustris	-	+	+	+	2	IV
Silene alba	-	+	-	+	-	II
Trollius europaeus	-	-	+	-	-	I
Carduus crispus	-	-	+	-	-	I
Astrantia major	-	-	+	-	-	I
Cirsium waldsteinii	-	+	+	-	-	II
Aconitum paniculatum	-	-	+	-	-	I
Torilis arvensis	-	-	+	-	-	I
Ajuga reptans	+	-	-	-	-	I
Achillea millefolium	+	-	-	-	-	I
Taraxacum officinale	+	-	-	-	-	I
Trifolium repens	1	-	-	-	-	I
Agrostis stolonifera	+	-	-	-	+	II
Rumex obtusifolius	+	-	-	-	-	I
Lolium perenne	+	-	-	-	-	I
Lotus corniculatus	+	-	-	-	-	I
Prunella vulgaris	+	-	-	-	+	II
Bellis perennis	+	-	-	-	-	I
Lamium purpureum	+	-	-	-	-	I
Plantago media	+	-	-	-	-	I
Spiraea chamaedrifolia	-	-	-	-	+	I
Galium verum	-	-	-	-	+	I
Filipendula ulmaria	-	-	-	-	+	I
Chaerophyllum aureum	-	-	-	-	+	I
Aconitum calybotrion	-	-	-	-	+	I
Fragaria vesca	-	-	-	-	+	I
Hypericum maculatum	-	-	-	-	+	I
Equisetum arvense	-	-	-	-	+	I

Provenance des relevés: 1, 5 – Dragoș; 2, 4 – Lunguleț; 3 – Deia



3a

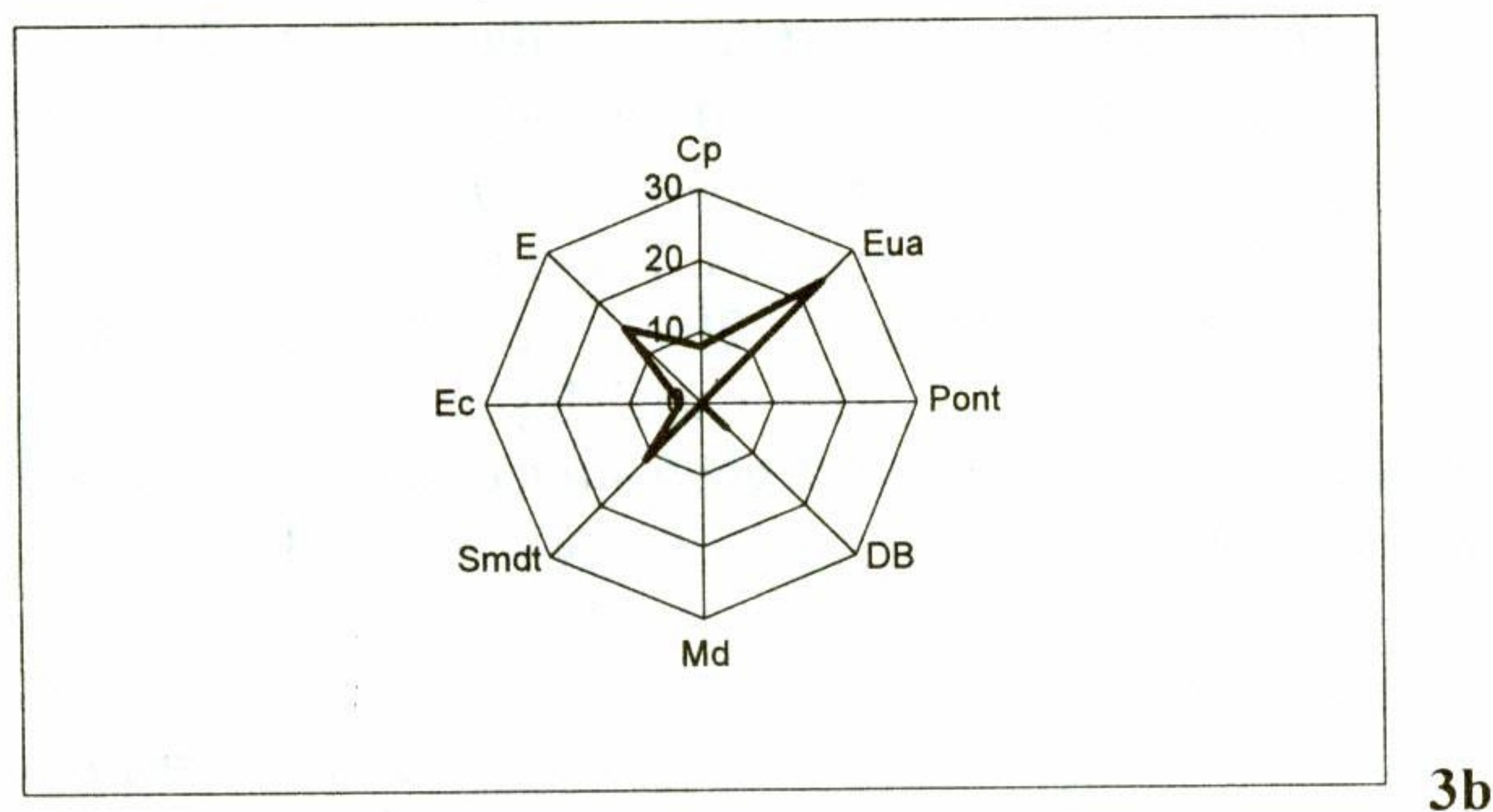


Fig. 3 – Écogramme (a) et corogramme (b) de l'association *Telekio speciosae* – *Alnetum incanae* Coldea (1986) 1990.

Légende:

L – Lumière, T – Température, K – Continentalité, U – Humidité, R – pH du sol, N – Trophicité du sol
Cp – Circonpolaire, Eua – Eurasiatiques, Pont – Pontiques, DB – Daco-Balkaniques, Md – Méditerranéennes, Smdt – Sous méditerranéennes, Ec – Centrale-européennes, E – Européennes

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VIABILITY AND SURVIVAL HALOPHILIC SPECIES *BASIPETOSPORA HALOPHILA* IN NATURAL AND ARTIFICIAL MEDIA

IOANA GOMOIU*, MARIUS ATAMAN**

Basipetospora halophila is the only fungal species isolated from Grota Miresei Lake and organic matters from its shore and can survive 12 months still having germination potential both on them and in an oligotrophic medium. Germination process in water from Grota Miresei Lake and suspensions from mixed feathers, leaves, root plants and grassy plants shows that the higher ratio was obtained on the feather suspension and the lowest in the water from Grota Miresei Lake. Low germination ratio and fungal contents in Grota Miresei Lake but high on the feathers and leaves demonstrate that the colonization process is started by hyphae and spores provided from water or from salt crystals. *Basipetospora halophila* is involved in the degradation of plant and animal debris from the shore improving the organic content of hypersaline lake. *Basipetospora halophila* together with halophilic bacteria are the main decomposers in this ecosystem and very important in biogeochemical cycles.

Key words: fungal contents, germination, hypha and spores, water, halophilic, hypersaline lake, ecosystem.

INTRODUCTION

Microorganisms can live in saline terrestrial or aquatic natural environments instead of the fact that they find a double stress: increased electrolyte concentration and lower water potential. They have own strategy meaning both excluding the ions from the cytoplasm and an alternative osmotic to assure a normal cell volume, spore germination and growth process (1, 3, 9).

Yeast and fungi, which are living in saline environments, are true halophilic or adapted microorganisms (2, 8, and 10).

The main factors of selection in saline environments are salts, temperature and pH. Type of salts and their concentration maintain only halophilic fungi and exclude those which cannot adapt. Geographical distribution of fungi is under control first of all by temperature and secondary by salt concentration. *Wallemia sebi*, which can degrade salted fish, is exceptionally isolated from the tropical area because it needs 10–15 °C for growth.

Basipetospora halophila, *Exophiala werneckii* and *Polypaecium pisce* have been isolated only from salted or dried fish having spoilage potential but not from the hypersaline lakes (10). We have isolated a moderate strain of *Oospora* from Techirghiol Lake (5–7) and two halophilic strains from Grota Miresei Lake (Prahova area). The paper contains proofs that a hypersaline lake as Grota Miresei and organic substrates from it are natural environments for *Basipetospora*

halophila. Also this fungus can survive in laboratory on nutritive media containing the same salts like in the lake.

MATERIAL AND METHODS

Some of researches have been done on the following types of samples:

- Feathers from the shore of the Grota Miresei Lake brought by wind;
- Leaves from the shore of the Grota Miresei Lake brought by wind;
- Root plants from the shore of the Grota Miresei Lake brought by wind;
- Grassy plants from the shore of the Grota Miresei Lake brought by wind.

All of them had salt crystals on the surface or were covered by a layer of 1–2 mm salt. Samples were kept at +4 °C during deliver, then in the lab, crystals or salt layer were taken off and have been worked by dilution series. Using the same methods samples kept at +4 °C for 12 and 18 months were analyzed.

Other researches have been done on a culture of *Basipetospora halophila* after conservation for 12 and 18 months on nutritive media (yeast extract, peptone, glucose and 190 g/l salt from Grota Miresei).

In addition, feathers, leaves, root plants and grassy plants were used to assay ergosterol content of their mycelium (4). Ergosterol was extracted with methanol, saponified by refluxing in methanol/ethanol/potassium hydroxide. Non-saponifiable lipids were extracted with pentane, transferred to methylene chloride/isopropanol, filtered, dried under nitrogen. Finally, it was taken up in methanol/methanol chloride and injected into a high-performance liquid-chromatograph system. Ergosterol content (at 282 nm) means two replicates.

Germination process was assayed by inoculation of suspensions from mixed feathers, leaves, root and grassy plants into water from Grota Miresei Lake with spores and counting at Thoma chamber.

Colonization of natural substrates such as: feathers, leaves, roots and grassy plants has been done by spreading *Basipetospora halophila* spores and incubation at 28 °C. It was appreciated as ratio after 3, 6, 12 months.

RESULTS AND DISCUSSIONS

Basipetospora halophila is the only fungal species isolated from Grota Miresei Lake and organic matters from its shore. The type of substrate does not matter but this fungus can survive 12 months still having germination potential both in them and into an oligotrophic medium (Table 1).

Organic substrates are very difficult to decompose because of their content of lignin, cellulose and chitin. In the lake and the other surfaces, the germination

process is initiated by the addition of chemical compounds but maintaining oligotrophic conditions.

Table 1

Basipetospora halophila survival in natural environment

Type of sample	Survival (%)		
	Initial	12 months	14 months
Feathers	100	98	97
Leaves	100	98	98
Root plants	100	92	90
Grassy plants	100	96	94

Ergosterol content of natural substrates (feather, leaves, roots and grassy plants) confirms results obtained in survival experiments. All samples have contained ergosterol from the time of collecting till the end of experiment (Table 2). Very thick wall of spores give resistance to survive for a long time in dried conditions. Germination and growth processes at very low available water suggest that intracellular pressure is higher than at other fungi as a result of accumulation of some compounds which balances those outside. The same results were obtained by Alder (1), Tanaka (8) and Tohuoka (9).

Table 2

Ergosterol content of samples from Grota Miresei Lake

Type of sample	Ergosterol content ($\mu\text{g}\cdot\text{g}^{-1}$)		
	Initial	12 months	14 months
Feathers	2.2	2.1	2.1
Leaves	2.0	2.1	2.1
Root plants	2.4	2.2	2.2
Grassy plants	2.5	2.4	2.4

Results obtained in case of strains from culture collection (after 12–14 months of conservation) suggest the same idea. Although the tube collection is covered by a paraffin layer, water was evaporated and salts crystallized. Spores survived but when they found optimal conditions, germination started (Table 3).

Table 3

Basipetospora halophila survival in artificial nutritive medium

Strain	YPG medium		
	Initial	12 months	14 months
O ₁	100	98	96
O ₂	100	97	94
O ₃	100	98	93

O₁ = *Basipetospora halophila* isolated from Techirghiol Lake

O₂ = *Basipetospora halophila* isolated from Grota Miresei Lake

O₃ = *Basipetospora halophila* isolated from mud of Baia Baciului Lake

To confirm these results it was tested the germination process in water from Grota Miresei Lake and suspensions from mixed feather, leaves, root plants and grassy plants (Table 4). The higher ratio of germination meaning 100% was obtained on the feather suspension and the lowest in water meaning 82%, from Grota Miresei Lake.

Table 4

Spores germination of *Basipetospora halophila* on different substrates

Strain	Substrates			
	Water from lake	Feathers	Leaves	Grassy plants
O ₁	65	100	82	85
O ₂	72	100	91	95
O ₃	82	100	79	85

O₁ = *Basipetospora halophila* isolated from Techirghiol Lake

O₂ = *Basipetospora halophila* isolated from Grota Miresei Lake

O₃ = *Basipetospora halophila* isolated from mud of Baia Baciului Lake

Basipetospora halophila was isolated by Ander and Pitt in 1987 (2) from dried salt fish and by Gomoiu in 1999 (5) from Techirghiol Lake as halophilic and halotolerant fungus. Strains isolated from Grota Miresei Lake belong to halophilic fungi. They are resistant to low temperature during the winter and on substrates maintained at +4 °C.

Colonization of natural substrates by *Basipetospora halophila* was also demonstrated. Feathers and leaves sterilized and inoculated with spores were covered with mycelium ¼ after 3 months of incubation at 26 °C (Table 5) and ½ after 12 months. Hardly are colonized root and grassy plants: only ¼ from surface were covered with mycelium in 12 months.

Table 5

Colonization of natural substrates by *Basipetospora halophila*

Type of sample	% surface colonized after		
	3 months	6 months	12 months
Feathers	< 1/4 from surface	< 1/4 from surface	< 1/2 from surface
Leaves	< 1/4 from surface	< 1/4 from surface	< 1/4 from surface
Root plants	Not visible at the microscope	< 1/4 from surface	< 1/4 from surface
Grassy plants	Not visible at the microscope	< 1/4 from surface	< 1/4 from surface

Table 6

Ergosterol content of different substrates inoculated with *Basipetospora halophila*

Substrate	Time after inoculation			
	Initial	3 months	6 months	12 months
Water from G.M. Lake	0	0.8	1.0	1.0
Feathers	0	1.2	1.7	2.0
Leaves	0	1.6	1.9	2.2
Root plants	0	1.0	1.6	2.0
Grassy plants	0	1.3	1.8	1.9

Ergosterol content of all substrates colonized reveals that fungi are grown (Table 6). On the microscope both mycelium and spores were noticed. Low ergosterol content was registered in water where the germination process was also very down.

Low germination ratio and fungal contents in Grota Miresei Lake but high on the feathers and leaves demonstrate that the colonization process is started by hyphae and spores provided from water. *Basipetospora halophila* is involved in degradation of plant and animal debries from the shore improving organic content of hypersaline lake. *Basipetospora halophila* together with halophilic bacteria are the main decomposers in this ecosystem and very important in biogeochemical cycles.

CONCLUSIONS

1. *Basipetospora halophila* can survive as spores in natural and artificial environments.
2. Salts crystals from Grota Miresei Lake shore allow conservation for at least I year of *Basipetospora halophila* spores.
3. Natural substrates such as feathers, leaves, roots and grassy plants can be colonized by *Basipetospora halophila* spores.
4. *Basipetospora halophila* isolated from Grota Miresei Lake is a true halophilic fungus.

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MYCELIAL FORAGING STRATEGIES AND FRACTAL BEHAVIOR OF MYCELIA IN THE LITTER OF *QUERCUS CERRIS*

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Samples of *Quercus cerris* litter were incubated in damp chambers using cover slips and mica chips to mimic natural spatial discontinuities in litter. Foraging strategies and fractal behavior of explorative mycelia were investigated by image analysis of camera lucida drawings. Fractal geometry of mycelia was determined by box-counting method of black/white digitized, masked image. Results show that in such natural substrata as litter, foraging mycelia of micromycetes display a mixture of fractal and non-fractal behavior of multipolar and directional mycelia assigned to one or several fungal species. Branching in foraging mycelial systems serves to metabolite reallocation, positional information, lateral consolidation of the mycelial exploratory front and a foretic, facilitation function.

Key words: litter, foraging mycelia, fractal behavior, facilitation.

INTRODUCTION

During the past decades, the growing interest in mycelial growth models stimulated various approaches designed to explain the fungal strategies in substrate colonization and exploitation. Several models were proposed using fractal geometry (5, 8, 15, 19), cellular automata theory (9), foraging theory (4, 5) and deterministic models (13).

Filamentous fungi are modular iterative, indeterminate organisms (1, 2). Modularity is a frequent growing strategy in many organisms as plants, sessile animals, fungi that permit the parts separation without compromising the vitality and viability of the whole organism (10, 14), the module being a repeating unit of form. It is a prerequisite condition for a successful foraging in the case of passive organisms. In fungi, such a strategy permits the absorption, translocation and resource capture in a discontinuous environment (17). Mycelia are also nonlinear, hydrodynamic systems and assimilative, conservative, exploitative and redistributed structures (18).

Essentially, modular organisms (plants and fungi) exhibit extensive branching in nutrient rich substrata and poor branching in depleted substrata.

Fractals are used in the measurement of the complexity of an object and describe the inherent regularities through power laws. Natural pattern, especially natural objects frequently appear irregular, complex and hard to measure, even at very small scales. Fractals are geometric objects, which exhibit scale-invariance

that leads to a class of scaling rules – power laws – characterized by scaling exponents. The fractal dimension D represents a measure of heterogeneity and branching of the mycelia in the case of fungi (19). Fractal dimension is both a quantitative and qualitative parameter that estimates the evolution of a system by estimating a graphic dimension, namely the geometry of a system. Fractals apply to structures that are self-similar, that is repeated over magnification ad infinitum. Under different magnification, such natural objects as mycelia are not identical and because similarity is scale dependent, they are considered only statistically self-similar (12). Complexity provides a greater possibility for adaptation to dynamic environment (19). When assigning a fractal dimension to complex objects it is assumed that a power law relationship exists between parameters used to measure the structure. A complementary explanation for fractal behavior of mycelia gives the foraging strategy theory that states: the resource capture is foraging between resource poor and resource rich substrata which modifies the modular model of the foraging blocks. Fractal growth optimizes nutrient capture optimizing the balance between exploratory and exploitative modes of growth.

The present work is a preliminary study concerned with growing strategies of litter fungi in resource depleted areas of *Quercus cerris* litter using light microscopy and fractal analysis of the obtained images. The litter is a heterogeneous, discontinuous and exposed to disturbance habitat. Disturbances that affect the entire fragment may occur more frequently on smaller fragments (11) The driven experiments were designed to mimic the natural discontinuities in litter that represent major disturbances in such a fragmented habitat.

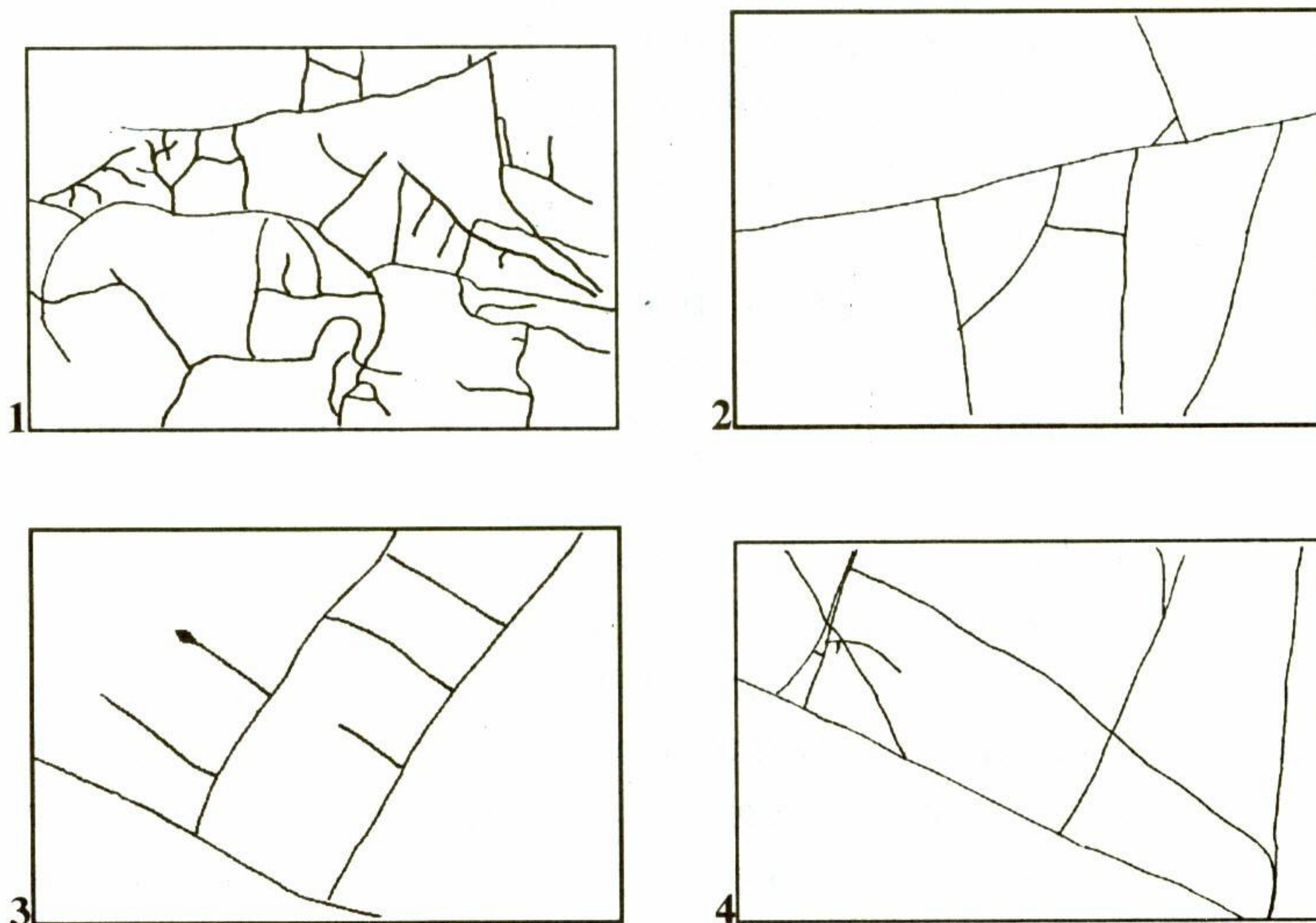


Fig. 1

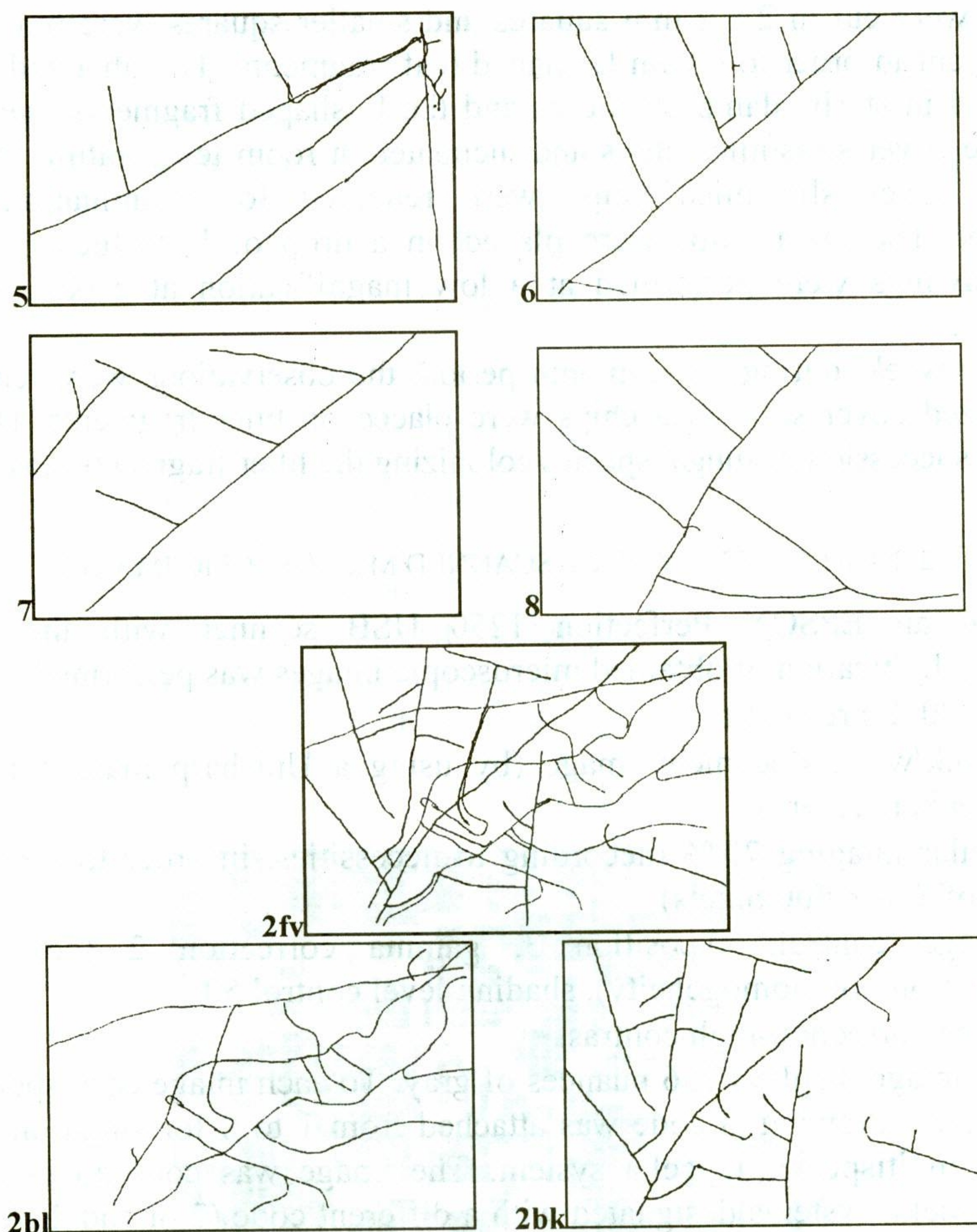


Fig. 1 – Scanned images of *Q. cerris* litter foraging mycelia on mica chips and identification by alphanumeric code (256 color palette).

MATERIAL AND METHODS

1. COLLECTION OF LITTER SAMPLES AND LABORATORY PROCESSING

Litter samples of *Q. cerris*, first decomposition layer, were collected in a mixed broad-leaved forest at Băile Felix, near Oradea, Bihor County, in northwestern Romania, in March 1998. The mixed broadleaved forest vegetates in a mesic habitat; the soil type belongs to brown forest soils, poorly drained.

The leaves were repeatedly washed under tap water. Eight fragments from central area were excised and surface sterilized with peroxide 3 %. These

fragments were cut in 2×2 mm squares and smaller squares were removed from each fragment in order to obtain U shaped leaf fragments. The obtained fragments were placed in sterile damp chambers and the U shaped fragments were covered with sterile cover slips/mica chips and incubated at room temperature. After three days, the cover slips/mica chips were removed for examination in light microscopy. The mica chips were placed on a drop of lacto-fuch sine. Camera Lucida drawings were performed at a low magnification at a Rathenow light microscope.

Every week, during a two-month period, the observations were repeated and new sterilized cover slips/mica chips were placed on litter fragments. During this period, the succession of fungal species colonizing the litter fragments was recorded.

2. DIGITAL ANALYSIS OF SCANNED MICROSCOPIC IMAGES

Using an EPSON Perfection 1250 USB scanner with the following parameters, digitization of obtained microscopic images was performed:

- A 200 dpi resolution
- Black/white document image (by using a Unisharp mask for activated image enhancement)
- Scalar imaging 70 % (according to necessities, in order to obtain a final image of 800×600 pixels)
- Image control: exposition 3, gamma correction 2 (correction for illumination non-homogeneity), shading level control 50
- Tone correction-high contrast

Final image displays 256 nuances of gray. To each image corresponding to a mycelia system drawing, a code was attached from 1 to 8 for single mycelia and 2 fw for a multispecies mycelia system. The image was consequently split for separate mycelia systems designated with a different code (2 bl and 2 bk). A post-digitizing processing operation included processing different color palettes in order to obtain a final image in 16 different colors (Fig. 2).

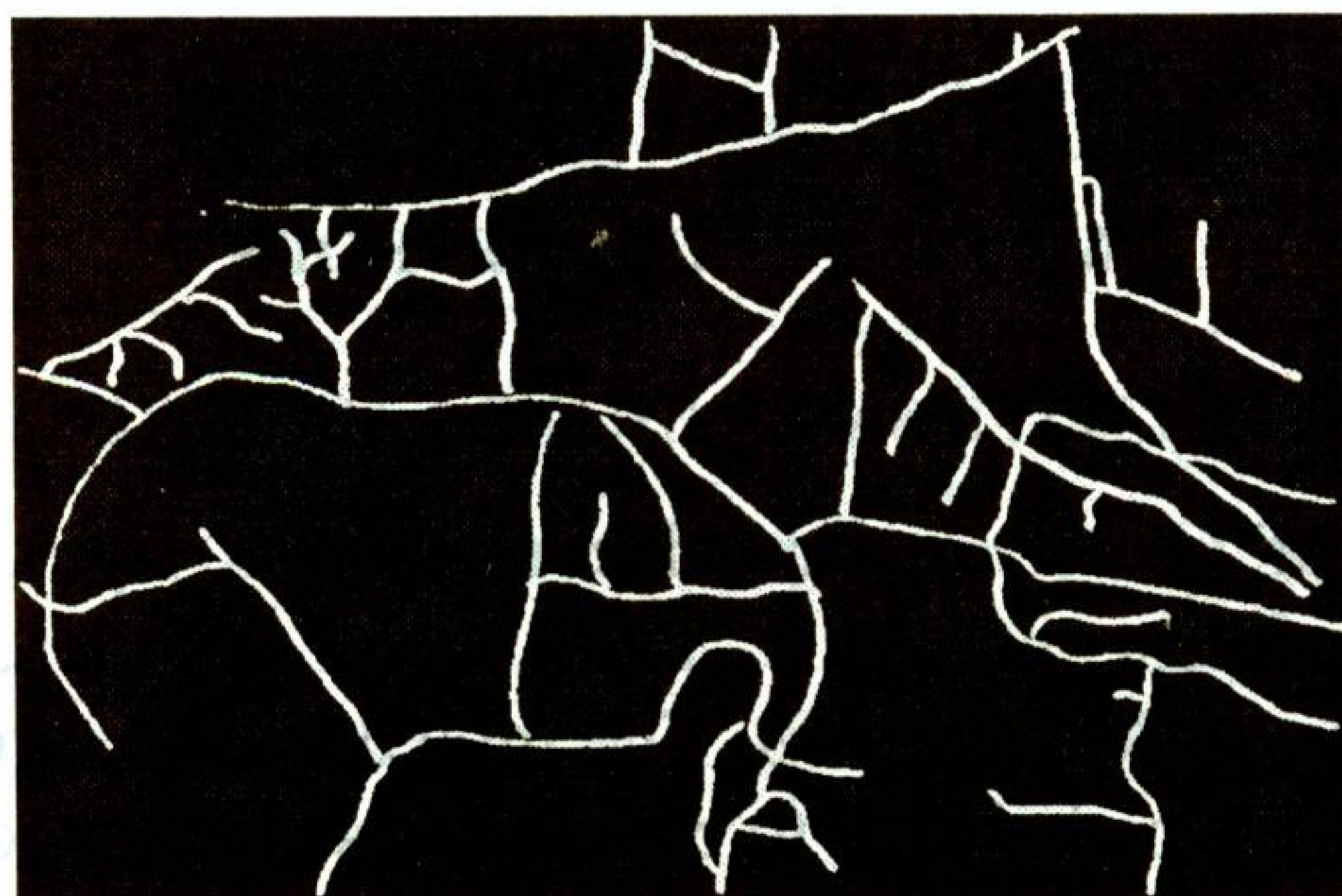


Fig. 2 – Example of scanned and post-processed (16 color palette) mycelia, developed on mica chip.

In choosing image background, black is recommended to be avoided. The remaining colors are also recommended to be different of white. The choice of the foreground is recommended to be light, almost white. The term color discussed in the context of image analysis refers to Boolean color designating an arbitrarily chosen color for image description.

3. FRACTAL ANALYSIS-BOX-COUNTING METHOD

Fractal analysis was performed on drawings of developing fungal structures by the box-plot method (12) using Fractal Vision soft (16) determined for masked image. The method determines the fractal dimension of black/white digitized image covering a fractal with boxes then evaluating how many boxes are needed to cover the fractal completely. Repeating this measurement with different sizes of boxes results into log function of box size (on x-axis) and number of boxes needed to cover the fractal (on y-axis). A rectangular grid is superimposed on the digital image of the mycelia with different dimensions of the unit box ($r = 2, 4, 6$ pixels). The counting of boxes that contain at least a pixel of a hypha is considered as function of N (Fig. 3).

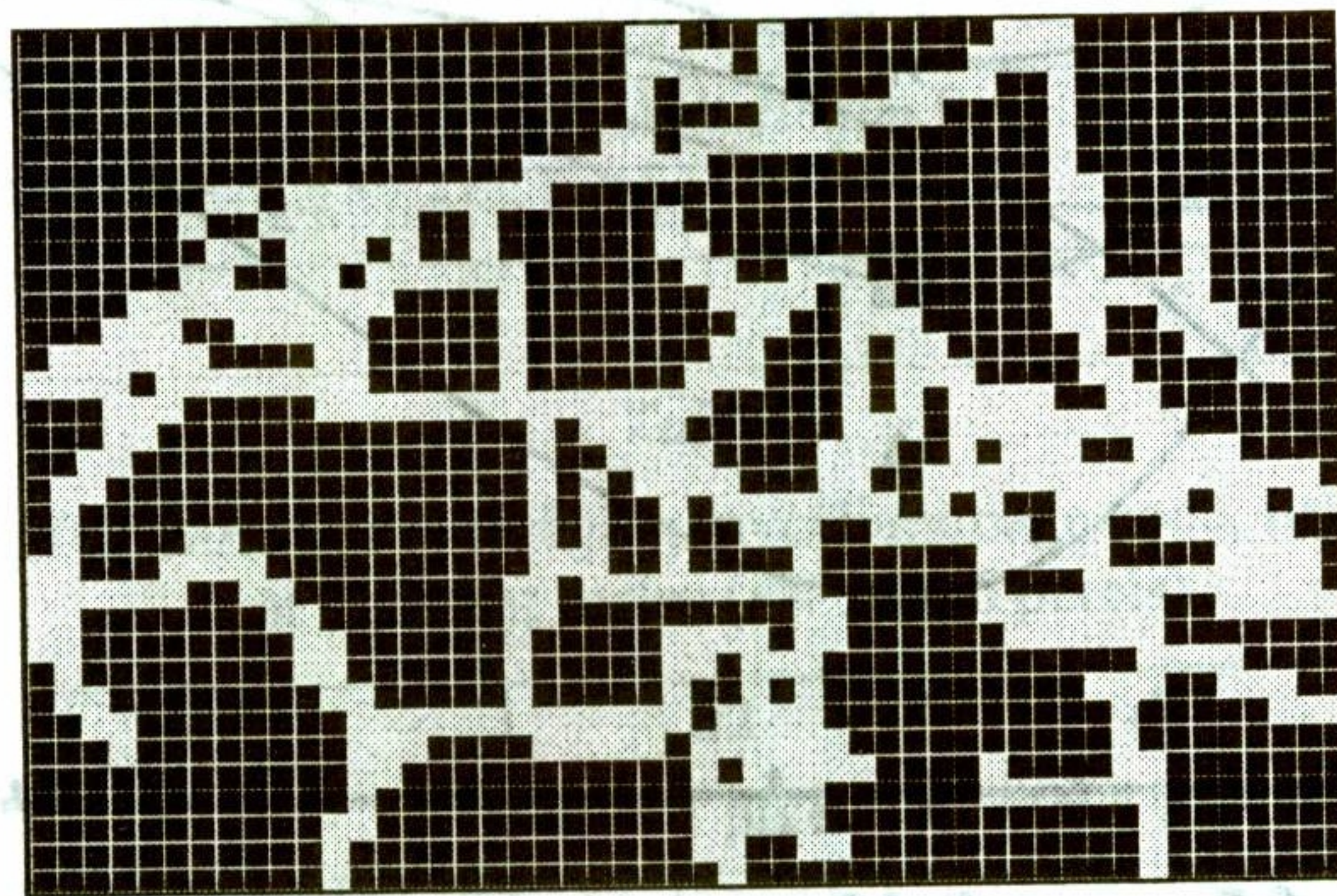


Fig. 3 – Example of a superimposed grid on mycelia in box-counting method protocol.

Fractal dimension represents the slope of a regression line of $\log n(r)$ on $\log(1/r)$ for at least 2 values of r . The slope of the function is an appropriate approximation of fractal dimension (6). The considered power law was:

$$N(r) = C \cdot r^{-Df}$$

where r is number of boxes of size $1/r$, Df is the fractal dimension, C is a proportionality constant, $N(r)$ is the number of boxes which include the image.

Box-counting method implies to consider many values for box dimension in order to obtain a great precision. A supplementary analysis is performed to display graphically the functional dependence $\log(Df) = f(\log 1/r)$ for each digitized image

and for the considered r values (2, 4, 6 pixels). The normal situation is encountered when all three dependence points are placed on a line, which is parallel to the abscissa, meaning a small slope. The slope is determined by linear regression 9 (Fig. 4, Table 3).

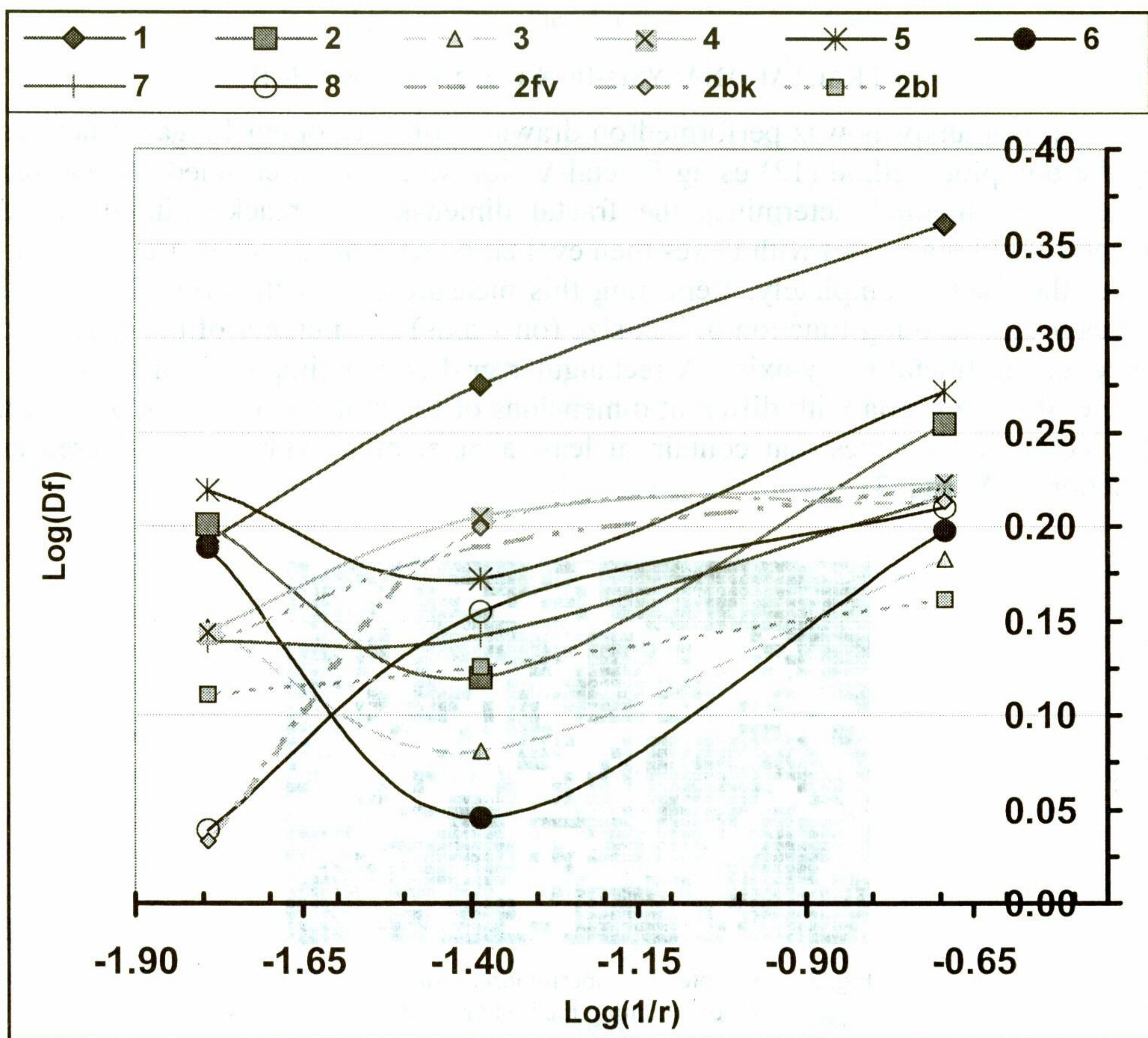


Fig. 4 – Supplementary analysis of fractals-graphical representation of the results.

A correlation coefficient of the relationship is also calculated. If the value of the slope is small, close to zero and the correlation coefficient is high, close to 1, then fractal dimension of the image is to be considered and the object shows fractal geometry. A correlation coefficient, greater than 0.995, shows that data are consistent with power law (19). If the value of the regression slope is high and the correlation coefficient is low, it is possible that the studied object is not fractal, but Euclidean (of finite dimension).

A total number of 11 images were selected for fractal analysis, corresponding to different moments of cover slips' colonization. Too early stages of colonization and too exposed substrata were excluded because of scarce or abundant and overlapping mycelia.

Table 1

Identified species of micromycetes in *Quercus cerris* litter incubated in damp chambers (Băile Felix forest, March 1998)

<i>Alternaria alternata</i> (Fr.) Keissler
<i>Arthobotrys robusta</i> Duddington
<i>Cladosporium cladosporioides</i> (Fre.) de Vries
<i>Epicoccum nigrum</i> . Link.
<i>Fusarium oxysporum</i> Schleht.
<i>Harzia acremonioides</i> (Harz.) Gost.
<i>Helicosporium roseus</i> (Bon.) Saccardo
<i>Microthyrium illicinum</i> De Not.
<i>Monacrosporium</i> sp.
<i>Monochaetia dimorphospora</i> Yokohama
<i>Myrothecium rorida</i> Tode ex Fr.
<i>Polyscitalum foecundissimum</i> Riess
Unidentified mycelia

Table 2

Fractal dimension of *Q. cerris* litter foraging mycelia: image code, final and partial D values at different scales ($r = 2, 4, 6$ pixels)

Image code	<i>D_f</i>	<i>d₂</i> ($r = 2$ pixels)	<i>d₄</i> ($r = 4$ pixels)	<i>d₆</i> ($r = 6$ pixels)
1	1.320682	1.433331	1.316889	1.211828
2	1.213057	1.290143	1.126925	1.222103
3	1.147666	1.200622	1.084726	1.157650
4	1.210603	1.249822	1.227470	1.154517
5	1.248260	1.312650	1.187794	1.244335
6	1.157511	1.218566	1.046162	1.207804
7	1.180973	1.240333	1.153178	1.149407
8	1.146692	1.233416	1.166908	1.039751
2fv	1.201168	1.247760	1.208191	1.147552
2bk	1.163600	1.237313	1.219947	1.033539
2bl	1.141768	1.174798	1.133471	1.117035

Table 3

Supplementary analysis of fractals. In gray, excluded curves due to low correlation coefficient

Code hypha	r (pixels)	2	4	6	Slope $\text{Log}(Df)=f[\text{Log}(1/r)]$	R
	$\log(1/r)$	-0.69315	-1.38629	-1.79176		
1	Log(Df)	0.36000	0.27527	0.19213	0.1495	0.9896
2		0.25475	0.11949	0.20057	0.3268	0.5306
3		0.18284	0.08133	0.14639	0.7546	0.4902
4		0.22300	0.20496	0.14368	0.0464	0.8983
5		0.27205	0.17210	0.21860	0.3079	0.6547
6		0.19767	0.04513	0.18880	1.4926	0.2005
7		0.21538	0.14252	0.13925	0.2573	0.9442
8		0.20979	0.15436	0.03898	1.5672	0.9394
2fv		0.22135	0.18912	0.13763	0.4835	0.9605
2bk		0.21294	0.19881	0.03299	2.2488	0.8237
2bl		0.16110	0.12528	0.11068	0.1998	0.9961

RESULTS

The incubation in damp chambers stimulates the development of fungal colonies in litter fragments as well as their sporulation. Cover slips/mica chips mimic the natural discontinuities that make the litter a heterogeneous, anisotropic substrate. Table 1 presents the list of the recorded species of micromycetes sporulating on incubated litter fragments.

During a two-month period, changes in community composition were recorded. The dominating species as *Arthobotrys robustus* with *Helicosporium roseum*, known as saprotrophic species that can switch to predatorism on small invertebrates, mostly nematodes, are characteristic for early successional stages of decomposition. *Polyscytalum foecundissimum* together with *Monacrosporium* are dominating in a short sequence of succession. More restricted and ephemeral growths were recorded in the case of *Fusarium oxysporum* and *Cladosporium cladosporioides*, known as cosmopolitan saprotrophic species developing on ephemeral resources. *Aureobasidium pullulans* which formed satellite microcolonies as is the case of *Cladosporium cladosporioides* is also an oligotrophic species capable to scavenge in extreme habitats (Fig. 2: B.)

Mycelial growths that occurred on exposed cover slips were assigned to four morphological types. Only in few cases, the mycelia could be identified at species level, mostly in the presence of sporulating structures. These areas were confined to cover slips' edges.

1. Hyaline or pigmented unidentified mycelia that cross the disruption area represented by cover slips, with rapid growths and extension of the main axis by means of apical domination. Rare branching at right angles, distance between two consecutive branches of more than 50 μm .

Frequent scalariform anastomoses, also frequent hyphal association in bundles of 2 or 3 hyphae (Fig. 1: A. B).

2. Mycelia of a restricted growth pattern, confined to the edges of the cover slip. Frequently they associate with first type hyphae that gain a phoretic property.
3. Mycelia of a restricted growth pattern that coil around the first type hyphae on short distances. A rapid sporulation occurs after a restricted mycelial growth with scarce branching (Fig. 2: A).
4. Multipolar mycelial growth pattern, restricted, initiating a rapid sporulation. The inocula were carried by abundantly developing nematods, during the incubation period. Rare cases with phoretic hyphal behaviour.

Fig. 1 presents the digitized images of mycelial systems and Table 2 the Df values obtained for analyzed images.

These values vary between a minimum of 1.1417 and a maximum of 1.3206, considerably low Df corresponding either to initial phases of mycelial growth, or to explorative mycelia. Mycelial systems of image 6 and 3 are not fractal as low values of correlation coefficients show, meaning that these objects are described by Euclidean geometry. The graphical dependence of $\log Df$ (log of fractal dimension) and $\log (1/r)$ meaning box dimension is displayed in Fig. 2. Non fractal objects 3 and 6 display a particularly curved regression line, stating once again for non-fractal nature of analyzed mycelia.

DISCUSSION

As foraging theory states, foraging strategies support the maximisation of the net production (biomass) (Conivaux, 1982). Foraging in fungal mycelia permits the reallocation of mycelial biomass in a patchy environment as litter or soil. Particularly effective foragers able to cover large areas in the forest floor are basidiomycetous mycelia of cord and rhizomorph forming fungi (Boddy, 1993). Most of pioneer micromycetes in litter exhibit a restricted mycelial growth and a rapid sporulation. However, these mycelia forage in a similar manner as cord forming species on more restricted areas. Unlike mycelia growing on culture media under laboratory conditions, that are uniform and determine a multipolar growth pattern, these growing on natural substrata are apically dominated. This growing pattern is characterised by a leading hypha and lateral branching systems that are abundant on rich substrata and poor on depleted substrata. Poor branching together with the tendency to form bundles of 2 and 3 hyphae are consistent with exploratory foraging strategy (Fig. 2). As results show, the hyphal systems are laterally consolidated by hyphal anastomoses in a scalariform manner that permits the translocation of metabolites and gives a positional information to whole

mycelium (Fig. 1). It is worth mentioning that same type of mycelia is encountered in such epiphytic obligate parasites as powdery mildews, namely, *Microsphaera alphitoides*. Hyphal leading systems are monospecific or plurispecific of 2 or 3 interacting species. The interaction is of a facilitative type (sensu Bertness & Calloway, 1994).

The fractal dimension of foraging mycelial systems in depleted areas approaches the topological dimension of the line due to the exploratory strategy with poor branching. The cover slips mimic a transition zone between rich and depleted substrates or disruption areas characterised by drastic microclimatic changes with respect to humidity, clearly an anisotropic habitat, very different from laboratory media that are isotropic with respect to resources and microclimate, supporting a different type of mycelial growth. Fractal structure of such mycelia implies that there is no preferred center of growth, all points within the colony display same hyphal distribution. In mycelia growing in a gradiental habitat, branching is abundant in points where resources are concentrated. The foraging leading hyphae are satellized by microcolonies of fast growing species as *Cladosporium cladosporioides* and *Aureobasidium pullulans* adapted to oligotrophic or scarce resources (Fig. 2:B.). Propagules of these species were carried by litter invertebrates but also existed on leaves during the vegetation period as phylloplane species. The colonies exhibit a particular growing pattern: few hyphae that coil around the other species leading hyphae. Few and scattered conidiophores show a rapid switch to sporulation of the microcolonies. Able to cross the disruption zone represented by the cover slips are only few bundles of hyphae. Obviously, their function as foragers is doubled by a phoretic function for other species mycelia. A question raises whether the relationship between such microcolonies and leading hyphae is also of an exploitative nature. The leading hyphae function as feeders in depleted areas of the habitat, an additional resource for scavengers as pioneer species that utilize litter as nutrition substrate. Such a system is hardly branching, being geometrically close to topological dimension of the line (code 3 and 6 of Fig. 1). A special remarque deserves the zoophagous species that are able to cover larger areas of the substrate including disruption zones due to their particular nutrition requirements, that is litter invertebrates as additional source of nitrogen. Fractal behavior of combined of two or more species mycelia are not cumulative but integrative as our results show: a dual system (code 2 fv) displays D of 1.2011. Separated mycelia of the system have 1.1417 and 1.1636, respectively (Fig. 1).

Comparing literature data, one reaches the conclusion that exploitative mode of growth determines low D values, which are also obtained for initial phase of growth in young colonies where D is close to 1 (15) and in exploratory mycelia of naturally growing rhizomorphs of *Hypholoma fasciculare* and *Phanerochaete gigantea* (5). However, authors consider that this initial growth phase is not fractal because fractal behavior is associated with a branching process. Exploitative

mycelia in natural substrata and a later stage development of fungal colonies on laboratory media display higher D values as 1.81 in *P. velutina*, and 1.93 in *H. fasciculare*. In our opinion, there is a fractal/non-fractal mosaic of mycelia in natural substrata due to resource discontinuities.

This preliminary study shows that in litters interspecific interactions of facilitation type take place between species that play a phoretic role and species that take advantage of the phoresy. Phoretic capacity exhibits mycelia with exploratory autonomy, capable to cross disruption and resource depleted areas and characterized by low D values or even a non-fractal behavior. Hyphen systems are monospecific or plurispecific. The fractal analysis gives little information in systems with scarce ramification due to depletion of nutrients or disruption of the substrate. The branching in such systems serves to metabolite reallocation, positional information, lateral consolidation of the mycelial exploratory front and phoretic, facilitation function.

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