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ULTRASTRUCTURAL CHANGES IN *HELIANTHUS ANNUUS* L. COTYLEDON CELLS DURING GERMINATION AND AFTER SEED GAMMA IRRADIATION

BY

AURELIA BREZEANU, FL. TĂCINĂ and I. CIOBANU

Doses between 40 and 70 Kr gamma radiations inhibit the cellular differentiation process of *H. annuus* L. cotyledon storage cells, but they do not produce the ultrastructural alterations of cell organelles. The protein bodies are strongly affected and their function is similar to lysosome systems. The number, morphology and catalase content of microbodies are not affected by higher doses of radiations, which explains only the slowing down of the metabolical processes they are involved in and not their inhibition.

Ultrastructural and biochemical studies on different species of cotyledon tissues have proved that during germination, storage cells are extensively modified in point of morphology and metabolism [2], [7], [8], [14], [15], [16—19], [25]. These modifications induce the gradual digestion of food reserves.

During cellular differentiation hydrolasic digestion processes occur alongside profound alterations in the morphology of cytoplasmic organelles.

GRUBER and co-workers [6] described the successive steps of cellular alterations which occur in sunflower cotyledon cells during germination with special reference to microbodies (glyoxysomes and peroxisomes) and their enzymatic content.

The present study is aimed at distinguishing the effects of various doses of ^{60}Co gamma rays on the ultrastructure of the cotyledon storage cell in different successive stages of cell differentiation, during seed germination and cotyledon greening.

MATERIAL AND METHODS

Different treatments of gamma radiation (1,40, 50, 60, 70 Kr) were applied to *H. annuus* L. dry seeds. Five days after treatment, the seeds were germinated in tap water at room temperature. At various stages of sunflower seedling development (e.g., 1, 4, 7 days after seed imbibition) the portions of cotyledon tissue were fixed for 2h at room temperature in a solution of 3% glutaraldehyde and 0.05M potassium phosphate buffer (pH 6.8). The tissues were next postfixed in buffered 2% OsO_4 for 2h, dehydrated in acetone followed by propylene oxide and embedded in Epon resin. Cytochemical location of catalase in microbodies was determined by using FREDERIK and NEWCOMB' s method [5]. After glutaral-

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dehyde fixation and rinsing, the tissue was incubated in 3.3′ diaminobenzidine (DAB) for 60 min., at 37°C.

Silver-gray sections were stained by Reynolds method and examined in a Jem 7 electron-microscope at an accelerating voltage of 75 KV.

RESULTS

a) SUCCESSIVE ULTRASTRUCTURAL ALTERATIONS IN THE CELL OF CONTROL TISSUE DURING GERMINATION

The cotyledon storage cells of *H. annuus* undergo rapid and distinctive ultrastructural alterations during the first week of germination. One day after the onset of imbibition, the ultrastructural aspect is a largely dormant tissue. All cells are loaded with protein and lipid bodies. The protein bodies appear as large formations (Pl. 1 A), which are separated by a unit membrane and contained an amorphous matrix. Globoidal structures and sometimes small vacuole formation can be seen in young protein bodies. These might represent an incipient stage of hydrolasic digestion which generally begins very early about 1—2 hours since imbibition (Pl. 1 B).

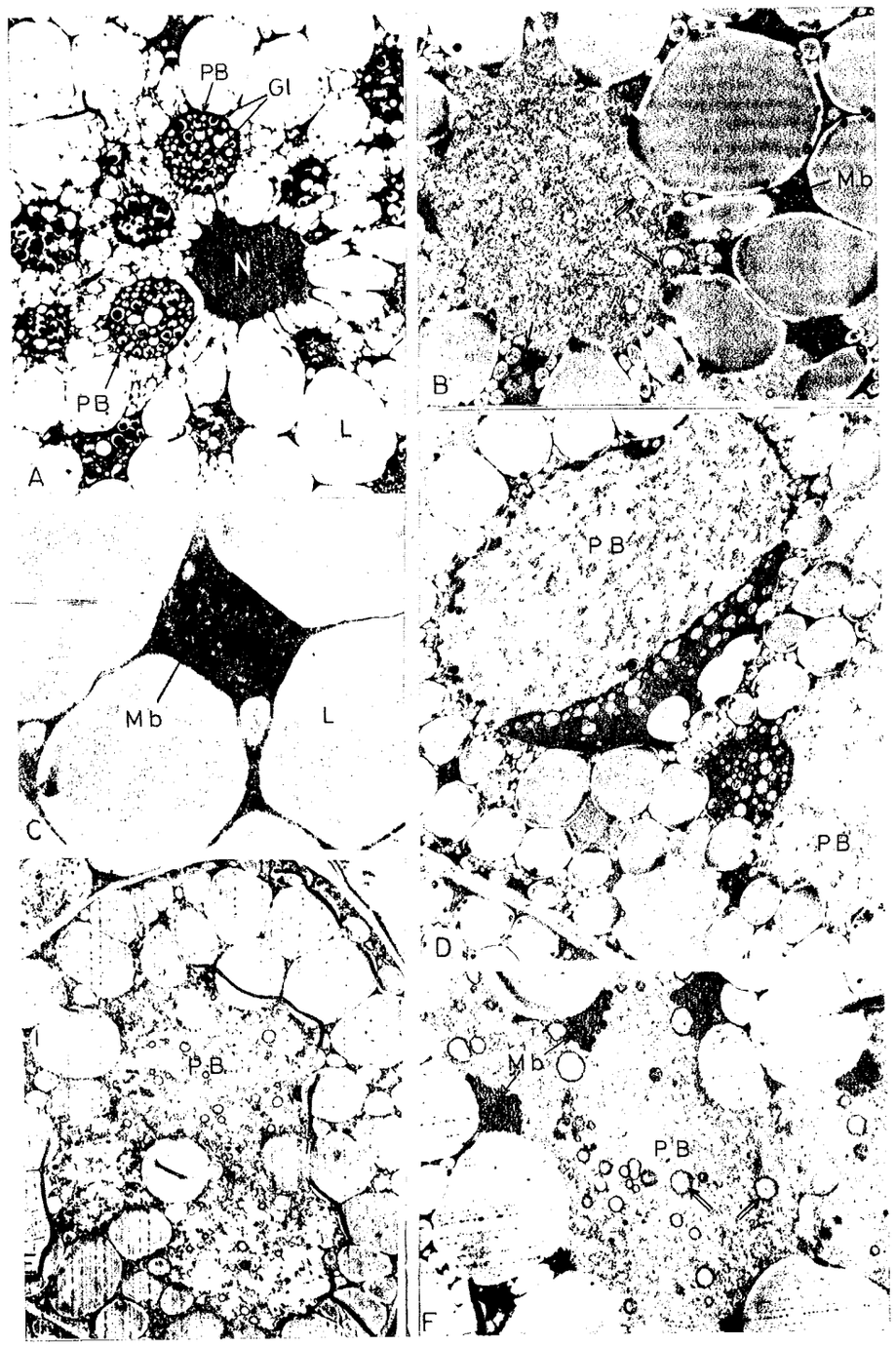
The lipid storage, which is seen as electron-transparent lipid droplets (L), (Pl. 1B) is probably separated by membranes and regularly displayed near the cell wall and between protein bodies.

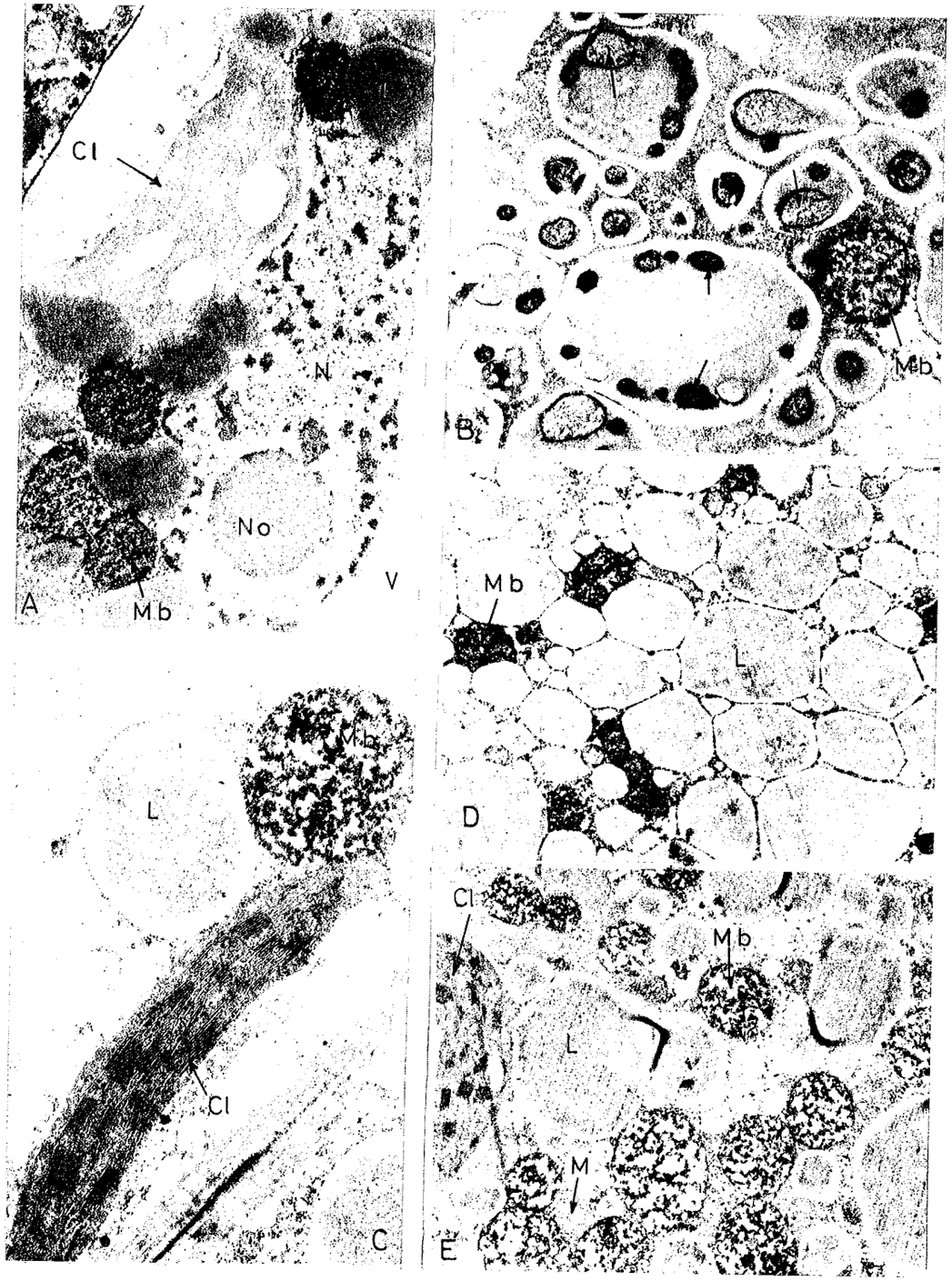
The nuclei are large and irregular in shape (Pl. 1A). Other cellular organelles as plastides, mitochondrias and microbodies are in early differentiation stages and sometimes it is quite difficult to identify them and to observe fine structural details.

Microbodies are numerous, closely associated with lipid bodies and they can be safely identified only after specific incubation in DAB medium (Pl. 1B, C).

Four days after the onset of imbibition the storage cells are characterized by active metabolization of proteic reserves and moderate metabolization of lipidic ones and by cell organelle differentiation. Protein bodies were thoroughly digested and, in the middle of the cell, a large central vacuola is getting differentiated (Pl 2A). The disappearance of the storage protein from protein bodies without the apparent destruction of the limiting membrane suggests that these organelles might be capable of autolysis.

PLATE 1. — Fine structure of storage cells in *Helianthus annuus* L. cotyledon, 1 day after imbibition :
A, B, C — Ultrastructural details of cells in non-irradiated tissue : A — General aspect of the cell : N = nucleus, PB = protein bodies, Gl = globoid, L = lipid bodies (× 3,500) ;
B — Detail of a proteic bodies : the arrow indicates the unit membrane between protein bodies ; the double arrow indicates vacuoles within the amorphous matrix ; Mb = microbodies after incubation in the DAB medium (× 9,900) ;
C — Detail of protein body structure (× 22,000) ;
D — Protein bodies in different stages of enzymatic digestion (× 10,250) ;
E, F — Ultrastructural modifications of protein bodies induced by irradiation (40—70Kr) (E — 3 000 ; F — × 9,100) ;





Most lipid droplets have numerous lytic areas within (Pl. 2B, see the arrows). Plastids develop their inner lamellar system — grana and intergrana — and starch granules appear in some chloroplasts (Pl. 2A). All cells are rich in mitochondrias, the internal structure of which is well-differentiated. The cytoplasm is also rich in ribosomes. Microbodies are very frequent ; part of them are still associated with lipid bodies and many are attached to chloroplast envelope (Pl. 2A, B) or to nuclear envelope.

Seven days after the onset of imbibition, the cotyledons become photosynthetic organs and the characteristics of storage cells resemble those of mesophyll cell. In highly vacuolated cells, chloroplasts, as fully developed organelles, are predominant. Microbodies are also frequent and seem to be preferentially associated with chloroplasts. Their matrix showed little differentiation and no amorphous or crystalline inclusions were observed in our material. At the same time, microbodies are also observed in contact with mitochondrias, endoplasmic reticulum and chloroplasts. In all differentiation stages, the response of microbodies to the DAB test was positive which demonstrated the active presence of catalase.

b) THE EFFECTS OF DIFFERENT DOSES OF ^{60}Co GAMMA RADIATIONS ON STORAGE CELL DIFFERENTIATION IN COTYLEDONS

Our experiment showed that lower doses of radiations (1 Kr) affected positively seed germination, seedlings growth and meristem cell differentiation. Considering the great number of similarities between these variants and the control material, we do not consider necessary to insist on this problem.

The higher doses, especially those of 40—70 Kr, inhibited seed germination and seedling growth and decreased the rhythm of progressive ultrastructural changes which usually appeared in non-irradiated material during germination. They did not induce morphological alterations in the inner structure of organelles. This fact points to some modifications occurring at molecular and sub-molecular level which are not reflected in cell ultrastructure.

The most important effects in early germination (1 day) were the disappearance of the surrounding membrane of protein bodies, followed by the confluence of several protein bodies and appearance of numerous vacuoles within their matrix. An intense lytic process seems to occur which affects part of lipid bodies and other neighbouring organelles. Thus, in the middle of the cell a large lytic area develops like a vacuole, which could be interpreted as the development of lysosome formations (Pl. 1E). The digestion of lipid droplets seems to be inhibited, since they are still in large number.

PLATE 2. — Ultrastructural characteristics of storage cells after 4 days since imbibition (A, B, D), respectively, 7 days (C, E) ; A, B, C—aspects of non-irradiated tissue (4 days): The arrow indicates the lytic areas within lipid bodies : No =nucleolus, Cl = chloroplast, M — mitochondrias ; Plate A, B represent non-contrasted sections (A = × 10, 100, B = × 6, 100, C = ×, 18,000) ;
D — Part of the cell from irradiated material after 4 days of imbibition (× 6,900) ;
E — Part of the cell from irradiated material after 7 days of imbibition (×, 9, 100)

On the 4th and 7th day of imbibition, respectively, the cells are much richer in lipid storage than the control and we cannot detect any lytic areas within (Pl. 2 C, F). In all differentiation stages, the nucleus has not been altered after treatment. Generally, higher doses of radiation inhibited the differentiation of cell organelles. The number of microbodies and their catalase content tested by the DAB reaction were not affected by higher doses of radiation. It was only at 70 Kr that their slight swelling was observed. Most microbodies were mainly associated with lipid droplets and less with chloroplasts (Pl. 2 D, E). It is difficult to specify without specifical biochemical analysis wether they are glyoxysomes or peroxisomes or whether both kind of organelles are present.

DISCUSSIONS

The study of the effects of various irradiation sources and doses on different plant tissues and cells has gained special interest since knowledge of their action at cell level has not been elucidated so far. Ionizing radiations have long been thought to affect mainly the nucleus and the genetic material. Lately, much information has been collected on the influence of ionizing radiations on many other cytoplasmic organelles and cellular processes. Proliferation of the endoplasmic reticulum, proplastid swelling, anomalous mitochondrias and the development of cytoplasm vacuolarization have been described.

LANE and NOVIKOFF [10] pointed out the proliferation of the endoplasmic reticulum, the increase in lysosome number, numerous connections between the endoplasmic reticulum and lysosomes and the continuity of acid phosphatase reaction product in these organelles after X and ultraviolet irradiatoions, on the KL cells. The participation of the endoplasmic reticulum in the formation of autophagic vacuole within cytoplasm was also pointed out.

HUGON et al [9] described alterations of mitochondrias and abundance of lysosomes as induced by irradiation.

WANGENHEIM [24], [25], COULON [3] also showed that with different plant cells, changes similar to those produced in the anoxia or during bacteria infection or symbiosis occur. Irrespective of the destructive agent, the cells act for the mobilization of their lysosomal and cytomembranar systems. Many other enzyme systems may also be involved.

In our experiment, we have first observed alterations in protein bodies ultrastructure. Their hydrolasic digestion started very early in the irradiated material and was almost completed 1 day after the onset of imbibition with 70 Kr doses.

The capacity of protein bodies to function as lysosomes and digest both the storage proteins within and part of the cytoplasm with neighboring cell organelles was reported by MATILE [11—13], COFFEY et al [4], VILLIERS [22], ÖPIK [18], [19], and HARRIS and CHRISPEELS [7]. Our results stand proof of the active participation of lytic enzymes in cellular processes after irradiation.

The process starts in protein bodies which function as lysosomes and after the destruction of their limiting membrane, they may a affect other neighbouring cell formations.

It was interesting to notice that in point of number, morphology and catalase content, microbodies were not affected by higher doses of radiations. It is possible that other enzymes which are directly involved in lipid metabolism may be affected. That explains also why the metabolical processes for which they are so important have been only slowed down and not completely inhibited.

In our experiment, plant growth was strongly inhibited after treatment with doses between 40 and 70 Kr but this process cannot be related to any cytomorphological alteration at electron-microscope level. The data in the literature and some of our previous results [21] allow us to consider that inhibition of plant growth is induced by modifications in phytohormones content (auxin and gibberellin) which have not been reflected in cell ultrastructure.

Our results support the idea of the active participation of enzymatical processes, alongside other metabolical processes, in the complex plant cell modifications which occur after radiation treatment.

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ELECTRON-MICROSCOPIC RESEARCH CONCERNING THE EFFECT OF PROCAINE ON THE EPIDERMIC CELL VACUOLES WITH NENUPHAR PETALS *(NYMPHAEA ZANZIBARIENSIS)*

BY

DORINA CACHIȚĂ-COSMA and C. CRĂCIUN

Procaine has a peculiar action when applied on vegetal epidermal cells (of floral petals) in concentration exceeding 100 ppm; the result is the appearance in the vacuole of hyaline spheres which are strongly refringent. The images in electron microscopy show that at the limit between tonoplast and vacuole, a dense mass of material from the vacuolar sap is deposited, or fine particles of vacuolar sap group together, forming globulose corpuscles. Both the deposit and the corpuscles are electron-dense. Aspects of the penetration of the corpuscles through the tonoplast into the cytoplasm were also observed. In certain conditions, the corpuscles may undergo structural modifications, e.g., in vacuole formation.

In medical practice, procaine is known for its anaesthetic properties or, in certain therapeutic doses, as a biostimulating substance of metabolic functions. In plants, besides the stimulating action, a special influence was observed when procaine was administered to tissues in super-optimal concentration (1000 ppm). When penetrating in cells, it determines a lack of balance in the physico-chemical stage of vacuolar colloids; as a consequence, minute corpuscles appear in the vacuolar sap which are in Brownian movement. With longer conservation of tissues in procaine solution, or with increased concentration of the latter, the corpuscles are gradually fused into a kind of big spheres floating in the vacuole, or they stop in the tonoplast or penetrate the cytoplasm [1], [2], [3],[4], [5], [6]. The chemical composition of these corpuscles is supposed to be complex. Generally, it seems that lipid molecules (phospholipides or lipoproteins) and water are involved. The corpuscles have special affinity for vacuolar dyes (natural—athocyans — or basic vital dyes, such as neutral red) [1], [3], [5]. In certain experimental conditions, an alteration of corpuscle structure was observed, especially in the big ones, which led to emptying the content and their transformation from optically homogeneous corpuscles into spheres arranged in globulose cavities. This phenomenon develops gradually. The cavities delimited within the corpuscles fuse and tend to become a sphere with a wall of various thickness, strongly refringent, and with less dense a content. The modification of corpuscle structure may be caused by the plasmolysis and deplasmolysis of tissues, by cell trauma, or by washing of preparations with ethyl alcohol. Such researches have been carried out on epidermal cells of different plant species,

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treated with procaine and examined in optical microscope, in normal light or in polarized light [5].

This work is aimed at reproducing this phenomenon also in the epidermic cells of other plant species and analysing the modifications caused by procaine using electron microscopy.

MATERIAL AND METHODS

The effect of the 1000 ppm concentrated procaine on the ultrastructure of the epidermal cells of nenuphar petals was studied. They have been infiltrated in distilled water, and part of them have been treated in procaine solution for 48 hours. Out of the procaine-treated petals, some fragments have been plasmolysed (in 1 M saccharose, for one hour) and deplasmolysed. Some of the fragments treated with procaine for 48 hours have been lightly traumatized through repeated pressure. In all cases, the preparations have been examined in optic microscopy, and then the tissues have been fixed using electron microscopy techniques [7]. In order to fix the tissues for electron microscopy, they have been immersed in 3% glutaraldehyde solution, in 0.15 M phosphate buffer, at 7.4 pH, followed by successive washings in this buffer, and then a further fixation of tissues in 1% osmic acid. The tissues have been dehydrated in acetone bathes and included in vestopal W. The sections obtained with a LKB-Ultratome III were contrasted with uranyl acetate solution and lead citrate. The preparations have been examined in a TESLA-BS-613 electron-microscope at 80 kv.

RESULTS AND DISCUSSIONS

By treating the nenuphar petals with procaine (1000 ppm), corpuscles of different forms and sizes were formed in the vacuoles of the epidermic cells (Figs. 2, 3 and 4). In the vacuoles of cells from the control lot, tissues without treatment, such corpuscles were not identified (Fig. 1). The corpuscles are in very clear contrast, and by sectioning, the dense material they are composed of suffered an undulating process. In Fig. 2, a huge corpuscle may be analysed. In a previous phase, the corpuscle had a slightly wavy aspect at the level of section surface ; when subject to a concentrated electron flux, a sublimation process of some compounds of corpuscle content was observed ; this sublimation was not uniform on the entire surface, but on certain zones, clear-cut areas where sublimation did not occur being delimited (with apparently smaller density). By electronic transparence, corpuscles with areas of different electronic density were observed, which suggested a peculiar internal structure. It seems that at these levels, which correspond to less dense areas, the emptying of corpuscles occurs when supplementary treatments are applied. In Fig. 3, corpuscles migrated in the cytoplasm and a vacuole fragment are observed. In the vacuolar sap of this cell, some molecular aggregates, floating into the vacuole, and a deposit covering the inside of the tonoplast are seen. With Fig. 4, we re-

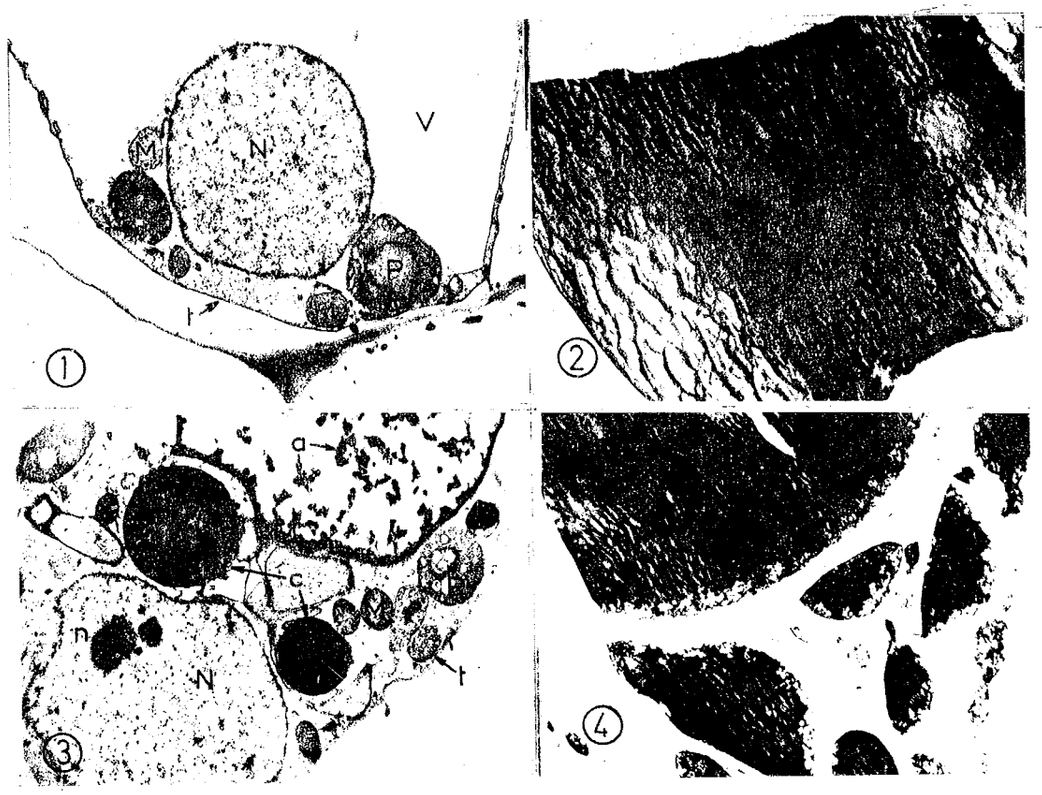
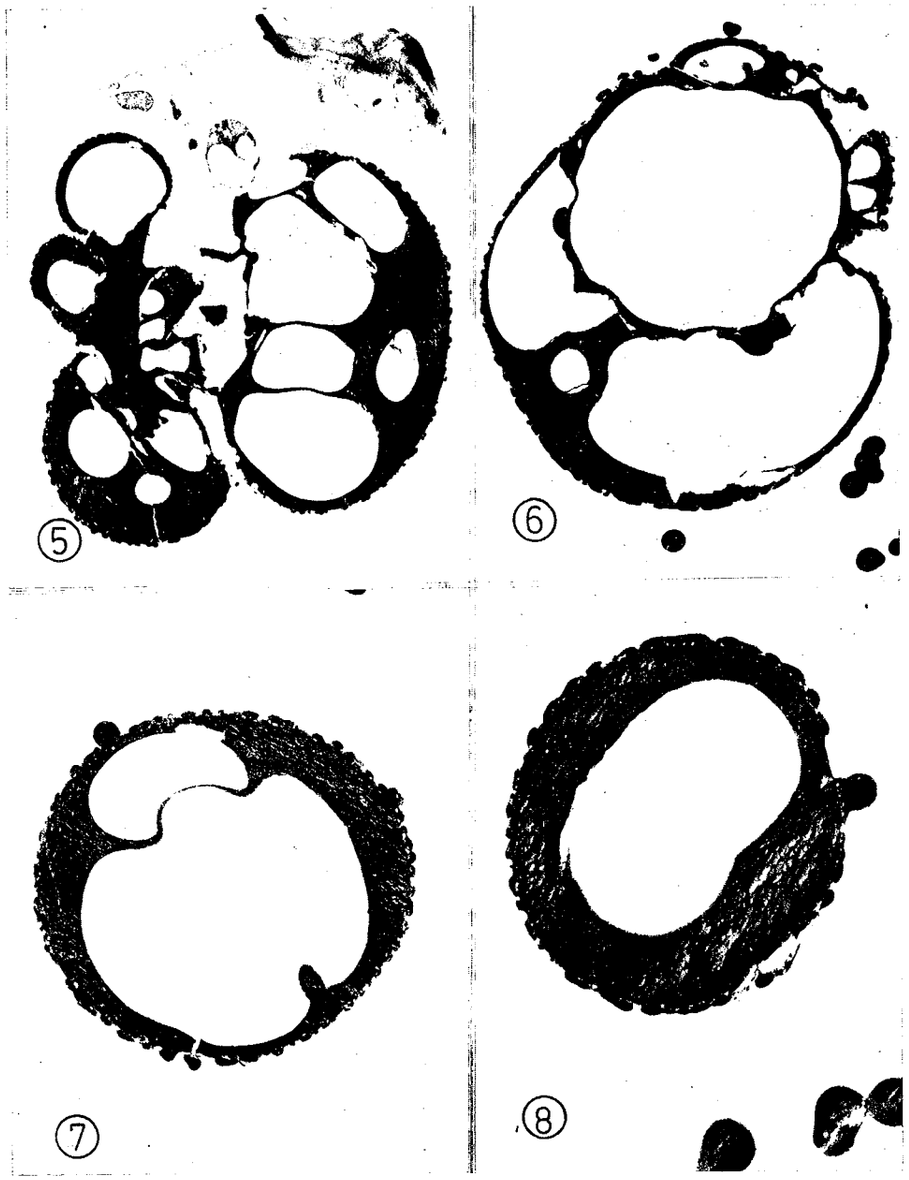


Fig. 1. — Epidermic cells of nenuphar petals — the control tissues (N = nucleus ; V = vacuoles ; M = mitochondrion ; P = plastid ; t = tonoplast) (× 16, 00)

Fig. 2. — Huge corpuscle formed in the vacuole of nenuphar petals after 48-hour-procaine treatment (1000 ppm solution). The nonhomogeneous structure of the corpuscle is observed (×16,500).

Fig. 3. — General aspect of the epidermic cells in nenuphar petals after procaine treatment (N = nucleous ; n = nucleolus ; t = tonoplast ; a = molecular aggregates in the vacuolar sap ; c = corpuscles migrated in the cytoplasm ; M = mitochondrion ; P = plastids) (× 16,500).

Fig. 4. — Corpuscles of different sizes formed in the vacuoles of nenuphar petals after procaine treatment. The edges of corpuscles are fissured by mechanic trauma (× 16,500).



Figs. 5, 6, 7, 8 — Induced modifications in the structure of big corpuscles as a consequence of saccharose or potassium nitrate plasmolysis and water deplasmolysis. The corpuscles gradually change their inner structure, as shown in the stages plotted in Figs 5—8; Fig. 8 illustrates a stable stage (Figs 5 and 6 = 5,000 ×; Fig. 7 = 6,650 ×; Fig. 8 = 13,400 ×).

mark the "fissure" process of the corpuscles as a consequence of their trauma. A similar process naturally happens in older corpuscles. Unlike the other epidermic cells, the stomatic ones do not produce corpuscles when treated with procaine.

Through plasmolysis, the viability degree of cells was verified. We should mention that the cells have been viable for some days. As mentioned in previous works [5], [6], plasmolysis and deplasmolysis have caused the release of an emptying phenomenon in the large corpuscles, within their initial dimensions, without modifying the volume of corpuscles. It is assumed that within the corpuscles, there occur a division of phases and an alteration of the equilibrium of the molecule complex (which have grouped into corpuscles) from the vacuolar sap composition. This separation of phases induces the appearance of cavities of myelinic aspects, with a more consistent partition wall (a sort of "stroma"), which accumulates the anthocyanic dye; this system is of relative stability and tends towards continuous modification of its structure in fusing the cavities until the stage of a sphere with a thick wall and less dense a content is reached. In optical microscopy, these modifications can be seen by the investigator. The electron-microscopic images are revealing. We have obtained sections in such corpuscles during various phases of their structural modifications. Figure 5 presents a section through a multi-divided corpuscle and Figs. 6, 7, and 8, the fusion (resorption of the partition walls) of many compartments into a stage of non-divided sphere. These aspects are not the result of sections in various planes and depths of a corpuscle; the described phases may be easily observed in optic microscopy. In electron microscopy, when supplementary treatments were applied, the surface of the corpuscle was no longer even, but sinuous. If the cell dies, the presence of a mummified "stroma" in place of the former corpuscle is identified but it does not resemble its structure when the cell was alive. It is possible that alongside the death of the cell, certain substances are desorbed in the vacuoles, disorganizing the range of molecules in corpuscles.

We cannot be precise on how far procaine is itself a compound of the corpuscle, but it is sure that with its penetration into the vacuole, a phenomenon of physico-chemical imbalance in the colloids of the vacuolar sap is released; the colloids range in a certain way, forming spheric corpuscles and tonoplast deposits. It is possible that in the macromolecule organization, an important part is held by hydrophilous or hydrophobe groups of the compounds which form corpuscles, and the electrical charge of the molecular aggregates floating in the vacuolar sap. It is sure that a certain ranging of water molecules and their interference with different organic substances are essential for the stability of the corpuscle sphere. All the descriptions of the metamorphosis of corpuscles refer to a new concept on the place of the molecules inside the corpuscle, within the limits of the same shape and volume. It is worth mentioning that with similar tissues of other monocotyledonous plants, the phenomenon could not be generally reproduced, even when procaine concentration into the medium was increased (neither when crystalline powder of procaine was applied on the tissue); the colloids of the vacuolar sap are likely to be more stable than in dicotyledonous plants. However, this complex phenomenon still requires further investigations.

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ULTRASTRUCTURE OF PEROXISOMES IN CUCUMBER COTYLEDONS (*CUCUMIS SATIVUS* L.) TREATED WITH ^{60}Co GAMMA IRRADIATIONS

BY

H. TIȚU and INNA SUSHROVA BORȘAN

In this paper the ultrastructure of peroxisomes in cotyledonary cells of seedlings resulted from irradiated seeds with 2.5 Kr, 5 Kr, 10 Kr and 15 Kr doses are analysed. The doses of 2.5 Kr have stimulating action on peroxisomes, the larger ones being more numerous than the unirradiated. Alongside the increase of irradiation dose, peroxisomes and mitochondria remain unchanged while plastids suffer important alterations. With the 15 Kr dose, although the differentiation in the assimilatory tissue was blocked, the presence of numerous microbodies was noticed, where catalase was cytochemically detected by the DAB method.

The investigations regarding the morphology and biochemistry of peroxisomes in plants had a rapid development as a consequence of the role that these cell organelles have in the metabolic usage of some substances produced by chloroplasts [2]. The participation of peroxisomes in the photorespiration phenomenon is also mentioned [12]. These discoveries were obtained especially by the study of peroxisomes in leaves of higher plants [5], [10], but the category of microbodies in cotyledonary cells during greening are also considered as organelles of similar function [14].

The ultrastructure of peroxisomes consists of a single membrane and a matrix featuring a granular or fibrillar texture that may contain an amorphous or crystalline nucleoid [10], [16]. By biochemical analyses in some plants, the main peroxisomal enzymes (namely, glycolate oxidase, catalase and hydroxypyruvate reductase) were studied, their activity being stimulated by light [5–6]. Still, there are papers where the name of microbodies is kept for peroxisomes although in plants the glyoxysomes also belong to the category of microbodies, because besides catalase they contain the enzymes necessary for the conversion of lipids into carbohydrates [1–2].

In this paper we shall analyse the ultrastructure of peroxisomes in the cotyledons with differentiated assimilatory tissue, from cucumber seedlings resulted from seeds treated with different doses of ^{60}Co gamma irradiation.

MATERIAL AND METHODS

The cucumber seeds (*Cucumis sativus* L.) moistened in tap water were treated with 2.5, 5, 10 and 15 Kr in a ^{60}Co irradiator. Immediately after irradiation, the seeds were put for germination in Petri dishes, on

filter paper, wet with tap water, under normal 12—12 hr light-dark cycle, at + 24°C. The germination began after 24 hours from seed irradiation. The seedlings grown from unirradiated seeds were used as a control.

For ultrastructural studies, small portions of cotyledonary tissue were taken out, from 3, 4, 5, 7 and 10-day old plants and were fixed with 2.5% glutaraldehyde in 0.5 M cacodylate buffer, at pH 6.8, for 2 hours at + 4°C. In order to make a cytochemical detection of catalase in the peroxisomes of cotyledonary cells, beginning with the 5th day since the germination, they were incubated in the medium suggested by FREDERICK and NEWCOMB [7] which consists of 10 mg 3,3' — Diaminobenzidine tetrahydrochloride (DAB), 0.1 ml 3% H_2O_2 and 0.5 ml 2 amino-2 methyl 1,3 propandiol, pH 9, at 37°C for 60 min. The material was post-fixed with 1% osmium tetroxide in a cacodylate buffer for 2 hours at + 4°C and embedded in Epon 812. The control samples were preincubated with 0.02 aminotriazol and embedded in the same epoxy resin. All fixation and embedding products were supplied by SERVA *Feinbiochemica*, D-6900, Heidelberg 1. The ultrasections were made with an ultramicrotome TESLA BS-490 and, after staining with uranyl acetate and lead citrate, they were examined by the electron-microscope JEM-7.

RESULTS

24 hours after irradiation the seeds in variants 2.5 Kr, 5 Kr and the control ones began germination, and after 48 hours the cotyledons appeared white-coloured; on the 3rd and 4th days they were yellow-greenish and only after the 5th day they became green. These observations correspond to the diagram of seedlings growth in the same species studied by other authors [14]. In 5-day plants of variant 2.5 Kr, the cotyledons were a little bigger than the control ones. In the case of variant 15 Kr, the inhibition of plant growth was noticed with the 5th day of germination when the activity of the root meristem ceased; the cotyledons were green-yellowish presenting evident signs of etiolation.

Figure 1 represents the electron-micrograph of some relatively large areas in the two palisadic cotyledonary cells, in a cross section in 5-day-old plants; in this stage, the large central vacuole is still undergoing formation because almost all lipidic inclusions converted into other metabolic products. A reduced number of lipidic inclusions is found in the thin layer of cytoplasm, near the cellular walls, together with other organelles, such as mitochondria, chloroplasts and peroxisomes. The cytoplasm is mainly made of free ribosomes and wholly isolated fragments of granular endoplasmic reticulum. The central vacuole contains large fragments from proteic bodies. At the same time, residual proteic bodies closed in small vacuoles of autophagic type are noticed in the cytoplasmic zone. One may presume that in the disintegration process of some portions of proteic bodies, the autophagic vacuoles play an important role; this was evident because of the tonoplast evaginations in the direction of the residual fragments of proteic bodies from the central vacuole (Fig. 1, arrows).

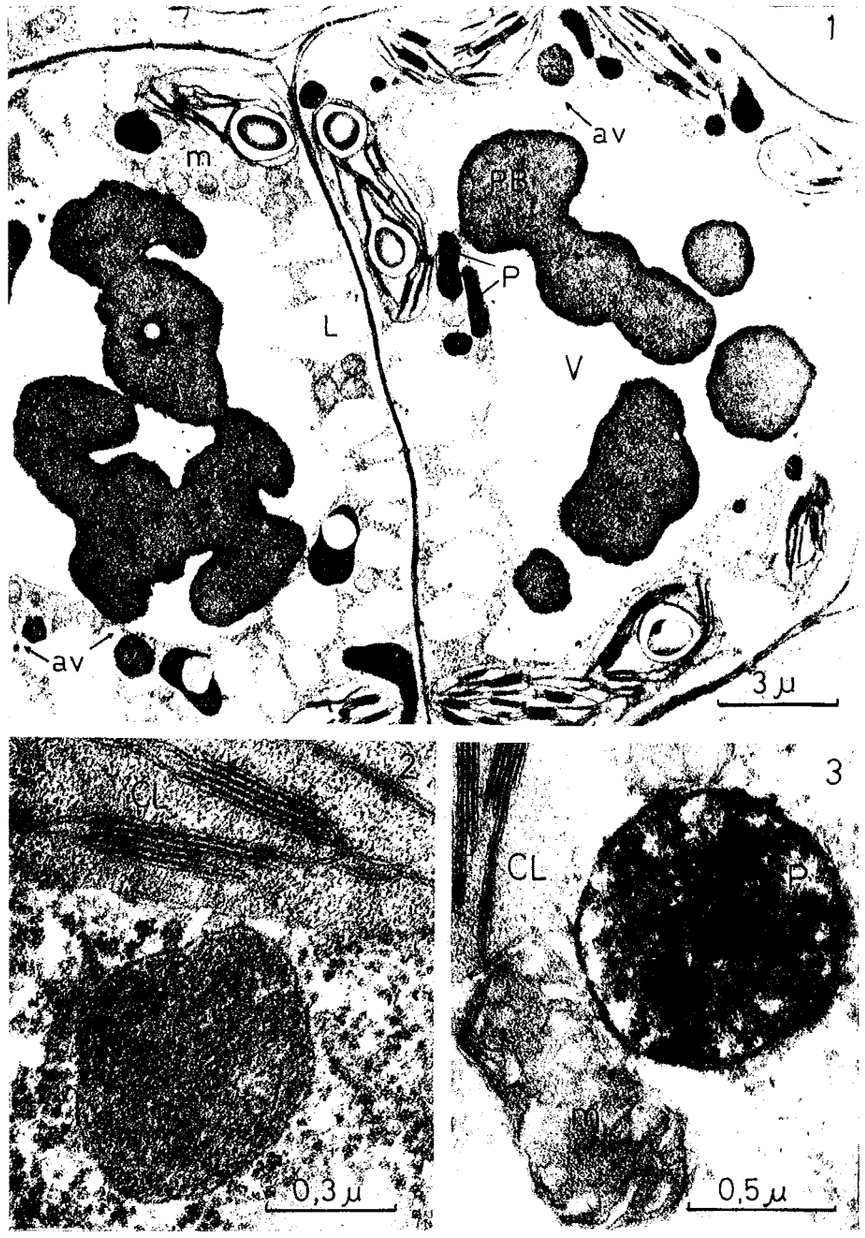

Fig. 1—3. — Ultrastructure of peroxisomes in cucumber cotyledons. Unirradiated. material P, peroxisomes; L, lipidic inclusions; m, mitochondria; av, autophagic vacuoles; PB, fragments of proteic bodies; V, central vacuole undergoing formation at 5 days after germinaton; CL, chloroplast. For details, see the text.

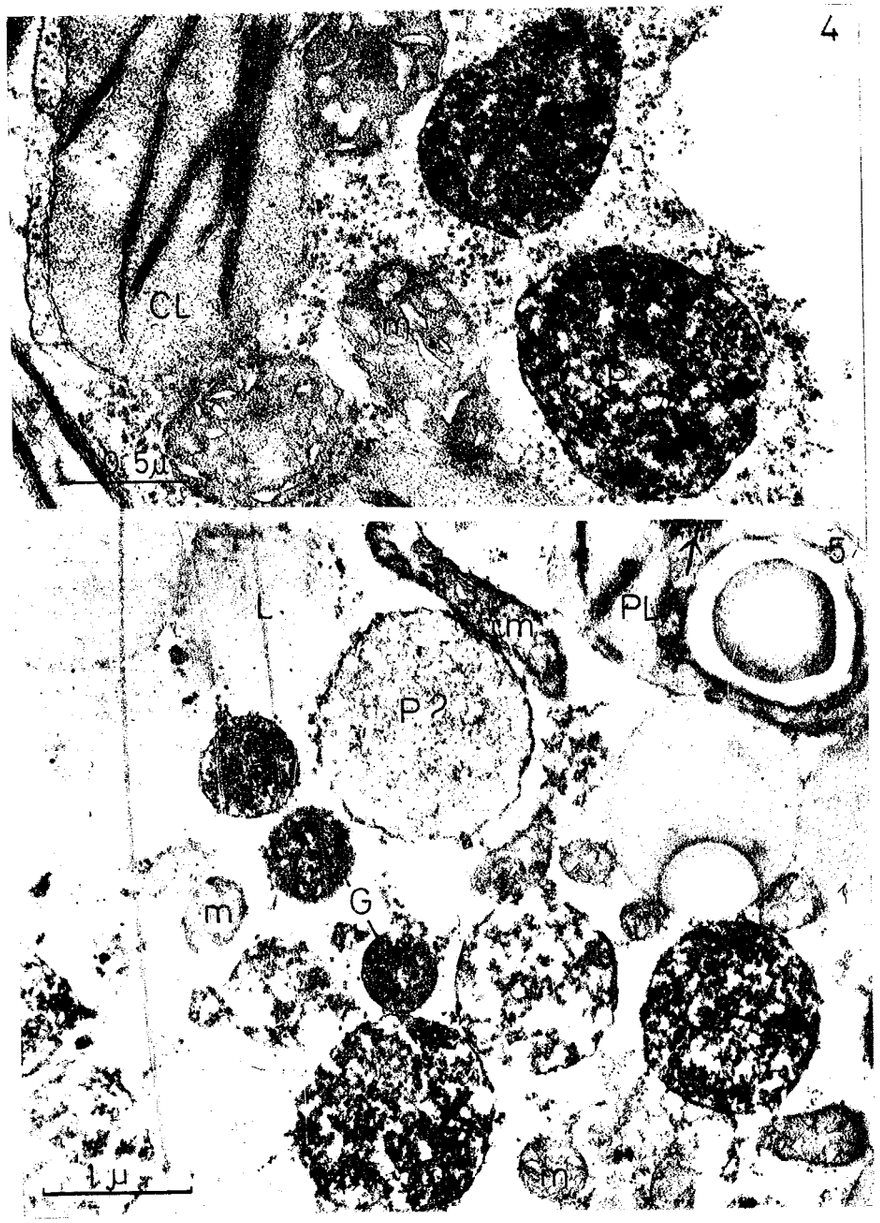

Fig. 4. — Ultrastructure of peroxisomes in cotyledons from 10 Kr variants ; 10-day-old seedlings.

Fig. 5. — Portion of a cotyledonary cell from 10-day-old seedlings 15 Kr variant ; for details, see the text.

As regards peroxisomes, we shall mention that they are quite frequently found in the cotyledons of 5-day-old seedlings in the close neighbourhood of chloroplast. Other microbodies of similar size are in close contact with the lipidic inclusions and that is why we consider them to be glyoxysomes. The intensity of the reaction product with DAB in both types of microbodies is similar. In 7-day-old seedlings, the aspect of typically assimilatory cells is recognized in the structure of cotyledons, characterized by large chloroplasts containing grana made of a large number of thylakoids. The proteic formations are wholly disintegrated, and the lipidic inclusions are occasionaly met so that most microbodies found in this stage may be considered to be peroxisomes. The mitochondria are very frequent and are generally grouped with peroxisomes at the chloroplast extremities where cytoplasm layer is thicker. Ultrastructurally, peroxisomes in the control material have a matrix with homogeneous fine granular texture (Fig. 2). The reaction product with DAB, in the same unirradiated seedlings, is regularly widespread in the whole matrix, and even in the single membrane because it appears more intensely coloured and thicker in the control samples (Fig. 3).

In variant 2.5 Kr a clearer development of cell organelles is noticed in comparison with control samples from unirradiated cotyledons. The stimulating effect on cell differentiation is observed although, to a lesser extent, it is also met in variant 5 Kr. In both situations, the peroxisomes are larger and lipidic inclusions are less than in the similar organelles from the unirradiated samples ; at a dose of 10 Kr, small values are recorded as regards peroxisome dimensions (Table 1). Occasionally, in the last variant, the peroxisome single membrane presents interruptions, sometimes on important regions (Fig. 4).

Table 1

Average dimensions of peroxisomes from cucumber cotyledons in 7-day-old seedlings

Variants	Diameter of peroxisomes µm
Control	0.5±0.07
2.5 Kr	0.8±0.03
5 Kr	0.6±0.05
10 Kr	0.4±0.06

The ultrastructural study of the samples from variant 15 Kr shows that in 5-10-day-old seedlings, the cotyledonary cells contain an important number of lipidic inclusions and microbodies of different sizes (Fig. 5) ; in this case, most microbodies have a coarsely granular matrix where the reaction product with DAB appears and because these organelles are interspersed among the lipidic inclusions we consider them glyoxysomes (G) ; in the same electron-micrograph, microbodies have a matrix of flocculent texture where catalase was not cytochemically detected (P). TRELEASE and col. [14] have carried out a remarkable study on the ultrastructure and biochemistry of microbodies in the same tissue, but in other experimental conditions they pointed out a flocculent texture of matrix especially at peroxisome level. We underline the fact that we deal with a mixt population of microbodies appreciated according to the heterogeneity of the matrix noticed in one and the same cell ; these data confirm the assumption that in cotyledonary cells both types of microbodies may coexist (glyoxysomes and peroxisomes), each of these organelles being active at different stages of plant growth [14].

In variant 15 Kr, plastids do not differentiate in chloroplasts; the structure of such plastids consists of 1—2 prolamellar bodies (Fig. 5, arrow). Plastid envelope often undergoes disintegration. Numerous small mitochondria are observed, whose internal structure is less damaged by irradiations.

DISCUSSIONS

The results in this paper show that the dose 2.5 Kr contributes to a more rapid exaustion of the lipidic substances in cotyledons and therefore in a faster differentiation of the assimilatory tissue. This is in accordance with the data reported in the literature which mention the stimulating effect of small irradiation doses on some enzymatic processes carried out during germination and growth periods [3], [8].

The analysis of cell ultrastructure in the cotyledons of seedlings resulted from seeds irradiated with 2.5 Kr, beginning with the 5th day from the germination, reveals that under such conditions, the peroxisomes have larger dimensions than in the unirradiated material. At higher doses of irradiation, a series of ultrastructural alterations occur at the chloroplast level, while the microbodies and mitochondria remain unchanged, i.e. keep their integrity. A series of previous investigations demonstrate that the noxious effect of ionizing irradiations applied in high doses produce modifications of the chloroplast structures [9], [18], proliferation of the endoplasmic reticulum [19] in higher plants. In the green alga *Brachiomonas submarina*, UNDERBRINK and col. [17] notice that after irradiation with 4 Kr (X-rays) and 15 Kr (gamma rays) a series of modifications occur: dilatations of the endoplasmic reticulum, swelling of mitochondria, disintegration of nuclear envelope.

We may conclude that peroxisomes are relatively radioresisting organelles as they keep their integrity even when seedling growth is blocked at high doses of irradiations; under the same conditions, important modifications are produced at the level of chloroplast ultrastructure. The mitochondria have higher frequency and do not undergo modifications visible at the cristae level, suggesting the idea that, in such cases, we may speak of compensatory phenomena in the degenerative processes [11] caused by irradiations.

The authors wish to thank the staff of the Oncological Institute in Bucharest, and particularly Dr. physicist T. Săndulescu, for their kind assistance in supplying us with the ^{60}Co irradiator used in our seed irradiation experiments.

We are also grateful to SERVA *Feinbiochemica* for their valuable gift, the diaminobenzidine, which was used throughout our cytochemical investigations.

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PALYNOLOGICAL STUDIES ON THE *EUPHORBIACEAE* FAMILY

BY

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The paper covers the morpho-pollinic analysis of 37 taxa of *Euphorbiaceae*, native as well as cultivated.
The pollen grains are of medium size; they are generally tricolpate and present features which are characteristic of the various species. By their features, the pollen grains impart to the family of *Euphorbiaceae* a stenopalynous character.

The family of *Euphorbiaceae* of the order *Tricoccae* is represented in the world flora by 290 genera with 7500 species. These spread for the greater part throughout the tropical and subtropical regions. Thus, many species of *Euphorbiaceae* are to be found in tropical America and Africa, whereas in the northern and southern parts of Australia this family has but a few representatives.

In our country, the family of *Euphorbiaceae* is represented by three genera with 44 species [4]; of these, 37 units have been analysed morpho-palynologically. The number of analysed units is smaller because some of our species are synonyms [3]. We have also studied the pollen morphology of several exotic species as, for example, *Andrachne colchica*, Fisch et Mey., and *Synadenium grantii*, Hook F., cultivated in the hot-houses of the Botanical Garden in Bucharest, as well as *Phyllanthus angustifolius*, Sw., in the Herbarium generale of the Botanical Garden in Cluj-Napoca. These species have been analysed in view of gathering data for the comparison between their morpho-palynous characters and those observed in the native species of the Romanian flora.

From the botanical literature [7], we know that the *Euphorbiaceae* is one of the largest and most polymorphous families of Angiosperms; their classification, based upon natural criteria, always implied some difficulties. Hence, new contributions are of peculiar value in establishing the phylogenetic relationships within this family.

The analysis of pollen-grain morphology, even when carried out on a smaller number of taxa, has proven its usefulness for this purpose.

In *G. Erdtman's* works [1], [2], there are very important data concerning the morphology of the pollen grains from representatives of the family of *Euphorbiaceae*. Although these indications deal with 225 species from 150 genera, they comprise no representative of the Romanian flora, not even the cultivated ones.

Köhler [5] gives many data on the morphology of pollen, but he does not refer to representatives of the Romanian flora.

Punt [8] und *Kuprianova, Alyoshina* [6] give some data and characteristics of the morpho-palynous peculiarities of some members of the

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family which are components of our flora. Thus, the present research contributes to the knowledge of pollen-morphology in the family of *Euphorbiaceae*.

The morphological characters of pollen grains have been analysed for the greater part on material from herbaria, only a few analyses being made on fresh material. The pollen from the analysed taxa of Euphorbiaceae was of yellow colour and presented orange to brown hues in water; in chloral-hydrate, the colour was much lighter, ranging from greenish-yellow to colourless.

As far as the dimensions of the pollen grains are concerned, these are of medium size and diameter 24.7 to 41.6 μ: their shape varies from spheroid prolate to prolate. A general characteristic is given by the presence of colpi, the pollen grains being tricolpate.

Some representatives have the sporoderm ornamented with verruculi which are irregularly distributed with *Euphorbia nutans*, Lag., *E. Humifusa*, Willd., *E. Chamaesyce*, L. The pollen grains of *Ricinus communis*, L. possess a tectum covered by hardly visible verruculi. With other taxa, the sporoderm is reticulate as for example with *Euphorbia peplis*, L. var. *erythrocaulos*, Delph., *Mercurialis ovata*, Sternb. et Hoppe; the difference in mesh-size of the reticulum which decorates the surface of the pollen grain allows us to identify easily the different taxa of the family.

In optical section, the structure of the sporoderm is also suited for the identification of species; it appears to be pilate and pilate-sympilate.

From our observations it results that within a genus the pollen grains, its species may be generally ranged into the same type, the differences being merely in size and proportions. It is worth while mentioning that even different genera usually present the same type of pollen; this is illustrated in plate 1 for the genera *Andrachne*, *Mercurialis*, *Ricinus* and *Euphorbia*. Owing to this circumstance, one cannot tell at first glance to which genus certain pollen grains belong, a detailed morphologic analysis being necessary.

In the material studied by us, the most frequent pollen grains are those with strongly narrowed colpi.

As a whole, the morpho-pollinic characters of pollen grains from the analysed genera allow a systematic stenopalynous grouping which facilitates precise diagnosis of genera and species of the *Euphorbiaceae*.

Plate I

Fig. 1. — *Andrachne colchica*, Fisch et Mey., *a*, pollen grain in polar view, outer aspect and sporoderm in optical section; *b*, sporoderm in optical section; *c*, ornamentation (*a* = 1280 X; *b*, *c* = 2525X, orig.).

Fig. 2. — *Mercurialis annua*, L., pollen grain in equatorial view (cf. Pl. XX, fig. 3 in W. Punt).

Fig. 3. — *Ricinus communis*, L., pollen grain in polar view (cf. Pl. XIX, fig. 5, in W. Punt).

Fig. 4. — *Euphorbia nutans*, Lag., pollen grain in polar view, outer aspects and sporoderm in optical section (2525x, orig.).

Fig. 5. — *Mercurialis ovata*, Sternb. et Hoppe, pollen grain in polar view, outer aspect and sporoderm in optical section (2525X, orig.).

Fig. 6. — *Euphorbia peplis* var. *erythrocaulos*, Delph., pollen grain in polar view, outer aspect and sporoderm in optical section (1280X, orig.).

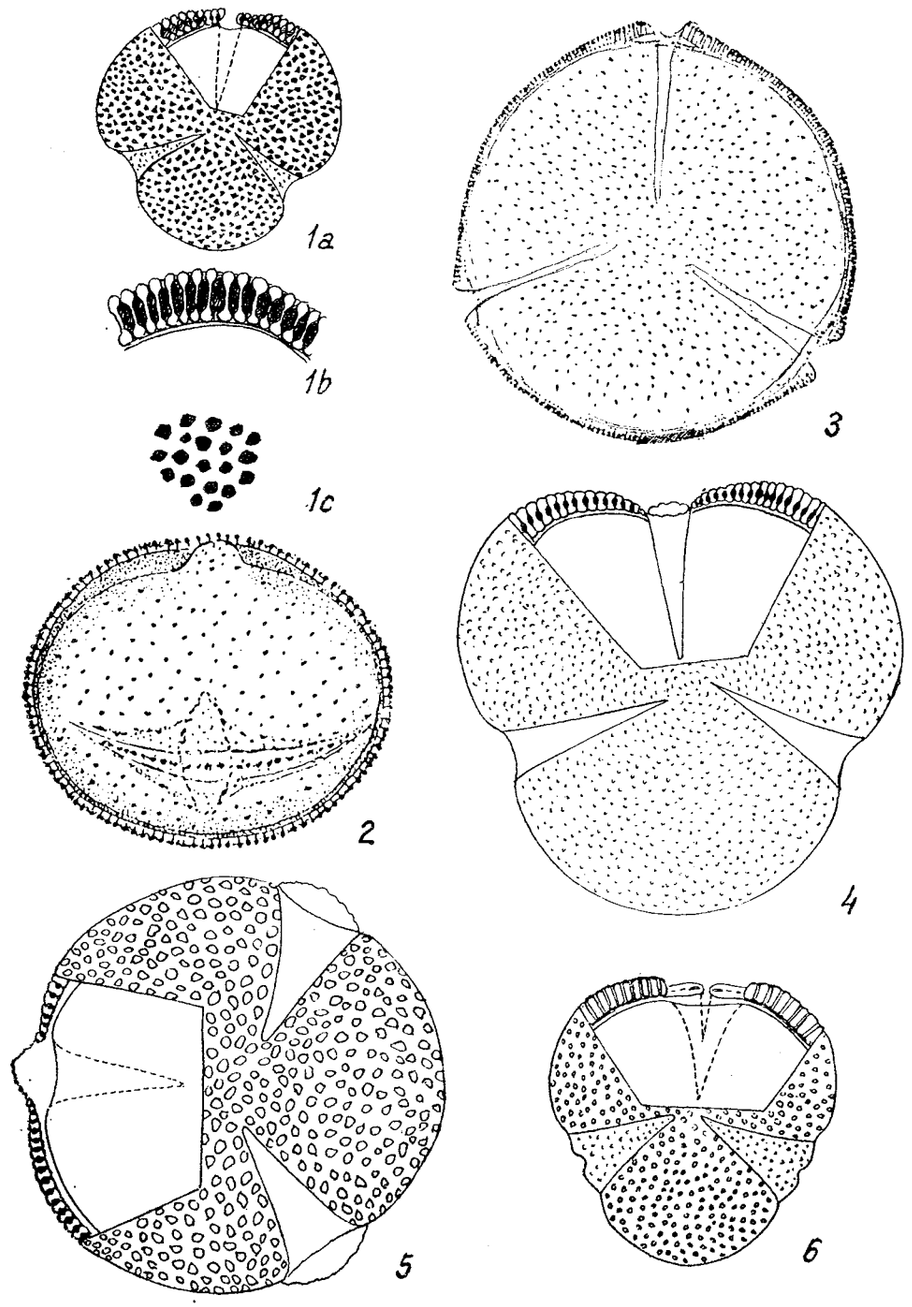

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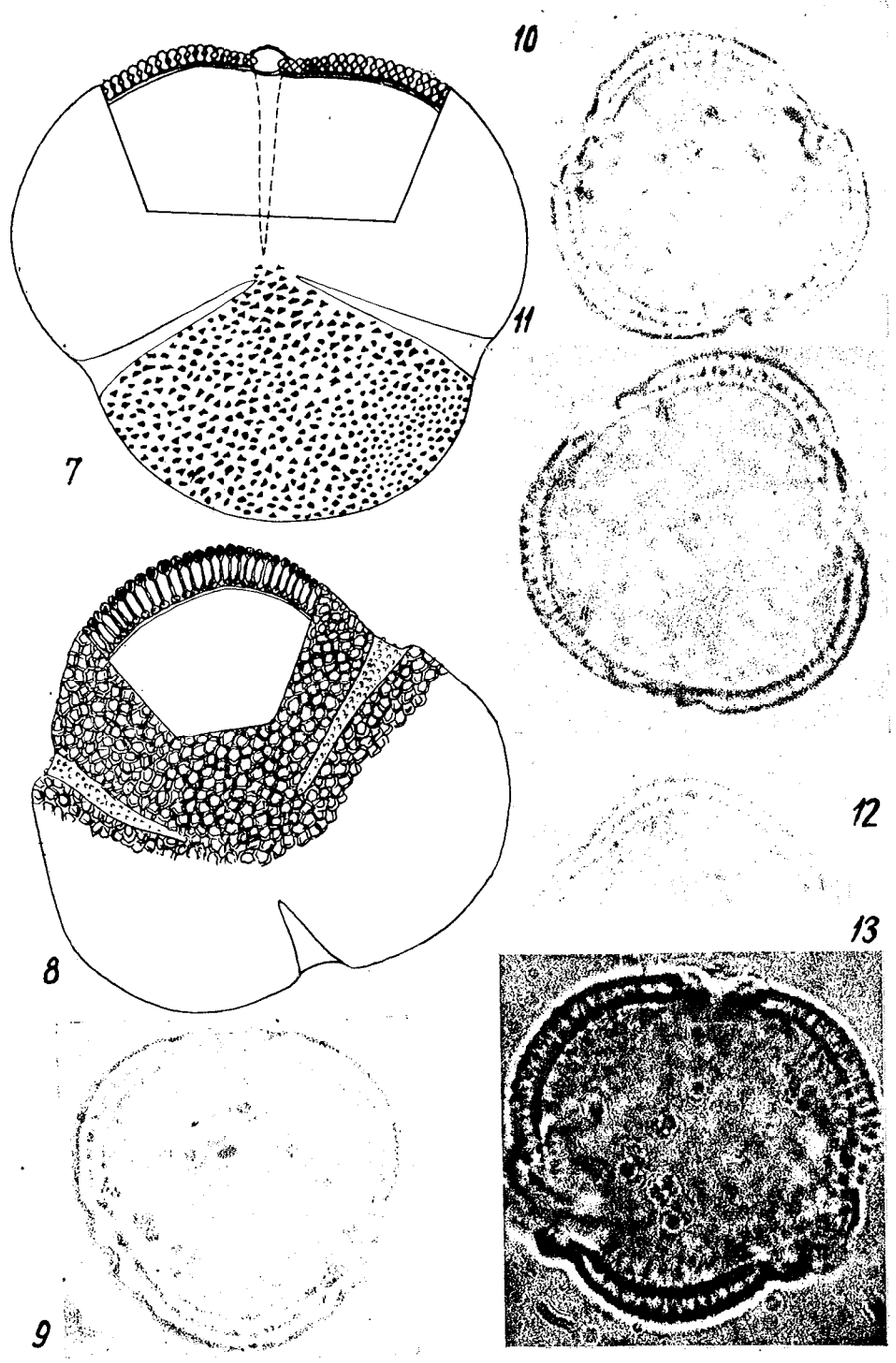
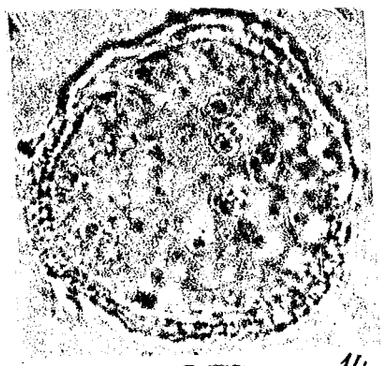
Plate II

Fig. 7. — *Euphorbia maculata*, L., pollen grain in polar view, outer aspect and sporoderm in optical section (2525 × orig.).
Fig. 8. — *Euphorbia valdevillosocarpa*, Arvat et E. I. Nyarady, pollen grain in polar view, outer aspect and sporoderm in optical section (1120 × orig.).
Fig. 9. — *Euphorbia dulcis*, L., pollen grain in polar view, sporoderm in optical section (1500 × orig.).
Fig. 10. — *Euphorbia carniolica*, Jacq., pollen grain in polar view, sporoderm in optical section (1500 × orig.).
Fig. 11. — *Euphorbia angulata*, Jacq., pollen grain in polar view with sporoderm in optical section (1500 × orig.).
Fig. 12. — *Euphorbia brittingeri*, Opiz et Samp., sector from sporoderm in optical section (1500 × orig.).
Fig. 13. — *Euphorbia palustris*, L., pollen grain in polar view, outer aspect and sporoderm in optical section (1500 × orig.).





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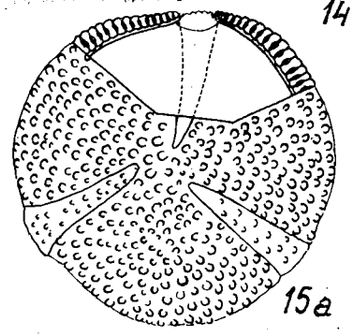

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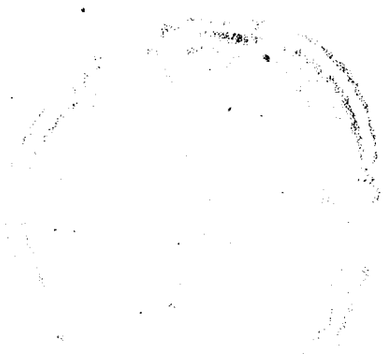

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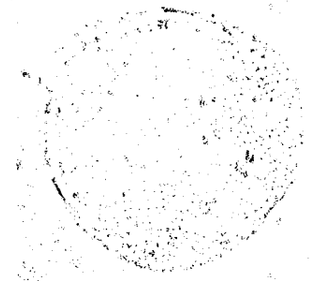

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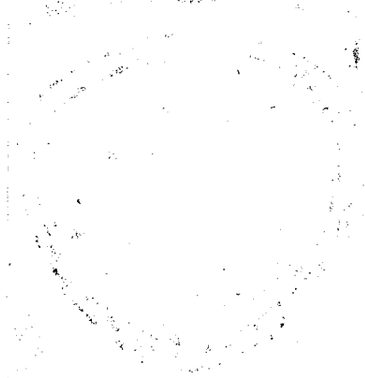

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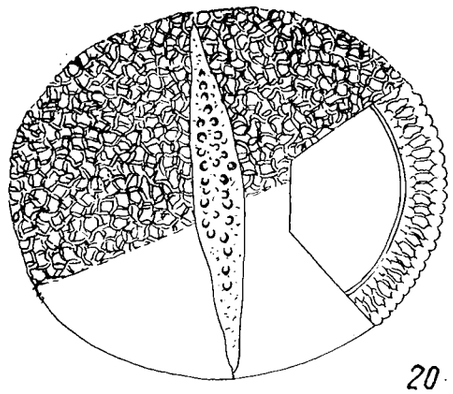

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Plate III

Fig. 14. — *Euphorbia segetalis*, L., pollen grain in polar view, outer aspect and sporoderm in optical section (1500 ×, orig.).

Fig. 15. — *Synadenium grantii*, Hock F. (*Euphorbia grantii* Oliv.) *a*, pollen grain in polar view, outer aspect and sporoderm in optical section ; *b*, sector from sporoderm' magnified (*a* = 1280 × ; *b* = 2525 ×, orig.).

Fig. 16. — *Euphorbia myrsinites*, L., pollen grain in polar view ; outer aspect (1500 ×, orig.).

Fig. 17. — *Euphorbia paralias*, L., pollen grain in polar view, with sporodermin o ptical section (1500 ×, orig.).

Fig. 18. — *Euphorbia platyphyllos*, L., pollen grain in polar view with sporoderm in optical section (1500 ×, orig.).

Fig. 19. — *Euphorbia nicaeensis*, All., pollen grain in polar view, outer aspect and sporoderm in optical section (1500 ×, orig.).

Fig. 20. — *Euphorbia agraria*, Bieb., pollen grain in equatorial view, outer aspect and sporoderm in optical section (1500 ×, orig.).

STIPA TENACISSIMA L., UNE ESPÈCE À GRANDE VALEUR ÉCONOMIQUE

PAR

P. RACLARU

The paper makes some chorological, ecological, morphophysiological and phytocenological remarks upon the *Stipa tenacissima* species, characteristic to the dry and half-dry regions in mediteranean Africa and meridional Spain.
Stipa tenacissima is appreciated for its economic value, especially in the paper industry.

Stipa tenacissima, connue dans les langues vulgaires de plusieurs peuples sous le nom d'*alfa* ou *halfa*, est une graminée caractéristique des régions arides et semi-arides de l'Afrique méditerranéenne et de l'Espagne méridionale, appréciée pour sa valeur économique dans l'industrie papetière et la sparterie.

RÉPARTITION GÉOGRAPHIQUE ET ÉCOLOGIE

Stipa tenacissima est un élément ibéro-maurétanien, répandu dans le nord de l'Afrique (Maroc, Algérie, Tunisie et Lybie) et le sud de l'Espagne, en climat méditerranéen aride et semi-aride. Elle est peu exigeante aux facteurs du milieu, à l'exception de la pluviométrie, pouvant faire appel à des facteurs compensateurs, tels que la pente du terrain dans le cas d'une précipitation abondante. L'alfa résiste tant au froid qu'à la chaleur estivale prolongée. L'optimum de développement est entre les isohyètes 200 et 400 mm, pouvant supporter jusqu'à 600 mm et respectivement 150 mm des précipitations moyennes annuelles.

L'alfa est sensible à la nature du substrat non pas tant par la composition du sol que par l'humidité du sol, l'humidité persistante étant un facteur défavorable de son développement. Il préfère un sol léger, sablonneux-caillouteux, qui assure un bon drainage. L'abondance des sels minéraux solubles, surtout des chlorures, constitue aussi un facteur défavorable du développement des touffes d'alfa.

Parmi les pays où cette espèce croît, l'Algérie occupe la première place, à une surface de 5 millions ha, dans la zone de steppe, à pénétrations irrégulières aussi dans le nord du Sahara.

Stipa tenacissima a été introduite aussi en Roumanie, par les jardins botaniques, où elle se développe et fructifie bien.

CARACTÈRES MORPHO-PHYSIOLOGIQUES

Stipa tenacissima croît en touffes, issues d'un rhizome, d'abord compactes et homogènes, puis elles deviennent annulaires par dépérissement

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des rameaux anciens centraux du rhizome. Les rameaux périphériques s'isolent au fur et à mesure que le cercle s'agrandit, donnant naissance à de nouvelles touffes.

Le rhizome est très rameux, présentant des entre-nœuds courts et des nœuds à racines adventives très ramifiées, courtes ; certaines racines s'allongent considérablement et pénètrent à grande profondeur. À la surface du sol, des groupes de 2 ou 3 rameaux (innovations) apparaissent enveloppés, sur une longueur de 15 cm environ, par les gaines foliaires successives des feuilles, qui camouflent l'axe caulinaire.

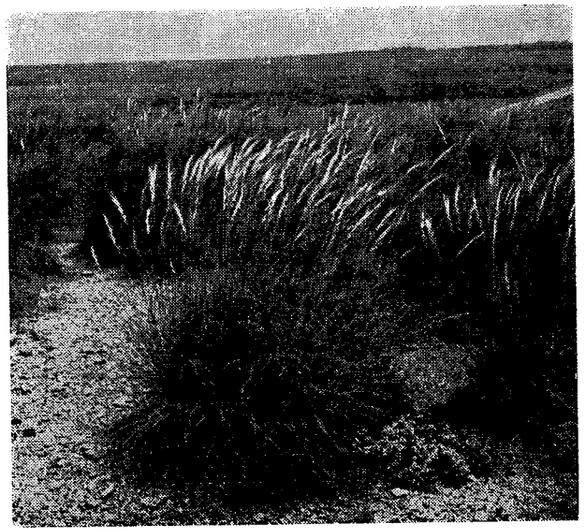

Fig. 1. — Touffes de *Stipa tenacissima* de la steppe d'Algérie.

Les feuilles présentent une gaine lisse, glabre ou plus ou moins velue, une ligule bi-auriculée, velue et un limbe de 25 à 125 cm de longueur, déterminant généralement un critère de qualité ; le limbe est presque plan en temps humide, condupliqué et junciforme par temps sec, aigu piquant. La face inférieure est lisse, à tissu hypodermique (sclérenchyme) continu, la supérieure est scabre, à 7 côtes très saillants (sclérenchyme développé). Le limbe peut être assez facilement détaché de la gaine, ce qui est mis à profit dans la cueillette manuelle.

Le chaume florifère se développe sur les rameaux les plus anciens du rhizome et peut atteindre 60 à 120 cm de hauteur.

L'inflorescence est une panicule allongée, longue de 25 à 35 cm, étroite, à épillets indépendants, comportant une fleur fertile. La glumelle supérieure (lemme) porte un arête genouillée, longue de 6 cm. L'alfa fleurit abondamment d'avril à juin. Les caryopses mesurent à maturité 5 à 8 mm de longueur.

La fécondation ne se fait que d'une manière très irrégulière, un petit nombre de fruits se développant dans une inflorescence. Il arrive aussi que les fruits se développent souvent incomplètement, ils sont ridés

et comme desséchés avant maturité, signe d'épuisement de la plante qui manque de réserves nécessaires pour la maturation des ovaires.

La faible fertilité de l'alfa est due soit à des facteurs climatiques, soit à un vieillissement génétique de l'espèce, soit aux effets néfastes provoqués par la blessure due à l'arrachage des feuilles à certaines époques de l'année. On peut ajouter la constatation que la diminution du nombre de fruits a lieu dans les régions à pluviométrie inférieure à 200 mm.

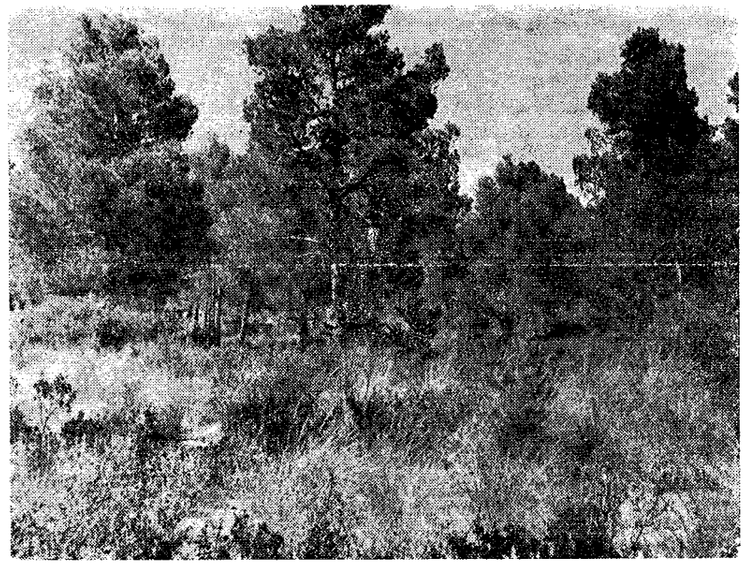

Fig. 2. — Transition entre la forêt de *Pinus halepensis* et *Stipetum tenacissimae* à Djelfa (Algérie).

PHYTOCÉNOLOGIE

Les nappes alfatières résultent dans leur évolution de la dégradation progressive de la forêt à *Pinus halepensis*. À présent on peut voir des transitions entre la forêt de *Pinus halepensis* et les phytocénoses de *Stipa tenacissima*, représentées par un mélange de plantes à forêt (*Pinus halepensis*, *Juniperus oxycedrus*, *J. phoenicea*, *Quercus ilex*, *Rosmarinus officinalis*, *R. tournefortii*, *Globularia alypum*, *Phyllirea angustifolia*, *Cistus salvifolius*, *C. libanotis*, *Pistacia lentiscus*, etc.) et plantes à steppe, parmi lesquelles domine *Stipa tenacissima*.

L'aspect actuel des phytocénoses à *Stipa tenacissima* est très monotone, celles-ci étant dominées par *Stipa tenacissima*, d'où le nom vulgaire de « mers d'alfa ». En printemps on développe parmi les touffes d'alfa des espèces annuelles et géophytes, comme : *Micropus bombycinus*, *Atractylis cancellata*, *Eruca vesicaria*, *Valerianella coronaria*, *Alyssum linifolium*, *A. scutigerum*, *A. macrocalyx*, *Xeranthemum inapertum*, *Echinaria capitata*,

Medicago hispida, *Thalaspi perfoliatum*, *Chrysanthemum fuscatum*, *Pallenis spinosa*, *Tulipa silvestris*, etc. Celles-ci disparaissent rapidement, restant seulement certaines espèces vivaces, xérophyles, comme : *Artemisia herba-alba*, *Atractylis humilis*, *Koeleria vallesiana*, *Thymelea tartonraira*, *Alyssum*

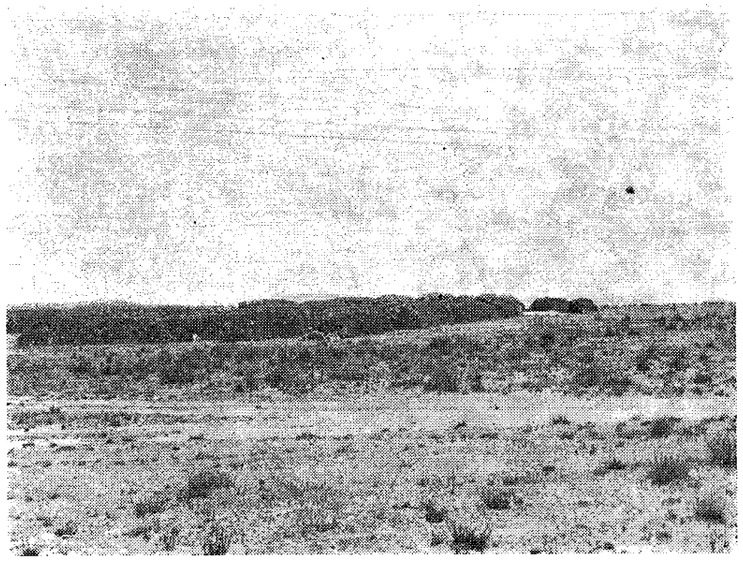

Fig. 3. — Stocks d'alfa dans la steppe d'Algérie.

serpyllifolium, *A. cochleatum*, *Erysimum grandiflorum*, *Launea acanthoclada*, *Polycnemum fontanesii*, *Phagnalon rupestre*, *Thymus ciliatus*, *Helianthemum pilosum*, *H. cinereum* ssp. *rubellum*, *Arabis nova*, *Pituranthos scoparius*, *Dianthus caryophyllus*, etc.

IMPORTANCE

Stipa tenacissima est une excellente plante industrielle, les fibres cellulosiques que les feuilles contiennent étant appréciées tant sous l'aspect quantitatif que sous celui qualitatif. Les feuilles d'alfa fournissent la matière première de qualité supérieure pour l'industrie papetière. La production annuelle de l'alfa vert en tonnes est la suivante : l'Algérie 100 000, 20 kg/ha en moyenne, le Maroc 80 000, la Tunisie 65 000, l'Espagne 25 000, la Lybie 10 000.

Dans les conditions d'une exploitation plus rationnelle et de l'amélioration des terrains, la production annuelle sera doublée et même triplée.

La cueillette des feuilles d'alfa est faite par l'arrachage des feuilles, rarement par fauchage, le dernier affectant la régénération de l'alfa ; la cueillette par machines à cueillir est faite surtout dans les conditions de la culture d'alfa (Espagne).

Les feuilles d'alfa sont utilisées aussi pour la sparterie, pouvant substituer le jute *(Corchorus olitorius, C. capasularis)*, le kenaf *(Hibiscus cannabinus)*, le chanvre *(Cannabis sativa)*, le chanvre de Sisal *(Agave sisalana)*, etc.

En certaines régions l'alfa est cueilli dans le but de l'utilisation comme nourriture des chèvres et des chameaux pendant la saison défavorable, ou comme combustible.

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TRANSPIRATION AND WATER CONTENT IN SOME FLOOD PLAIN SPECIES

BY

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The paper presents the day and season dynamics of transpiration and water content in some herbaceous and woody species in the Ciorogîrla flood plain. The saturation deficit and the influence of different quantities of underground water on the plant hydric regime are calculated.
The populations of *Rubus caesius* and *Phragmites australis* keep their polyhydric and poikilohydric character, irrespective of their distance from the water and the species *Salix alba*, *Amorpha fruticosa*, *Lysimachia nummularia* and *Lolium perenne* are oligohydric and isohydric.
All species present a diurnal curve of transpiration type with more maximum periods.

The water intake at plant level reflects the possibilities to supply water from underground layers and plants capacity to absorb the necessary quantity according to the existing conditions. On the river flood plains the sandy underground retains water but the level of the underground water is very high and assures good water supply even within periods of reduced rains.

Ecophysiological investigations in the flood plains of our country are reduced in number [8], [4] and the results require much discussion.

In the present paper we intend to consider whether transpiration and water content of flood plain plants under field conditions indicate good supply for the station or secondary ecological factors of restrictive effects are involved.

MATERIAL AND METHODS

The investigations were carried ont in the Ciorogîrla flood plain near the Domnești village (Ilfov). The flood plain forest is a narrow piece of land of 10—15 m in the neighbourhood of agricultural fields.

Different individuals at various distance from the water were investigated :

a) in the flood plain forest, 1 m from the water ;

b) in the flood plain forest, 5 m from the water ;

c) the land of the usual vegetation of fallow between the flood plain forest and agricultural fields.

For testing the hydric regime the following were used :

— transpiration in characteristic species, the most abundant being : ***Phragmites australis*** and ***Rubus caesius*** in the two zones of the flood plain

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(a, b), *Salix alba* in zone *a*, *Amorpha fruticosa* and *Lysimachia nummularia* in zone *b*, and for the periphery of the culture *Lolium perenne*;

— determination of water content was made in a large number of plants, about 20.

Transpiration was determined by the Huber-Ivanov method and the water content by the gravimetrical method, drying at 85°C and comparing the result with the fresh weight.

The microclimatical measurements were made concomitantly with the determination of physiological processes air temperature at 50 cm height, soil temperature at 10 cm depth, air humidity measured by psychrometer Assmann, Piché evaporimeter measured evaporation and the degree of light using the luxmeter with the photo-electrical compensated cell of L.A.P. type.

Soil relative humidity was determined by the gravimetrical method drying at 105°C and comparing the result with the fresh weight. The determinations were made monthly, from May until September 1976.

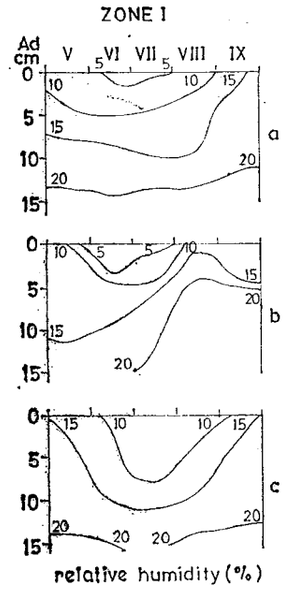


Fig. 1. — Chronoisopleta of underground in the Ciorogîrla flood plain: a) near water; b) middle of flood plain; c) margin of cultures.

RESULTS AND DISCUSSIONS

1. SOIL HUMIDITY

The three microstations, differentiated by the depth of the underground water and distance from the shore, illustrate the active and positive dynamics of the water retained at the surface of the underground (Fig. 1).

Although the rains were sufficient this year, the underground humidity was reduced at the surface and higher with depth.

Water quantity is much more reduced at the surface of the underground zones *(a)* and *(b)* as compared to *(c)*, because of sandy soils; the herbaceous vegetation, which is better organized in zone *(c)*, protects the surface against excessive evaporation.

During the vegetation season in June and July, the most reduced values of underground humidity are recorded because of intense evaporation and absorption since precipitations were average and permanent.

In the conditions analysed, the supply of the underground at the surface is less due to precipitations and mainly to the supply from the underground water, by the capillary ascension produced on 80—150 cm height.

2. WATER CONTENT IN PLANTS

2.1. Average level

The values of plants water content with plant saturation and permanent lack of saturation (table 1) are rather contradictory.

Table 1

Some hydric characteristics of flood plain plant species

Species	Station (distance to the river)	Mean value: Water content in saturation state %	Mean value: Water saturation deficit %	Mean value: Transpiration average/day mg/g/h
I. *Herbaceous and semibush species in flood plain*				
— Phragmites australis	A	75.50	12	463.1
— Phragmites australis	M	79.30	17	402.1
— Lysimachia nummularia	M	85.10	10	253.8
— Aegopodium podagraria	M	89.00	3	—
— Galium palustre	M	85.45	—	—
— Aristolochia clematites	M	80.50	17	—
— Rubus caesius	A	75.90	18	622.0
— Rubus caesius	M	76.10	25	420.0
II. *Woody and semiwoody species in flood plain*				
— Populus nigra	M	63.60	—	—
— Populus alba	M	62.80	—	—
— Alnus glutinosa	A	65.70	10	—
— Salix alba	A	67.70	—	356.2
— Amorpha fruticosa	A	65.70	4	275.4
— Robinia pseudacacia	M	65.60	—	—
— Cornus sanguinea	M	76.30	—	—
— Crataegus monogyna	M	63.10	—	—
— Humulus lupulus	M	76.10	—	—
III. *Herbaceous species on the margin of cultures*				
— Lepidium draba	m	80.00	50	—
— Asparagus tenuifolius	m	78.86	11	—
— Chaerophyllum temullum	m	81.00	—	—
— Salvia nemorosa	m	78.50	49	—
— Euphorbia cyparissias	m	78.79	—	—
— Lolium perenne	m	87.00	44	323.9
— Setaria glauca	m	79.00	—	—
— Cochorium intybus	m	77.81	—	—
— Veronica chamaedrys	m	77.24	—	—

A = near water M = middle of flood plain m = margin of cultures

Under saturation conditions, the values of flood plain plants and those on the margin of cultures in the Ciorogîrla flood plain forest are high and very close to water content; herbaceous plants have by 15% more water in their leaves than woody plants. Herbaceous plants in the flood plain, which are included into a wide variation range of this para-

meter, have high values : 89% in *Aegopodium podagraria* and only 75% in *Phragmites australis* because of the differences in chemical composition and cellular structure.

The values of water content with the saturated species investigated in the Ciorogîrla flood plain are comparable with those found in other plants in the Prahova flood plain [4], being by 2% smaller in *Populus* and *Salix* and by 7% larger in *Rubus*.

The herbaceous plants in the vicinity of the cultures on the Ciorogîrla flood plain present greater uniformity than those in the river-side forest, most values being about 79%.

2.2. Saturation deficit

The saturation deficit reflects a clear difference between the herbs of the river-side forest and those in the vicinity cultures; the latter reach strong dehydration (more than 40%) as compared to the average values (15%) in the former (table 1) although the possibility of water supply is practically identical in the two microstations. The saturation deficit is smaller in individuals situated right on the river-side with both *Phragmites australis* and *Rubus caesius*.

As compared to other forest species, those investigated by us have high saturation deficit in the river-side forest. W. Larcher [5] quotes *Pulmonaria officinalis* with only 6% or *Oxalis acetosella* with 14% saturation deficit and *Fragaria vesca* with 25%.

2.3. Diurnal dynamics of water content

The dynamics of water content in river-side forest species is obtained at large amplitude during a whole day, between 5—10% (Fig. 2), especially in the graminea. Maximum dehydration is obtained during a day by 2 p.m. in all species and its transient character is marked by a complete rehydration at 5 a.m.

The diurnal variation of water quantity is more reduced in individuals on the border river-side than in those in the river-side forest (Fig. 2).

2.4. Season dynamics of water content

Along the vegetation period, the end of July marks the driest stage of the plant this year. *Lolium perenne* is remarked by very strong dehydration which is not reversible to the same offsprings (Fig. 3a).

In August and September the species present an increased process of humidity, which corresponds to the newly formed offsprings but it does not equal the one in spring months.

In the woody species, where the water content was recorded only in leaves, dehydration continues until autumn months at slower rate and with reduced amplitude (Fig. 3b).

The analysis of plant water content indicates an insufficient supply in water, at least in the late vegetation period, and the phenomenon is stronger with cultures on the margin.

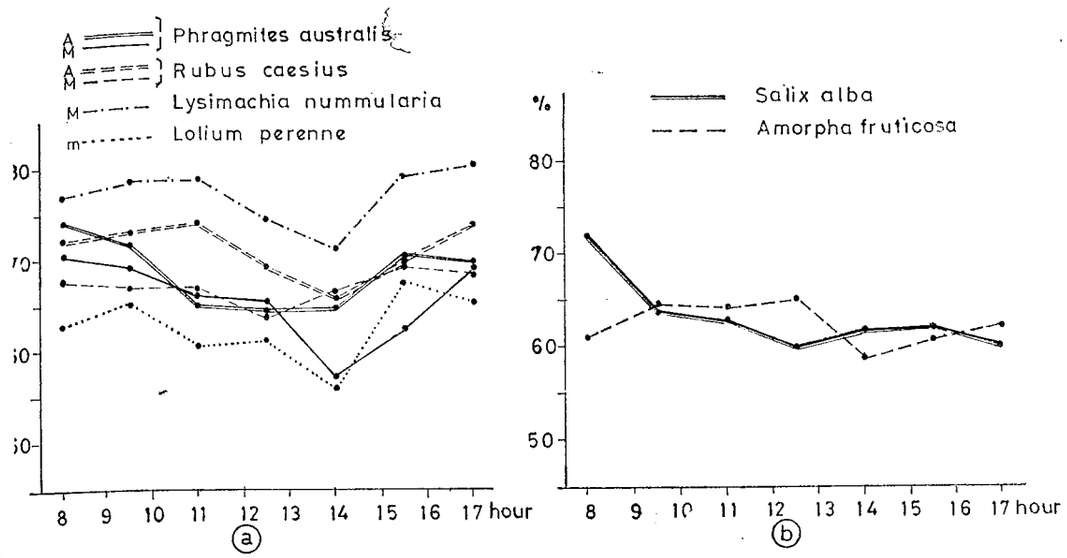


Fig. 2. — Diurnal dynamics of water content in leaves in the summer time A — near water; M — middle of flood plain; m — margin of cultures.

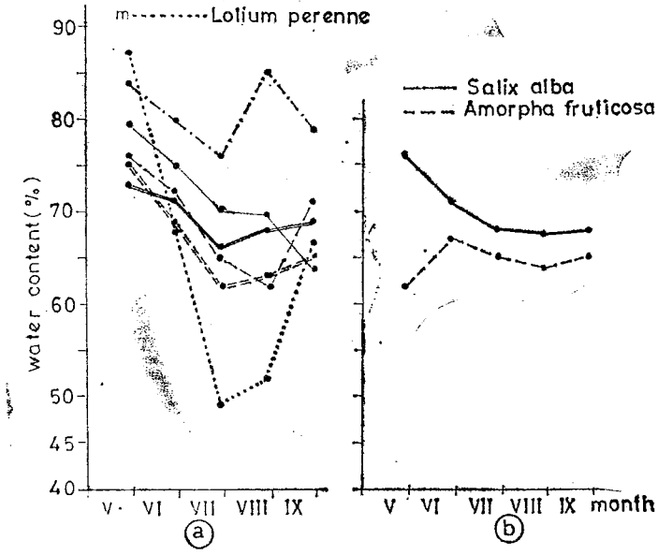


Fig. 3. — Season dynamics of water content in years by offsprings A — on the border of the river; M — in the middle of the flood plain; m — margin of cultures.

It also shows us that the water physically existent, as measurements show, should be kept in the underground by other factors that hinder absorption and the reduction of water in offsprings at the end of summer is an effect of their ageing.

3. PLANT TRANSPIRATION

3.1. Average level

The transpiration of plants investigated in the river-side forest and on the vicinity of cultures presents a generally reduced and very differentiated level, function of species and microconditions (Table 1).

The highest values of transpiration are observed in *Rubus caesius* and *Phragmites australis*, especially when close to the river.

Under the same conditions, the woody species have transpiration values by 1/3 lower. *Lysimachia nummularia* is remarked for its large water content, good water supply for the underground and best economy in the use of transpiration; even the representative species for the vicinity cultures *(Lolium perenne)* has an average value higher than the former.

This phenomenon is pointed out in other species and in stations with excessive humidity; Dihoru and Brezeanu [3] in *Schoenoplectus lacustris*, the average diurnal transpiration being 211 mg/g/h.

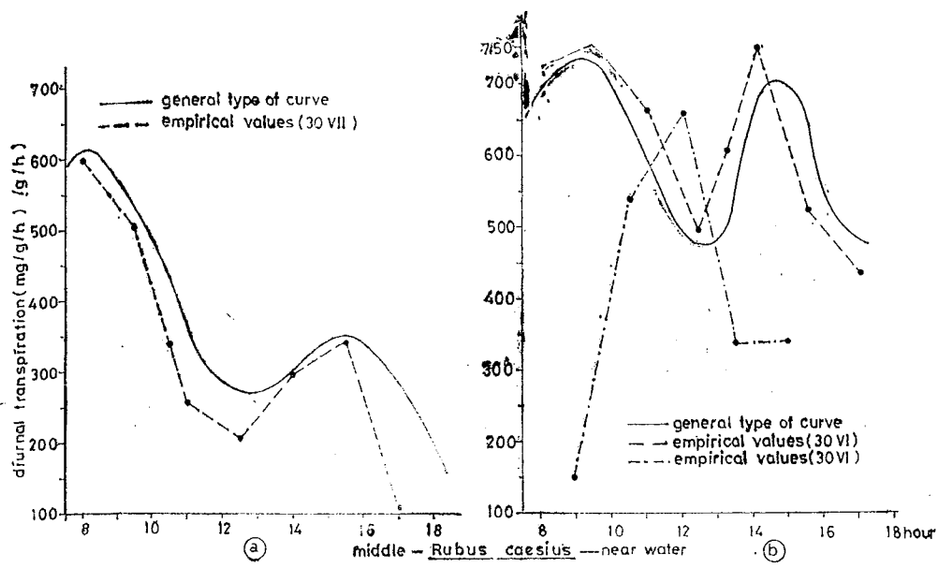
Similar investigations carried out in the Prahova flood plain showed higher level of transpiration in herbaceous species, as compared to woody ones. This level is also reflected in our data [4]. All investigated species may belong to the category of oligohydric species with transpiration under 450 mg/g/h [1] excepting *Phragmites australis* and *Rubus caesius*, which, because of their high transpiration values, 450 mg/g/h, belong to polyhydric species.

We remark that in other stations [3], *Phragmites australis* has higher transpiration values.

3.2. Diurnal dynamics

Throughout a day, transpiration dynamics illustrates the intensity of exchanges with environment in the period of light. The physiological phenomenon is well known but it presents a specific character for any changed parameter. Fig. 4 presents graphically the theoretical curve of transpiration for some species investigated in the flood plain together with more representative empirical curve. The measurement of diurnal transpiration was made in June and July 30, the typical summer days, for which we present the curves of main climatical factors (Fig. 5). Their specific and almost regular dynamics were observed. In these conditions, the transpiration curve reflects the reaction of plants according to their necessity and possibility of adaptation.

We can see that all species belong to plants that restrict their transpiration; *Rubus caesius* (Fig. 4a, b). *Lolium perenne* (Fig. 4c) and *Amorpha fruticosa* (Fig. 4d) present the type of curve with 2 maxima, the first at about 8—9 a.m. and the second at 12 a.m.—1 p.m.; *Salix alba* (Fig. 4f)



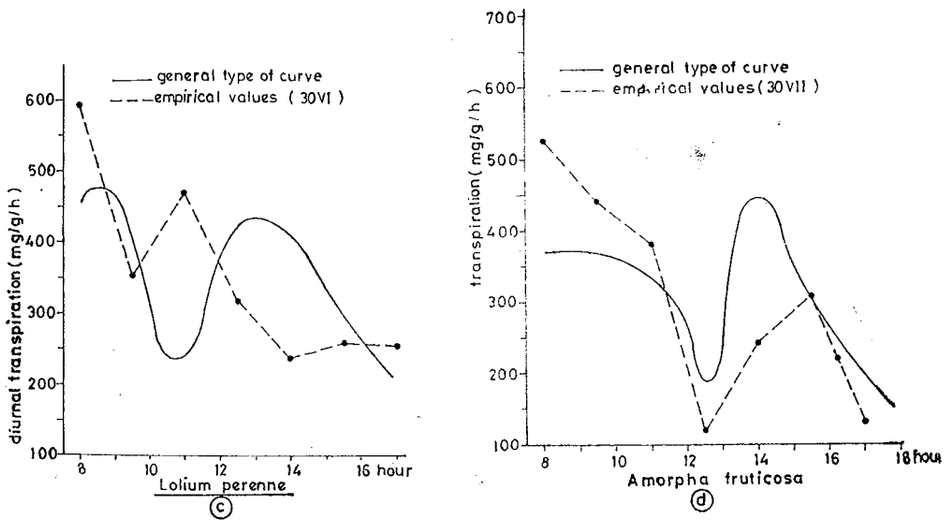


Fig. 4. — Diurnal dynamics of transpiration in the Ciorogîrla flood plain.

presents a curve with smooth maxima which are closer to the type of typical isohydric curve with temporary intensification of transpiration, at 9 a.m. in *Salix*, at 3 p.m. in *Lysimachia*, after the danger of loosing water by evaporation was removed.

Phragmites australis presents a special type of curve with evident instability of transpiration (Fig. 4a, b) having a supplementary maximum of transpiration in the afternoon hours (4—5 p.m.).

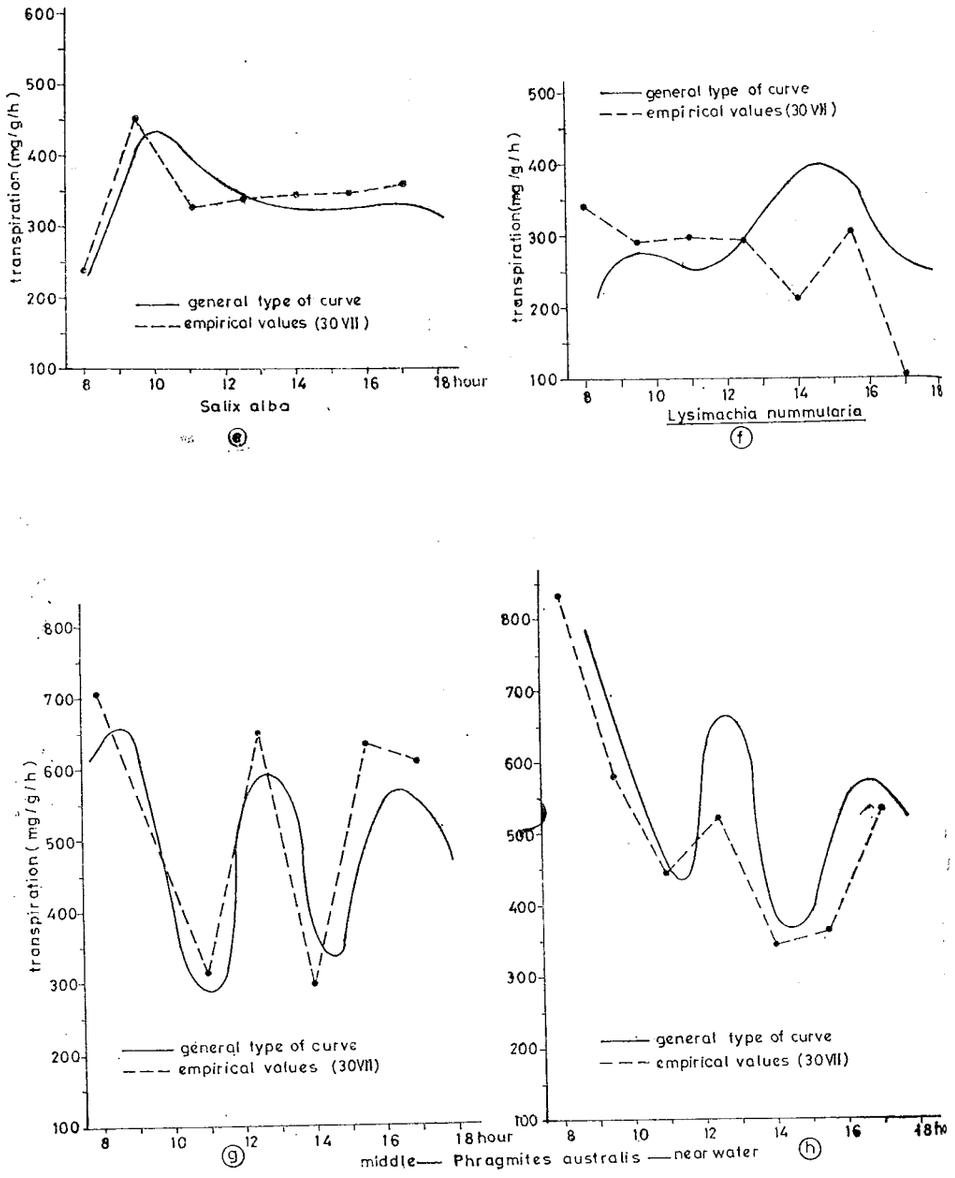


Fig. 4

The presence of a permanent source of water may be a factor that allows rapid rehydration of cells. In some species, during the turgidity period, transpiration may present uniform variation, with only one maximum, following the evaporation curve (Fig. 4b).

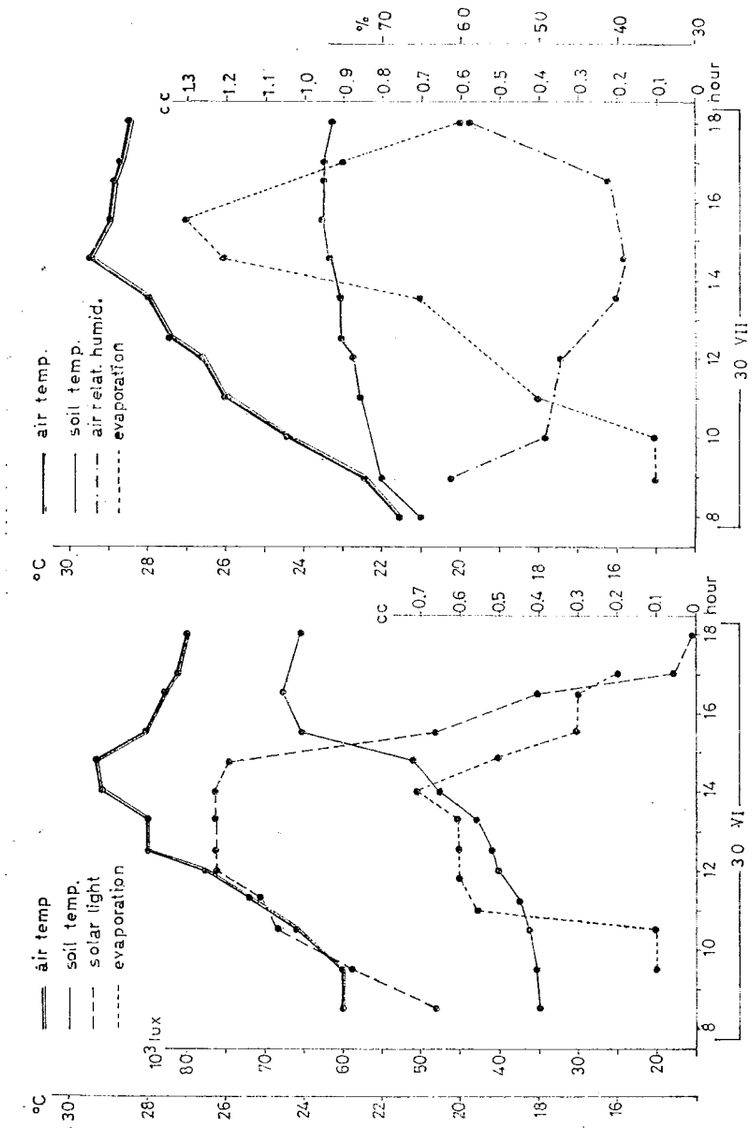


Fig. 5. — Diurnal dynamics of the main microclimatic factors.

3.3. Relative transpiration

Transpiration of the investigated species should be considered high, at least in the morning, because as we have notice the relative transpiration $\left(\frac{T}{E} \times 100\right)$ has very high values (Fig. 6). *Rubus caesius* and *Phragmites australis* are remarked, as they may have high values, equal to evaporation in the hours with maximum transpiration.

The experimental research in the Danube Delta showed that in marshes covered with reed, the quantity of water that evaporates is larger that in independent lakes [8] but the primary cause does not appear so evident.

Lysimachia nummularia and *Lolium perenne* present the most reduced relative transpiration among the tested species, having however high values. So, in this respect, transpiration was a little lower as compared to evaporation.

Similarly, as evaporation was quite uniform, transpiration had minimum periods, that pointed out the capacity of these species to control water intake (Fig. 6).

Transpiration of flood plain forest species is not higher than that of other species in forest or plain zones [3], but they exceed those in the mountain zone [1], [6].

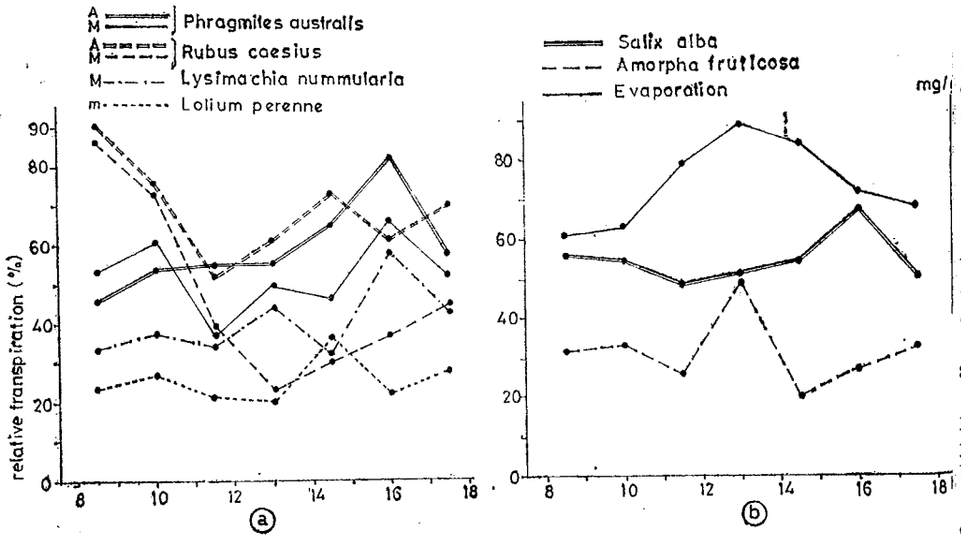


Fig. 6. — Diurnal dynamics of relative transpiration
a) herbaceous species; *b*) woody species.

3.4. Season dynamics of transpiration

During the vegetation season, transpiration develops like other physiological processes, with an estival maximum and a decrease in intensity to the end of the vegetation period (Fig. 7).

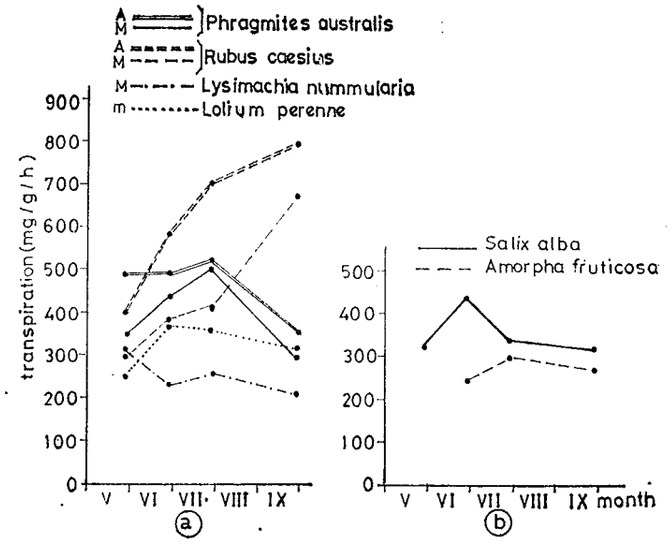


Fig. 7. — Seasonal dynamics of transpiration
a) herbaceous species; *b*) woody species.

The maximum transpiration was generally obtained in July, but in *Lolium perenne* and *Salix alba* the value is higher in June. A special case is represented by *Rubus caesius*, that records an abnormal value in September, perhaps because of premature osmotic pressure intensification [7].

CONCLUSIONS

1. In the flood forest species with high transpiration, that is high water intake, the temporary saturation deficit is more advanced but it is quickly compensated as a consequence of higher absorption *(Phragmites australis)*.

2. In species with reduced transpiration all processes are moderate and the saturation deficit is less advanced *(Lysimechia nummularia)*.

3. Transpiration character, namely, the type of diurnal curve, is more constant in comparison with the species than in comparison with microstational differences, and process intensity is higher in populations better provided with water *(Rubus caesius)*.

4. The limits imposed on transpiration are in climatically stable days caused by physiological limits of absorption (the underground water is enough but is retained under the soil by strong forces because of solubilized substances).

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LA DYNAMIQUE DE L'INTENSITÉ DE LA RESPIRATION ET SES RELATIONS AVEC L'INTENSITÉ DE LA TRANSPIRATION ET AVEC LE DÉFICIT HYDRIQUE, CHEZ QUELQUES ESPÈCES DE PLANTES

PAR

ELENA JEANRENAUD

In 34 species of plants from different ecophysiological groups, the dynamics of respiration, water deficit and transpiration intensity which depends on age has been studied. With some species, this process has also been investigated during the day. The relationships established among these processes and their importance are discussed, as well as the dependency of the transpiration degree on respiration intensity.

Ces derniers temps l'idée que la transpiration est un phénomène de transport actif, qui se réalise avec dépense d'énergie procurée par la cellule, gagne du terrain [2] [3] [7] [11]. C'est pourquoi il faudrait qu'il existe un parallélisme entre la variation de l'intensité de la transpiration et celle de la respiration, processus qui fournit l'énergie libre nécessaire pour l'accomplissement des fonctions vitales. Mais A. M. Alekseev [1] constate l'existence d'une corrélation négative entre le mouvement de translation de l'eau et l'intensité respiratoire et conclut que le courant de transpiration ne dépend pas de l'énergie de la respiration, mais de l'énergie solaire. En même temps, le degré de satisfaction des tissus en eau, qui est indiqué par le déficit hydrique, se répercute sur les processus de respiration [4] [5] [6]. En partant de ces données nous nous sommes proposé d'investiguer les relations de dépendance qui s'établissent entre l'intensité de la respiration, d'une part, et le déficit hydrique et l'intensité de la transpiration, d'autre part, dans la dynamique de ces processus dans la nature.

MATÉRIEL ET MÉTHODES

On a déterminé le déficit hydrique (DH) par la méthode de Stocker [10] (exprimé en % de la réserve d'eau à la saturation), l'intensité de la transpiration (IT) par la méthode Huber-Ivanoff (exprimée en mg eau perdue par 1 g p.fr./1′) et l'intensité de la respiration (IR) investiguée par la méthode manométrique avec l'appareil Warburg, à la température de 25°C (exprimée en ml O_2/g p.s./30′) ; les données ont été enregistrées sur 34 espèces de divers groupes écophysiologiques du littoral de la Mer Noire, spécialement des dunes. Ces trois processus ont été investigués dans des conditions naturelles, en diverses phases ontogéniques, le matin, entre 7—8h, et pour quelques espèces, le matin, à midi et le soir ; pour 10 espèces on a poursuivi la dynamique quotidienne (d'une heure à l'autre, de 6—18h) des processus respectifs.

RÉSULTATS ET DISCUSSION

Les résultats sont inscrits dans les tableaux 1, 2, 3, et les figures 1, 2, 3, 4, 5, 6, 7, 8.

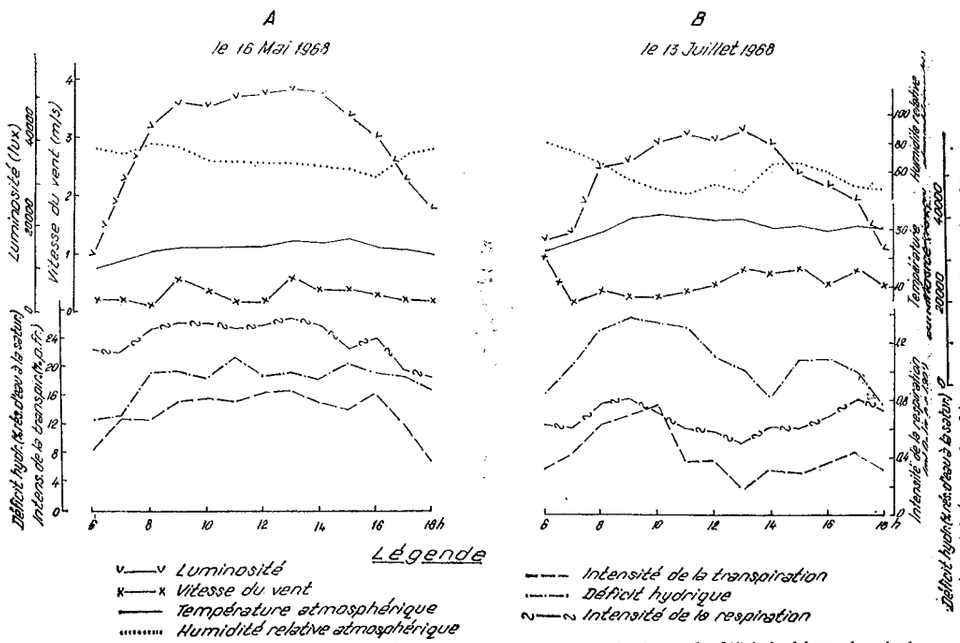

Fig. 1. – La dynamique journalière de l'intensité de la respiration, de la transpiration et du déficit hydrique chez *Anchusa procera* Bess.; A – phase de boutons, B – floraison – fructification

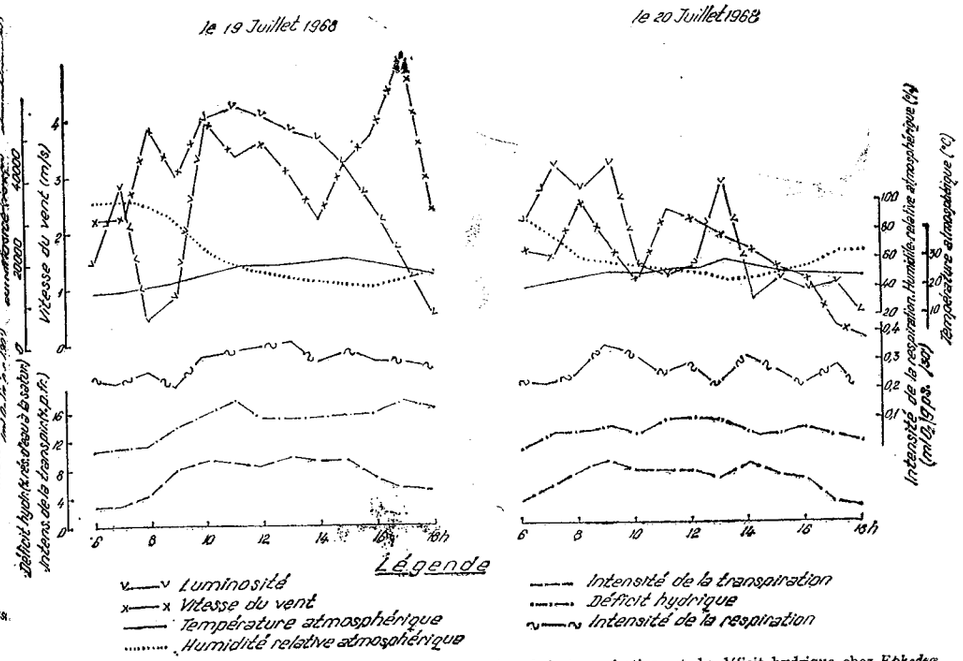

Fig. 3. – La dynamique journalière de l'intensité de la respiration, de la transpiration et du déficit hydrique chez *Ephedra distachya* L. pendant la floraison; – A – pieds femelles, B – pieds mâles.

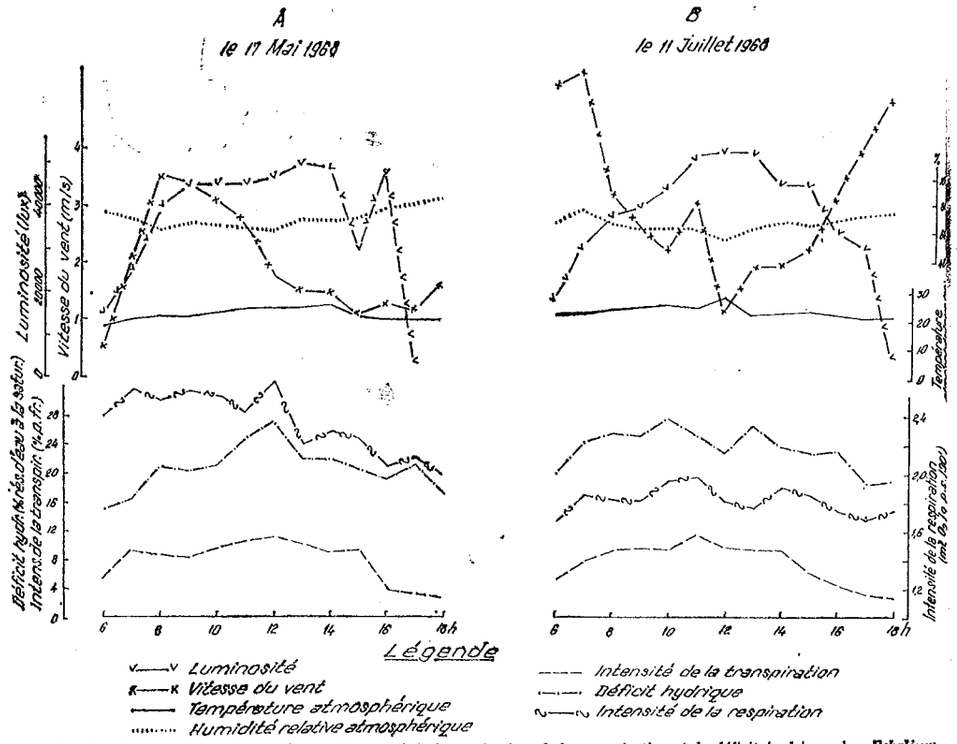

Fig. 2. – La dynamique journalière de l'intensité de la respiration, de la transpiration et du déficit hydrique chez ***Ecballium elaterium*** (L.) Rich.; A – phase végétative, B – floraison.

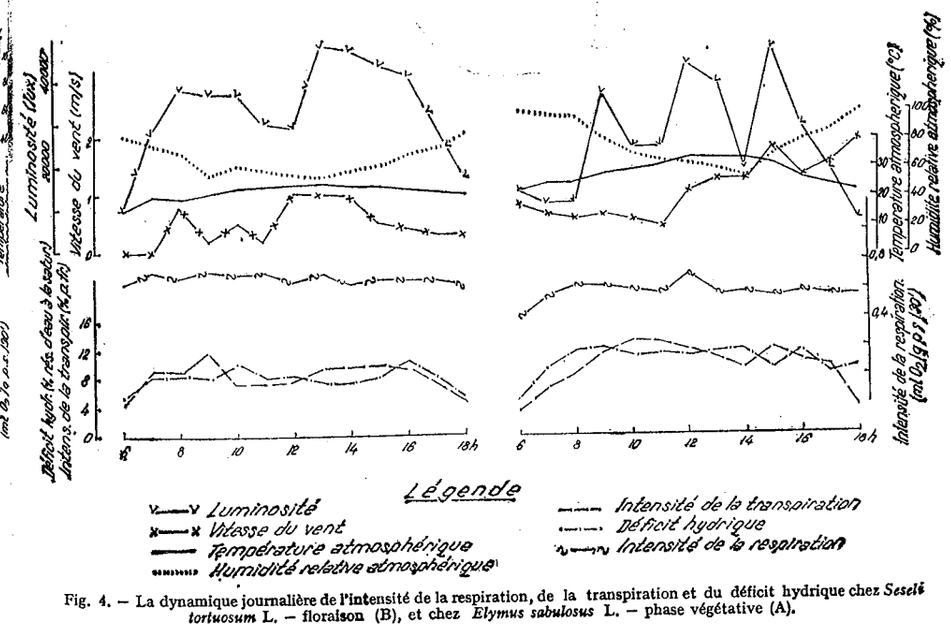

Fig. 4. – La dynamique journalière de l'intensité de la respiration, de la transpiration et du déficit hydrique chez *Seseli tortuosum* L. – floraison (B), et chez *Elymus sabulosus* L. – phase végétative (A).

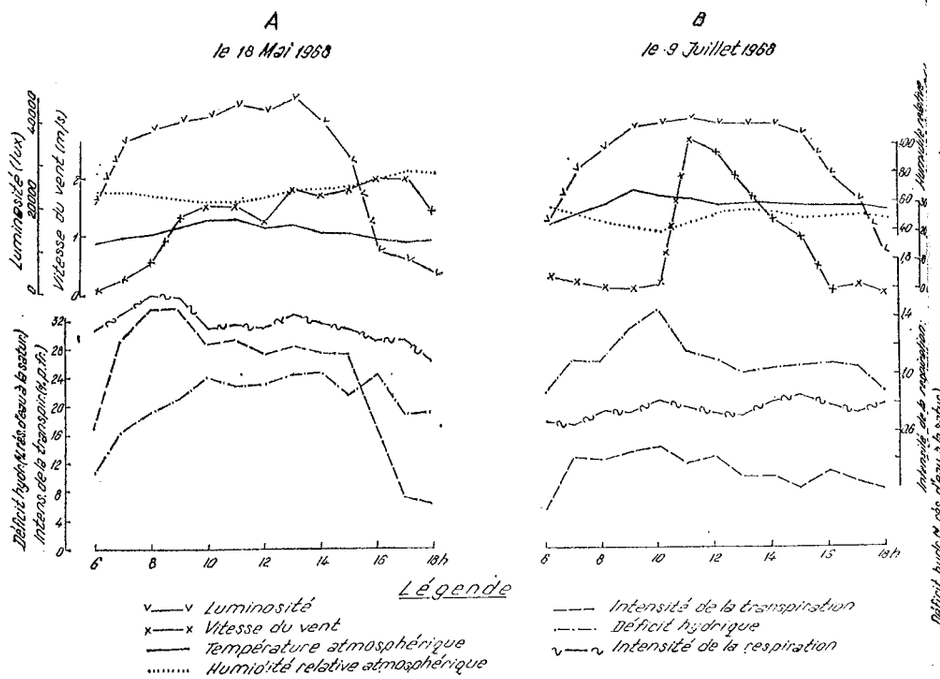

Fig. 5. — La dynamique journalière de l'intensité de la respiration, de la transpiration et du déficit hydrique chez *Marrubium peregrinum* L.; A — phase végétative, B — floraison.

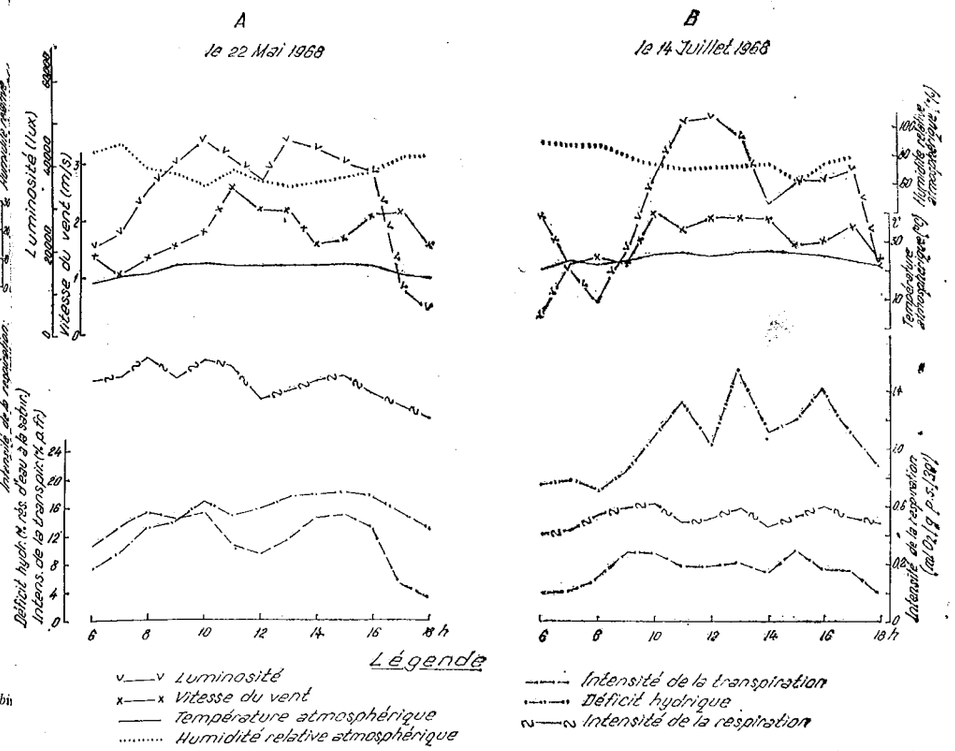

Fig. 7. — La dynamique journalière de l'intensité de la respiration, de la transpiration et du déficit hydrique chez *Teucrium chamaedrys* L.; A — phase végétative, B — floraison.

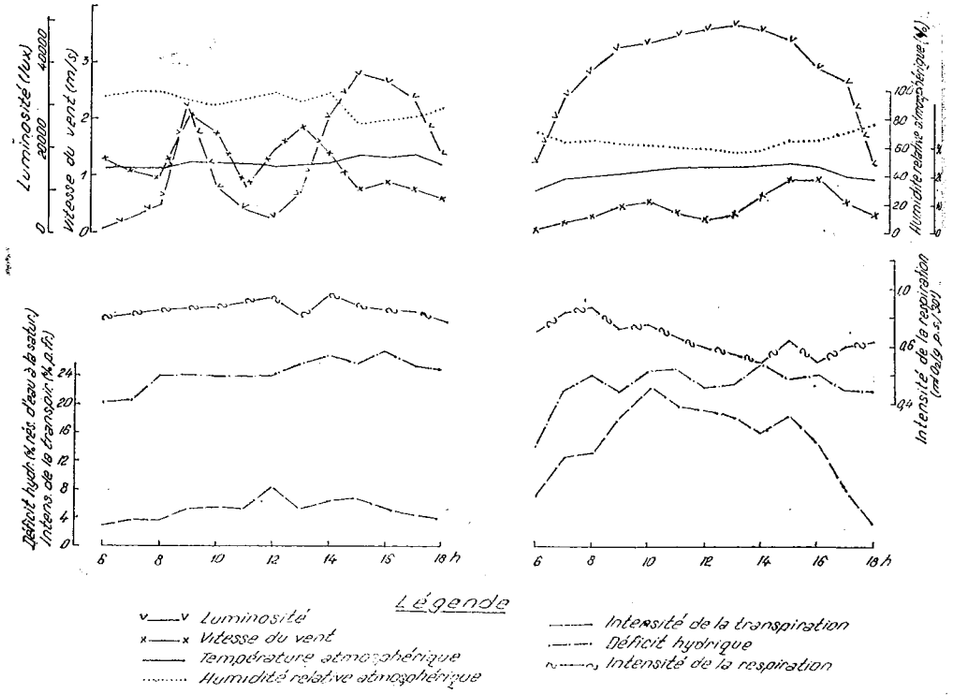

Fig. 6. — La dynamique journalière de l'intensité de la respiration, de la transpiration et du déficit hydrique chez *Atriplex nitens* Schk. — floraison (A), et *Convolvulus persicus* L. — phase végétative (B).

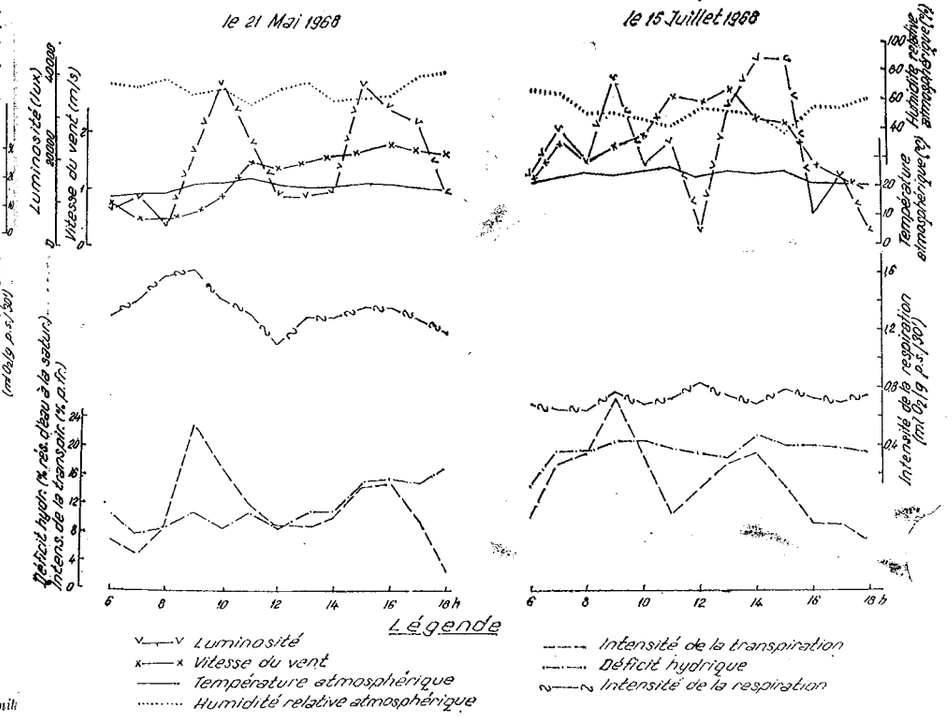

Fig. 8. — La dynamique journalière de l'intensité de la respiration, de la transpiration et du déficit hydrique chez *Inula germanica* L.; A — phase végétative, B — floraison.

Tableau

L'intensité de la respiration et ses corrélations avec le déficit hydrique

L'espèce	Date et phénophase	Intens. de la respir. (ml O_2/g p.s./30′ à 25°C).			
		moyenne diurne	le matin	à midi	le soir
Ecbalium elaterium L.	mai 1968, végét.	2,36	2,40	2,43	2,01
	juillet 1968, flor.	1,82	1,66	1,85	1,75
	moyenne	2,09			
Anchusa procera Bess.	mai 1968, bout. de flor	1,40	1,31	1,51	1,16
	juillet 1968 flor.-fruct.	0,87	0,82	0,81	0,96
	moyenne	1,14			
Marrubium peregrinum L.	mai 1968, végét.	1,37	1,33	1,39	1,11
	juillet-1968, flor.	1,77	0,68	0,76	0,81
	moyenne	1,07			
Convolvulus persicus L.	mai 1968, végét.	0,97	1,02	0,90	0,95
Verbascum banaticum Roch.	1965 juillet. flor.	0,92	0,84	0,85	0,89
Inula germanica L.	mai 1968, végét.	1,38	1,32	1,14	1,22
	juillet 1968, flor.	0,77	0,73	0,80	0,79
	juillet 1965 flor.-fruct.	0,53	0,39	0,53	0,60
	moyenne	0,90			
Atriplex nitens Schk.	juillet 1968, flor.	0,86	0,81	0,89	0,79
Elymus sabulosus L.	mai 1968, végét.	0,71	0,68	0,72	0,65
	août 1968, fruct.	0,52	0,45	0,42	0,44
	moyenne	0,61			
Seseli tortuosum L.	juil. 1968, flor.	0,59	0,42	0,63	0,58
Teucrium chamaedrys L.	mai 1968, végét.	1,44	1,49	1,43	1,25
	juillet 1968, flor.	0,51	0,39	1,52	0,52
	août 1967, fruct.	0,41	0,34	0,45	0,33
	moyenne	0,79			
Ephedra distachya L. fem.	juillet 1968, fruct.	0,31	0,27	0,37	0,30
mâle	,, ,, , fruct.	0,27	0,23	0,31	0,23
,,					

1

et l'intensité de la transpiration chez diverses plantes

Rapport entre les val. moyennes diurnes max. et min. de la respir.en diverses phases.	Déficit hydrique (% rés. d'eau à la sat.)				Intens. de la transpir. (mg/g p.fr./1′)			
	moyenne diurne	le matin	à midi	le soir	moyenne diurne	le matin	à midi	le soir
1,3	20,5	16,0	24,4	17,5	7,9	5,6	10,6	2,5
	23,6	20,1	25,2	19,1	6,4	5,0	8,3	2,6
	22,1				7,2			
1,6	18,4	12,7	20,2	17,5	13,8	8,0	16,0	7,8
	21,3	16,6	23,1	16,4	8,4	6,0	6,1	6,6
	19,9				11,2			
1,8	20,9	10,3	23,7	19,5	23,9	11,2	28,5	6,1
	26,6	21,9	29,9	21,8	10,7	8,8	12,0	8,8
	23,7				17,2			
	22,8	14,0	23,7	22,2	15,5	7,3	19,8	3,5
	13,8	8,4	16,7	12,7	8,6	5,9	11,6	4,2
2,5	11,9	11,2	10,9	17,1	11,3	7,2	9,8	3,6
	19,8	14,6	19,6	20,0	15,2	10,5	14,5	7,1
	19,0	12,0	19,8	24,7	9,6	5,6	13,7	4,9
	16,9				12,0			
	24,5	20,6	24,8	24,9	5,3	3,7	6,3	4,1
1,3	7,9	5,0	8,0	5,3	8,1	4,4	8,1	4,4
	8,0	4,5	8,6	4,4	8,1	3,2	5,0	2,3
	8,0				8,1			
	10,3	4,9	11,3	9,4	9,6	3,4	12,0	4,2
3,5	14,4	7,4	15,6	12,9	11,6	10,5	10,7	3,5
	25,1	18,9	32,6	21,5	6,9	3,7	8,2	3,9
	26,9	17,6	28,3	25,7	5,6	3,4	6,2	4,3
	22,1				8,0			
	14,6	10,3	15,9	16,2	6,6	2,9	9,0	4,8
	12,7	10,1	14,4	12,2	6,1	3,0	7,9	2,9

Tableau 2

L'intensité de la respiration (déterminée entre 7—8h) et ses corrélations avec le déficit hydrique et avec l'intensité de la transpiration chez des plantes de divers groupes écophysiologiques

L'espèce	Intensité de la respiration ($ml/O_2/g$ p.s./30′ à 25°C)				rapport des val. max.	Déficit hydrique (% rés. d'eau à la sat.)				Intensité de la transpiration (mg/g p.fr./1′)			
	moyenne de diverses phases	avant la floraison	floraison	fructification	val. min. de la resp. en diverses phases	moyenne de diverses phases	avant la floraison	floraison	fructification	moyenne de diverses phases	avant la floraison	floraison	fructification
MÉSOPHYTES													
Ecbalium elaterium (L.) Rich.	2,23	2,58	1,87	—	1,4	19,6	16,2	22,9	—	8,4	9,3	7,5	—
Pisum sativum L.	—	—	2,23	—	—	—	16,8	—	—	—	3,7	—	—
Avena sativa L.	1,38	1,43	1,32	—	1,09	13,2	11,2	14,5	—	8,5	6,2	10,9	—
Anchusa procera Bess.	1,05	—	1,29	0,80	1,6	17,3	—	13,5	21,1	10,5	—	12,8	8,2
Cichorium intybus L.	—	—	1,01	—	—	—	—	12,0	—	—	—	4,9	—
Papaver rhoeas L.	—	—	0,93	—	—	—	—	15,2	—	—	—	11,3	—
SUCCULENTES et HÉLIOPHYTES						SUCCULENTOÏDES							
Cakile maritima Scop.	1,50	1,93	1,07	—	1,8	16,4	19,2	13,8	—	3,2	2,0	4,3	—
Crambe maritima L.	—	—	1,79	—	—	—	—	16,6	—	—	—	2,5	—
Salsola ruthenica Iljin	1,26	1,39	1,13	—	1,2	11,0	13,4	8,7	—	5,2	6,9	3,5	—
Eryngium maritimum L.	1,18	—	1,15	1,21	1,05	7,1	—	7,8	6,3	3,5	—	2,6	4,3
Atriplex nitens Schk.	—	—	0,85	—	—	—	—	21,9	—	—	—	3,7	—
Silene otites (L.) Wib.	—	—	0,78	—	—	—	—	18,5	—	—	—	4,3	—
HÉMIXÉROPHYTES													
Medicago falcata L.	1,26	1,10	1,41	—	1,3	18,3	20,4	16,2	—	10,2	8,2	12,2	—
Astragalus virgatus Pall.	1,16	—	1,14	1,17	1,03	16,5	19,6	13,3	—	10,0	—	8,2	11,8
Marrubium peregrinum L.	1,03	1,45	0,88	0,75	1,9	21,5	19,6	26,7	18,1	21,1	29,4	11,8	—
EUXÉROPHYTES													
Kochia prostrata (L.) Schrad.	—	—	1,13	—	—	—	—	17,7	—	—	—	2,3	—
Verbascum banaticum Roch.	1,04	0,98	1,54	0,62	2,5	17,5	19,4	17,8	14,8	12,0	—	16,7	7,7
Medicago marina L.	—	—	1,03	—	—	—	—	16,8	—	—	—	5,8	—
Alyssum borzeanum Nyar.	0,84	—	0,66	1,01	1,5	22,6	—	28,6	16,5	8,1	—	4,9	11,2
Convolvulus persicus L.	0,72	1,11	0,56	0,58	2,0	15,5	20,5	13,9	13,1	12,5	14,5	10,6	—
Chrysanthemum millefolium L. Mant.	0,71	—	0,70	0,72	1,03	25,2	—	24,7	25,7	7,4	—	9,6	5,2
Asparagus verticillatus L.	—	—	0,70	—	—	—	—	19,9	—	—	—	6,6	—
Ephedra distachya L. pieds. fem.	0,64	—	1,03	0,25	4,1	12,1	—	14,0	10,2	3,3	—	3,5	3,1
,, ,, ,, ,, mâles	0,65	—	1,06	0,23	4,6	12,4	—	14,6	10,2	3,9	—	2,9	5,5
Artemisia austriaca Jack.	—	—	0,64	—	—	—	—	25,1	—	—	—	4,9	—
Elymus sabulosus L.	0,62	0,71	0,60	0,55	1,2	11,2	8,1	7,8	17,7	8,3	9,0	6,3	9,5
Seseli tortuosum L.	0,53	—	0,58	0,48	1,2	11,5	—	9,2	13,7	—	—	8,4	—
Carex ligerica Gay.	0,44	—	0,49	0,39	1,1	11,2	—	9,6	12,7	10,4	—	6,7	14,2
HELIOPHYTES XÉROPHYTOÏDES						(MÉSOXÉROPHYTES)							
Convolvulus arvensis L.	1,2	—	1,60	0,79	—	16,5	—	13,0	20,0	—	—	12,0	—
Vicia cracca L.	—	—	1,44	—	—	—	—	26,4	—	—	—	13,4	—
Salvia nemorosa L.	1,12	—	1,20	1,04	—	12,1	—	14,7	9,5	11,5	—	12,5	11,4
Centaurea arenaria M. B.	—	1,14	—	—	—	—	19,3	—	—	—	10,4	—	—
Agropyron repens L.	—	1,05	—	—	—	—	3,1	—	—	—	7,8	—	—
Inula germanica L.	0,83	1,50	0,54	0,50	2,85	18,7	9,2	17,8	29,0	10,2	5,6	12,5	—
Teucrium chamaedrys L.	0,78	1,41	0,51	0,41	3,5	17,0	14,4	18,8	17,7	7,7	13,0	3,8	6,3

RELATIONS ENTRE l'IR ET LE DH

Des données consignées dans les tableaux 1 et 2, il résulte qu'entre la variation des valeurs de l'IR et du DH, en fonction de la nature des plantes, s'établissent des rapports qui n'ont pas un caractère de lois, valable pour toutes les espèces étudiées. Il est vrai que chez quelques espèces on remarque une corrélation positive entre ces deux indices ; des espèces avec un DH élevé enregistrent une respiration intense *(Ecbalium elaterium, Anchusa procera, Pisum sativum, Marrubium peregrinum, Medicago falcata, Alyssum borzaeanum, Verbascum banaticum)* et d'autres avec un DH réduit ont une respiration d'intensité toujours petite *(Elymus sabulosus, Seseli tortuosum, Carex ligerica)*. Mais il existe aussi des espèces qui, pour un DH élevé, ont la capacité de réduire l'IR *(Teucrium chamaedrys, Inula germanica, Silene otites, Atriplex nitens, Convolvulus persicus, Chrysanthemum millefolium)*; d'autres, au contraire, pour un DH réduit ou modéré, enregistrent une respiration intense *(Eryngium maritimum, Salsola ruthenica, Crambe maritima)*.

Entre la dynamique de l'IR et les valeurs du DH par rapport à la phase ontogénique, chez la majorité des espèces s'établissent des corrélations inverses ; chez la plupart *(Ecbalium, Anchusa, Avena sativa, Marrubium, Inula, Teucrium, Convolvulus arvensis*, etc.), à mesure que le DH augmente, par rapport à l'âge, l'IR diminue (tableaux 1 et 2). C'est une réaction à caractère adaptatif, vu que les plantes réussissent à réduire la consommation des substances plastiques et à économiser l'énergie, quand l'état d'hydratation des tissus s'aggrave, à cause de la réduction de l'humidité, au milieu et à la fin de l'été. Dans les années, où par déviation du cours normal, l'humidité pédologique est plus élevée au milieu de l'été, le DH diminue dans la phase de fructification par rapport à la floraison et l'intensité de la respiration, augmente ; il s'agit des cas rencontrés en 1969 chez *Medicago falcata, Astragalus virgatus, Alyssum, Eryngium*. Chez très peu d'espèces *(Convolvulus persicus, Ephedra distachya* — pieds mâles, *Salvia nemorosa)* s'établissent des corrélations directes ; la réduction de l'IR, à mesure que l'âge avance, est accompagnée de l'abaissement du DH.

RELATIONS ENTRE L'IR et L'IT

Les relations entre ces deux indices, par rapport à la nature des plantes, sont très variées. Certaines euxérophytes *(Ephedra, Asparagus verticillatus, Artemisia austriaca, Chrysanthemum, Elymus* — tableau 2) réunissent une respiration réduite avec une transpiration de faible intensité, et d'autres espèces *(Anchusa, Avena, Medicago falcata, Marrubium, Convolvulus persicus, C. arvensis)* enregistrent pour une respiration intense une IT de valeurs élevées aussi. Mais il y a en même temps des corrélations inverses ; des espèces oligohydriques (moyenne diurne de l'IT en général sous 4 mg/g p. fr./1′) comme les succulentes et les succulentoïdes, de même *Pisum* et *Kochia* (tableau 2) enregistrent des valeurs élevées de l'IR, et d'autres, chez une IR réduite, inscrivent une transpiration active *(Inula, Seseli* — tableau 2).

Nous avons constaté que 64% des plantes étudiées enregistrent une corrélation directe entre la dynamique de l'IR et de l'IT, par rapport à l'âge (tableaux 1, 2). Chez quelques espèces *(Eryngium, Medicago falcata, Astragalus, Alyssum* — tableau 2), les deux processus augmentent avec le passage de la phase de floraison à celle de la fructification ; le phénomène est la conséquence de l'accroissement de l'humidité pédologique qui dévie de là normale dans les années respectives. Mais chez la plupart des espèces, l'IR et l'IT diminuent à mesure que les plantes vieillissent *(Ecbalium, Anchusa, Salsola, Marrubium, Verbascum, Convolvulus persicus, Salvia*, etc. — tableaux 1 et 2). En quelques cas, des corrélations inverses par rapport à l'âge s'établissent entre la dynamique de ces deux indices *(Avena, Cakile, Ephedra*—pieds mâles).

L'existence, dans la majorité des cas, des corrélations directes entre la variation de l'IR et de l'IT en fonction de l'âge, et souvent en fonction de l'espèce aussi, représente un argument à l'appui de l'idée que la respiration fournit l'énergie pour les processus de transpiration. Mais les autres rapports qui s'établissent en fonction de l'âge et de la nature des plantes, en prouvent que les processus respiratoires ne représentent pas toujours le facteur qui détermine le niveau de l'IT.

PÉRIODICITÉ JOURNALIÈRE DE L'IR ET SES RELATIONS AVEC LA DYNAMIQUE DU DH ET DE L'IT

Du tableau 3 on constate qu'il y a certaines concordances entre la variation, en fonction de l'espèce, des rapports des valeurs de l'IR, du DH et respectivement de l'IT enregistrées à midi, par comparaison à celles du matin. En même temps pour les dernières 8 espèces inscrites dans le tableau, la réduction du rapport : IR à midi/IR le matin, en fonction de la nature des plantes, est liée à la diminution graduelle du rapport : IT à midi/IT le matin (tableau 3). Ce comportement justifie la supposition que l'énergie dégagée dans les processus respiratoires détermine le niveau de l'IT.

L'enregistrement de la dynamique diurne de ces indices nous a permis d'apporter certaines contributions à la connaissance de la marche journalière des processus respiratoires dans la nature et à l'élucidation des relations de la respiration avec la dynamique du déficit hydrique et de la transpiration.

En général la périodicité journalière de l'IR varie avec l'espèce et avec la phase du développement ontogénique, une importante influence ayant l'état d'hydratation des tissus, qui dépend des conditions d'humidité du milieu. En beaucoup de cas, l'IR enregistre les valeurs maxima à mi-journée *(Anchusa* — fig. 1A, *Ecbalium* — fig. 2A, *Ephedra* — fig. 3A, *Seseli* — fig. 4B), ce que Meyer et Deleanu [8], Popescu [9], Kraft, cité par Stocker [10] trouvent aussi pour diverses plantes. *Marrubium* (fig. 5A) inscrit les valeurs maximales de l'IR à 8h. Plus fréquemment, dans les conditions du littoral, la marche diurne de l'IR enregistre une dépression dans les heures de tension maximale des facteurs météorologique, à midi *(Anchusa*—fig. 1B, *Ecbalium* — fig. 2B, *Marrubium* — fig. 5B, *Atriplex* — fig. 6A, *Convolvulus* — fig. 6B, *Teucrium* — fig. 7A, *Inula* — fig. 8A) ; c'est une réaction adéquate des plantes, celles-ci réussissant à faire économie

Tableau 3

La variation de la grandeur des rapports entre les valeurs enregistrées à midi et le matin de l'intensité de la respiration (IR), du déficit hydrique (DH) et de l'intensité de la transpiration (IT) chez diverses espèces

L'espèce	Rapport IR à midi / IR le matin	Rapport DH à midi / DH le matin	Rapport IT à midi / IT le matin
L'IR déterminée à la température du milieu au moment du prélèvement des échantillons			
Echinops ruthenicus (Fischer) M. B.	2,1	2,2	2,6
Ecbalium elaterium (L.) Rich	2,0	2,1	2,1
Eryngium campestre L.	2,0	2,1	2,5
Crambe maritima L.	1,9	1,6	1,4
Salsola ruthenica Iljin	1,6	1,3	1,4
Kachile maritima Scop.	1,5	1,7	1,2
Verbascum banaticum Roch.	1,4	2,0	2,0
Eryngium maritimum L.	1,4	2,0	2,0
Inula germanica L.	1,4	1,65	2,2
Convolvulus persicus L.	1,4	1,2	2,2
L'IR déterminée à la température de 25°C			
Seseli tortuosum L.	1,5	2,3	3,5
Ephedra distachya L. pieds fem.	1,4	1,5	3,1
Ephedra distachya L. „ mâles	1,36	1,47	2,6
Inula germanica L.	1,36	1,6	2,4
Teucrium chamaedrys L.	1,35	1,7	2,2
Ecbalium elaterium (Fischer) M. B.	1,1	1,25	1,6
Atriplex nitens Schk.	1,1	1,2	1,7
Marrubium peregrinum L.	1,1	1,36	1,36

d'énergie en conditions non favorables de milieu, à mi-journée. L'interruption de cette dépression par un accroissement passager de l'IR à 12 h, chez *Ephedra* — fig. 3B et *Teucrium* — fig. 7B, donne la possibilité aux tissus de produire, dans les conditions d'un haut déficit, l'énergie nécessaire à la synthèse de certains biopolymères qui jouent un rôle dans la retention de l'eau [5]; en effet à cette heure chez les deux espèces l'IT a baissé.

Chez quelques espèces *(Ecbalium* — fig. 2, *Anchusa* — fig. 1, *Marrubium* — fig. 5, *Inula* — fig. 8, *Convolvulus* — fig. 6B, *Teucrium* — fig. 7) la marche diurne enregistre des oscillations à amplitudes larges, chez d'autres *(Ephedra* — fig. 3, *Seseli* — fig. 4B, *Elymus* — fig. 4A) plus réduites, ce qui atteste un métabolisme stable. En général, l'amplitude des oscillations diurnes de la respiration diminue à mesure que l'âge avance, comme l'IR aussi.

En ce qui concerne les relations qui s'établissent entre l'IR et le DH nous remarquons que chez les espèces qui enregistrent des déficits hauts ou relativement élevés, dans la plupart des cas, il y a une corrélation inverse entre la dynamique diurne du DH et de l'IR. Ainsi chez *Marrubium* — fig. 5, *Teucreium* — fig. 7, *Inula* — fig. 8, *Ecbalium* fig. 2A et *Convolvulus* — fig. 6B, la respiration enregistre les valeurs les plus élevées le matin, quand le DH est modéré; dans l'après-midi, quand le déficit augmente, les processus respiratoires diminuent en intensité. Ce comportement démontre qu'à certaines limites supérieures du DH, les tissus entrent dans un état de défense, réduisant les dépenses d'énergie, ce qui représente

une réaction à rôle adaptatif. En très peu de cas, chez les espèces à hauts déficits, on constate une corrélation directe entre la dynamique diurne de ces 2 indices *(Anchusa* — fig. 1, *Ecbalium* — fig. 2B). Chez les espèces qui enregistrent dans la nature des valeurs petites ou modérées du DH, on constate que l'abaissement ou l'accroissement du déficit hydrique au cours de la journée provoquent les mêmes modifications dans la variation de l'IR, ou que la dynamique de ces indices n'est pas concluante *(Ephedra* — fig. 3, *Seseli* — fig. 4B, *Elymus* — fig. 4A).

Les relations qui s'établissent entre la périodicité journalière de l'IR et de l'IT sont variées. Dans la plupart des cas on constate une concordance évidente, même un parallélisme, entre les oscillations diurnes de ces deux processus *(Teucrium* — fig. 7, *Anchusa* — fig. 1, *Inula* — fig. 8A, *Marrubium* — fig. 5A, *Ephedra*, pieds mâles — fig. 3B); en d'autres cas, la concordance est moins visible, ou elle est limitée seulement à certaines heures de la journée *(Ecbalium* — fig. 2, *Marrubium* — fig. 5B, et *Ephedra* — fig. 3A).

Ces comportements plaident en faveur de l'idée d'une liaison énergétique entre les processus respiratoires et la transpiration. En moins de cas on ne peut distinguer aucune corrélation à caractère de lois entre la dynamique diurne de ces deux processus *(Elymus* — fig. 4A, *Seseli* — fig. 4B, *Inula* — fig. 8B); c'est pourquoi on peut affirmer que l'énergie libérée par la respiration n'est pas toujours le facteur déterminant du niveau de la transpiration. Les relations entre ces processus sont complexes; elles dépendent de facteurs endogènes qui réagissent différemment par rapport à l'espèce et à l'âge, aux changements des conditions du milieu.

CONCLUSIONS

— Entre les valeurs de l'intensité de la respiration et celles du déficit hydrique il existe des corrélations directes ou inverses en fonction de la nature des plantes; entre la dynamique de ces deux indices par rapport à la phase ontogénique s'établissent des corrélations inverses.

— Les relations entre l'intensité de la respiration et l'intensité de la transpiration sont variées, mais entre les dynamiques de ces deux processus par rapport à l'âge, chez la plupart des espèces on enregistre des corrélations directes.

— La marche diurne de l'intensité de la respiration inscrit des courbes variées, par rapport à l'espèce, à l'âge et aux conditions d'humidité, mais dans la plupart des cas, dans les conditions du littoral, une dépression de la respiration a lieu à mi-journée, les plantes ayant la capacité de faire économie d'énergie à midi, quand les facteurs météorologiques sont défavorables.

— La diminution de l'intensité respiratoire à mesure que le déficit hydrique s'accroît au cours de la journée chez les plantes à hauts déficits représente une réaction adaptative qui permet de réduire la dépense d'énergie quand l'état d'hydratation des tissus devient défavorable.

— L'existence en beaucoup de cas d'un parallélisme ou d'une certaine concordance entre les oscillations diurnes de la respiration et celles de la transpiration, ainsi que la constatation des corrélations directes entre

ces deux indices, parfois en fonction de l'espèce, souvent par rapport à l'âge, plaident en faveur de l'idée que la respiration fournit l'énergie necéssaire aux processus de transpiration.

— Le manque de concordance chez quelques espèces entre la marche diurne de la respiration et de la transpiration démontre que l'intensité de la respiration ne détermine pas toujours le niveau de la transpiration; celui-ci dépend souvent de l'influence directe des facteurs extérieurs.

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GERMINATION OF SOME SAND DUNE SEEDS FROM KONYA, KARAPINAR AREA, TURKEY

BY

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Seeds from the sand dunes of the area were collected and their germination grades under controlled conditions at normal 0°C, −2°C, −5°C, at darkness and light were studied in the laboratory; *Agropyron elongatum* showed 100% germination at normal (untreated), *Onobrychis hypargyrea* 95% at −2°C and 100% at −5°C, *Prangos meliocarpoides* 100% at 0°C, *Thymus squarrosus* 10% at −2°C, and at −5°C, respectively, while *Scabiosa ucranica* showed 50% germination at −2°C.

The study of abundant seeds is very important for stabilization of sand dunes in an area like Karapinar. As a matter of fact, much work has been done on germination.

It is known that seeds of many plants of practical importance do not germinate easily. The reasons are threefold. There are special substances present in seeds and fruits which inhibit germination. The seed or fruit coat is impermeable to water or air. The seeds especially the embryo needs after-ripening. In some cases after ripening this is necessary because the embryo is not fully developed when the fruit ripens [4]. Evenari and co-workers worked on the germination of some rosaceous seeds of apples. They concluded that the seeds of all varieties used are unable to germinate without after-ripening. After-ripening must be done at low temperature and in moist conditions. After-ripening can be effectively achieved when the seeds are still inside the fruit by placing the whole fruit in the refrigerator.

Warming [9] stressed the need for further studies in this field. However, the germination of desert seeds for the stabilization of sand dunes provides another important field in this regard. Hammouda and Bakr [5] worked on some aspects of germination in desert seeds. They studied the role of different factors like temperature, rainfall, light and salinity on 29 species collected from desert and semi-desert areas. Warming [10] studies the ecological aspects of seed dormancy and germination. Vardar and Ahmad [8] studied the physiology of seeds germination and its importance. They have described how the effects of water, chemicals like gibberellic acid, kinetin, coumarin, nitrates, temperature and light play an important role in germination.

Bakir et al. [2] worked to prove the longevity of some important range seeds. They found out that leguminous seeds have longer germination power than grasses. Çetik and Düzenli [3] performed germination experiments on seeds of some steppe plants in the surroundings of Ankara. They carried out their works on 70 seeds of different species.

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MATERIAL AND METHODS

The authors have used same method as that adopted by Çetik and Düzenli [3]. Germination iron cups 50×40×8 cm were used. On their upper sides glass bars were kept parallel to one another, which were later wrapped with filter papers. 20 gms seeds from different species collected were put on the paper-covered glass bar of the cup. Before putting on the bars, the authors have carried them to the plant protection department of the Agricultural Faculty for different treatment like 0°C, −2°C, −5°C, darkness and light for 24 hours. The experiment lasted for 2 months. Distilled water was used during the tests. Daily observations were made. Some of the seeds collected from Karapinar on 19th July, 1974, are *Artemisia scoparia, Astragalus micropterus, Centaurea pulchella, Thymus squarrosus, Isatis tinctoria, Agropryron elongatum, Scabiosa ucranica, Gypsophila anatolica, Amygdalus orientalis, Lepidium latifolium, Prangos meliocarpoides, Onobrychis hypargyrea, Peganum harmala, Scirpus holoschoenus, Verbascum cheiranthifolium.*

RESULTS AND DISCUSSIONS

The results obtained present evidence of ecological significance. In the sandy habitat short-lived, longeval and dormant seeds with germination chances for some years are present.

Different seeds require different temperatures for germination. The range of temperature for germination may be wide (the majority of species), or otherwise narrow and more defined. Optimum temperature may be low, a feature characterizing many seeds germinating in cold weather, e.g., *Onobrychis hypargyrea, Thymus squarrosus* and *Agropyron elongatum.*

Table 1

Percentage of germinated seeds under different conditions

Seed of	Conditions for germination			
	Normal	0°C	−2°C	− 5°C
1. *Agropyron elongatum*	100	15	0	0
2. *Onobrychis hypargyrea*	90	90	95	100
3. *Prangos meliocarpoides*	50	100	80	0
4. *Thymus squarrosus*	12	10	100	100
5. *Astragalus micropterus*	0	5	10	15
6. *Centaura pulchella*	5	0	0	0
7. *Salvia cryptantha*	0	0	0	0
8. *Scabiosa ucranica*	20	0	50	30
9. *Scirpus holoschoenus*	0	0	0	0
10. *Peganum harmala*	0	0	0	0
11. *Verbascum cheiranthifolium*	0	0	0	0
12. *Astragalus christianus*	5	0	0	5
13. *Lepidium latifolium*	5	0	10	0
14. *Amygdalus orientalis*	0	15	30	0
15. *Gypsophila anatolica*	0	5	0	0
16. *Astragalus lydius*	0	5	0	0
17. *Isatis tinctoria*	10	0	5	0

The importance of water for germination as a limiting and important factor cannot be overlooked at any stage, Kahn [6], [7]. However, the authors used distilled water. In the field, 5 mm rainfall has been found insufficient for germination but 10 mm and 20 mm rainfall fulfil germination requirements of many seeds [5]. Dormancy in desert seeds could be considered as an adaptation to the hard conditions of natural habitats. Hard seed coat cause dormancy in many seeds. Generally, in many leguminous plants prolonged dormancy is very clear. *Astragalus micropterus, A. christianus* and *A. lydius* showed very slow rate of germination. In such cases mechanical sacrification or some chemical treatment may give positive results [1]. However, we did not apply the above treatment. That is why our results may not be satisfactory in the case of the above species.

The results obtained are shown in Table 1.

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THE EFFECT OF GAMMA RAYS ON DNA AMOUNT WITHIN MAIZE ROOT MERISTEM

BY

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The dried caryopses of maize (*Zea mays*) were irradiated with gamma rays in the following doses : 1000, 3000, 7000, 20,000, 30,000, 40,000, 60,000 and 80,000 R. When the maize seedlings were 5-days the root top was fixed in Carnoy mixture. The nuclei were stained through Feulgen reaction and the relative amount of DNA was measured by the double-wave-length method. The results show that relative amount of DNA within the maize root meristem decreased according to a negative exponential relation. The dose-effect curve shows a similar relationship. The lowering of the DNA amount may be related to the decrease of cell division number.

The research performed so far, using the cytophotometrical method [3], [9], [10], have shown that after irradiation with various ionizing rays the amount of DNA per nucleus decreased according to the applied doses and a strong disorganization of the heterochromatine occurred. We must stress that most investigations carried out on DNA syntheses during and after irradiation usually aimed at the rate of tritiated thymidine incorporation [7]. The cytophotometrical methods have scarcely been used for the study of the effect of irradiation on the nuclear DNA.

MATERIAL AND METHODS

The plant material used, dried caryopses of maize *(Zea mays)*, were irradiated with gamma rays at the Institute of Atomic Physics (Bucharest-Măgurele) in the following doses : 1000, 3000, 7000, 20,000, 30,000, 40,000, 60,000 and 80,000 R/25 sec. The caryopses were irradiated for the first time (I generation) or they originated from plants the seeds of which were also irradiated (II generation) in dried stage. No significant differences can be established between these two generations.

The germination of caryopses occurred on filter paper, daily moistened with water in Linhard dishes and on circadyne variations of temperature within the laboratory. On the 5th day the top of the root was cut off and fixed for 24 hours in Carnoy mixture. After fixation, the roots were washed with running water for 24 hours. The plant material was kept in 70% alcoholic solution and then stained by Feulgen's method.

The hydrolyses of the material were performed at room temperature for $1^1/_2$ hour in 5 N HCl. The staining with Schiff's reagent lasted for $2^1/_2$ hours.

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The specimen was made by squash technique according to BERLYN and MIKSCHE [1].

The measurement of the relative amount of DNA was performed at a Leitz Ortholux Microscop Photometer, using the double wave-length method, according to PATAU [8] and MENDELSOHN [6]. The used pair-wave-lengths were 500 and 479 nm. Only interphase nuclei were measured and for each variants (control and treated ones) about 200 nuclei were reckoned. All the obtained data were submitted to statistical and mathematical calculation [4].

RESULTS AND DISCUSSIONS

Figure 1 shows the effect of various gamma rays doses on the relative amount of DNA within meristemic cells of maize. With the applied doses no stimulation of DNA syntheses occurred in both the first and second generations in the two repetitions. The second repetition consists in the reckoning of all nuclei (Feulgen stained and unstained nuclei, i.e. the so-called Feulgen negative nuclei [2], [5]) and the first repetition in the reckoning of stained nuclei only. By Fig. 1, it is obvious that between the applied doses and the lowering of the DNA amount, expressed in arbitrary units, there is a particular relation.

We have counted (Fig. 2) the number of cells in telophase (i.e. the last phase of cell division) for 60 microscopic fields and we observed that the rate of cell division decreased with the increase of gamma ray doses. The two processes, the lowering of the DNA amount and the decreasing of mitoses, are likely to be closely related. All these findings prove that irradiation inhibits the DNA syntheses and, as a consequence, cellular division.

Figure 3 mathematically proves the dose-effect relationship computed by the formula :

$$\frac{N}{N_0} = \exp\ (-\alpha D) \text{ or } N = N_0 \cdot e^{-\alpha D}$$

where N_0 = the nuclear DNA amount at the control;
N = the nuclear DNA amount after irradiation with a particular dose;
α = the probability of hitting the target;
e = the base of natural logarithms (2.71828 ...) [4].

The relation between the applied doses and the amount of nuclear DNA is therefore negative-exponential.

Figure 4 shows the probability of DNA syntheses inactivation. The probability of inactivation was computed by the formula :

$$W(D) = 1 - N/N_0$$

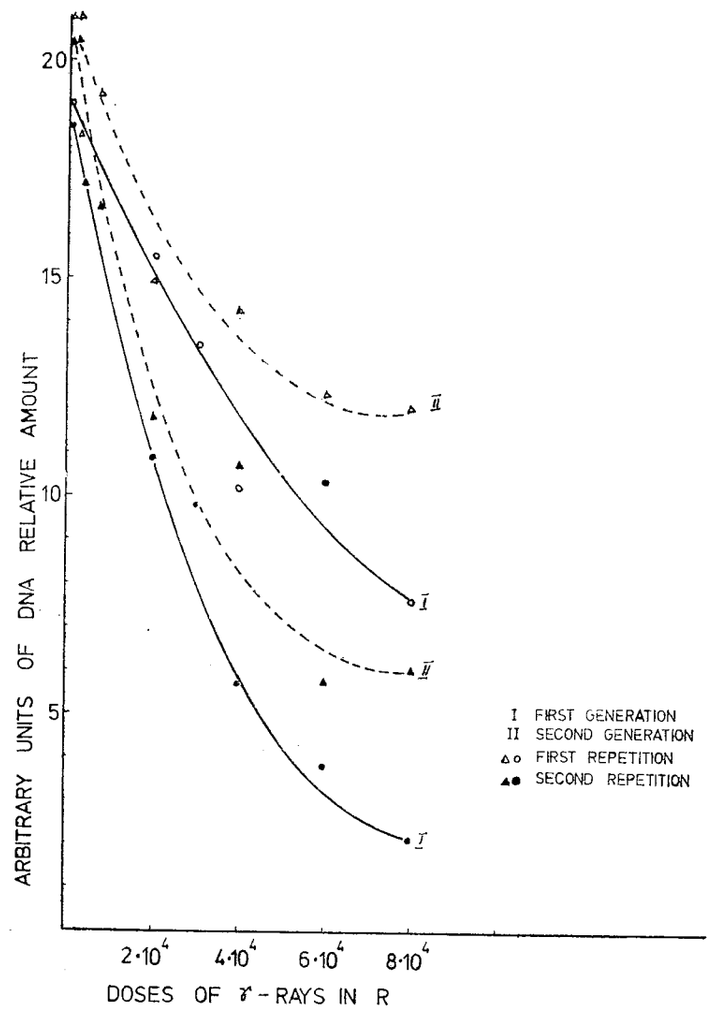


Fig. 1. — The dependence between relative DNA amount (in arbitrary units) and the irradiation with gamma rays in different doses.

where $W(D)$ = the probability of DNA syntheses inactivation depending on the applied doses;
N = the relative amount of nuclear DNA after irradiation with a particular dose;
N_0 = the nuclear DNA amount at the control.

From the data in Fig. 4 it is obvious that the inactivation of DNA syntheses within meristemic nuclei usually increased in linear relation with the increase of the applied doses.

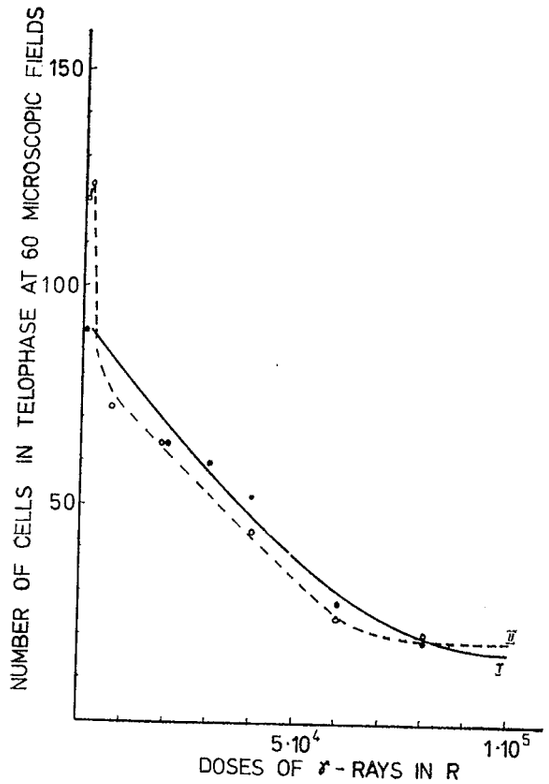


Fig. 2. — The decrease of cell divisions after gamma irradiation with various doses.

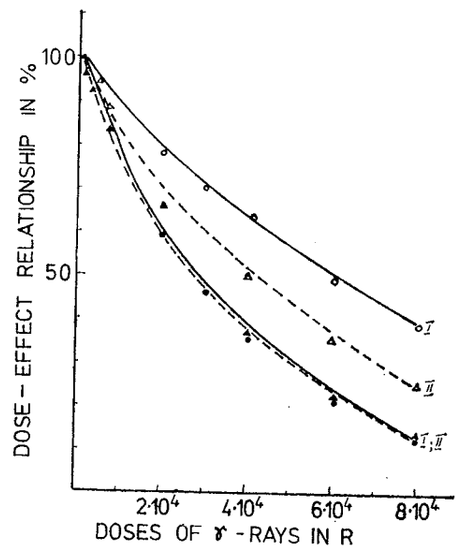
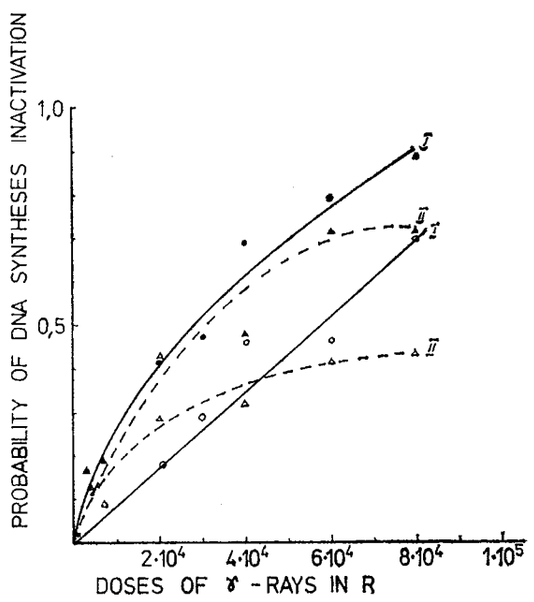


Fig. 3. — The dose-effect relationship between the applied gamma ray doses and nuclear amount of DNA.

Fig. 4. — The probability of DNA syntheses inactivation after irradiation with various gamma ray doses.



The inactivation of nuclear DNA syntheses may also be related to the increase of the microscopic fields which contain Feulgen negative nuclei (Fig. 5). The number of these fields usually increases with an S-shape (logistic) curve.

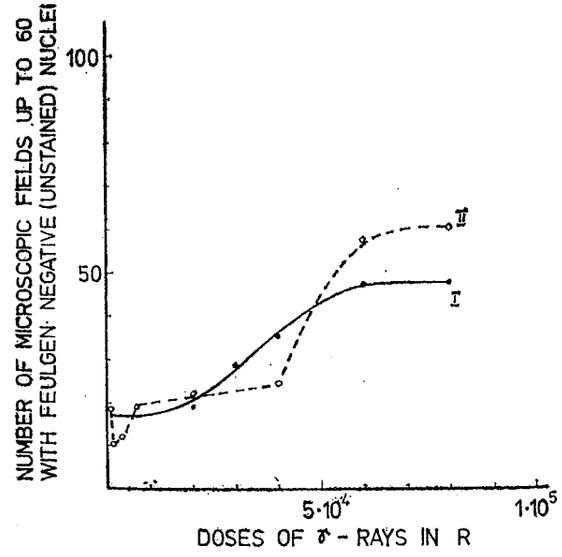


Fig. 5. — The increase of microscopic fields with Feulgen negative (unstained) nuclei after irradiation with various gamma ray doses.

CONCLUSIONS

1. With the applied doses of gamma rays only inhibition of nuclear DNA syntheses within meristemic cells of maize can be obtained.
2. The relationship between the amount of nuclear DNA and the increase of gamma rays doses is negative-exponential.
3. The probability of inactivation of nuclear DNA synthesis increases with the increase of gamma ray doses.

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THE EFFECT OF X RAYS ON ROOT MERISTEM OF BROAD BEAN *(VICIA FABA)*

I. THE RELATIVE AMOUNT OF NUCLEAR DNA AFTER IRRADIATION

BY

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Broad bean *(Vicia faba)* root meristem of 5-day-old seedling was irradiated with X rays in the following doses : 50, 100, 150, 200, 300 and 500 R. The relative amount of DNA was measured by cytophotometrical techniques using the Feulgen reaction. The double wave-length method was used. The results show a slight increase of the DNA amount at 50 R, after 24 and 48 hours. At 100, 150 and. 200 R, the relative amount of DNA per nucleus decreased in exponential manner. At 300 and 500 R, the amount of nuclear DNA increased again. This increase may be related to the formation of new meristemic cells from the "quiescent zone" of the root.

The relationship between the DNA amount per nucleus, the synthesis of DNA and the changes of cellular cycle after ionizing radiation is known [1], [9], [13]. The research carried out so far has proved that ionizing radiations (X and gamma rays) inhibit the DNA synthesis within the nucleus and causes the retardation of cell division, thus changing the duration of cell cycle.

The researches on the DNA synthesis were usually made with tritiate thymidine. However, some old and new investigations [7], [9], [10] proved that Feulgen reaction may also be used for the nuclear DNA amount estimation after ionizing radiation.

Our investigations deal with the variations of nuclear DNA content within the root meristem of broad bean *(Vicia faba)* after X-ray irradiation. The amount of DNA was measured by cytophotometry of Feulgen reaction.

MATERIAL AND METHODS

The seeds of broad bean *(Vicia faba)* were soaked for 24 hours, in tap water. They were germinated on moistened filter paper, on Linhardt dishes. When the primary root was 2—3 cm in length, i.e. 5 day old, the root tops were irradiated with the following doses : 50, 100, 150, 200, 300 and 500 R. The irradiation was performed with the therapeutical apparatus TUR I, 180 kv, 10 mA, 1 Cu, CD, DFO-40 cm.

After 24 and 48 hours of irradiation, the meristemic zone of the root was fixed in Carnoy fluid for 24 hours. After fixation, the plant material was washed for 24 hours in continuous running water.

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The hydrolysis was made at room temperature (18—20°C) with 5 N HCl; the hydrochloric acid was removed by washing the plant material thrice with distilled water for 10—15 minutes each time. The staining lasted for $2^1/_2$ hours at dark in Schiff reagent. After staining, the root top was squashed on a microscopic slide of 0.6—0.8 mm thickness.

The relative amount of DNA per nucleus was expressed in arbitrary units. The measurements were performed at Leitz Ortholux Cytophotometer MPE, using the double-wave-length method according to Patau [15], Ornstein [14] and Mendelsohn [11]. We used 500 and 479 nm as pair wave-lengths.

The obtained data were statistically reckoned involving the arithmetical mean, standard deviation and significance of the difference between various data according to Student parameter.

In some cases, histograms analyses after various doses of irradiation were made.

RESULTS AND DISCUSSIONS

The results of our investigations are shown in Figs. 1—5. Figures 1 and 2 show the effect of X-ray irradiation in various doses on nuclear DNA amount within meristemic cells of broad bean root, after 24 and 48 hours of irradiation. The data are given separately for 3 kinds of nuclei : *a*) prophase nuclei, *b*) telophase nuclei (corresponding to the end of cell division) and *c*) interphase nuclei. It was proved that the smallest doses of irradiation, i.e. 50 R, caused a slight increase of the relative amount of DNA. This increase is sometimes significantly different from the control, and probably it is due to some perturbation induced by irradiation. We tried to calculate the probability of DNA synthesis inactivation using the formula : $W(D) = 1 - N/N_0$ in which W(D) is the probability of DNA synthesis inactivation depending on the applied doses, N is the relative amount of DNA after irradiation and N_0 is the relative amount of DNA in the control [8]. The result suggests that no inactivation of DNA synthesis occurred at 50 R, the probability of inactivation being close to zero.

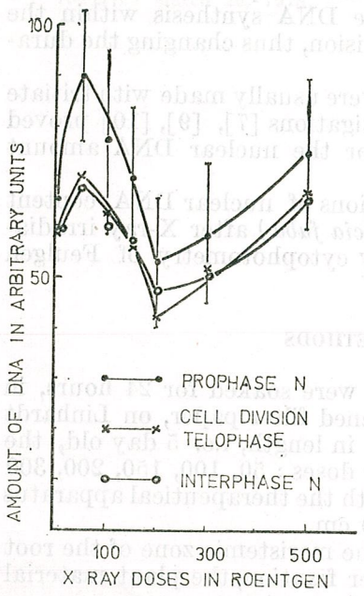

Fig. 1. — The action of various doses of X-ray on the nuclear amount of DNA within root meristemic cells of broad bean *(Vicia faba)* after 24-hour irradiation.

At 100, 150 and 200 R, the amount of DNA per nucleus decreased according to a dose-effect curve and the decrease of values may be related to a negative exponential curve according to the formula : $N = N_0 \cdot e - \alpha.D$ in which N_0 is the relative DNA amount in the control, N is the relative DNA amount in the irradiated material, D are the applied doses, $-\alpha$ is the probability of hitting the "target", and e is the base of natural logarithms (2.71828).

Quite unexpectedly, at 300 and 500 R, both after 24 hours and 48 hours of irradiation, the relative amount of DNA increased again. It was mainly at 500 R that the relative amount of DNA reached the values of the control. This result suggests that something had happend within the broad bean meristemic cells. We notice (unpublished data) that such an increase of the DNA amount per nucleus has never occurred when the dried seeds were irradiated. The only possible explanation for this increase is given by Clowes' data [2], [3], [4], [5], [6] concerning the role of the so-called "quiescent centre" in the renewing of the active cells of the root meristem, damaged or destroyed by irradiation. We think that after irradiation, at 300 and 500 R, most cells from our squash belonged to former quiescent zone cells. However, the nuclei of these cells are not normal because their volumes show continuous increase, depending on the applied doses (Fig. 3). The histograms in Figs 4 and 5 explain this increase of the nuclear volume by the increase of chromosome number and tetraploidy. Therefore, the cells from the quiescent zone have suffered

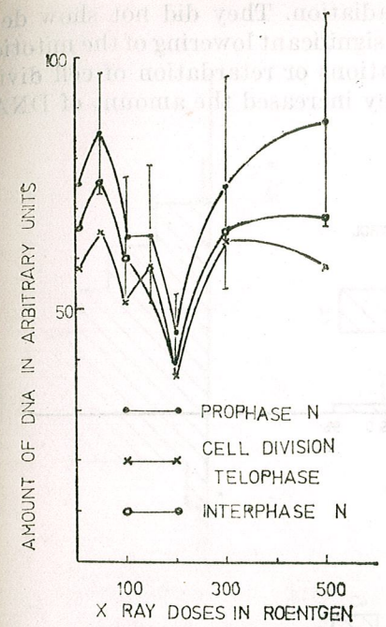

Fig. 2. — The action of various doses of X-ray on the nuclear amount of DNA within root meristemic cell of broad bean *(Vicia faba)* after 48-hour irradiation.

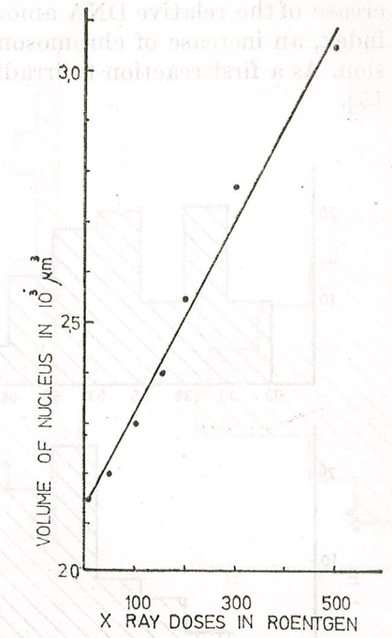

Fig. 3. — The relationship between applied doses of X-ray and the volume of nuclei.

some damage or influence caused by irradiation. They did not show decrease of the relative DNA amount, but a significant lowering of the mitotic index, an increase of chromosomal aberrations or retardation of cell division. As a first reaction to irradiation, they increased the amount of DNA [5].

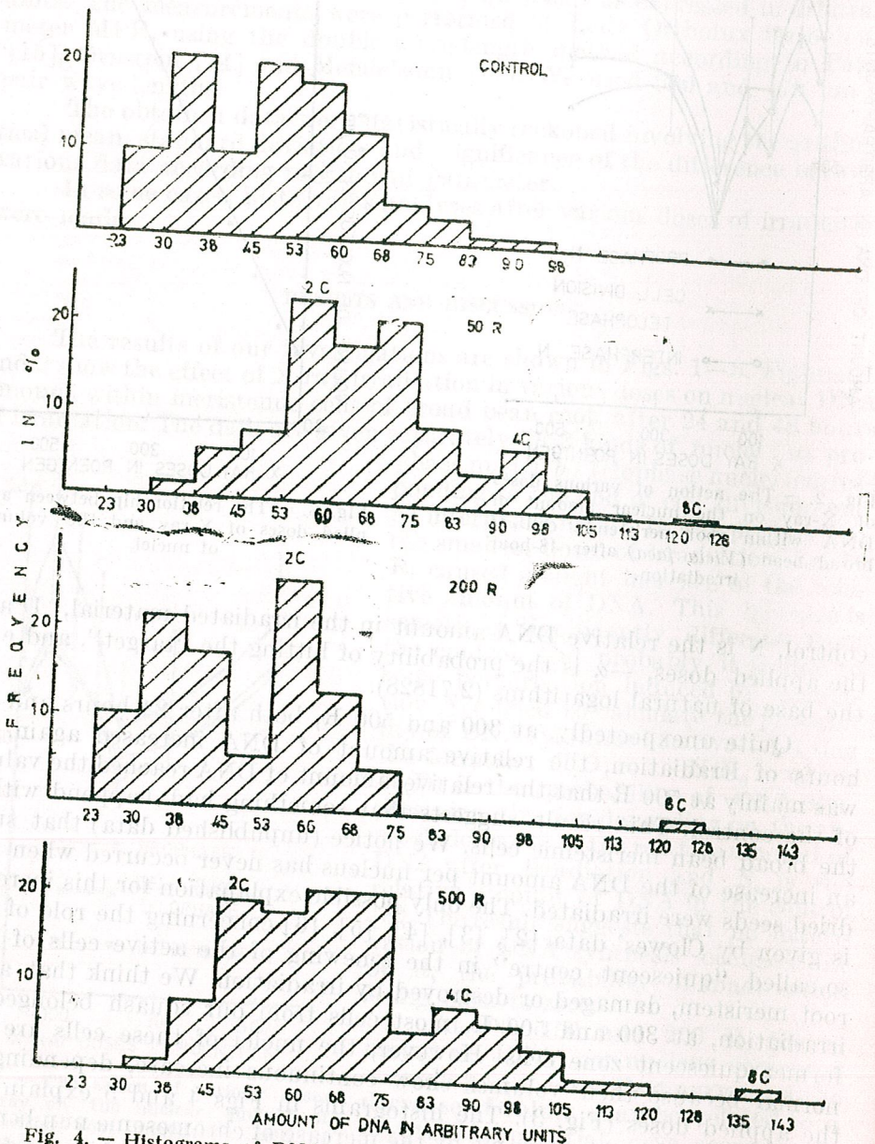

Fig. 4. — Histograms of DNA distribution at several doses of irradiation by X-ray, after 24 hour irradiation.

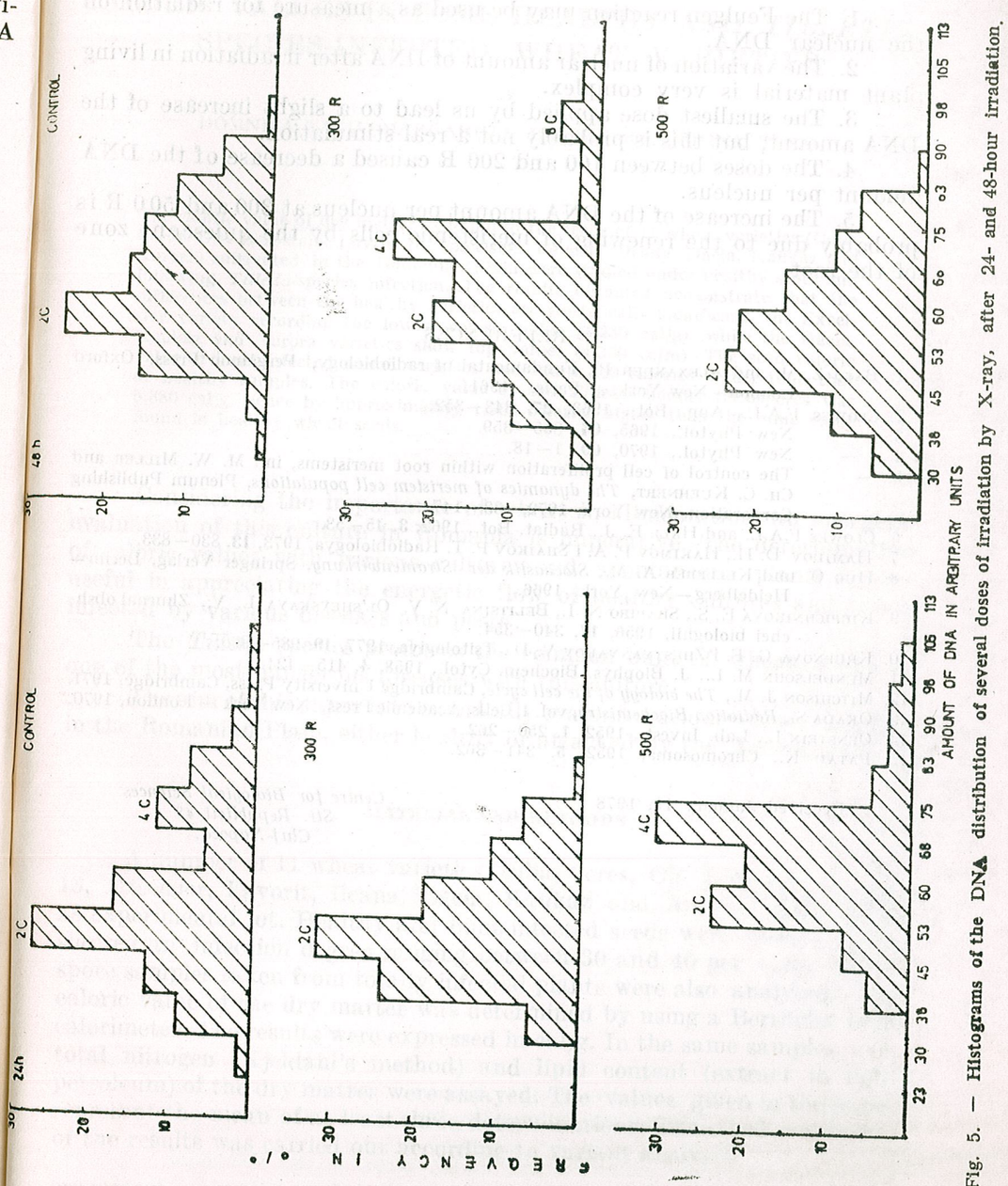

Fig. 5. — Histograms of the DNA distribution of several doses of irradiation by X-ray, after 24- and 48-hour irradiation.

CONCLUSIONS

1. The Feulgen reaction may be used as a measure for radiation on the nuclear DNA.

2. The variation of nuclear amount of DNA after irradiation in living plant material is very complex.

3. The smallest dose applied by us lead to a slight increase of the DNA amount, but this is probably not a real stimulation.

4. The doses between 100 and 200 R caused a decrease of the DNA amount per nucleus.

5. The increase of the DNA amount per nucleus at 300 and 500 R is probably due to the renewing of meristemic cells by the quiescent zone of the root.

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CALORIC VALUES IN SOME HEALTHY AND *TILLETIA*-SPECIES-INFECTED WHEAT VARIETIES

BY

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The caloric value of dry matter as found in the seeds of 11 wheat varieties (Iulia, Ceres, Olt, Bezostaia, Lovrin 13, Excelsior, Favorit, Ileana, Dacia, Kaukaz and Aurora) cultivated in the Romanian Plain were studied under healthy state and following *Tilletia*-species infection. The results obtained demonstrate that the differences between the healthy varieties are statistically significant, the Excelsior variety recording the lowest caloric value (4,230 cal/g), while the Dacia, Kaukaz and Aurora varieties show top values (4,320 cal/g). The seed caloric value of bunt-infected wheat varieties was about 300 calories higher than that of healthy samples. The caloric value of the *Tilletia* fungus teleospores was 5,880 cal/g, hence by approximately 1,500 calories higher than the same value found in healthy wheat seeds.

Considering the importance of wheat in alimentation, an energetic evaluation of this culture in Romania is imperative. The determination of caloric values with various cultures and spontaneous plants is also useful in appreciating the energetic flow of healthy cultures and those infected by various diseases and pests.

The *Tilletia* species produces the common bunt of wheat, which is one of the most dangerous diseases with this culture.

The aim of this paper is to calorically evaluate various wheat varieties in the Romanian Plain, either healthy or attacked by the *Tilletia* species.

MATERIAL AND METHODS

A number of 11 wheat varieties (Iulia, Ceres, Olt, Bezostaia, Lovrin 13, Excelsior, Favorit, Ileana, Dacia, Kaukaz and Aurora) were taken as experimental lot. Healthy and bunt-infected seeds were studied which showed an infection degree ranging between 30 and 40 per cent. Teleospore samples taken from totally infected plants were also analysed. The caloric value of the dry matter was determined by using a Berthelot type calorimeter. The results were expressed in cal/g. In the same samples, ash, total nitrogen (Kjeldahl's method) and lipid content (extract in light petroleum) of the dry matter were assayed. The values given in the paper represent the mean of at least three determinations. Statistical evaluation of the results was carried out according to variant analysis.

RESULTS

The caloric values found in healthy and bunt-infected wheat are given in tables 1 and 2. Comparison between the caloric values of dry matter in healthy varieties (Table 1) reveals a distinctly significant difference

Table 1

Caloric values in several healthy wheat varieties

Variety	Water %	Ash %	Extract in light petroleum (lipids) %	Total nitrogen (N) %	Caloric values of dry matter cal/g
Iulia	7,14	1,64	1,12	1,96	4.290±47
Ceres	6,90	1,52	1,07	1,73	4.270±26
Olt	7,06	1,51	2,18	1,79	4.310±33
Bezostaia	7,17	1,73	0,99	1,86	4.280±15
Lovrin 13	6,88	1,59	1,24	1,90	4.300±24
Excelsior	7,38	1,63	1,34	1,82	4.230±106
Favorit	7,47	1,66	1,26	1,84	4.300±48
Ileana	6,98	1,58	1,34	2,19	4.300±52
Dacia	7,08	1,62	1,24	2,12	4.320±53
Kaukaz	7,12	1,66	0,99	1,86	4.320±18
Aurora	7,40	1,61	1,10	1,56	4.320±23

Table 2

Caloric values in several bunt-infected wheat varieties

Variety	Water %	Ash %	Extract in light petroleum (lipids) %	Total nitrogen (N) %	Caloric values of dry matter cal/g
Iulia	7,28	2,19	1,26	2,33	4.700±93
Ceres	6,84	2,30	1,17	2,12	4.730±31
Olt	7,36	2,15	2,39	2,46	4.600±23
Bezostaia	7.20	2,24	1,25	2,35	4.700±83
Lovrin 13	8,96	2,11	1,46	2,58	4.680±92
Excelsior	7,08	2,26	1,98	2,28	4.770±117
Favorit	7,64	1,84	1,31	2,06	4.470±63
Ileana	8,16	2,21	1,65	2,25	4.670±40
Dacia	8,60	2,07	1,89	2,46	4.710±28
Kaukaz	8,40	2,35	1,75	2,46	4.760±51
Aurora	8,52	2,07	1,79	2,32	4.660±20
Teliospores of *Tilletia* sp.	8,20	3,46	0,83	3,60	5.880±88

(F = 3.2 ; 10 and 71 d.f.), the Excelsior variety recording the minimal value (4,230 cal/g) while the Dacia, Kaukaz and Aurora varieties show maximal values (4,320 cal/g). Among the bunt-infected varieties (Table 2), distinctly significant differences are also recorded (F = 16.9 ; 10 and 55 d.f.), the maximal caloric value being found in the Excelsior variety (4,770 cal/g) while the Favorit variety shows the lowest value (4,470 cal/g).

By comparing the caloric values of healthy wheat to those found in the infected one, a difference of about 300 calories more is found in the infected wheat (F = 123.2 ; 21 and 126 d.f.), a distinctly significant difference. The energetic value of the teleospores is by about 1,500 calories higher than in healthy seeds (F = 539.0 ; 11 and 76 d.f.). The other parameters analysed (lipid and especially ash and total nitrogen content) recorded higher values in the infected seeds, which is the more so in the teleospores in which the ash and nitrogen content is twice as large as in healthy wheat. Emphasis should be laid on the Olt variety which, as compared to the other varieties, contains higher lipid percentage in both healthy and infected plants.

DISCUSSIONS

The results obtained in the present study demonstrate that the caloric value of bunt-infected wheat seeds is higher than the caloric value of healthy seeds. The larger concentration in both lipids and especially total nitrogen found in the infected wheat seed somehow justifies the findings reported in the work. When correlated with the high energetic value of the *Tilletia* teleospores, the high percentage of total nitrogen assayed in the latter seems to be due to their chemical composition which is rich in proteins favouring infections.

With respect to the parasite-host plant relationships it appears that, according to our results, the host plant (wheat) is inferior from the energetic viewpoint as against the parasite (*Tilletia* sp.). This energetic capacity of *Tilletia* spp. has an adverse effect on wheat plants since it assists the onset of the disease. The readiness with which this disease spreads and the high damages caused are probably correlated with this capacity. Bunt-infected wheat crops cannot turn into good account the consumption of energy from ecosystems, a fact of undesired consequences on the quantity and quality of yields. The data presented reveal the scarcely studied aspect of the pathological process determined by one of the commonest diseases of wheat, which is a basic plant crop. Sound knowledge of the physiological aspects of host plant-pathogen system contributes to the development, within the framework of wheat cropping technologies, of an adequate control based on modern scientific principles.

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LA VALORISATION DE LA FLORE MÉDICINALE DU DÉPARTEMENT DE BOTOȘANI (ROUMANIE)

PAR

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The work deals with the spontaneous medicinal flora in the Botoșani district, which can be exploited in large quantities, without disturbing local biological equilibrium. Many of the plants existing in this district, are of fine quality and some species represent kinds of raw valuable material.

Dans le cadre du problème d'importance scientifique et économique concernant la cartographie des plantes médicinales de la flore spontanée de Roumanie, on a continué à étudier et à mettre en valeur celles du département de Botoșani, situé à l'extrémité nord-est du pays, entre 48°15′06″ latitude nord et 26°42′05″ longitude est, ayant une superficie de 4,965 km carrés.

Données générales géographiques. Une grande partie du territoire du département est occupée par la dépression Jijia-Bahlui, qui au nord est entourée d'une partie montueuse, coupée de collines ne dépassant pas 200 m d'altitude. Vers l'est il y a une zone de plaine et de prairie le long du Prut et vers l'ouest la zone de hautes terrasses de la rive gauche du Siret, qui fait partie de la zone sud-est du Plateau de Suceava, avec des hauteurs dépassant environ 300 m, culminant au sud-ouest avec le prolongement nord de la cime de Dealu Mare (593 m).

Le réseau hydrographique est orienté en général du nord-ouest vers le sud-est. Deux vallées principales traversent le centre du département : Başeu et Jijia [7] où se réunissent de petits ruisseaux qui souvent tarissent en été, et deux autres grandes rivières, le Prut et le Siret, délimitent le département à l'est et à l'ouest.

Le sous-sol est constitué principalement de roches sédimentaires calcaires avec des insertions de grès, marnes, argiles et sables sarmatiques et pliocènes.

Le sol est formé particulièrement de tchernozioms lévigés et de tchernozioms typiques, surtout dans les parties plus basses du département. Toujours dans les régions basses apparaissent des lopins de solonetz, groupes de sols très fertiles, contenant à un haut degré du sodium et de solontcheacs, groupes de sols interzonaux avec un contenu élevé de sels solubles — chlorures, sulfates, carbonates, formés par l'accumulation des sels résiduels des roches.

Dans les parties plus hautes prédominent les sols gris foncé et brun-gris. La partie occidentale qui appartient au Plateau de Suceava comporte des sols bruns-podzoliques.

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Dans les vallées du Siret et du Prut, riches en végétation spontanée et en cultures, il y a des sols alluvionnaires très fertiles.

La flore et la végétation. La flore spontanée du département de Botoşani est caractéristique de la sylvo-steppe, où l'on peut rencontrer de petits groupes de forêts de chênes et de rouvres *(Quercus robur* et *Q. petraea)* et seulement quelques prés de steppe xéromésophiles secondaires avec *Festuca valesiaca* et *Stipa joanis*. Dans la partie de l'ouest, centrale-nord et sud, là où le terrain n'est pas cultivé, sont présents encore des prés secondaires avec *Festuca pseudovina*, *F. rupicola*, *Botriochloa ischaemum*, etc. Dans la vallée du Prut, du côté nord du département, il y a un bassin riche en *Inula helenium*, au nord-ouest quelques groupes de rouvraies et au sud-ouest des hêtraies de colline, mêlées au rouvre.

Par l'extension graduelle des zones arables dans cette région ont été prises des mesures pour protéger certaines plantes rares par la création de nombreuses réserves naturelles.

Dans ce département sont protégés le nénuphar jaune et le nénuphar blanc de la vallée du Siret, de même que le lys sylvestre *(Lilium martagon)* dans toutes les forêts du département. Il faut mentionner aussi l'espèce relicte *Schivereckia podolica* de Ştefăneşti — Truseşti, comme un élément rare pour la flore de la République Socialiste de Roumanie.

Quant à la flore médicinale du département de Botoşani, on a identifié 158 espèces appartenant à 134 genres et à 58 familles ; les plantes médicinales, à l'exception de celles rudérales, se trouvent surtout dans les vallées du Siret et du Prut, dans les forêts limitrophes de celles-ci, vers l'ouest et le sud du département.

On peut récolter, des cultures de céréales, sur tout le territoire du département, les espèces : *Papaver rhoeas*, *Delphinium consolida* et *Centaurea cyanus*.

Les méthodes de travail sont celles utilisées pour les travaux de cartographie antérieurs.

L'énumération des principales espèces médicinales et les quantités proposées en vue de leur valorisation sont présentées en ordre systématique dans le tableau 1.

Outre cette évaluation quantitative de la matière première d'origine végétale avec des applications dans la thérapeutique, on a effectué aussi l'évaluation qualitative du matériel récolté dans différentes périodes de l'année, par des recherches phytochimiques concernant le contenu de principes actifs, calculé au pourcentage. A cet effet, on a analysé des échantillons de divers organes de plantes médicinales fréquemment utilisées en thérapeutique, telles que : *Flores Millefolii*, *Flores Chamomillae*, *Herba Serpylli*, *Radix* et *Folium Belladonae*, *Cortex Frangulae*, *Fructus Cynosbati* et autres. Les résultats des déterminations quantitatives des principes actifs sont exprimés dans le tableau 2.

De ces données il résulte ce qui suit : tous les échantillons de *Flores Millefolii* ont une teneur en huile volatile entre 0,28—0,40 %, résultat qui s'intègre dans les limites moyennes à l'échelle du pays, en ce qui concerne ce paramètre. Mais, vu que, pour obtenir le chamazulen à l'échelle industrielle par l'entraînement avec des vapeurs d'eau, l'huile volatile doit, pour être rentable, contenir au moins 30 % de chamazulens, tous les échantillons n'ont pas réalisé cette condition. Ainsi, les échantillons

Tableau 1

Dénomination de la plante	L'organe récolté	La quantité en kilos
1. *Equisetum arvense* L.	Herba	3 700
2. *Equisetum maximum* Lam.	Herba	2 200
3. *Dryopteris filix-mas* (L.) Schott	Rhizoma	4 050
4. *Corylus avellana* L.	Folium	27 250
5. *Betula verrucosa* Ehrh.	Cortex	16 100
6. *Quercus* sp.	Cortex	10 500
7. *Juglans regia* L.	Folium	84 500
8. *Salix* sp.	Cortex	99 750
9. *Populus nigra* L.	Gemmae	3 500
10. *Morus* sp.	Folium	7 000
11. *Urtica dioica* L.	Folium	29 500
12. *Poligonum hydropiper* L.	Herba	3 700
13. *Polygonum aviculare* L.	Herba	700
14. *Lychnis flos-cuculi* L.	Herba	600
15. *Mercurialis perennis* L.	Herba	4 450
16. *Asarum europaeum* L.	Radix	15 250
17. *Aristolochia clematitis* L.	Radix	6 600
18. *Corydalis cava* (L.) Schw. et Koerte	Tuber	500
19. *Alliaria officinalis* Andrz.	Herba	1 700
20. *Dentaria bulbifera* L.	Herba	1 050
21. *Viola odorata* L.	Radix	2 400
Viola silvestris Lam.	Radix	
22. *Viola arvensis* Murr. *V. tricolor* L.	Herba	1 100
23. *Hypericum perforatum* L.	Herba	2 550
24. *Crataegus monogyna* Jacq.	Fructus	11 700
25. *Fragaria vesca* L.	Folium	17 680
26. *Potentilla anserina* L.	Herba	3 600
27. *Geum urbanum* L.	Rhizoma	9 450
28. *Filipendula hexapetala* Gilib.	Flores	2 700
29. *Agrimonia eupatoria* L.	Herba	5 900
30. *Rubus plicatus* Weihe et Ness	Folium	19 000
31. *Rosa* sp.	Fructus	10 500
32. *Prunus spinosa* L.	Fructus	8 250
33. *Cerasus avium* (L.) Mnch.	Stipites	1 450
34. *Melilotus officinalis* (L.) Medik.	Herba	2 150
35. *Trifolium repens* L. *T. pratense* L.	Flores	6 300
36. *Robinia pseudacacia* L.	Flores	200 550
37. *Lythrum salicaria* L.	Herba	2 650
38. *Malva neglecta* Wallr.	Folium	1 350
39. *Malva silvestris* L.	Folium	700
40. *Tilia cordata* Mill.	Flores	8 200
41. *Tilia platyphyllos* Scop.	Flores	2 600
42. *Tilia tomentosa* Mnch.	Flores	14 700
43. *Geranium robertianum* L.	Herba	1 050
44. *Euonymus europaeus* L.	Cortex	750
45. *Cornus sanguinea* L.	Cortex	7 900
46. *Cornus mas* L.	Fructus	8 600
47. *Hedera helix* L.	Folium	2 850
48. *Sanicula europaea* L.	Herba	7 450
49. *Eryngium campestre* L.	Herba+Radix	3 700
50. *Eryngium planum* L.	Herba	3 350
51. *Cicuta virosa* L.	Herba	600
52. *Aegopodium podagraria* L.	Herba	1 100
53. *Lysimachia nummularia* L.	Herba	3 150
54. *Convolvulus arvensis* L.	Herba	1 850
55. *Calystegia sepium* (L.) Ro. Br.	Herba	1 550
56. *Symphytum officinale* L.	Radix	1 300

Dénomination de la plante	L'organe récolté	La quantité en kilos
57. *Pulmonaria officinalis* L.	Herba	8 500
58. *Pulmonaria montana* Lej.	Folium	14 500
59. *Lithospermum officinale* L.	Herba	
60. *Echium vulgare* L.	Herba	500
61. *Atropa belladonna* L.	Radix + Folium	2 700
62. *Physalis alkekengi* L.	Fructus	750
63. *Linaria vulgaris* Mill.	Herba	1 200
64. *Scrophularia nodosa* L.	Herba	1 700
65. *Melampyrum bihariense* Kern.	Herba	3 200
66. *Teucrium chamaedrys* L.	Herba	650
67. *Marrubium vulgare* L.	Herba	1 050
68. *Glecoma hederacea* L.	Herba	1 250
69. *Prunella vulgaris* L.	Herba	6 000
70. *Leonurus cardiaca* L.	Herba	750
71. *Salvia nemorosa* L.	Herba	4 800
72. *Salvia glutinosa* L.	Folium	1 150
73. *Betonica officinalis* L.	Herba	550
74. *Origanum vulgare* L.	Herba	7 850
75. *Thymus* sp.	Herba	5 500
76. *Mentha longifolia* (L.) Nathh.	Herba	2 600
77. *Plantago major* L.	Folium	3 100
78. *Plantago lanceolata* L.	Folium	12 100
79. *Plantago media* L.	Folium	9 000
80. *Cynanchum vincetoxicum* (L.) Pers.	Rhisoma + Radix	9 100
81. *Fraxinus excelsior* L.	Folium	1 600
82. *Fraxinus ornus* L.	Folium	20 600
83. *Ligustrum vulgare* L.	Flores	8 750
84. *Asperula odorata* L.	Herba	4 450
85. *Sambucus nigra* L.	Flores	6 500
86. *Sambucus ebulus* L.	Radix	13 650
87. *Viburnum opulus* L.	Cortex	12 900
88. *Viburnum lantana* L.	Folium	3 000
89. *Valeriana officinalis* L.	Cortex	7 600
90. *Dipsacus silvester* Huds.	Rhisoma + Radix	3 700
91. *Eupatorium cannabinum* L.	Herba	750
92. *Bellis perennis* L.	Flores	11 600
93. *Inula helenium* L.	Rhisoma + Radix	600
94. *Achillea millefolium* L.	Herba	24 000
95. *Chrysanthemum vulgare* (L.) Bernh.	Flores	13 300
96. *Artemisia absinthium* L.	Herba	5 850
97. *Artemisia vulgaris* L.	Herba	8 850
98. *Tussilago farfara* L.	Folium	1 850
99. *Petasites hybridus* (L.) G. M. Sch.	Rhisom	6 200
100. *Arctium lappa* L.	Radix	11 400
101. *Taraxacum officinale* Weber	Radix	15 500
102. *Cichorium intybus* L.	Radix	13 500
103. *Altium ursinum* L.	Herba	8 700
104. *Asparagus officinalis* L.	Herba	1 550
105. *Polygonatum officinale* L.	Herba	950
106. *Convallaria majalis* L.	Folium	4 850
		6 360

récoltés près de Horlăceni, à la lisière de la forêt de Vorona, de la forêt de Vlădeni, de Băbiceni, des forêts de Pădureni, Soldănești et Cerbu ont été complètement dépourvus de chamazulen, la majorité des échantillons ont eu de petites quantités de chamazulen, qui ont imprimé à l'huile végétale une couleur faiblement bleuâtre ; un seul échantillon, celui provenant

Tableau 2

Dosage des principes actifs de certains échantillons récoltés en 1976 dans différentes localités du département de Botoșani

Dénomination de l'échantillon	Stade de la végétation	Date de la récolte	Localité	Principe actif dosé	Contenu %	Humidité	Observations sur les principes actifs
1	2	3	4	5	6	7	8
Flores Millefolii	Floraison	24.VI.	Oroftiana Vallée du Prout	Huile volatile	0,40	7,61	faiblement bleu
Flores Millefolii	Floraison	20.VI.	Balta Teilor Vallée du Siret	-,,-	0,40	7,02	bleu
Flores Millefolii	Floraison	5.IX.	Petricani Săveni	-,,-	0,41	4,94	faiblement bleu
Flores Millefolii	Floraison	26.VI.	Forêt de Vorona près de Horlăceni	-,,-	0,40	7,13	incolore
Flores Millefolii	Floraison	7.IX.	Forêt de Rădeni	-,,-	0,39	5,61	faiblement bleu
Flores Millefolii	Floraison	7.IX.	Forêt de Vlădeni Deal	-,,-	0,39	6,43	incolore
Flores Millefolii	Floraison	8.IX.	Forêt de Copălău	-,,-	0,30	5,49	faiblement bleu
Flores Millefolii	Floraison	9.IX.	Forêt de Băbiceni	-,,-	0,42	6,21	incolore
Flores Millefolii	Floraison	10.IX.	Forêt de Drislea	-,,-	0,39	6,01	faiblement bleu
Flores Chamomillae	Floraison	24.VI.	Oroftiana Vallée du Prout	-,,-	0,44	7,58	bleu verdâtre
Herba Thymi serpylli	Floraison	25.VI.	-,,-	-,,-	0,60	6,32	
Herba Thymi serpylli	Floraison	20.VI.	Balta Teilor Vallée du Siret	-,,-	0,44	6,32	
Herba Menthae	Floraison	17.VI.	Forêt de Gorovei	-,,-	0,60	6,80	
Herba Menthae	Floraison	3.IX.	Forêt de Tudora Pleșa	-,,-	0,38	6,11	
Folium Belladonnae	Avant la floraison	19.VI.	Forêt de Pustoaia	Alcaloïdes totaux	0,38	6,2	
Folium Belladonnae	Avant la floraison	18.VI.	Forêt de Baranca	Alcaloïdes totaux	0,27	7,81	
Radix Belladonnae	Fructification	18.VI.	-,,-	-,,-	0,40	7,0	
Radix Belladonnae	Fructification	8.IX.	Forêt de Stahna	-,,-	0,42	6,09	
Cortex Frangulae	Fructification	28.VI.	Forêt de Mlenăuți à Hudești	Oxyméthylanthrachinones	2,30	8,30	
Fructus Cynosbati	Fruits mûrs	4.IX.	Cristești-Oneaga	Vitamine C	0,86	5,48	
Fructus Cynosbati	Fruits mûrs	9.IX.	Forêt de Băbiceni	-,,-	0,82	6,12	
Fructus Cynosbati	Fruits mûrs	11.IX.	Forêt de Doina	-,,-	0,84	5,44	
Fructus Cynosbati	Fuits mûrs	12.IX.	Forêt de Zahoreni	-,,-	0,85	6,34	

de la vallée du Siret, de Balta teilor, a contenu des quantités appréciables de chamazulen.

Comme on le sait, la teneur en chamazulen varie beaucoup en fonction de l'espèce, du moment de la floraison, du caractère génétique et de la manière de séchage. Bien que l'on n'ait pas étudié tout spécialement ces facteurs, on peut conclure que les échantillons de *Flores millefolii* du département de Botoşani ne présenteraient pas de valeur économique quant à l'obtention du chamazulen, mais seulement pour les principes actifs qu'ils contiennent. Pas même le seul échantillon de *Flores Chamomillae* récolté dans ce département n'a contenu des chamazulens caractéristiques de la camomille d'autres départements, mais cela ne présente pas d'importance, puisque la flore spontanée du département de Botoşani ne dispose pas de réserves importantes de camomille.

— En ce qui concerne *Herba Serpylli*, le contenu d'huile volatile s'intègre dans les limites moyennes.

— Parmi les échantillons de *Mentha longifolia*, un échantillon récolté avant la floraison dans la forêt de Gorovei, près de Horlăceni, retient l'attention par la teneur très élevée en huile volatile : 1,2%.

— Un fait qui vaut bien être mis en évidence pour cette région, c'est la teneur relativement élevée en alcaloïdes dans certains échantillons de *Atropa belladonna*, par exemple ceux de la Forêt de Baranca et de la Forêt de Stahna, qui, bien que récoltés dans une période moins optimale pour les racines, contiennent 0,40—0,42% d'alcaloïdes. Certains échantillons de feuilles provenant de cette espèce, tels ceux de la Forêt de Pustoaia, en ont eu un contenu dépassant les prévisions. C'est pourquoi nous considérons que dans cette zone il serait rentable de rendre spontanée cette importante espèce médicinale, d'autant plus que dans le département de Botoşani, *Atropa belladonna* est assez peu répandue, dans des endroits isolés sporadiques.

— La teneur en dérivés anthracéniques, dans l'écorce de *Rhamnus Frangula*, ne diffère pas beaucoup de la moyenne, s'intégrant dans les limites habituelles.

— La teneur en acide ascorbique des fruits d'églantier récoltés dans la première moitié du mois de septembre n'a pas dépassé 0,90% de vitamine C, se situant entre 0,66—0,86% dans des fruits récemment cueillis et analysés après séchage dans des conditions naturelles, à l'humidité de 5,11—6,34%.

CONCLUSIONS

Bien que le département de Botoşani ne soit pas l'un des départements de grande importance en ce qui concerne la flore spontanée à cause de l'extension des surfaces cultivées ou boisées dans cette région, on peut y mettre en valeur dans des quantités appréciables, comprises entre 2—80 tonnes, les espèces médicinales suivantes :

Equisetum arvense, Betula verrucosa, Juglans regia, Salix sp. Asarum europaeum, Crataegus monogyna, Corylus avellana, Rosa sp., Robinia pseudacacia, Tilia sp., Plantago sp., Sambucus nigra, Inula helenium, Artemisia absinthium, Fragaria vesca, Pulmonaria montana, etc.

Bon nombre des espèces médicinales identifiées dans la flore spontanée du département de Botoşani sont de bonne qualité et certaines espèces fournissent des assortiments de matière première importante qui, à l'heure actuelle, est déficitaire dans d'autres départements de Roumanie. Compte tenu des voies d'accès praticables et des possibilités locales, nous estimons que la valorisation des plantes médicinales spontanées de ce département est rentable pour certains assortiments.

Mais l'identification de nouveaux bassins de plantes médicinales dans ce département aussi exige en même temps des mesures de protection du patrimoine végétal naturel par une exploitation rationnelle, de même que par le réensemencement des espèces dont on récolte la plante entière ou les organes souterrains.

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THE INFLUENCE OF AIR-POLLUTING GASES ON SOME PLANT METABOLISM

BY

ILEANA BUICULESCU, D. POPESCU, MARGARETA IORDAN, I. M. PEICEA and G. ŞERBĂNESCU

The article presents the effects of atmosphere pollution with SO_2 and fluorides on some physiological indicators (content of free and bound water, intensity of respiration and photosynthesis, content of chlorophylls *a* and *b*), their seasonal variation in the three wooden species (*Elaeagnus augustifolia*, *Populus nigra*, and *Ulmus procera*) near a fertilizer factory. Compared to plants in the control zone, important modifications are noticed at the level of all the parameters analysed.

Our aim in this paper is to approach problems related to the seasonal variation of some parameters for establishing the physiological state of some plants in the surrounding area of a fertilizer factory, under the permanent influence of noxae (SO_2, fluorides, CO, dust).

As stated in the literature, the action of air-polluting gases on the physiological processes of plants was studied mainly in laboratory conditions (fumigation rooms) with controlled regime for a few hours and days, while the investigations in field conditions during the whole vegetation period were sporadic.

MATERIAL AND METHODS

We have examined the leaves of *Elaeagnus angustifolia*, *Populus nigra* and *Ulmus procera* in an industrial area and a control station outside the influence of the chemical plant, 35 km southwards.

The following methods were used : the Boysen-Jensen for determining the intensity of respiration, the Ivanov-Kossovici for photosynthesis intensity [2], the Okunţov-Marincik for dosing the free water in leaves [6], and the Mackinney for establishing the chlorophyll quantity [5]. At the same time, measurements were made on some environmental factors implied in the processes investigated (light intensity, air and soil temperature, soil humidity) ; they did not present significant differences between the control and the polluted zone.

RESULTS AND DISCUSSIONS

THE CONTENT OF FREE AND BOUND WATER IN LEAF TISSUES

According to the theory of biostructure established by E. Macovschi [3, 4], living cells contain water in three main forms, namely : *assimilated water* integrated in the biostructure, which has not the characteristics of

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a solvent, *bound water* connected to hydrophyll colloids, and *free water* present in the vacuoles and interstitial content. As this theory shows, in the material treated with metabolical inhibitors, an alteration of the intracellular biostructures and a supplementary release of water is produced, so that the ratio between free and bound water in the affected tissues should be larger than in the control ones. Since the impact on plants of noxae released in the atmosphere by the fertilizer factory — SO_2 and fluorine — is similar to impact of metabolical inhibitors, we have checked the applicability of this theory with the plants *in situ*, which grow in a permanently polluted area.

By the determinations made in spring (May), summer (July) and autumn (September), the seasonal variation of the content in free and bound water in the leaf tissues of the three investigated wooden species was pointed out. From Table 1, it results that the leaves of *Populus nigra*

Table 1

Water percentage (free, bound and total) in leaves in the control (C) and polluted (P) zones

Species	Zone	May			July			September		
		Free	Bound	Total	Free	Bound	Total	Free	Bound	Total
Elaeagnus angustifolia	C	18.5	81.5	73.1	9.3	90.7	64.1	7.4	92.6	63.1
	P	20.7	79.3	73.2	10.5	89.5	71.8	10.1	89.9	71.3
Populus nigra	C	15.2	84.8	73.7	11.7	88.3	65.4	8.1	91.9	62.5
	P	25.9	74.1	75.6	18.0	82.0	69.0	13.0	87.0	67.0
Ulmus procera	C	—	—	73.2	15.5	84.5	61.2	11.4	88.6	62.0
	P	—	—	69.8	18.1	81.9	65.2	12.9	87.1	58.7

and *Elaeagnus angustifolia* in the polluted zone contain a larger quantity of total water and those of *Ulmus procera* a smaller one (excepting July), in comparison with the control samples. In all species in the factory neighbourhood, an increase of the quantity of free water and reduction of the content of bound water is noticed (Fig. 1). Thus, the relationship free-bound water is larger in the polluted zone throughout the vegetation period; its value reduces from spring to autumn in both stations simultaneously with the ageing process in leaves (Table 2).

The increase of free water content in the vegetal tissue of plants that live in impure environment demonstrates that the impact of noxae is similar to that of metabolical inhibitors, the structure of vegetal cells being more or less affected according to Macovschi's theory of biostructure.

RESPIRATION INTENSITY

We could notice that with all species studied, the seasonal respiration curve reaches maximum values during early vegetation period, followed by a gradual decrease towards autumn. Although the seasonal

Table 2

The value of relationship free/bound water in the leaves

Species	Month	Station	
		Control	Polluted
Elaeagnus angustifolia	May	0.22	0.26
	July	0.10	0.12
	Sept.	0.07	0.11
Populus nigra	May	0.18	0.35
	July	0.13	0.22
	Sept.	0.08	0.14
Ulmus procera	May	—	—
	July	0.18	0.22
	Sept.	0.12	0.14

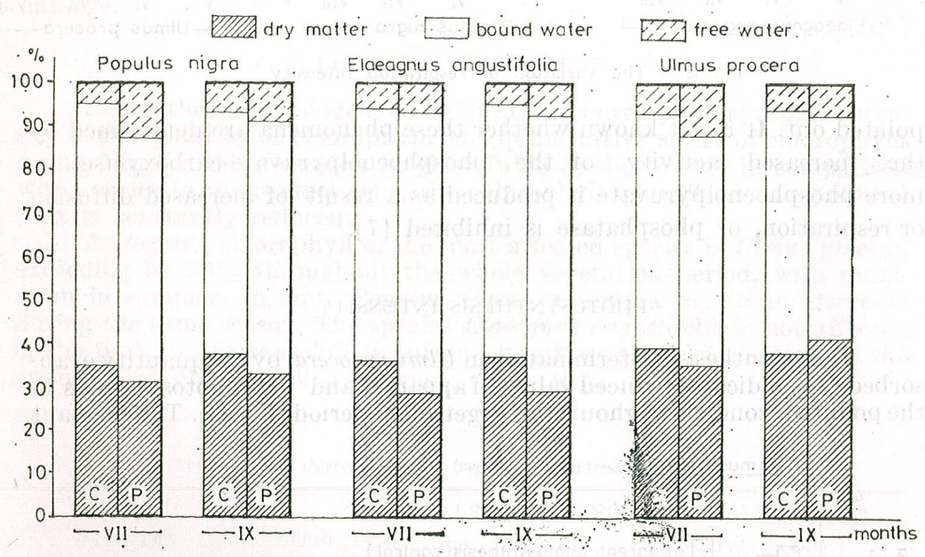

Fig. 1. — The relationship between dry matter, bound and free water, in leaves in the control (C) and polluted (P) zones.

rate of this process is similar in both stations, still, there are remarkable differences as regards its intensity : in the polluted zone, plants have more intense respiration (about 6 times higher in *Elaeagnus angustifolia*) than in the control samples (Fig. 2). Although respiration stimulation had already been pointed out in the case of fluorine and SO_2 fumigations, it may be a nonspecific phenomenon, just a general damage symptom [7].

After fumigations with HF, a high accumulation of malonic acid, malic acid and citric acid, in the incipient stage of necrosis formation was

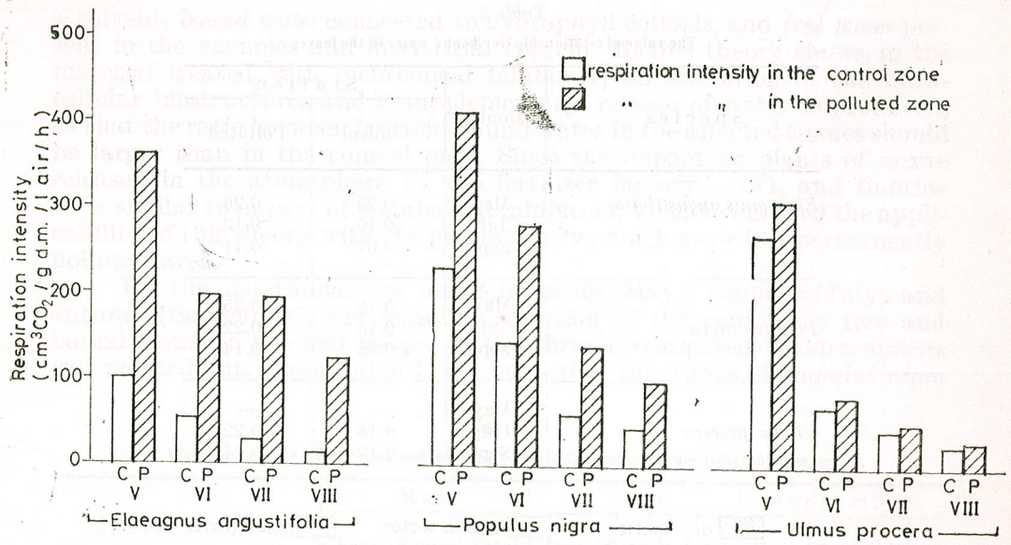

Fig. 2. — The variation of respiration intensity

pointed out. It is not known whether these phenomena are determined by the increased activity of the phosphoenolpyruvate-carboxylase or more phosphoenolpyruvate is produced as a result of increased diffusion or respiration, or phosphatase is inhibited [7].

PHOTOSYNTHESIS INTENSITY

Photosynthesis determination in *Ulmus procera*, by the quantity of absorbed CO_2, indicates reduced values of apparent and real photosynthesis in the polluted zone, throughout the vegetation period (Fig. 3). The constant

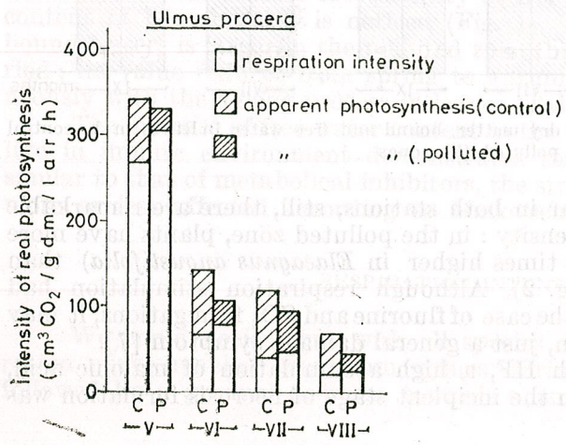

Fig. 3. — The variation of photosynthesis intensity

diminish is due to the high quantities of fluorides and SO_2 in the neighbouring zone of the factory.

Laboratory experiments show that with low concentrations of HF, photosynthesis is not affected, while with higher concentrations, it is reduced by about 40% [7]; with SO_2 concentrations that are too strong for the metabolical capacity of plants, reduced assimilation also occurs.

Although many studies have been carried out by now, especially as regards SO_2, the inhibitio n mechanism of photosynthesis has not been fully elucidated. We do not know whether SO_2 acts directly on the photosynthesis reactions or the membranes are first damaged, whether the effect is produced because of protoplasm acidification or by altering the redox system.

The electron-microscopic study of young maize plant tissues with visible damages, caused by fumigations with SO_2 [1], points out an alteration of cell organelle structure and ,in case of a prolonged action of SO_2, a total and irreversible disaggregation of the cell structure. The ultrastructural modifications solve only partially the process of photosynthesis damage.

CHLOROPHYLL CONTENT

One of the main endogenous factors that control photosynthesis intensity is the quantity of chlorophyll. The quantitative study of chlorophylls *a* and *b* in the three wooden species (Table 3) shows that in comparison with species in the control zone, the chlorophyll quantity in the polluted area is constantly reduced.

As regards chlorophyll *a*, the most affected species is *Ulmus procera*, exceeding by 50% throughout the whole vegetation period, with maximum in summer. In fact, *Populus nigra*, presents a maximum decrease during the same season. The species *Elaeagnus angustifolia* is not affected in the first two seasons, but in autumn it suffers a sudden decrease in the quantity of chlorophyll *a* by more than 40%.

Table 3

The variation of chlorophyll content (mg chlorophyll/g fresh leaves)

Species	Month	Chlorophyll *a*				Chlorophyll *b*			
		Control	Polluted	Difference	%	Control	Polluted	Difference	%
Elaeagnus angustifolia	May	6.87	6.82	0.05	0.73	3.92	3.02	0.90	22.96
	July	9.63	9.46	0.17	1.76	5.41	3.63	1.78	32.90
	Sept.	9.19	5.48	3.71	40.37	3.47	2.13	1.34	38.62
Populus nigra	May	6.96	6.08	0.88	12.64	3.10	2.82	0.28	9.03
	July	8.65	5.35	3.30	38.15	3.66	3.17	0.49	13.39
	Sept.	7.03	4.74	2.29	32.57	2.48	2.07	0.41	16.53
Ulmus procera	May	9.83	4.60	5.23	53.20	3.60	2.58	1.02	28.33
	July	9.46	4.04	5.42	57.30	3.05	2.57	0.48	15.74
	Sept.	6.46	2.84	3.62	56.04	3.68	2.08	1.60	43.48

Referring to chlorophyll *b*, the values are smaller, especially in autumn in all the three species, the difference growing progressively from one season to another, excepting *Ulmus procera* with which the smallest difference is in summer. The variation of the total quantity of chlorophyll is constantly revealed in all species and both stations as a continuous reduction process from spring to autumn, with smaller values in species in the polluted zone (Fig. 4). Thus, the direct relationship between chlorophyll quantity and photosynthesis intensity is confirmed.

Many studies pointed out that the synthesis of chlorophylls *a* and *b*, and especially protochlorophyll, is inhibited in plants exposed to fluoride fumigation. McNulty and Newmann suppose that simultaneous inhibition of caroten and chlorophyll synthesis under fluoride effect is due to the decomposition of the chloroplast membrane [7], a phenomenon also noticed in case of fumigation with SO_2 [1].

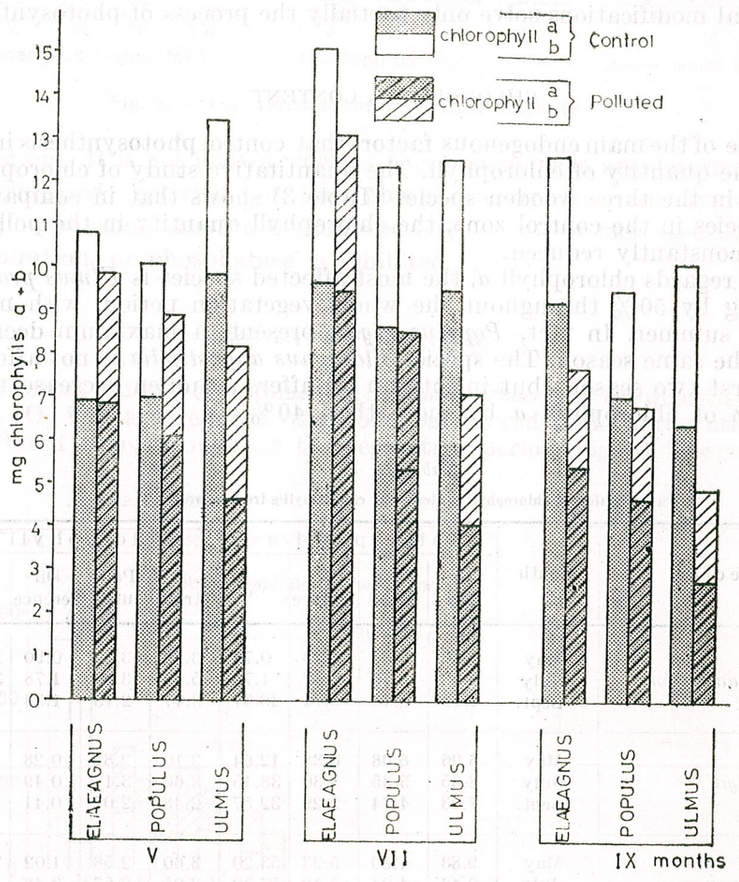

Fig. 4. — Chlorophyll quantity expressed in mg/g fresh leaves.

CONCLUSIONS

As pedoclimatic conditions in the two stations are quite similar, plant metabolism perturbations in the polluted area are due to the main noxae (fluoride and SO_2) produced by the fertilizer factory. The modifications consist in the increase of free water percentage in tissues, decrease of the quantity of chlorophylls *a* and *b* and photosynthesis intensity, and increase of respiration intensity.

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ȘTEFAN PÉTERFI, MEMBER OF THE ACADEMY

(1906 — 1978)

Professor Ștefan Péterfi, an outstanding personality of the Romanian biology and public life, died suddenly on May 6, 1978, while his prolific creative and guiding activity was in full swing. His unexpected death is a great loss not only for the Romanian scientific and cultural life to which he devoted himself for more than half a century, but to the whole scientific world as well.

He was born on March 8, 1906 in the town of Deva, Hunedoara county, as the son of Martin Péterfi, a school teacher who later became a well known bryologist, working at the Botanical Museum of Cluj.

He completed his studies in Cluj, the scientific center of Transylvania, obtaining his diploma in Natural Sciences in 1933. While still a student he started his scientific and didactic activity as a member of the Botany Department led by the distinguished Romanian algologist Ioan Grințescu, who introduced him to the study of algae. His first paper, bearing on the effect of microelements on some filamentous green algae, was the fruit of their collaboration. In 1937 he obtained his doctor's degree with *Magna cum laude* for his thesis on the biology of the green alga *Microthamnion kützingianum*.

Starting with 1929 till his death Ștefan Péterfi faithfully served the Cluj University as preparator, then assistant, lecturer and finally professor of plant physiology from 1943 till 1976. He retired from the didactic activity after being many years head of the Plant Physiology Department, dean of the Faculty of Natural Sciences and pro-rector of the University

(1959 — 1976), so that more than thirty generations of students have benefitted from his high competence and warm generosity.

The scientific field to which he was most attached remained that in which he started his activity in 1932 : the study of the Romanian freshwater algae. His interest was not limited to a methodical investigation of freshwater algae, especially those growing in the peat bogs of the Carpathians (he discovered and described many new species and varieties), but extended to the thorough research of their biology, particularly their development, nutrition, growth conditions, etc. His scientific works on algal physiology, well known and appreciated in phycological circles, were based on his own pure alga cultures. The collection he succeeded in keeping up for many years was the first of that kind in Romania. The scientist of national and international fame and leader of an algological team initiated large-scale phycological investigations, founding a modern algological school in Cluj-Napoca. Fully aware of the importance of freshwater algae for the environment and human life, he introduced the mass cultivation of microscopic algae in view of practical purposes. He was the chief editor of the first Romanian treatise on algology in 4 volumes, of which 2 have already been published, and of a cryptogamic flora of Romania.

Professor Péterfi also manifested a keen interest for plant physiology, especially for the development of flowering plants. In his treatise *The Physiological Bases of Plant Growth and Development* (1954), as well as in those published subsequently (1956, 1960, 1974) he was among the first plant physiologists to emphasize the difference between plant growth and plant development, a conception generally accepted nowadays. Although he was aware of the prevailing role of specific substances in the control of the succession of characteristic phases in the development of plants he did not admit florigen as the only substance of this kind. On the contrary, he recalled Klebs' data concerning the proportion of photosynthesized carbohydrates and the available inorganic nutrients, and following this line he emphasized the mutual action of the hormone-like substances. Both his own interest and that of his co-workers was focused on the alternation of the main characteristic substances (carbohydrates, proteins, inhibitors) synthesized during the life-history of the plants (wheat, vine, pine, hip rose, etc.). Together with his co-workers he carried out a comprehensive research on the biological properties of the fruits of autochthonous and introduced fruit-trees (apples, pears, plums, nuts, etc.), which contributed finally to the elaboration of the first Romanian pomology.

He was likewise deeply concerned with the protection of nature in Romania, his successful activity in this field being well known. Even a hobby — pigeon breeding — became with him a true scientific activity. He published several books in this domain, in which most of the Romanian and East-European breeds were studied and described for the first time.

Professor Ştefan Péterfi, the author of more than two hundred scientific papers, treatises and handbooks, was rewarded for his indefatigable efforts in the field of science by being elected in 1955 as corresponding member and in 1963 as full member of the Romanian Academy.

For three years he was a member of the Praesidium and from 1974 he became vice-president of the same Academy. He was member of several scientific societies and organizations such as the national commissions for IBS, UNESCO and IUBS, vice-president of the Society of Natural Sciences and Geography — Cluj-Napoca Branch, and president of the Subcommission of Natural Monuments of the Academy. For some of his works he was awarded the "Emil Racoviţă" Prize of the Academy (1956) and the First Class Prize of the Ministry of Education (1972), besides many orders and medals given for his scientific and public activities.

He was editor-in-chief or member in the editorial board of several scientific periodicals published by both the University and the Academy.

He was also highly appreciated abroad, being member of the International Phycological Society, as well as of the Scandinavian Society for Plant Physiology. In 1973 he was elected in the Executive Committee of the International Union of Biological Sciences.

Ştefan Péterfi will remain in the memory of all those who knew and appreciated him as a remarkable representative of Romanian biology.

Fr. Nagy-Tóth and *L. Péterfi*

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