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# REVUE ROUMAINE DE BIOLOGIE

## SÉRIE DE BIOLOGIE VÉGÉTALE



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# PROFESSOR NICOLAE SĂLĂGEANU, MEMBER OF THE ACADEMY, ON HIS 80th BIRTH ANNIVERSARY

On December 12, 1987, one of the most distinguished phytophysiologists of Romania, professor Nicolae Sălăgeanu, member of the Academy, celebrated the 80th anniversary of a fruitful life.

Born in 1907 in the Idicel commune, Mureş county, Nicolae Sălăgeanu was educated at the Reghin gymnasium and "Al. Papiu-Ilarian" Highschool of Tîrgu-Mureş.

Between 1928 and 1932, he attended the courses of the Faculty of Sciences at the Bucharest University, studying the natural sciences as first specialty and physico-chemistry as secondary specialty.

After graduating from the university, he was appointed in 1933 as preparator at the Laboratory of Vegetable Physiology of the Faculty of Sciences at the Bucharest University.

Nicolae Sălăgeanu has never ceased working in the laboratory created by Professor Em. C. Teodorescu, the father of Romanian vegetable physiology, standing out by his steady activity and remarkable perseverence, both in scientific research and in education.

The results of his work were highly appreciated and he became an assistant in 1938, an associate professor in 1943 and a professor of vegetable physiology in 1948. In this capacity, he carried out an intense didactic activity, sparing no effort in organizing the students thorough instruction. Together with Prof. E. Pop, Prof. St. Peterfi and Prof. H. Chirilei he wrote a handbook of plant physiology in two volumes, published in 1957 by "Editura Didactică şi Pedagogică". Another handbook, signed by him and Prof. St. Peterfi, was published in 1972 and got high appreciations

Prof. Nicolae Sălăgeanu has been equally interested in his didact activity and in scientific research, which he started as early as his firs appointment in higher education. He worked for 4 years for a doctora thesis entitled "On nutrition in Rhinanthaceae", under the guidance of late professor Em. C. Teodorescu and he contributed to the elucidation of the nutrition of these semiparasitic plants, about which several illustrious physiologists made contradictory statements.

Subsequently, his scientific activity has become more embracing extending to the most varied domains of vegetable physiology. Availing himself of updated scientific information, he has always succeeded in grasping issues at stake; by carrying out painstaking research he made important contributions to the knowledge of a great number of phenomena of plant life.

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From among the aspects of vegetable physiology which interested him, an important part is devoted to problems referring to the water regime of plants. There stand out the works dealing with the physiological criteria for establishing the optimum moment of applying watering norms in irrigated cultures. He has also followed the intensity of transpiration in the day-time and the vegetation period in some of the main varieties of cereals cultivated in Romania, also determining the economic coefficient of their transpiration. In another work of great practical importance, he analysed the value of different indirect methods for establishing the drought resistance of plants.

In addition to problems referring to the water regime, Prof. N. Sălăgeanu has also studied plant development. He has worked out the reaction of some cereal varieties to the duration of the light period and settled the duration of their photoperiodicity.

He has also studied the movement of voluble plants, using a high-sensitivity biological test to prove that auxin is nonuniformly distributed in the stems of these plants, both longitudinally and transversally. Based on these studies, he has offered an explanation for the twisting of voluble plants on their support, as a result of unequal growth on various surfaces of their stem under the action of auxin.

Throughout his long-standing scientific activity, studies concerning the phenomenon of photosynthesis have held pride of place. He has made important contributions to the knowledge of this process, following the influence of environmental factors.

He has pointed out that in fresh-water submersed plants photosynthesis goes on up to freezing temperature, and not only up to 6 — 10°C. By determining photosynthesis and respiration at ever lower light intensities, he has proved that these processes occur down to the lowest light intensities. Photosynthesis behaves the same as photochemical reactions and does not start from a certain threshold of light intensity. His studies on the compensation point in heliophilous, sciatophilous and golden leaves point out that its knowledge may serve to characterize the nature of various leaves and that it varies with the modifications of some external factors, such as temperature and carbon dioxide concentration. He has also dealt with the course of photosynthesis in plants with winter-persistent leaves, as well as with the influence of some mineral substances on photosynthesis.

His studies on photosynthesis have culminated in the working out of a monograph, "Photosynthesis", published by Editura Academiei in 1972, and of a book coauthored with Conf. dr. L. Atanasiu in 1981 in the same publishing house.

Prof. Nicolae Sălăgeanu has intensely sought for procedures aimed at obtaining high yields of mass-cropped algae.

At the National Conference of Physiology (Bucharest, 9—11 October 1967) he presented an extremely interesting work, contributing to the determination of mineral substances required by plants, with the help of alga cultures; this method permits one to determine in a short period of time and on small soil samples the necessary amounts of chemical fertilizers for plants cultivated on various soils.



An extremely important aspect of his scientific activity is the elaboration of original research methods or the improvement of known ones, turning to account his vast experience and his qualities of fine experimenter.

In this connection, we mention his contribution to a method for determining the intensity of photosynthesis and respiration in terrestrial plant leaves at brief time intervals. He has also elaborated a method for determining the intensity of photosynthesis by weighing $CO_2$ in an air current passed through the assimilation chamber, substantially improving the device of $CO_2$ absorption. He has created a new variant of the Warburg manometric method, advantageous for the determination of photosynthesis in leaves of terrestrial plants. These methods are characterized as a whole by great accuracy and possible use in field conditions, which is very important since pertinent results are, as a rule, obtained only in situ.

Some of his scientific works are published with his former students and present coworkers. These cooperation relations have been an efficient means in initiating young people in the ways and norms of scientific research. Prof. N. Sălăgeanu patiently mentored a great number of researchers who submitted their doctoral theses under his scintific guidance, working in higher education or in various research institutes with fine results.

Highly appreciated for his activity, Prof. N. Sălăgeanu was appointed to several senior positions. Between 1948 and 1953 he was the dean of the Biology Department at the Bucharest University, between 1953 and 1954 prorector of this University and between 1954 and 1957 its rector. Between 1956 and 1957 he was a deputee in the Grand National Assembly and a member in its Presidium. He was also the director of the Bucharest Institute of Biological Sciences and chairman of the Commission for the Environment Protection in Romania. He helped in science popularization by an intense activity of lecturing and publishing papers in journals and magazines and booklets on plant life.

As a member of the Presidium of the Academy of the Socialist Republic of Romania he carried out an intense activity as chairman of the Section of Biological Sciences and as editor-in-chief of the journals "Revue roumaine de biologie, Série de biologie végétale" and "Studii şi cercetări de biologie — Seria biologie vegetală", turning to account the most important results of Romanian biological research in the field of vegetable biology.

On the occasion of his 80th birth anniversary, the teaching staff of the Department of Plant Physiology at the Bucharest University, the researchers in plant physiology from biology research institutes and all his students, doctorands and coworkers are expressing their best wishes for good health and a long life!

*Dr. O. Boldor*
*Dr. Georgeta Fabian-Galan*
*Dr. L. Atanasiu*

# A NEW *HERPOTRICHIELLA* (ASCOMYCETES, HERPOTRICHIELLACEAE) ON *CALLUNA* FROM ROMANIA

A. RICHIŢEANU

*Herpotrichiella callunae* A. Richiteanu occurring on *Calluna vulgaris* (L.) Hull (Ericaceae) from Romania is described and illustrated as new.

The genus *Herpotrichiella* was described by Petrak (1914, p. 472) for *H. moravica* on rotting *Fagus* wood. As defined by Müller and von Arx (1962), Barr (1959, 1972), and von Arx and Müller (1975), this genus includes saprobic or hypersaprobic fungi developing a superficial mycelium composed of septate, branched, brown hyphae. The ascomata are uniloculate, globose or conic, thin-walled, setose or roughened by protruding cells. The ascospores are fusoid, elliptic, usually inequilateral, soon light olivaceous, brown or grayish brown, one- to several-septate, often with vertical septum in one or more cells.

*Herpotrichiella* belongs to the *Herpotrichiellaceae*, a family of bitunicate ascomycetes (Loculoascomycetes), erected by Munk (1953) to accomodate *Herpotrichiella*, *Capronia*, *Berlesiella*, and his new genera *Didymotrichiella* and *Dictyotrichiella*. Later, Müller and von Arx (1962) synonymized *Didymotrichiella* under *Herpotrichiella*.

According to von Arx and Müller (1975), the *Herpotrichiellaceae* differ from all other bitunicate ascomycetes by the elongated part of the asci, and by the dark, short setae or protuberances covering the ascomata.

The species of *Herpotrichiella* can easily be mistaken for a *Protoventuria*. *Protoventuria* (Venturiaceae), however, has a hypostroma deeply penetrating into the host tissue, while in *Herpotrichiella* the ascomata are quite superficial, without any hypostroma.

As far as we know, hitherto have been described 7 species of *Herpotrichiella* saprophytic on old wood, leaves and branches of different woody plants or parasitic on other fungi (Petrak, 1914 ; Munk, 1953, 1957 ; Müller and von Arx, 1962 ; Barr 1959, 1972 ; Eriksson, 1974).

Only *Herpotrichiella fusispora* Barr is known as occurring on members of Ericales (*Phyllodoce* and *Cassiope* spp.), both in North America (Barr, 1959, 1972) and Europe (Eriksson, 1974). *Herpotrichiella polyspora*, described by Barr (1959, p. 29) on *Cassiope tetragona* and *Empetrum nigrum* (Barr., 1972, p. 617) and reported by B. Eriksson (1974, p. 211) from Fennoscandia on *Calluna vulgaris*, *Cassiope tetragona* and *Empetrum nigrum*, deviates from the generic diagnosis in the polysporous condition of the asci and it is removed by Barr (1972, p. 617) in her new created genus *Polytrichiella*.

By its morphological characters our collection cannot be referred to any described species, and thus a new one must be described.

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**Herpotrichiella callunae** A. Richiteanu sp. nov. Figs. 1, 2, 3.

*Mycelium* paucum, superficiale, ex hyphis brunneis, irregulariter ramosis, reticulatis, septatis, 2 — 3,5 μm crassis compositum. *Ascomata* solitaria, atra, carbonacea, pyriformia vel globosa, non ostiolata, 80—120 μm crassa, 100—150 μm alta, in dimidio superiore setosa; paries

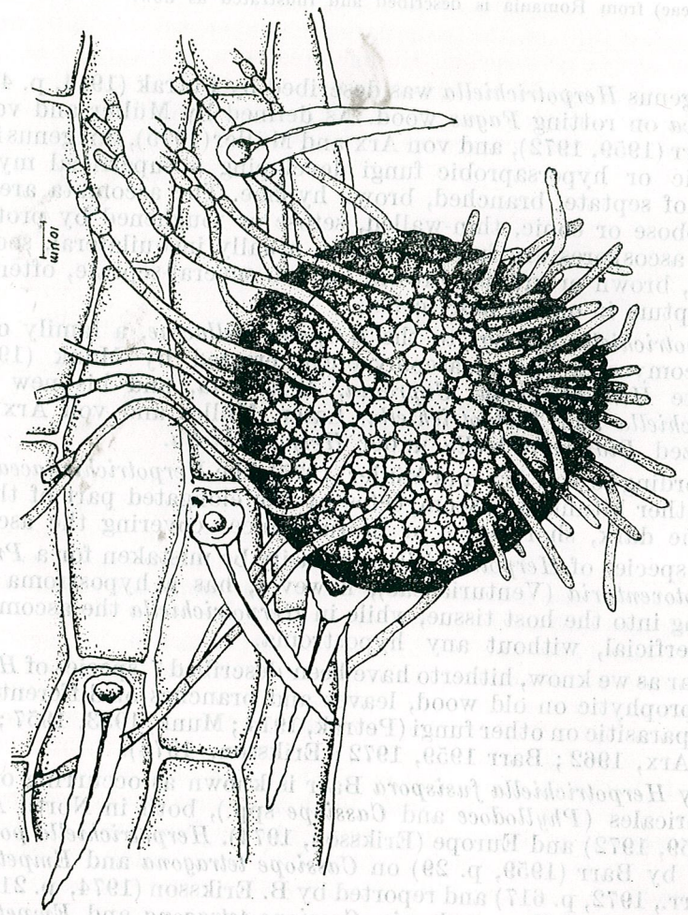


Fig. 1. — *Herpotrichiella callunae* — ascocarp and superficial mycelium sitting on leaf surface.

ascomatorum 1—2 stratosus, e cellulis brunneis, applanatis, extra obscuris et crassoparietalibus compositus; setae numerosae, castaneae, rectae vel curvatae, rigidae, (10—) 20 — 40(—50) μm longae, apicae versus obtusae. *Asci* octospori, cylindracei — clavulati, bitunicati, breviter stipitati, membrana sursum inspissata, 52—72 × 11 — 13 μm; pseudoparaphy-



ses nullae. *Ascosporae* plus minusque biseriatae, primo hialinae et 1 — septatae, deinde dilute virides vel pallide olivaceae et 3 transversaliter septatae, fusoideae vel ellipsoideae, rectae vel leniter curvae, utrinque rotundatae, (13—) 16 — 20 × (4—)5 — 6 μm. *Anamorphosis* ignota.

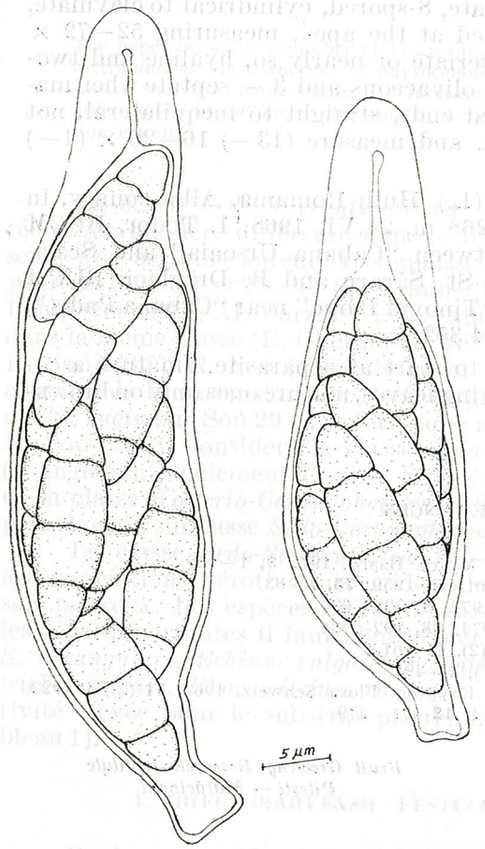


Fig. 2. — *Herpotrichiella callunae* — asci.

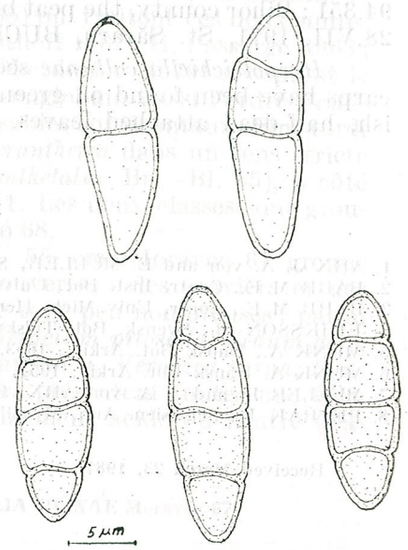


Fig. 3. — *Herpotrichiella callunae* — ascospores.

Hab. in foliis *Callunae vulgaris* (L.) Hull, Romania, distr. Alba, in pratis prope Detunatele, alt. cca 1268 m, 24. VI. 1968, leg. I. Todor, BUCM 94.350, holotypus.

The fungus appears as minute black dots on the lower surface, at the tips of the leaves, representing single ascomata. The *mycelium* is reduced to a few radial, superficial, brown, irregularly branched, septate hyphae which are 2 — 3.5 μm in diameter. The *ascomata* are superficial, born on the hyphae, scattered, pyriform to globose, with a short papillate apex, but without a distinct ostiole, 80—120 μm in diameter, 100—150 μm high, setose in the upper half; the ascocarp wall is composed of 1 — 2

layers of polygonal, flattened, dark brown cells, measuring 6 — 14 μm in diameter, which are thick-walled on the exposed surface; the setae are numerous, chestnut brown, one-celled or septate, straight or curved, (10—) 20 — 40(—50) μm long, 3—5 μm wide near base and gradually tapering to blunt points. The *asci* are aparaphisate, 8-spored, cylindrical to clavulate, short stalked, rounded and thickened at the apex, measuring 52—72 × × 11—13μm. The *ascospores* are biseriate or nearly so, hyaline and two-celled when young, greenish to pale olivaceous and 3 — septate when mature, fusoid to elliptic, with rounded ends, straight to inequilateral, not or slightly constricted at the septa, and measure (13—) 16—20 × (4—) 5 — 6μm. *Anamorph* unknown.

On leaves of *Calluna vulgaris* (L.) Hull, Romania, Alba county, in pastures near Detunatele, alt. c. 1268 m, 24.VI. 1968, I. Todor, BUCM 94.350, holotype; Alba county, between "Cabana Ursoaia" and Scărișoara, alt. c. 1000 m, 22.VII.1965, St. Săraru and B. Drăghici, BUCM 94.351; Bihor county, the peat bog "Tinovul Izbuc" near "Cabana Padiș", 28.VII. 1971, St. Săraru, BUCM 94.352.

*Herpotrichiella callunae* seems to start as a parasite. Imature ascocarps have been found on green, living leaves, mature ones only on brownish, half-dead attached leaves.

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# LA STRUCTURE DES GROUPEMENTS XÉROTHERMES DE LA CLASSE *SEDO-SCLERANTHETEA* Br. —Bl. 55 em. MORAVEC 67 DE ROUMANIE

V. SANDA, A. POPESCU et I. PEICEA

The paper presents a number of 17 xerothermic associations of the class *Sedo-Scleranthetea*. The structure of phytocenoses mentioned in the paper is revised on the basis of characteristic and indicatory species, classifying them into two orders and four alliances, changing in some cases the cenotaxonomical integration as well.

La classe *Sedo-Scleranthetea* Br. —Bl. 55 em. Moravec 67 est traitée d'une manière différente dans la littérature européenne de spécialité, selon que certains auteurs reconnaissent son existence près de *Corynephoretea* Br.—Bl. et Tx. 43 (J. Braun-Blanquet 1951, 1964; R. Tüxen, V. Lohmeyer 1962; Al. Borza, N. Boșcaiu 1965) ou traitent ces deux unités dans la même classe (E. Oberdorfer, W. Westhoff 1962; H. Passarge 1963; R. Soó 1968). R. Soó (1964, 1968) et E. Oberdorfer (1970) acceptent le contenu de la classe dans un sens élargi, en englobant aussi l'ordre *Festucetalia vaginatae* Soó 29 caractéristique aux sables continentaux. Plus tard, R. Soó (1973) considère la classe *Sedo-Scleranthetea* dans un sens stricte (y englobant seulement l'ordre *Sedo-Scleranthetalia* Br.—Bl. 55), à côté de la classe *Koelerio-Corynephoretea* Klika 41. Les deux classes sont groupées dans la surclasse *Sedo-Corynephorea* Soó 68.

La classe *Sedo-Scleranthetea* Br.—Bl. 55 em. Moravec 67 groupe les associations xérothermes acidophiles « ouvertes » des graviers et des sols pierreux. Les espèces de reconnaissance sont peu nombreuses; parmi les plus importantes il faut mentionner: *Hieracium pilosella*, *Sedum acre*, *S. sexangulare*, *Echium vulgare*, *Potentilla argentea*, *Veronica verna*, *Syntrichia ruralis*, *Rhacomitrium canescens*. Elles présentent toutes une électivité élevée pour le substrat pierreux, faiblement acide ou neutre (Tableau 1).

### 1. TRIFOLIO(ARVENSI) - FESTUCETALIA OVINAE Moravec 67

Espèces caractéristiques : *Festuca ovina*, *Trifolium arvense*, *Jasione montana*, *Cladonia rangiferina*, et, en tant que différentielle *Galium verum*.

#### 1.1. HYPERICO(PERFORATO)-SCLERANTHION PERENNIS MORAVEC 67

Espèces caractéristiques : *Scleranthus perennis*, *Dianthus deltoides*, *Hypericum perforatum*, et, en tant que différentielles *Carex caryophyllea* et *Potentilla tabernaemontani*.

1.1.1. *Genisto(spathulatae)— Agrostietum coarctatae* E. Schneider-Binder 75

Formes des prairies bien constituées dans des endroits sablonneux, avec du gravier grossier ou plus fin, sur des pentes ensoleillées, de pré-

*Tableau 1*

Noyau d'espèces à valeur cénotaxonomique des associations de la classe *Sedo-Scleranthetea* de Roumanie

| No du rang | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No du relevé | 23 | 6 | 8 | 5 | 7 | 4 | 10 | 6 | 14 | 5 | 1 | 7 | 10 | 14 |
| No d'espèces | 91 | 43 | 105 | 94 | 80 | 27 | 57 | 47 | 101 | 15 | 13 | 101 | 56 | 97 |
| *Trifolio-Festucetalia ovinae* | | | | | | | | | | | | | | |
| *Trifolium arvense* | IV | III | II | II | V | | IV | | III | | | | | |
| *Galium verum* (D) | | | | | | | II | | | | + | | | |
| *Hyperico-Scleranthion* | | | | | | | | | | | | | | |
| *Scleranthus perennis* | I | | | | I | | III | | II | IV | | III | | III |
| *Hypericum perforatum* | | | | | | | V | | | | | | | |
| *Petrorhago-Scleranthion* | | | | | | | | | | | | | | |
| *Rumex acetosella* | III | IV | IV | IV | | | IV | | II | | | | | |
| *Sedum acre* | | | | | | | | | II | | | V | | II |
| *Rhacomitrium canescens* | | II | | | | | | | | | | | | |
| *Vulpia myuros* (D) | I | IV | V | III | IV | | | | IV | | | | | |
| *Petrorhagia saxifraga* | V | V | | | V | | | | III | IV | | | | |
| *Scleranthus annuus* | III | V | I | I | I | | II | | IV | | | I | | |
| *Sedum rubens* | V | | | | I | | | | V | | | II | | |
| *Erysimum cuspidatum* | | | | | | | | | II | | | | | |
| | | | | | | | | | IV | | | | | |
| *Thero-Airion* | | | | | | | | | | | | | | |
| *Trifollum incarnatum* var. *molinerii* | I | III | III | V | I | | | I | I | | | | | |
| *Dasypyrum villosum* | I | | II | V | III | | | I | I | I | I | | | |
| *Ventenata dubia* | | | IV | V | | | | I | | | | I | | |
| *Aira elegans* | | | IV | I | | | | | | | | | | |
| *Bromus tectorum* | | V | I | II | | | V | III | V | | | I | | I |
| *Logfia arvensis* | III | I | III | | | | | V | III | III | | | | |
| *Achillea collina* | | | | | | | | | | | | | | |
| *Alysso-Sedetalia* | | | | | | | | | | | | | | |
| *Alyssum-alyssoides* | I | V | | | | | | | | | | | | |
| *Chondrilla juncea* | IV | V | II | III | | | II | | IV | | | | | |
| *Minuartia hamata* | I | | | | | | | | II | | | | I | |
| *Syntrichia ruralis* | V | | | | | | | | | | | | | |
| *Alysso-Sedion* | | | | | | | | | | | | | | |
| *Sedum album* | | V | I | | | | | | | | | | | |
| *Arenaria serpyllifolia* | | | | | | | I | II | I | I | | II | | III |
| *Apera spica-venti* | III | | | | | | | | | | | II | | |
| *Polycnemum arvense* | III | V | I | | | | | | | | | | | |
| *Poa compressa* | | | | | | | | | | | | | V | III |
| *Sedum hispanicum* | | | | | | | | | II | | | | V | III |
| *Alyssum petraeum* | | | | | | | | | | | | | V | |
| *Alyssum murale* | | | | | | | | | | | | | IV | V |
| *Cymbalaria muralis* | | | | | | | | | | | | | | |
| *Sedo-Scleranthetea* | | | | | | | | | | | | | | |
| *Hieracium pilosella* | | | | | | | II | | | | III | | | |



| No du rang | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Sedum sexangulare* | | II | | | | | | | | | | | | |
| *Potentilla argentea* | II | IV | IV | IV | V | | III | I | V | | + | II V | I | I |
| *Plantago altissima* var. *androalbida* | | | | | | V | | | | | | | | |
| *Verbascum speciosum* | I | | | | V | | | | III | | | | | |
| *Aegilops cylindrica* | I | | II | III | | | | | | | | | | |
| *Poa bulbosa* | IV | V | III | IV | II | | | II | III | | | | III | II |
| *Petrorhagia prolifera* | I | | | | | | | | I | IV | | | | |
| *Alyssum desertorum* | I | | | | | | | | | | | | | |
| *Herniaria glabra* | | V | I | | | | | | III | III | | | | |
| *Sedum hillebrandtii* | | II | | | | | | | | | | | | |
| *Myosotis micrantha* | | | | II | | | | | | | | | | |
| *Erysimum cuspidatum* | | | | | IV | | | | | | | | | |
| *Agrostis coarctata* | | | | | | | V | | | | | | | |
| *Genista spathulata* | | | | | | | V | | | | | | | |
| *Euphrasia stricta* | | | | | | | IV | | | | | | | |
| *Silene armeria* | | | | | | | II | | | | | | | |
| *Teucrium polium* | | | | | | | | | | V | | | | |

*Note*: 1 — *Sedo-Petrorhagietum syxifragae* Roman 74; 2 — *Alysso-Sedetum* apud N. Boşcaiu et I. Resmeriţă 69; 3 — *Filagini-Vulpietum* apud. N. Boşcaiu et I. Resmeriţă 69; 4 — *Trifolio* (*molinerii*) — *Haynaldietum villosae* Boşcaiu et Resmeriţă 69; 5— *Petrorhago-Verbascetum speciosae* Dihoru et al. 73; 6— *Plantaginetum androalbidae* Popescu et Ştefureac 76; 7 — *Genisto* (*spathulatae*) — *Agrostietum coarctatae*. E. Schneider-Binder 75; 8 — *Achilleo-Brometum tectori* Csűrös et al. 68; 9 — *Scleranthо-Erysimetum* (*Syrenietum*) *cuspidatae* Csűrös et al. 68; 10 — *Scleranthetо-Teucrietum polii* Andrei et Popescu 67; 11 — *Filagini-Aperetum* Oberd. 57 apud Dihoru et al. 73; 12 — *Scleranthetо-Poaetum compressae* Borza 59; 13 — *Alysso petraei-Sedetum hispanici* E. Schneider-Binder et al. 71; 14 — *Alyssetum muralis* Pop et Hodişan 97.

férence dans les parties supérieures des versants et des collines, où le caillou des anciennes terrasses fluviales surgit à la surface par lévigation (Erika Schneider-Binder 1975).

Les phytocénoses de l'association s'installent dans les stations occupées antérieurement par les variantes xérophiles des rouvraies acidophiles appartenant à l'alliance *Veronico* (*officinalis*)-*Quercion* I. Pop 71. L'association présente des affinités pour les prairies de *Nardo-Calunetea*, et surtout pour celles des *Festuco-Brometea*. Elle est mentionnée jusqu'à présent seulement dans le territoire compris entre Mohu-Bungard et Veştem. La présence et l'abondance des espèces *Thymus comosus* et *Festuca valesiaca* dans ces phytocénoses nous détermine d'y encadrer, en tant que synonyme, le cénotaxon *Thymo-Festucetum valesiacae* Erika Schneider-Binder nom. prov. 74.

1.1.2. *Polytricho* (*pilifero*)-*Scleranthetum perennis* Moravec 67

L'association a été citée par N. Boşcaiu (1970) dans les Monts Ţarcu-Godeanu et Cernei. Dans l'acception de J. Moravec (1967) ce cénotaxon comprend un noyau d'espèces caractéristiques formé de *Scleranthus perennis*, *Festuca ovina*, *Veronica verna*, *Erophila verna*, *Dianthus deltoides*, *Jasione montana*, *Pimpinella saxifraga*, *Hieracium pilosella*, *Trifolium arvense*, *Thymus pulegioides*, *Plantago lanceolata*, *Myosotis stricta*, *Polytrichum piliferum*.

1.2. PETRORHAGO (TUNICO)-SCLERANTHION CSŰRÖS et al 68

L'alliance comprend les associations des alluvions de gravier, innondées le printemps et très tôt en été, avec une répandue générale dans le sud-est du pays, aux altitudes réduites. Elle s'apparente à *Hyperico (perforato)-Scleranthion perennis* Moravec 67 avec les suivantes espèces indicatrices communes : *Potentilla argentea, Scleranthus perennis, Rumex acetosella, Sedum acre, Trifolium arvense, Rhacomitrium canescens*, mais en diffère par la présence des espèces thermophiles du sud. En tant qu'espèces indicatrices différentielles, il faut mentionner : *Vulpia myuros, Petrorhagia saxifraga, Scleranthus annuus, Sedum rubens, Berteroa incana, Achillea crithmifolia, Erysimum cuspidatum, Galium erectum* f. *pseudocinereum.*

1.2.1. *Sclerantho-Erysimetum(Syrenietum) cuspidatae* Csűrös et al. 68

Représente un groupement pionnier des graviers des rivières Eşelniţa et Mraconia, en substituant l'association *Myricarietum germanicae* des ruisseaux montagnards. Le gravier représente un écotope aride quoi que l'eau fréatique se trouve à petite profondeur. Bien chauffé toute la journée, il détermine une évaporation intense ; c'est pour cela que dans la phytocenose les espèces xérophiles dominent.

Dans l'association on a décrit :

— la subassociation *tunicosum* Csűrös et al. 68 qui se développe sur les dépôts de gravier avec peu de sable. Elle se caractérise par l'abondance des espèces *Petrorhagia saxifraga, Sedum rubrum, S. acre, S. hispanicum.* En tant qu'espèces différentielles locales il faut mentionner : *Sanguisorba minor, Trifolium parviflorum* et *Cynoglossum officinale.* On remarque l'abondance des bryophytes *Rhacomitrium canescens* et *Ceratodon purpureus.*

— la subassociation *vulpietosum* Csűrös et al. 68 qui se caractérise par la dominance de l'espèce *Vulpia myuros* et la présence des espèces *Lychnis coronaria* et *Achillea distans.* L'existence du sable en proportion plus élevée est indiquée par les mésophyles *Galium mollugo* et *Achillea crithmifolia.*

— la subassociation *typicum* qui se développe dans des endroits plus hauts, pierreux, mais avec beaucoup de sable, dans un stade plus avancé du processus de solification. La quantité un peu plus grande de humus est signalée par la présence des nitrophytes *Erodium cicutarium, Viola tricolor* et *Anthemis arvensis* qui forment un facies distinct, ainsi que par celles praticoles *Poa pratensis, Alopecurus pratensis* et *Festuca rupicola.*

1.2.2. *Petrorhago-Verbascetum speciosae* Dihoru et al. 73

C'est un groupement nitrophile, caractéristique pour les dépôts rocailleux des cônes de déjection. Elle a été identifiée dans la vallée de Mraconia, là où débordent les eaux à cause de la crue. *Verbascum speciosum*, l'espèce édificatrice, forme à côté de *Petrorhagia saxifraga* des phytocénoses caractéristiques. L'association évolue vers *Festucetum valesiacae* ou *Chrysopogonetum grylli*, fait prouvé par la présence d'un grand nombre d'espèces de la classe *Festuco-Brometea.* L'association, même semblable de point de vue floristique à la précédente, représente des stades différentes d'évolution de la végétation.



1.2.3. *Plantaginetum androalbidae* Popescu et Ştefureac 76

Forme des phytocénoses compactes sur les graviers du Danube (Plavişeviţa et Svinița). C'est *Plantago altissima* var. *androalbida* qui domine, à côté de laquelle on rencontre aussi *Holoschoenus vulgaris, Agrostis stolonifera, Potentilla supina, Verbena supina, Inula britannica, Carex hirta,* etc.

1.2.4. *Sedo-Petrorhagietum saxifragae* Roman 74

Les phytocénoses de *Petrorhagia saxifraga* avec *Sedum rubens* sont installées sur les graviers des vallées torrentielles ou sur les pentes des terrasses, là où le gravier a été tiré au clair par l'errosion pluviale. La constitution des phytocénoses est faite d'habitude par l'installation du noyau cénotique sur des groupements pionniers de *Poa bulbosa* et *Syntrichia ruralis.* L'association occupe, d'une manière disséminée, les graviers des vallées de Bahna, Vodiţa, Slătinicu Mare, Oglănic, Husniţa, les pentes de Ghelmegioaia (N. Roman 1974). Excepté les deux espèces caractéristiques, les plus fréquentes sont : *Scleranthus annuus, Apera spica-venti, Polycnemum arvense, Trifolium arvense, Filago arvensis, Rumex acetosella, Scleranthus perennis,* ainsi qu'une série d'espèces caractéristiques pour la classe *Festuco-Brometea.*

L'association présente le même noyau d'espèces communes pour *Sclerantho-Syrenietum cuspidatae* Csűrös et al. 68 et *Petrorhago-Verbascetum speciosae* Dihoru et al. 73.

1.2.5. *Scleranthetо-Poaetum compressae* Borza 59

Est décrite de la vallé de Sebeş avec les suivantes espèces caractéristiques : *Sedum acre, Poa compressa, Bromus tectorum, Potentilla argentea* et *Centaurea micranthos.* Les espèces compagnes et celles occasionnelles diffèrent beaucoup. L'association a été encadrée au commencement dans l'alliance *Sedo-Scleranthion* Br. —Bl. 55. La manque de la majorité des espèces caractéristiques de cette alliance, à savoir *Sempervivum arachnoideum, Poa xerophila, Scleranthus biennis* des phytocénoses de la vallée de Sebeş, nous détermine d'encadrer l'association dans l'alliance *Petrorhago (Tunico)-Scleranthion.*

C'est des vieilles murailles de la ville de Sebeş et de celles de la cité de Laz qu'on a décrit

— la subassociation *cymbalarietosum muralis* Borza 59.

1.2.6. *Sclerantheto-Teucrietum polii* Andrei et Popescu 67 (As. *Teucrium polium-Scleranthus perennis* Andrei et Popescu 67)

L'association est signalée sur la pente de Pricopan, ou elle présente un stade initial d'enherbement et de fixation des sols en cours de formation. Parmi les espèces les plus significatives rencontrées dans cette association il faut mentionner : *Dianthus nardiformis, Siderites montana* et *Heliotropium suaveolens.*

1.3. THERO-AIRION OBERD. 57

Réunit les associations qui végétent dans les stations dont les sols sont pierreux et compactes, sur les pentes lentes, là où s'installent des phy-

tocénoses pionnières avec *Trifolium incarnatum* ssp. *molinerii*, *Dasypyrum villosum*, *Ventenata dubia*, etc. qui reflètent un stade plus avancé du processus de solification. Parmi les espèces de reconnaissance on mentionne : *Aira caryophyllea*, *Vulpia myuros*, *V. bromoides*.

1.3.1. *Filagini-Vulpietum* Oberd. 57 (*Vulpio-Airetum capillaris* Paucă 41 ; *Vulpio-Airetum* Borhidi 56 ; *Airetum elegantis* Soó 47)

Les sols alluvionnaires, sablonneux, ainsi que les jachères sont colonisés par cette association. La constance élevée de certaines espèces ayant un caractère méridional, comme *Ventenata dubia*, *Achillea crithmifolia*, *Dasypyrum villosum* par exemple, a permis de faire la distinction de la variante régionale *banaticum* Boşcaiu et Resmeriţă 69.

Dans la composition floristique de l'association on remarque la présence d'un grand nombre de caractéristiques transgressives de l'ordre *Festucetalia valesiacae* qui indiquent les possibilités d'évolution dans la direction de l'installation des groupements xérophiles de cet ordre.

Erika Schneider-Binder (1970) décrit la variante régionale *transilvanicum*, qui diffère par le nombre réduit d'espèces caratéristiques aux cénotaxa de rang supérieur et l'absence des espèces méridionales.

1.3.2. *Trifolio(molinerii)-Dasypyretum villosae* Boşcaiu et Resmeriţă 69

Le noyau d'espèces caractéristiques *(Dasypyrum villosum*, *Trifolium molinerii* et *Aegilops cylindrica*) est reconnu aussi dans la composition de l'association de *Haynaldia villosa* décrite par Al. Buia et al. (1960) d'Oltenia.

L'association présente des exigences trophiques plus élevées que *Filagini-Vulpietum*, en végétant surtout dans les sols sablonneux de la base des pentes, là où se produisent les colluvions riches en substances humiques. Elle se développe aussi sur les jachères en précédant la réinstallation des associations de *Festuca valesiaca* et *Chrysopogon gryllus*. C'est une association dominée par terrophytes, quoique les hémicryptophytes y participent en grand nombres, surtout par les espèces rudérales.

1.3.3. *Ventenato(dubiae)-Xeranthemetum(cylindraceum)* nomen novum (As. *Ventenata dubia-Xeranthemum foetidum* Borza 50)

Elle est cantonnée dans des endroits secs, sur les pentes ensoleillées, là où la couche d'eau fréatique se trouve en profondeur. En tant qu'espèces dominantes et caractéristiques nous mentionnons : *Xeranthemum cylindraceum*, *X. anuum*, *Ventenata dubia*, *Trifolium striatum*, *Medicago minima* et *Botriochloa ischaemum*.

Comme évolution, ces prairies dominées par térrophytes, suivent après les mauvaises herbes qui s'installent sur les terrains laissés noncultivés, et succédées par *Botriochloetum ischaemi* ou *Chrysopogonetum grylli*.

1.3.4. *Achilleo-Brometum tectori* Csűrös et al. 68

L'association a été décrite des abruptes méridionaux de la terrasse de la plaine du Danube et des pentes ensoleillées, méridionales, situées entre Orşova et Eşelniţa. *Bromus tectorum* forme, surtout sur les sables, des phytocénoses pionnières encadrées dans l'alliance *Bromion tectorum*.



L'association décrite par St. Csűrös et al. (1968) diffère de ces phytocénoses par le manque des psamophytes, ce qui représente le premier stade du processus de fixation des herbes sur les pentes méridionales fortement inclinées et défrichées. La composition floristique de ces phytocénoses est hétérogène, les terrophytes y dominant et les hémicryptophytes ayant une participation significatives. Parmi les espèces représentatives nous mentionnons : *Vulpia myuros*, *Bromus arvensis*, *Poa bulbosa*, *Botriochloa ischaemum*, *Ventenata dubia*, *Lotus corniculatus*, *Trifolium dubium*, *Eryngium campestre*, *Convolvulus arvensis*, *Sherardia arvensis*, *Chondrilla juncea*, *Plantago lanceolata*, etc.

1.3.5. *Rhacomitrio-Festucetum pseudovinae* (Balázs 41) Soó 44

L'association a été mentionnée par Al. Borza (1963) dans le conspectus des association des Carpates.

F. Balázs (1941) la décrit des Monts Mezeş sur la base de 9 relevés, en l'encadrant dans l'alliance *Salicion incanae*. À côté des espèces édificatrices on rencontre avec une abondance-dominance significative : *Hieracium pilosella*, *Centaurea micranthos*, *Erigeron canadensis*, *Verbascum phlomoides*, *Acinos arvensis*, *Vulpia myuros*, *Berteroa incana*, etc.

1.3.6. *Filagini-Aperetum* Oberd. 57

Elle végète sur les jachères, les lieux dénudés ou les prairies xérophiles. Elle se caractérise par l'abondance et la prédominance des espèces de *Sedo-Scleranthetea* mélangées avec celles de *Chenopodietea*, *Festuco-Brometea* et *Molinio-Arrhenatheretea*, ce qui démontre la concurrence entre ces catégories écologiques de plantes pour occuper le terrain "libre". Espèces de reconnaissance : *Logfia arvensis*, *L. minima*, *Apera spica-venti*, *Trifolium arvense*, *Scleranthus annuus*, *Aphanes arvensis*, *Vicia tetrasperma*, *V. hirsuta*, *Rumex acetosella*, *Bilderdykia convolvulus*, etc.

## 2. ALYSSO-SEDETALIA Moravec 67

Espèces caractéristiques : *Alyssum alyssoides*, *Saxifraga tridactylites*, *Thlaspi perfoliatum*, *Acinos arvensis*, *Teucrium botrys*.

### 2.1. ALYSSO—SEDION OBERD. ET TH. MÜLLER IN TH. MÜLLER 61

Espèces caractéristiques : *Alyssum alyssoides*, *Sedum album*.

2.1.1. *Alysso petraei-Sedetum hispanici* E. Schneider-Binder et al. 71

L'association a été décrite des Cazanele Mari, Valea Mraconiei, Dubova et Plavişeviţa. Les espèces édificatrices et caractéristiques sont : *Sedum hispanicum* et *Alyssum petraeum*. En tant qu'espèces accompagnatrices avec une signification cénotique et écologique il faut mentionner *Petrorhagia saxifraga*, *Poa bulbosa*, *Satureja kitaibelii*, *Ceterah officinarum*, *Stipa eriocaulis*, *Scabiosa columbaria*, *Melica ciliata* var. *flavescens*, *Achillea crithmifolia*.

### 2.1.2. *Alyssetum muralis* Pop et Hodişan 79

Les phytocénoses d'*Alyssum murale* ont été identifiées sur les schistes cristallins, couverts d'une couche mince de sol riche en cailloutis fin, entre les pentes des collines de Gilău et Tarnița (la partie gauche de la vallée de Someșul Cald). Parmi les espèces avec une grande fréquence s'imposent *Artemisia absinthium*, et *A. campestris* var. *psilophylla* qui forment des facies caractéristiques.

— Le facies *artemisiosum absinthii* Pop et Hodişan 79 se constitue sur les sols riches en azotates et ressemble apparemment à l'association *Sysymbrio-Artemisietum absinthii* Pop 69.

— Le facies *artemisiosum campestris* Pop et Hodişan 79 a des contingences floristiques avec les suivantes associations (desquelles manque *Alyssum murale*) : *Artemisio (campestris)-Corynephoretum canescentis* Kosinová-Kučerová J. 64 de la Bohémie centrale, *Artemisio-Melicetum ciliatae* Korneck 74 de la région de Rhin, et *Tuniceto-Artemisietum campestris* Br.—Bl.61 identifiée sur les rochers d'Adda (Italie).

L'association *Alyssetum muralis* est dominée par les éléments eurasiatiques et européens, à coté desquels on remarque les espèces méridionales qui confèrent à la communauté un spécifique floristique hétérogène. C'est une association saxicole héliophile, modérée thermophile, faiblement acidophile jusqu'à neutre basiphile. Cette association pionnière évolue vers les prairies de *Festucetum pallentis transsilvanicum* Soó 59.

### 2.1.3. *Alysso—Sedetum Oberd.* et Th. Müller 61

L'association est très répandue au sud du Banat. Sur la base de la relative constance et abondance des espèces *Petrorhagia saxifraga* et *Polycneum arvense*, N. Boşcaiu et I. Resmeriță (1969) ont décrit la variante régionale *banaticum*.

Le spectre biologique de l'association met en évidence l'optimum biologique des terrophytes. Sur les graviers de la bouche des vallées, grâce aux accumulation, l'association a un caractère stationnaire, en établissant sa composition par recolonisations successives. Sur les alluvions sablonneuses moins exposées aux inondations périodiques, l'association se présente de plus en plus forte, évoluant dans la direction de la constitution des groupements de *Filagini-Vulpietum*.

Les deux espèces caractéristiques et édificatrices de l'association, *Alyssum alyssoides* et *Sedum album*, auxquelles s'ajoutent un groupe hétérogène de plantes, parmi lesquelles nous mentionnons : *Poa bulbosa*, *Scleranthus annuus*, *Herniaria glabra*, *Rhacomitrium canescens*, etc., colonisent les alluvions sablonneuses et les cailloutis.

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# LA DIFFÉRENCIATION DES BOURGEONS DE LA VIGNE

ANCA ANTOHE

The present paper is a continuation of the previous investigation (1, 2, 3, 4) carried out in North-East Moldavia, on Aligoté vine types (the type is origited from France) and Fetească neagră (an autochthonous type).
The differentiation of the buds takes place concomitantly with the growth of the offshoots and with the phenophase of the flowering that precedes it or follows it. This phenomenon is influenced by the position of the buds along the offshoot, by the age of the bud, by the conditions of environment, by the biological nature of the soil, by the quantity and quality of reserve materials.
In the Fetească neagră type, the greater number of buds with a beginning of differentiation or with an ended differentiation at the end of the vegetal period, attest the ecological adaptation of this type.

Les bourgeons de la vigne contiennent tant des feuilles primordiales végétatives que des feuilles primordiales des fruits.

La différenciation a lieu dans les bourgeons nouvellement formés des drageons en cours de développement. Ce phénomène depend en premier lieu du degré de préparation biologique (de maturation biologique) des tissus d'où proviennent les organes de la production des fruits (9, 10). Selon Negrul, A., M., 1967, Tiutiunic, A., F., 1961, dans le processus de différenciation des bourgeons, le tissu méristématique du cône de croissance du drageon embryonnaire peut se restructurer en vue de la formation des rudiments des organes végétatifs ou génératifs. Lorsqu'il y a assez de substances plastiques, les monticules fertiles formés jusqu'à ce moment-là, se différencient dans l'inflorescence. Les modifications substantielles qui se produisent dans une direction ou l'autre mènent à la transformation des bourgeons dans des bourgeons à fruits ou dans des bourgeons foliaires.

Le passage du méristème du cône de croissance vers la séparation des monticules génératifs marque l'état critique ou le moment critique dans l'organogénèse. Ce moment intervient après la séparation produite par le cône de croissance d'un grand nombre de métamères végétatifs, ce qui est spécifique des drageons embryonnaires. Le moment critique se déplace sur la longueur du drageon à partir de la base vers le sommet.

Des études ont été effectuées concernant l'organogénèse dans les inflorescences et les bourgeons floraux. Winkler et Schemsetlni, 1963 (cités par Bessis) en Californie, montrent que la feuille primordiale d'inflorescence commence à se former environ trois mois après l'apparition du duvet sur les bourgeons.

La croissance des feuilles primordiales est d'abord rapide et ensuite diminue vers la fin de la période de végétation. Ces résultats sont pareils à ceux de Barnas et Bernon et Levadaux (cités par Bessis) qui montrent que les grappes se forment vers la fin du mois de juillet (Montpellier, 1934).

Les études effectuées par Barnard et Levadaux (cités par Bessis) sur les ceps de vigne Sultana, tendent à montrer que si l'inflorescence se forme au cours de l'année précédant le moment où les bourgeons sont couverts de duvet, la différenciation des fleurs ne commence qu'au moment

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où les bourgeons sont sur le point d'éclore. Ces résultats sont proches de ceux obtenus par Alleweldt G., 1963, qui montre que les inflorescences se forment au mois de juillet et au mois d'août et que la différenciation des feuilles primordiales ne finit que l'année suivante.

Huglin A. (cité par Bessis), travaillant sur les ceps de vigne d'Alsace constate que les feuilles primordiales de l'inflorescence commencent à se former vers la moitié du mois de juillet dans les yeux latents des drageons et, au mois d'août, les bourgeons primaires ont des feuilles primordiales d'inflorescence. Tout cela mène à la supposition qu'il y a une grande précocité de formation des grappes et que leur nombre est probablement définitivement fixé à la fin de la période de végétation.

Bessis R. montre qu'on peut affirmer, à titre d'hypothèse, que la quantité de fleurs qu'un bourgeon pourra porter est entièrement déterminée par le temps de la chute des feuilles.

Alleweldt, G., 1960, en faisant des recherches sur les relations entre la formation des fleurs et la croissance des drageons de la vigne, montre que la formation des feuilles primordiales d'inflorescence commence dans le bourgeon d'hiver, de la region médiane du drageon, après qu'il eût atteint l'âge physiologique de croissance définitive. Ainsi, pour les espèces Riesling et Ares, les feuilles primordiales d'inflorescence ont été observées cinq ou six semaines après le bourgeonnement.

Le même auteur, par les mêmes expériences, constate que le nombre des feuilles/drageons nécessaires à la formation de l' inflorescence s'est situé entre 11,4—21,8. Six ou sept semaines environ après le début de la différenciation de l'inflorescence dans un bourgeon, le procesus de la differenciation de l'inflorescence est presque terminé.

La fin de la différenciation a lieu après le repos d'hiver (Alleweldt, G., Balrema, 1965).

Blagonravov, P.P., 1961, est d'avis que la formation et la différenciation des inflorescences dans le bourgeon ont lieu pendant la période de végétation de l'année précédente.

Les bourgeons d'un drageon ne se trouvent pas dans les mêmes stades de développement et n'evoluent pas dans le même rythme (5, 6, 7, 9).

Sur un drageon, la quantité des bourgeons est inégale et dépend de la partie du cep de la vigne sur laquelle la corde du drageon s'est développée. En ce sens, Guzin, N., I., 1959, constate que les bourgeons sur les drageons de la corde médiane sont les meilleurs du point de vue quantitatif. Melkonean, A., S., 1964, montre que le volume de la fertilité potentielle des bourgeons du centre est soumis à une variation le long du drageon. Dans la partie inférieure des bourgeons 1—4, la quantité des inflorescences complètement formées et des formes transitoires est plus réduite que dans les zones supérieures des drageons. La zone de la plus grande fertilité comprend les bourgeons 6—14.

C'est aux mêmes conclusions qu'arrive Sfarean, D., L., 1960, et le même phénomène est saisi aussi dans les travaux de Negrul, A., M., 1969, où l'on montre qu'à partir des nœuds 3—4, jusqu'aux nœuds 12—15, les bourgeons atteignent un degré élevé de différenciation qui diminue vers le sommet du drageon.



Carolus, M., 1971, étudiant les stades de développement des feuilles primordiales des inflorescences pendant l'organogénèse des bourgeons latents de la vigne, a constaté 12 stades de développement : 4 stades végétatifs et 8 stades d'inflorescence. Dans les stades végétatifs apparaissent les feuilles primordiales foliaires et dans les stades d'inflorescence apparaissent les feuilles primordiales d'inflorescence.

Les recherches effectuées en Roumanie sur le processus de différenciation des bourgeons de la vigne (8, 9, 10, 11, 12, 13) ont mis à jour une série de problèmes liés au moment du début du processus de différenciation, notamment la modification du bourgeon par rapport à différents facteurs (conditions de milieu, nutrition, nature biologique de l'espèce, quantité et qualité de matière de réserve).

## MATÉRIEL ET MÉTHODE

L'étude a été effectuée dans le cadre de la Station Expérimentale Hortiviticole de Iaşi dans des conditions d'expérimentation identiques à celles rappelées dans les travaux antérieurs (1, 2, 3, 4).

Nous avons tâché d'établir « le moment de la différenciation physiologique » qui a été considéré comme la date où les feuilles primordiales d'inflorescence ont pu être observées pour la première fois pendant la disection des bourgeons au microscope. On a noté ainsi la différenciation : + = début de différenciation ; + + = différenciation.

## RÉSULTATS ET DISCUSSIONS

En observant la différenciation sur les drageons situés à la base de la corde à fruit, à l'espèce Aligoté (fig. 1) on constate les feuilles primordiales qui peuvent être observées au microscope, le premier juillet. Ce phénomène apparaît aux bourgeons 4—9 et 14—15, parmi lesquels seulement le bourgeon du rang 9 est différencié, les autres présentant seulement un début de différenciation.

Le phénomène de différenciation est poursuivi et observé chez les différéntes catégories de bourgeons. Ce phénomène est surtout rencontré chez les bourgeons situés sur les nœuds 4—20, donc au milieu du drageon. Les autres bourgeons sont des bourgeons végétatifs.

Vers l'automne s'accroît le nombre ds bourgeons qui présentent la différenciation et diminue le nombre de ceux qui présentent un début de différenciation. Pourtant, au mois d'octobre on a observé des bourgeons qui présentaient un début de différenciation.

Le nombre de feuilles sur les drageons nécessaire à la différenciation complète des bourgeons est en fonction de la variété. Pour Aligoté sont necessaires 27 feuilles/drageon et pour Feteasca neagră—29.

Aux drageons de la base de la corde à fruits, à l'espèce Feteasca neagră (fig. 2) les premiers bourgeons qui se différencient sont les bourgeons 7—13. Parmi eux, les bourgeons 9—11 sont complètement différenciés tandis que les autres commencent à peine la différenciation. Le pro-

cessus continue et comprend les bourgeons situés sur les nœuds 2—18. Nous remarquons un nombre plus grand de bourgeons à la différenciation achevée, par rapport à l'espèce Aligoté.

La différenciation comprend les bourgeons formés au plus tard à mi-juin. Le reste des bourgeons ne se différencient plus, restant des bourgeons végétatifs.

En poursuivant la différentiation des bourgeons sur les drageons situées au milieu de la corde à fruits, chez les deux espèces (fig. 3,4), on observe les feuilles primordiales d'inflorescence, le premier juillet, aux bourgeons 6—13 (l'espèce Fetească neagră) et aux bourgeons 10—15 (l'espèce Aligoté).

La différenciation comprend les bourgeons situés entre les nœuds 4—20 (l'espèce Aligoté) et les bourgeons situés entre les nœuds 2—24 (l'espèce Fetească neagră). Le reste des bourgeons sont végétatifs.

Sur les drageons du milieu de la corde à fruits, on observe un nombre plus grand de bourgeons qui ont commencé la différenciation ou sont complètement différenciés, ce qui nous conduit à l'idée d'une qualité supérieure de ceux-ci.

De la qualité meilleure de ces bourgeons témoignent aussi les résultats obtenus par Alleweldt, G., 1963, et Guzin, N., 1959, et d'autres. Pour ce qui est de la fertilité des bourgeons, Sfarean, D., L., 1966, conclut que les bourgeons situés plus bas sur les drageons, du point de vue morphologique, ont une moindre fertilité. La zone de la plus grande fertilité est comprise entre les nœuds 6—15. Cette constatation coïncide aussi avec nos résultats.

Pour l'espèce Fetească neagră, un nombre plus grand de bourgeons se caractérisent plutôt par une différenciation terminée que par un début de différenciation.

Etudiant le phénomène de différenciation des bourgeons des drageons situés au sommet de la corde à fruits, chez l'espèce Aligoté (fig. 5), on remarque ce phénomène à peine le 15 juillet et ce n'est que pour peu de bourgeons (cinq seulement). On observe des différenciations aux bourgeons des rangs 4—16 et seulement d'une façon incidente aux bourgeon des rangs 18—21 ou 24. Le reste des bourgeons non différenciés sont des bourgeons végétatifs.

À la différence de l'espèce Aligoté, chez l'espèce Fetească neagră (fig. 6), les bourgeons commencent leur différenciation le premier juillet. A cette date on a observé pour les bourgeons 6—11 et 12—17 des débuts de différenciation. Les analyses ultérieures ont mis en évidence que les bourgeons 5—20 sont des bourgeons fertiles. Parmi eux, la plupart ont la différenciation terminée. Le reste des bourgeons des drageons sont des bourgeons végétatifs.

## CONCLUSIONS

1. Le phénomène de différenciation des bourgeons a été observé 10—11 semaines environ après l'éclosion des bourgeons en croissance.

2. La différenciation a lieu au fur et à mesure de la croissance des drageons et de la phénophase de la floraison qu'elle précède ou suit.



3. La différenciation est influencée par la position des bourgeons le long du drageon. La formation des feuilles primordiales d'inflorescence commence aux bourgeons qui se trouvent au milieu du drageon, après que ceux-ci atteignent l'âge physiologique de croissance définitive.

4. Le matériel actif de la différenciation des bourgeons est produit dans les feuilles dont la présence est obligatoire dans le processus de différenciation.

a) Pour la différenciation des bourgeons des drageons situés à la base de la corde à fruits il est nécessaire un nombre de feuilles moindre par rapport aux drageons du milieu ou du sommet de la corde à fruits.

5. La formation des bourgeons sur un drageon n'a pas lieu au même temps, ce qui fait que les bourgeons aient des âges différents et un degré différent de différentiation.

a) Les bourgeons situés dans la partie inférieure du drageon et les bourgeons de la partie supérieure sont des bourgeons végétatifs.

b) Les bourgeons situés au milieu du drageon sont fertiles (porteurs des fleurs).

6. La différenciation comprend les bourgeons qui apparaissent sur les drageons au plus tard jusqu' à la fin du mois du juin. Le reste des bourgeons ne se différencient plus, ils restent des bourgeons végétatifs (foliaires). C'est ce phénomène qui est à la base du procédé qui consiste dans la suppression de la partie terminale des drageons trouvés dans le processus de croissance en longueur, le premier août.

7. Les bourgeons qui se développent sur les drageons situés au milieu de la corde à fruits, sans tenir compte de l'espèce, se caractérisent par une fertilité accrue.

8. Pour l'espèce Fetească neagră, on constate un nombre plus grand de bourgeons qui présentent un début du différenciation ou qui ont terminé la différenciation à la fin de la période de végétation.

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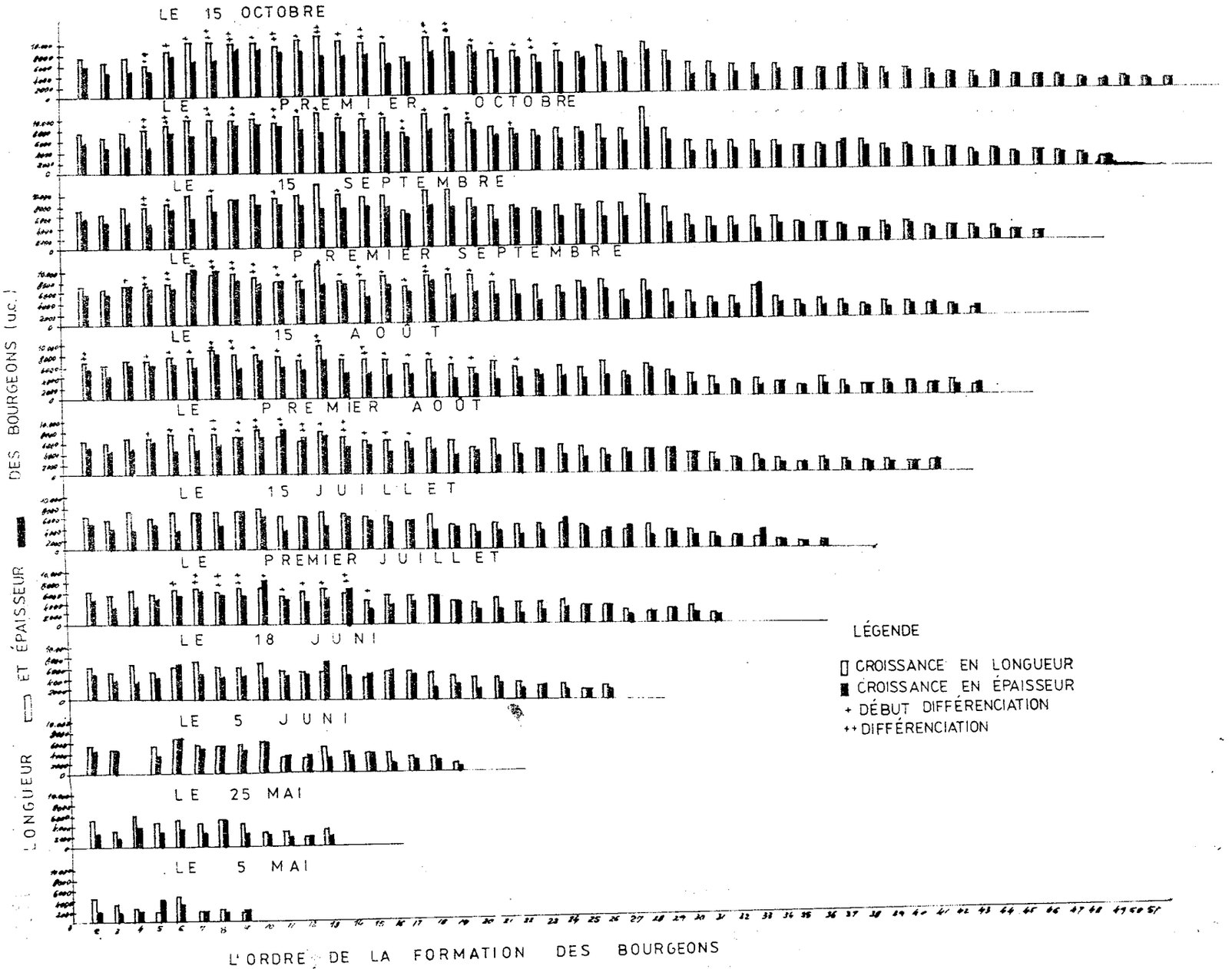
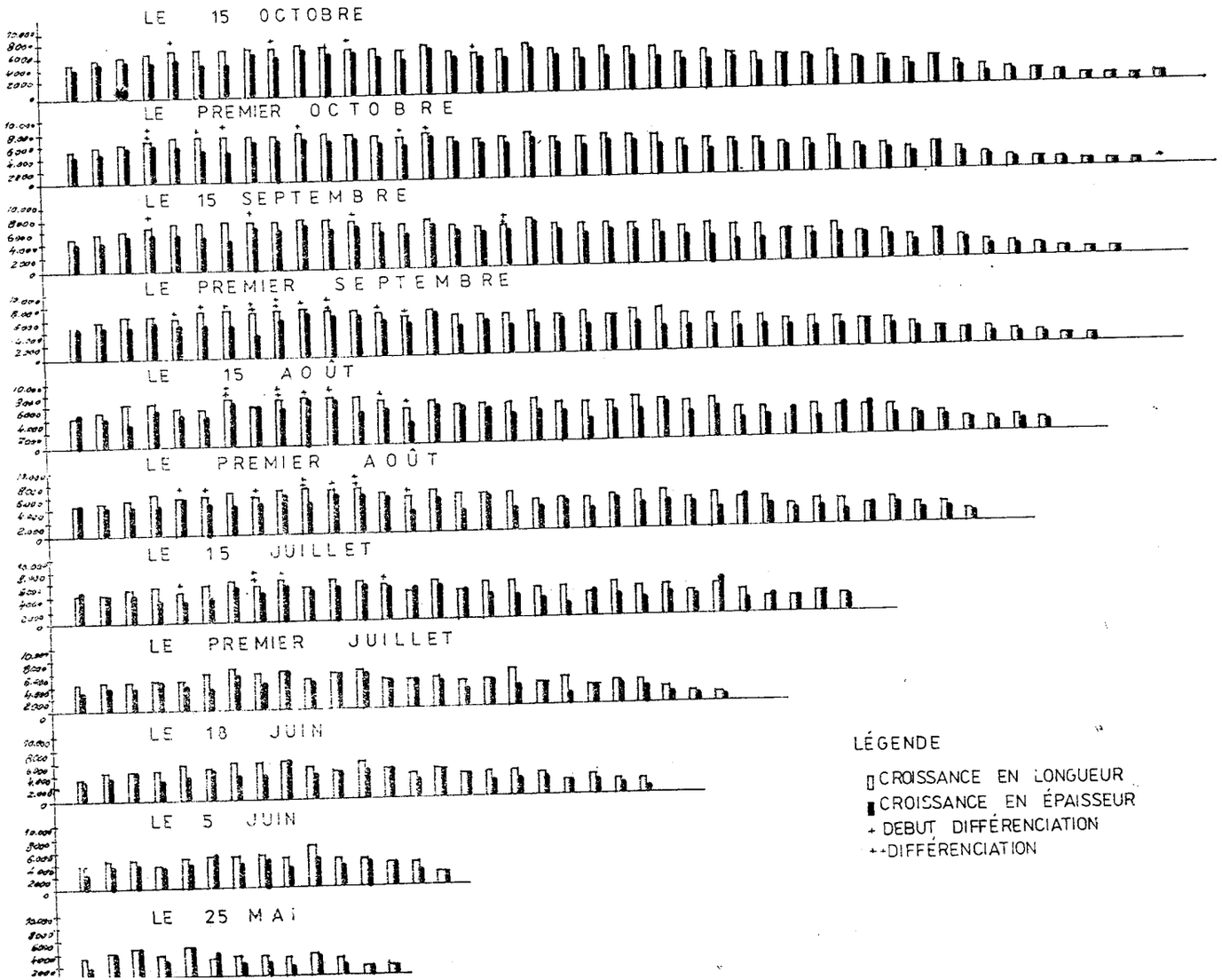



Fig. 4. — La croissance des bourgeons chez l'espèce Fetească neagră. Drageon du milieu du sarment.



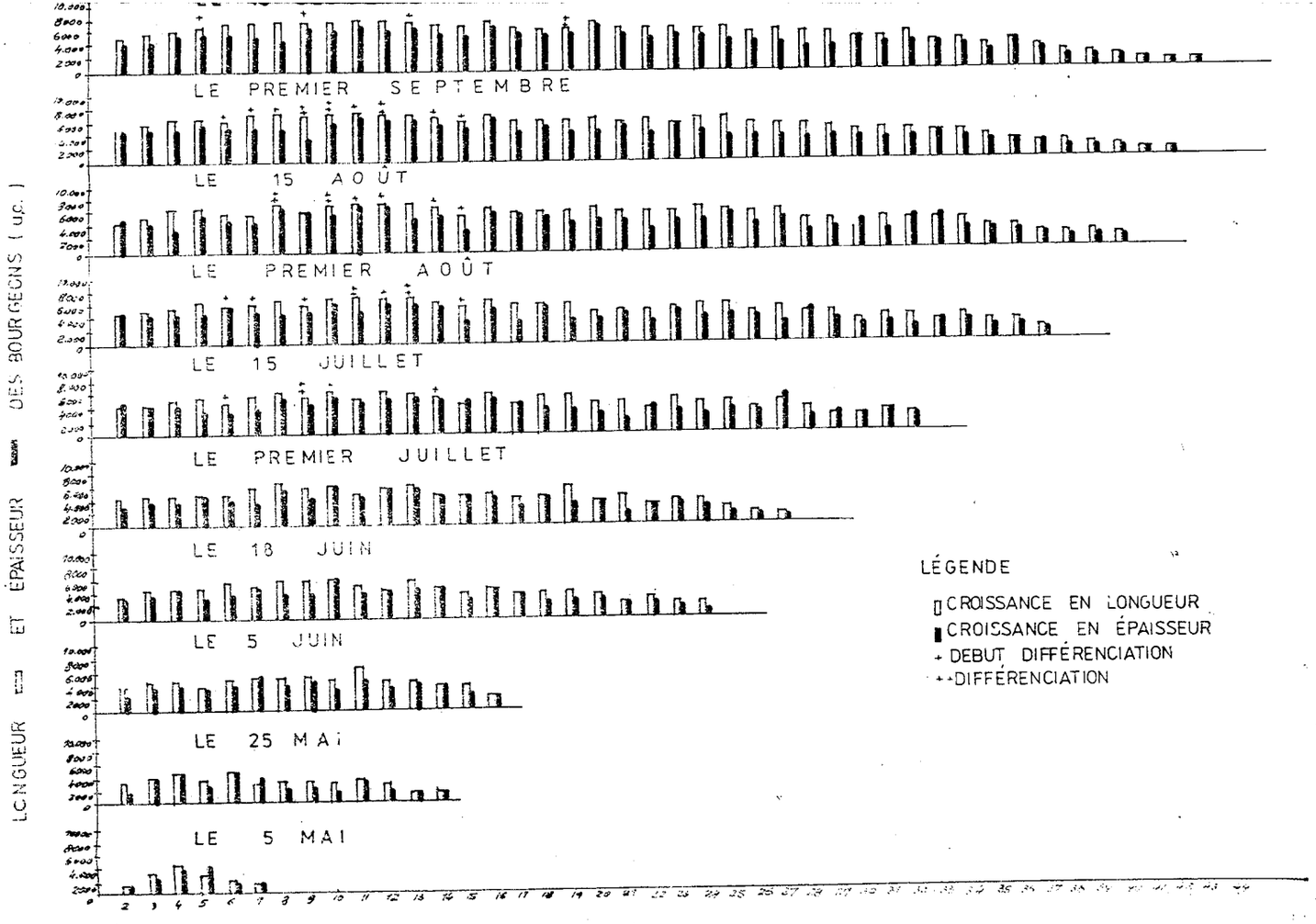



Fig. 5. — La croissance des bourgeons chez l'espèce Aligoté. Drageon du bout du sarment.

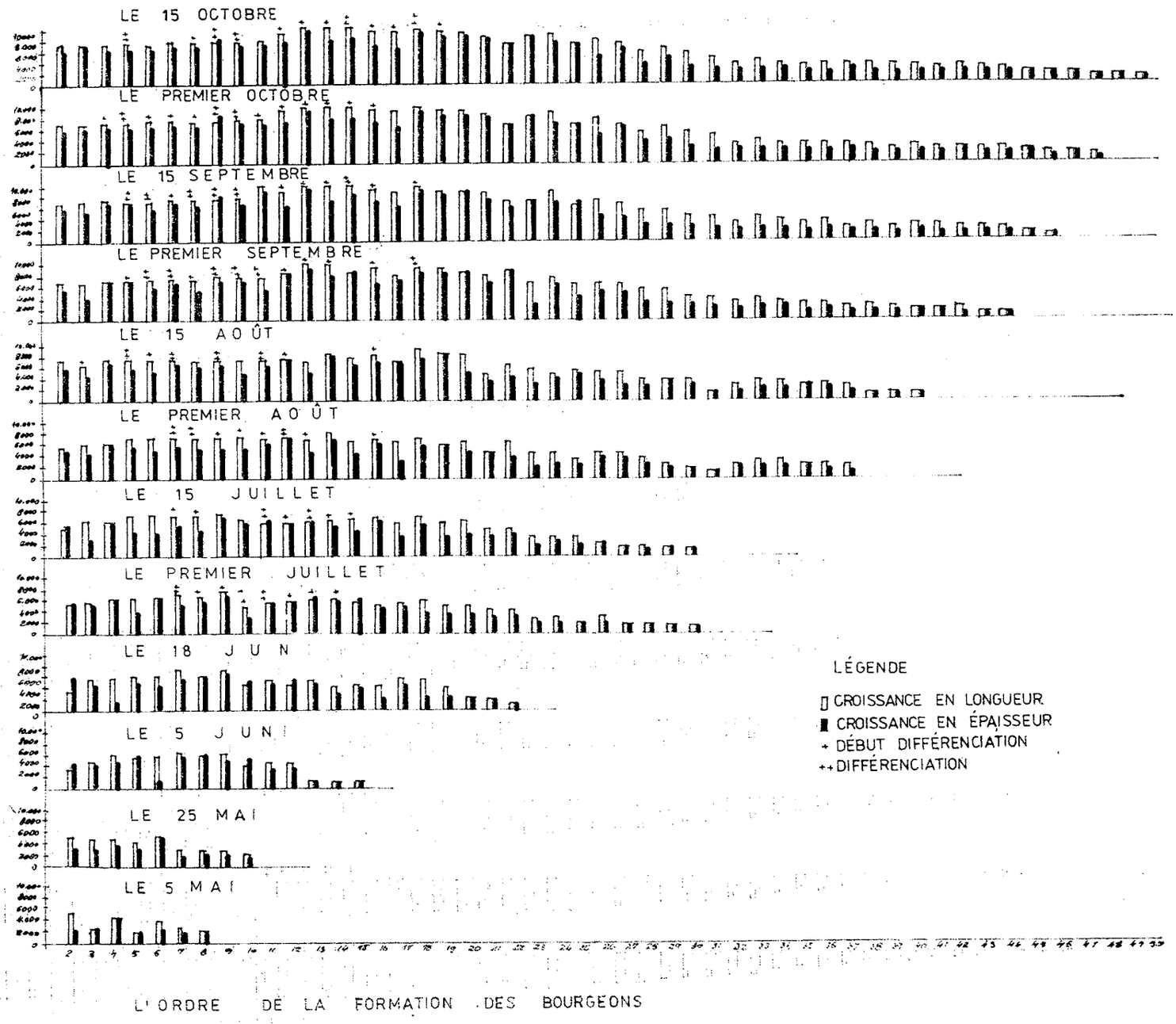



Fig. 6. — La croissance des bourgeons chez l'espèce Feteasca neagră. Drageon du bout du sarment.

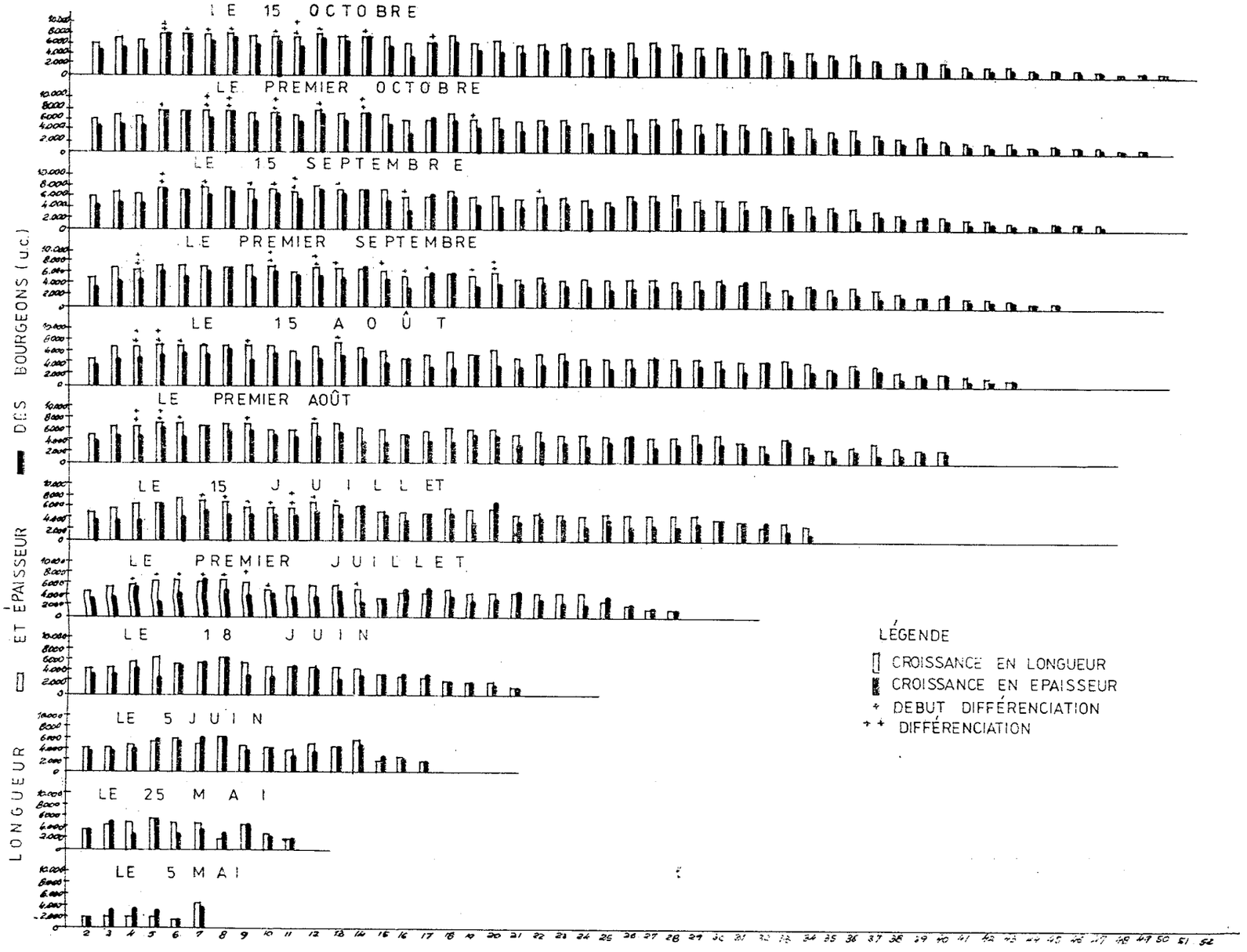
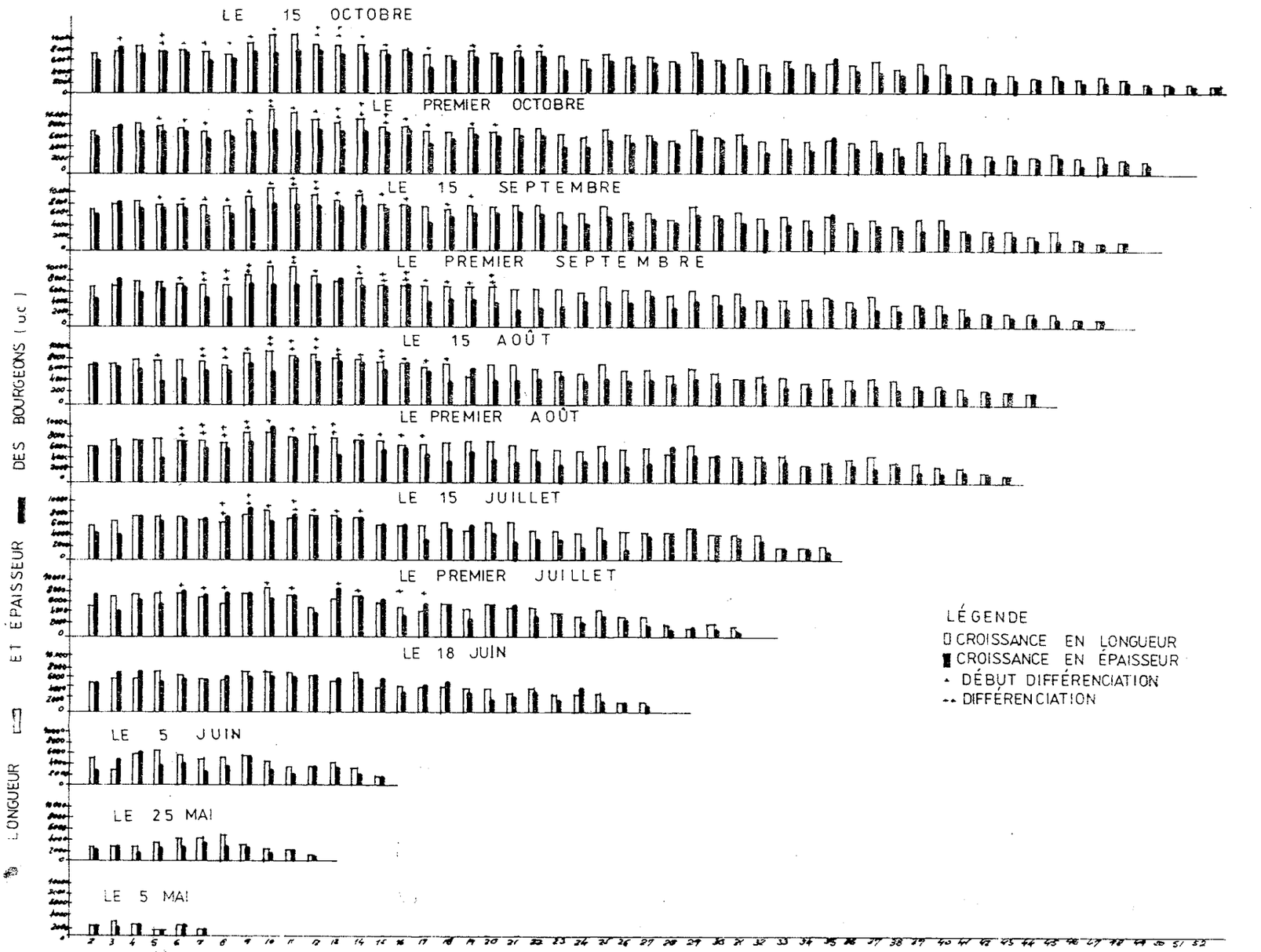



Fig. 1. — La croissance des burgeons chez l'espèce Aligoté. Drageons de la base du sarment.



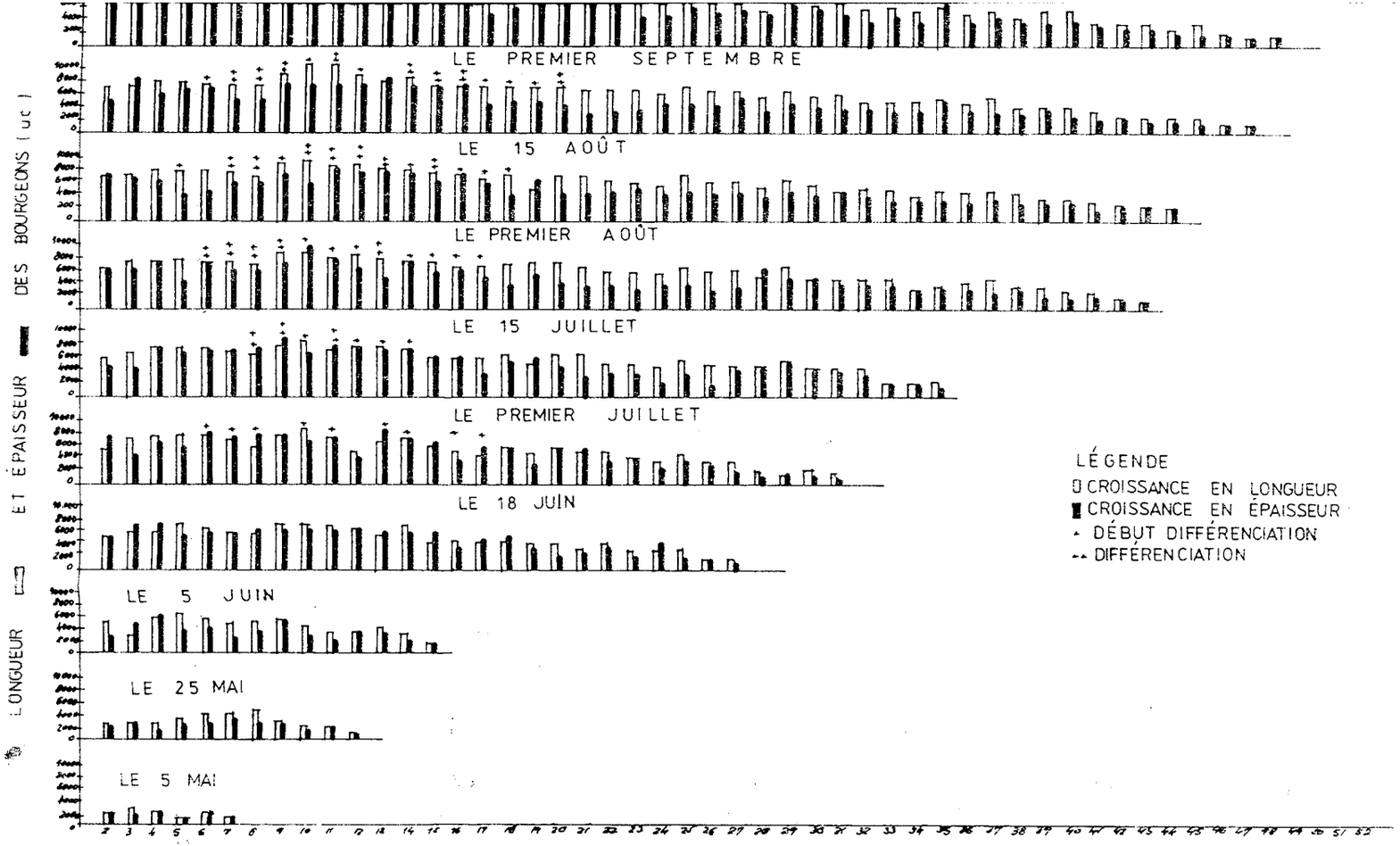



Fig. 2. — La croissance des bourgeons chez l'espèce Fetească neagră. Drageon de la base du sarment.

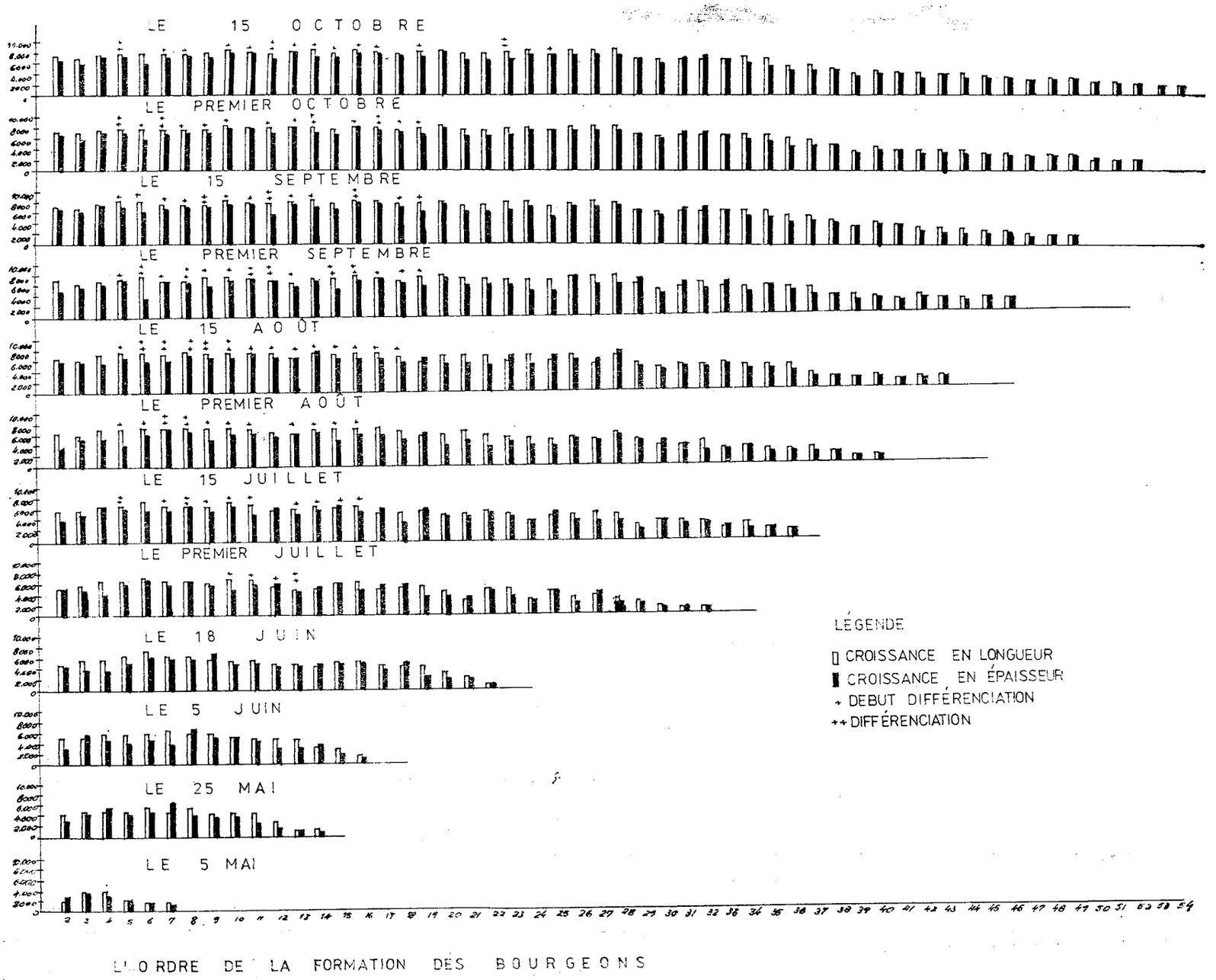



Fig. 3 — La croissance des bourgeons chez l'espèce Aligoté. Drageon du milieu du sarment.

# INTERACTIVE EFFECTS OF SALINITY AND PHYTOHORMONES ON GROWTH AND PLANT WATER RELATIONSHIP PARAMETERS IN MAIZE AND SAFFLOWER PLANTS

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The effect of various levels of NaCl salinization on seed germination, growth and plantwater relationships of maize and safflower plants was investigated. The role of $GA_3$ and IAA in modifying the salt-stress induced changes was also studied. The percentage germination and water content of stressed seeds were significatly reduced, but this inhibitory effect was eliminated by seed presoaking with $GA_3$ or IAA.

Salinity induced a considerable reduction in transpiration rate and stomatal frequency, but seed presoaking with $GA_3$ or IAA induced a significant increase in these plant-water relationships.

The dry matter yields of salt affected plants were significantly reduced. Such inhibitory action was decreased by treating the seeds with $GA_3$ or IAA, particularly in the case of maize plants.

Acknowledgement of the physiological effects of soil salinity and exogenously applied growth hormones on seed germination, plant growth and the relevant vital activities, provides a fundamental basis for the intelligent management of plant life for the good of mankind. In the majority of plants, germination of seeds is greatly retarded and seeding survival is difficult under saline conditions (Parmer and Moore, 1968; Williams and Ungar, 1972, Maftoun and Sepaskhah, 1978; Stout *et al.*, 1980 and Meikal *et al.*, 1982a). In addition, the response of plant growth to salinization treatments has been investigated. In this respect, some investigators reported a general reduction in plant growth (Shalhevet and Yaron, 1973; Stewart *et al.*, 1976; Verasan and Phillips, 1979, Ahmed *et al.*, 1980a and Heikal *et al.*, 1981a), while others recorded a promotion rather than inhibition in the growth of some salinized plants (Bernstein, 1975; Ahmed *et al.*, 1980b and Heikal *et al.*, 1980 and 1981b). According to Kessler (1961), Shah and Loomis (1965), the major effect of salinity in the roots environment was attributed to a reduced hormone delivery from root to leaves which could induce an inhibition of crop growth. Attempts have been made to overcome the growth suppression resulting from salinity and other factors such as genetic deficiencies or restrictive light and temperature conditions (Nieman and Bernstein, 1959). In this respect, presoaking seeds with optimal concentration of certain phytohormones has been shown to be beneficial to growth and yield of some plants grown under saline conditions (Kaufmann and Ross, 1970; Gary and Srivastava, 1970; Darra *et al.*, 1973 and Chhipa and Lal, 1978).

During the vegetative growth phase salinity has been recognized as one of the factors which induce significant changes in plant-water relationships. In this respect, many investigators recorded a reduced transpiration rate with the rise of salinity level (Tal and Gavish, 1973; Bozcuk,

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1975 ; Stewart *et al.*, 1976 and Ahmed *et al.*, 1980a). This reduction was found to be associated with reduced leaf surface area. Also, the stomatal number and movement were found to play an addition role (Bozcuk, 1975 and Ahmed *et al.*, 1979a). In accordance with this, Waisel (1972) and Shaddad and Heikal (1981) reported that the number, size and movement of stomata were considerably affected with the rise of NaCl concentration in the nutritive medium.

Changes in transpiration rate due to salinization treatments lead mostly to concomitant changes in plant water content (Wong and Jayer, 1978 ; Adams *et al.*, 1978 ; and Zidan, 1979). Poljakoff-Mayber and Gale (1975) attributed the increase in water content to an increase in abscisic acid concentration, which in turn induces stomatal closure, leading to an increase in leaf-thickness or succulence.

The present work was carried out to study the effect of various levels of salinity on seed germination, growth and plant-water relationships of maize (*Zea mays* L.) and safflower (*Carthamus tinctorius* L.) plants. Because of the various biological activities of $GA_3$ and IAA, their role in modifying the salt-stress induced changes was also investigated.

## MATERIALS AND METHODS

Preliminary tests were conducted to determine the optimum hormone concentration and optimum period of presoaking and drying which are effective in counteracting the effect of salt-stress on seed germination. The optimum conditions reached from these tests are shown in the following scheme :

| Test plant | The optimum conditions | | | | | |
|---|---|---|---|---|---|---|
| | $GA_3$ | | | IAA | | |
| | Conc. ppm. | Period of presoaking | Period of drying | Conc. ppm | Period of presoaking | Period of drying |
| Maize | 100 | 3 hours | one day | 50 | 3 hours | 3 days |
| Safflower | 100 | 5 hours | 7 days | 100 | 3 hours | 3 days |

Using NaCl the osmotic potential levels were chosen at 0 (control), −2, −4, −6, −8 and −10 bar. Twenty five seeds were placed on absorbant pads in Petri dishes to which 25 ml of the experimental saline solution was added. The Petri dishes were wrapped in two layers of aluminium foil and incubated at 25 °C. Seeds were considered to be germinated after the radicle emerged from the testa. To evaluate the interactive effect of salt—stress and $GA_3$ or IAA on seed germination, the seeds of the test plants were treated with the experimental phytohormones, air dried and germinated under the effect of various levels of salinity. The percentage germination was followed daily for a period up to 5 days.

The water content, soluble carbohydrate and soluble nitrogen of the germinated seeds were determined. The anthrone sulphuric acid method



(Fales, 1951 ; Schlegal, 1956 and Badour, 1959 ) was used for determining the carbohydrate content. For nitrogen determination, the microkjeldahl method was employed (Paech and Tracay, 1956).

To follow up the interactive effect of salinity and phytohormones on seeding growth, the phytohormone treated seeds were placed between folded paper towels in a beaker containing 80 ml of the salinized solution and incubated at 25°C for 10 days in darkness. Length of shoots and primary roots in case of maize and length of hypocotyl and roots in case of safflower were recorded.

For determining plant growth and plant-water relationship parameters, seeds of the test plants, after being presoaked and air dried were sown in plastic pots containing 2 kg air dried soil (sand/clay 1 :1 v/v). Stress levels were at −2, −4, −6, −8 and −10 bar in case of maize and at −3, −6, −9 and −12 bar in case of safflower. Plants were irrigated every other day with salinized nutrient Pfeffer's solution for two weeks. Thereafter, the test plants were irrigated every other day with N/10 non-salinized Pfeffer's solution. The soil moisture content was kept near the field capacity.

Transpiration rate was measured as described by Bazcuk (1975). Young seedlings were transferred to aerated 1/5 strength Pfeffer's nutrient solution for 5—9 days until several leaves were fully expanded. Then they were transferred to 250 ml conical flask covered with aluminium foil containing 200 ml of the experimental salinized nutrient solutions (0 to −10 bar in case of maize and 0 to −12 bar in case of safflower.) In order to prevent evaporation, the flasks were sealed with paraffin wax. The transpired water was then determined by weighing the plants together with the flask and experimental salinized nutrient solution at daily intervals for 15 days. After each weighing the level of the culture solution was brought to the initial level by addition of nutrient solution. Transpiration rate was calculated as g per unit leaf area per day (g/dm²/day) after subtraction of the amount of water lost from a control flask kept under the same conditions but without plants cultivated in.

For stomatal frequency determination, direct microscopic measurements were carried out on strips taken from the leaves under investigation and immediately immersed in absolute alcohol for fixation and preservation. The number of stomata per mm² (stomatal frequency) on upper and on lower epidermis was determined using the square occular micrometer.

At the end of the experimental period (45 days in case of maize and 60 days in case of safflower) the leaf area (dm²/plant) and dry matter yield were determined.

## RESULTS AND DISCUSSION

The seed germination of the test plants was generally reduced by salinization and the reduction was more pronounced with the rise of salinizing agent (Fig. 1). It seems that this reduction in seed germination is a general response to salinization treatments as realized by Parmer and Moore (1960) ; Ghorashy and Kheradnam (1972) ; Sionit *et al.*, (1973) and Heikal and Shaddad (1982b). The promotive effect of $GA_3$ and IAA on

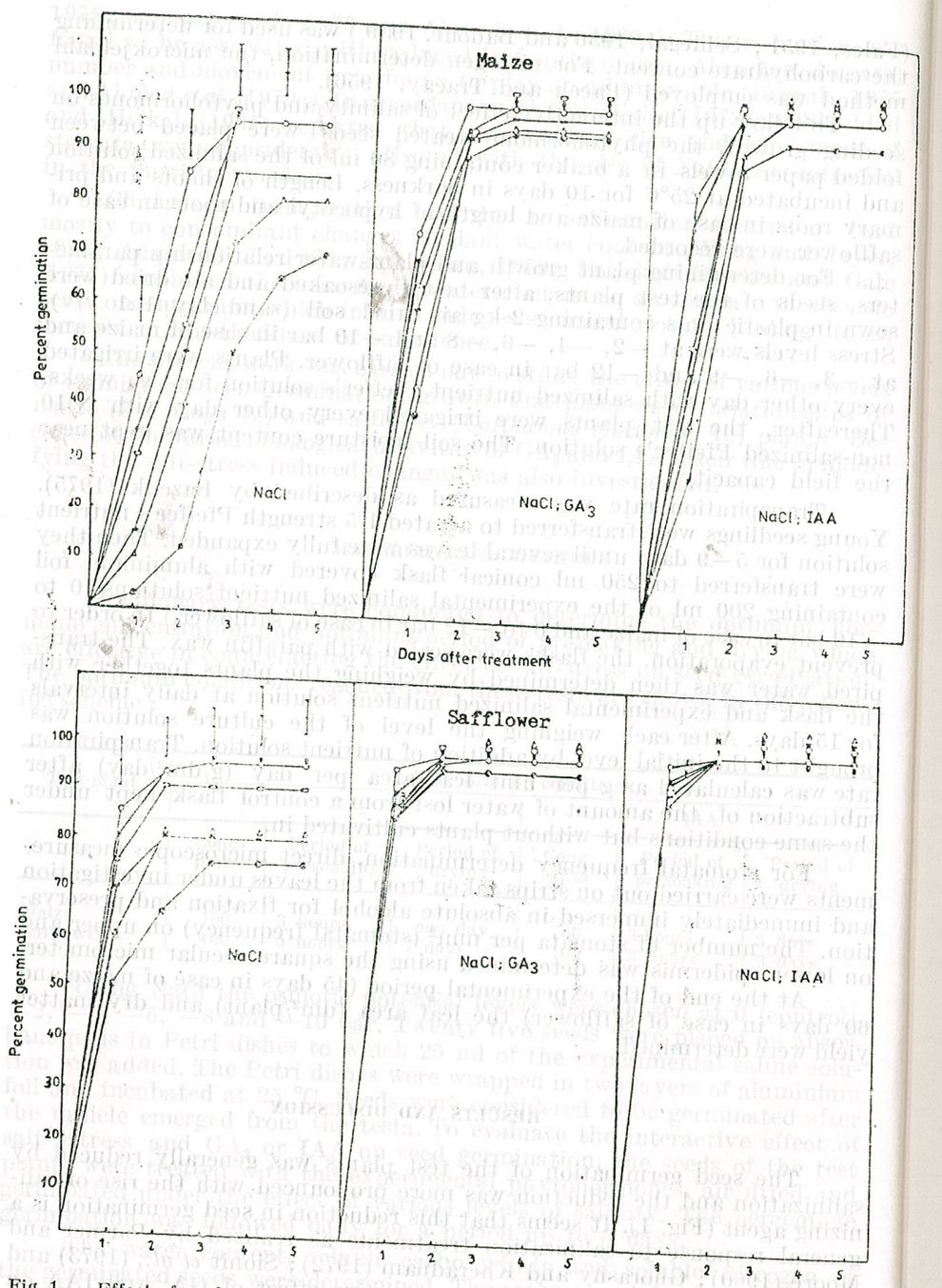


Fig. 1. — Effect of seed presoaking with $GA_3$ and IAA on percent germination of maize and safflower seeds stressed with different levels of sodium chloride
O = control, ◐ = −2 bars, □ = −4 bars
■ = −6 bars, △ = −8 bars ● = −10 bars



germination of salt-stressed seeds of the test plants is in agreement with the results obtained by other authors (Khan, 1960 ; Darra *et al.*, 1973 ; Boucaud and Unger, 1976 ; Stout *et al.*, 1980 and Heikal *et al.*, 1982a). This promotive effect was associated with an increase in water uptake (Table 1 and 2), which again confirms the suggestion that the beneficial effects of hormones are ascribed to their role in increasing water absorption (Darra *et al.*, 1973).

There is also a considerable reason to believe that the alteration in the production of soluble carbohydrates and soluble nitrogen in the germinated seeds may be one aspect of the role of salinity on the enzymatic activity in the overall phenomenon of seed germination. The obvious increase in the concentration of soluble carbohydrates in the stressed seeds as a result of presoaking with $GA_3$ (Tables 1 and 2) supports the opinion of Ikuma and Thimann (1963) who reached the conclusion that $GA_3$ promotes amylase activity which supplies the monosaccharides for embryo respiration. Also, seed presoaking with $GA_3$ was accompanied by an increase in the concentration of the soluble nitrogen fractions in the test seeds (Tables 1 and 2). From this point of view, it can be suggested that $GA_3$ does not only promote amylase activity but also acts as an activator of some hydrolases such as proteolytic enzymes. On the other hand, IAA, almostly induced a significant reduction in the contents of soluble nitrogen of salt-stressed seeds (Tables 1 and 2).

*Table 1*

Changes in water content (g/g dry weight), soluble carbohydrate and soluble nitrogen (mg/g dry weight), of maize seeds treated with $GA_3$, IAA and stressed with different levels of sodium chloride for three days

| Treatment | Water content | Soluble carbohydrates | Soluble nitrogen |
|---|---|---|---|
| Control | 0.83 | 42.10 | 6.85 |
| − 2 bar | 0.89* | 44.55* | 10.69** |
| − 4 bar | 0.63** | 31.59** | 8.77** |
| − 6 bar | 0.55** | 25.11** | 7.02 |
| − 8 bar | 0.49** | 17.01** | 6.56 |
| −10 bar | 0.47** | 14.58** | 6.12* |
| Control + $GA_3$ | 1.03** | 48.60** | 12.50** |
| − 2 bar + $GA_3$ | 0.89 | 47.36** | 11.05 |
| − 4 bar + $GA_3$ | 0.81** | 48.69** | 10.08** |
| − 6 bar + $GA_3$ | 0.72** | 46.70** | 9.17** |
| − 8 bar + $GA_3$ | 0.68** | 46.56** | 7.96** |
| −10 bar + $GA_3$ | 0.51 | 43.59** | 8.77** |
| Control + IAA | 0.95** | 65.12** | 7.26 |
| − 2 bar + IAA | 0.96** | 50.62** | 7.46** |
| − 4 bar + IAA | 0.71** | 42.77** | 7.86** |
| − 6 bar + IAA | 0.65** | 35.17** | 5.44** |
| − 8 bar + IAA | 0.58** | 35.96** | 5.44** |
| −10 bar + IAA | 0.55** | 23.33** | 6.75* |
| L.S.D. at 5% | 0.05 | 2.35 | 0.60 |
| L.S.D. at 1% | 0.07 | 3.15 | 0.81 |

* Significant differences.
** Highly significant differences as compared with the control.

*Table 2*

Changes in water content (g/g dry weight), soluble carbohydrates and soluble nitrogen (mg/g dry weight) of safflower seeds treated with GA, IAA and stressed with different levels of sodium chloride for three days

| Treatment | Water content | Soluble carbohydrates | Soluble nitrogen |
|---|---|---|---|
| Control | 3.35 | 67.23 | 7.25 |
| — 2 bar | 2.63** | 59.94** | 7.26 |
| — 4 bar | 1.82** | 57.51** | 6.71 |
| — 6 bar | 1.40** | 29.16** | 6.15** |
| — 8 bar | 1.32** | 21.06** | 6.15** |
| —10 bar | 1.38** | 21.06** | 6.47* |
| Control + $GA_3$ | 3.84* | 78.84** | 9.03** |
| — 2 bar + $GA_3$ | 3.30** | 81.61** | 11.99** |
| — 4 bar + $GA_3$ | 2.41** | 61.56** | 10.60** |
| — 6 bar + $GA_3$ | 1.85* | 39.69** | 8.77** |
| — 8 bar + $GA_3$ | 1.90** | 37.26** | 7.66** |
| —10 bar + $GA_3$ | 1.55 | 33.21** | 6.75 |
| Control + IAA | 1.20** | 26.63** | 6.75 |
| — 2 bar + IAA | 1.15** | 28.54** | 9.68** |
| — 4 bar + IAA | 0.84** | 39.69** | 9.96** |
| — 6 bar + IAA | 0.94* | 45.36** | 8.77** |
| — 8 bar + IAA | 0.83* | 43.74** | 7.66** |
| —10 bar + IAA | 0.79** | 41.31** | 6.75 |
| L.S.D. at 5% | 0.42 | 2.84 | 0.69 |
| L.S.D. at 1% | 0.57 | 3.80 | 0.92 |

* Significant differences.
** Highly significant differences as compared with the control.

The lengths of the root and shoot of maize seedlings were significantly increased at the lower levels of salinization (—2 and —4 bar). Thereafter, the values of these growth parameters decreased as the level of salinity increased (Table 3). In case of safflower the lengths of the hypocotyl and root were significantly decreased with the rise of salinization level, except at the lowest level (—2 bar) where these growth parameters were significantly decreased (Table 3). Presoaking with $GA_3$ induced a significant increase in seedling growth parameters of the two test plants, at all chosen stress levels (Table 3). Presoaking with IAA generally retarded the root growth of maize seedlings and hypocotyl and root growth of safflower seedling at various levels of salinization. On the other hand, the shoots length of salinized maize seedling was generally increased under the effect of IAA.

The reduction in stomatal frequency and rate of transpiration of the test plants at certain concentration of NaCl (Tables 4 and 5) is in agreement with the results obtained by some other authors (Meiri and Polijakoff-Mayber 1970; Tal and Gavish, 1973; Jensen, 1975 and Ahmed *et al.*, 1980a). Gale *et al.*, (1967) and Bozcuk (1975) attributed such inhibited transpiration to the partial closure of stomata. However, in the present investigation the reduction in the rate of transpiration of the test plants, in addition to relative stomatal closure, were associated by reduced stomatal frequency. Sead presoaking with $GA_3$ or IAA induced a significant increase in transpiration rate and stomatal frequency (Tables 4 and 5).



*Table 3*

Effect of seed presoaking with $GA_3$ and IAA on growth of seedlings of maize and safflower stressed for 10 days with different levels of sodium chloride

| Treatment | Maize | | Safflower | |
|---|---|---|---|---|
| | Shoot length (cm) | Root length (cm) | Hypocotyl length (cm) | Root length (cm) |
| Control | 9.61 | 15.73 | 9.34 | 14.40 |
| —2 bar | 13.89** | 20.70** | 10.67** | 18.67** |
| —4 bar | 12.43** | 19.96** | 8.22** | 14.29 |
| —6 bar | 10.06 | 14.23* | 6.36** | 9.68** |
| —8 bar | 7.35** | 10.95** | 5.46** | 9.55** |
| —10 bar | 5.32** | 6.75** | 3.69** | 3.19** |
| Control + $GA_3$ | 14.74** | 19.85** | 10.56** | 14.30 |
| —2 bar + $GA_3$ | 19.94** | 22.44* | 11.83** | 19.25 |
| —4 bar + $GA_3$ | 13.69* | 21.45* | 9.66** | 16.83** |
| —6 bar + $GA_3$ | 10.40 | 18.87** | 8.18** | 10.93* |
| —8 bar + $GA_3$ | 9.91** | 15.37** | 6.44** | 10.92** |
| —10 bar + $GA_3$ | 6.83** | 8.42* | 4.69** | 3.37 |
| Control + IAA | 12.18** | 15.51 | 8.22** | 6.64** |
| —2 bar + IAA | 14.89* | 20.60 | 7.02** | 6.67** |
| —4 bar + IAA | 12.19 | 14.53** | 5.85** | 5.32** |
| —6 bar + IAA | 11.13* | 11.58** | 4.17** | 1.87** |
| —8 bar + IAA | 8.53* | 8.41** | 2.52** | 1.04** |
| —10 bar + IAA | 6.56* | 6.55 | 1.47** | 0.80** |
| L.S.D. at 5% | 0.99 | 1.46 | 0.73 | 0.95 |
| L.S.D. at 1% | 1.33 | 1.95 | 0.98 | 1.27 |

* Significant differences.
** Highly significant differences as compared with the control.

*Table 4*

Effect of seed presoaking with $GA_3$ and IAA on leaf area ($dm^2$/plant), transpiration rate ($g/dm^2$/day) and stomatal frequency (number of stomata/$mm^2$) of maize plants stressed for 15 days with different levels of sodium chloride

| Treatment | Leaf area | transpiration rate | Stomatal frequency | |
|---|---|---|---|---|
| | | | Upper epidermis | Lower epidermis |
| Control | 1.85 | 4.39 | 162 | 266 |
| — 2 bar | 1.64* | 3.45** | 140** | 244** |
| — 4 bar | 1.49** | 3.26** | 103** | 207** |
| — 6 bar | 1.24** | 3.17** | 88** | 177** |
| — 8 bar | 0.95** | 2.66** | 66** | 140** |
| —10 bar | 0.57** | 1.98** | 44** | 10** |
| Control + $GA_3$ | 1.89 | 4.69* | 199** | 295** |
| — 2 bar + $GA_3$ | 1.82* | 3.92** | 169** | 244 |
| — 4 bar + $GA_3$ | 1.77** | 3.84** | 147** | 207 |
| — 6 bar + $GA_3$ | 1.29 | 3.72** | 140** | 199** |
| — 8 bar + $GA_3$ | 0.97 | 2.74 | 103** | 147 |
| —10 bar + $GA_3$ | 0.58 | 2.06 | 51 | 111 |

*Table 4 (continued)*

| Treatment | Leaf area | transpiration rate | Stomatal frequency | |
|---|---|---|---|---|
| | | | Upper epidermis | Lower epidermis |
| Control + IAA | 2.26** | 5.07** | 199** | 280 |
| — 2 bar + IAA | 1.82* | 4.72** | 169** | 258 |
| — 4 bar + IAA | 1.59 | 4.36** | 147** | 222 |
| — 6 bar + IAA | 1.32 | 4.24** | 133** | 199** |
| — 8 bar + IAA | 0.95 | 3.43** | 125** | 177** |
| —10 bar + IAA | 0.58 | 2.87** | 80** | 133** |
| L.S.D. at 5% | 0.18 | 0.28 | 13.92 | 15.22 |
| L.S.D. at 1% | 0.24 | 0.38 | 18.65 | 19.58 |

* Significant differences.
** Highly significant differences as compared with the control.

*Table 5*

Effect of seed presoaking with $GA_3$ and IAA on leaf area ($dm^2$/plant), transpiration rate ($g/dm^2$/ day) and stomatal frequency (number of stomata/$mm^2$) of safflower plants stressed for 15 days with different levels of sodium chloride

| Treatment | Leaf area | transpiration rate | Stomatal frequency | |
|---|---|---|---|---|
| | | | Upper epidermis | Lower epidemis |
| Control | 0.77 | 2.88 | 183 | 237 |
| — 3 bar | 0.68** | 2.85 | 158** | 215** |
| — 6 bar | 0.53** | 2.60** | 100** | 169** |
| — 9 bar | 0.36** | 1.77** | 66** | 158** |
| —12 bar | 0.29** | 1.14** | 50** | 83** |
| Control + $GA_3$ | 0.83* | 3.31** | 162* | 266** |
| — 3 bar + $GA_3$ | 0.70 | 3.10** | 169 | 236** |
| — 6 bar + $GA_3$ | 0.59* | 2.77* | 125** | 225** |
| — 9 bar + $GA_3$ | 0.43* | 1.77 | 100** | 183** |
| —12 bar + $GA_3$ | 0.39** | 1.18 | 50 | 100* |
| Control + IAA | 0.86** | 3.33 | 168 | 258** |
| — 3 bar + IAA | 0.71 | 3.29** | 158 | 258** |
| — 6 bar + IAA | 0.59* | 2.77* | 133** | 222** |
| — 9 bar + IAA | 0.43* | 1.79 | 100** | 147 |
| —12 bar + IAA | 0.37** | 1.31* | 50 | 107** |
| L.S.D. at 5% | 0.06 | 0.14 | 16.03 | 13.37 |
| L.S.D. at 1% | 0.08 | 0.19 | 21.03 | 17.92 |

* Significant differences.
** Highly significant differences as compared with the control.



The values of dry matter yield of the test plants were generally lowered by rising the salinizing agent concentration (Table 6). These inhibitory effects add more support to the results obtained by some other authors using various plants (Hutton, 1971 ; Lashin and Atanasiu, 1972 ; Heikal, 1975 ; Nassery *et al.*, 1979 ; Coughlan and Wynjones, 1980 ; Joshi and Kaik, 1980 ; and Singh and Singh, 1980). It was noticed that the growth of roots was severely retarded by salt-stress than the growth of shoots which could be clearly noticed from the increased values of the shoot/root ratio (Table 6). The close correspondence between the retarded growth and salinization was ascribed to the effect of NaCl on several factors of plant activities such as osmotic adjustment (Bernstein, 1963), protein and nucleic acid synthesis (Nieman, 1965), ion uptake (Greenway *et al.*, 1966), hormonal balance (Itai *et al.*, 1968), enzyme activities (Weimberg, 1970) and photosynthesis (Ahmed *et al.*, 1979b). The beneficial effect of seed presoaking with $GA_3$ or IAA on the growth of salinized plants was revealed in this work (Table 6). This may be attributed to the increased uptake of water which is the consequence rather than the cause of cell expansion induced by hormonal treatments. It is possible that under the influence of salt-stress the level of naturally synthesized growth hormones may be suppressed and that the exogenous application of phytohormones supplies more or less sufficient quantities which are involved in growth promotion. This opinion was favoured by Tagawa and Bonner (1957), Stewart (1959), Kassler (1961) and Shah and Loomis (1965).

*Table 6*

Effect of seed presoaking with $GA_3$ and IAA on the dry matter yield (g/plant) of maize and safflower plants stressed with different levels of sodium chloride (45 days in case of maize and 60 days in case of safflower)

| Treatment | whole plant | shoot/root ratio |
|---|---|---|
| Control | 2.10 | 0.46 |
| — 2 bar | 2.16 | 0.61** |
| — 4 bar | 1.62** | 0.71** |
| — 6 bar | 0.91** | 0.75** |
| — 8 bar | 0.75** | 0.67** |
| —10 bar | 0.68** | 0.58** |
| Control + $GA_3$ | 3.44** | 0.44 |
| — 2 bar + $GA_3$ | 2.47** | 0.72** |
| — 4 bar + $GA_3$ | 1.81* | 0.79* |
| — 6 bar + $GA_3$ | 1.22** | 0.72 |
| — 8 bar + $GA_3$ | 1.11** | 0.37** |
| —10 bar + $GA_3$ | 1.02** | 0.38** |
| Control + IAA | 3.13** | 0.51 |
| — 2 bar + IAA | 2.73** | 0.54 |
| — 4 bar + IAA | 1.69 | 0.66 |
| — 6 bar + IAA | 1.34** | 0.84* |
| — 8 bar + IAA | 0.87 | 0.36** |
| —10 bar + IAA | 0.06* | 0.34** |
| L.S.D. at 5% | 0.18 | 0.06 |
| L.S.D. at 1% | 0.24 | 0.10 |

| Treatment | whole plant | shoot/root ratio |
|---|---|---|
| Control | 1.11 | 1.70 |
| — 3 bar | 0.85** | 2.51** |
| — 6 bar | 0.38** | 3.13** |
| — 9 bar | 8.17** | 3.77** |
| —12 bar | 0.06** | 2.21* |
| Control + $GA_3$ | 0.91** | 1.84 |
| — 3 bar + $GA_3$ | 0.87 | 1.66** |
| — 6 bar + $GA_3$ | 0.54* | 3.77* |
| — 9 bar + $GA_3$ | 0.22 | 3.02** |
| —12 bar + $GA_3$ | 0.10 | 3.30** |
| Control + IAA | 1.73** | 2.04 |
| — 3 bar + IAA | 0.85 | 2.01 |
| — 6 bar + IAA | 0.54* | 2.38** |
| — 9 bar + IAA | 0.29 | 6.83** |
| —12 bar + IAA | 0.11 | 3.95** |
| L.S.D. at 5% | 0.13 | 0.51 |
| L.S.D. at 1% | 0.18 | 0.69 |

* Significant differences.
** Highly significant differences.

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# EFFECT OF FLUORINE AND SULPHUR INDUSTRIAL POLLUTION ON AGRICULTURAL CROPS AND EDAPHIC VEGETATION

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The biometric and quantitative analyses and the symptomatologic observation evidenced the reduction of the yields caused by the pollution level and the relation between the status of the edaphic vegetation (bacteria, algae and fungi), soil fertility and the production capacity of the agricultural crops.
The vegetation in the investigated polluted area contained 2—4 times more sulphur and fluorine and the number of edaphic organisms was reduced to a half by atmospheric pollution.

The action of noxes on plants is obvious in the areas bordering the industrial platforms which emanate polluted gases and dust in the air. It is manifested through burns, necroses, chlorosis and nanism phenomena, leading to the complete crop compromising (1), (3), (4), (5), (7). At the same time, xenic substances, being deposited in the soil, affect the edaphic flora and, thus, the soil fertility (2), (6), (8).

Our researches were performed in the proximity of an industrial zone that pollutes the surrounding environment, mainly with fluorine and sulphur dioxide; they were meant to determine the degree of vegetation damage and the relation between the pollution level, production and the condition of the vegetation in the soil.

## MATERIAL AND METHODS

The present studies were performed in a zone where the fluorine concentration varied between 0.026 and 0.135 mg/m³, the sulphur dioxide concentration between 0.15 and 0.45 mg/m³, the dust concentration between 720 and 815 g/m³.

Fluorine, sulphur and total nitrogen content in plants, the yield, the number of vegetal microorganisms in the soil, as well as certain soil physical and chemical qualities were checked in order to correlate the data and to understand the phenomenon exactly. Analyses have been performed in numerous localities in the polluted zone and compared to a zone located outside the noxes influence, considered a control zone. There were areas where F pollution was almost exclusive, others where $SO_2$ exceeded the allowed standard and, at last, areas where both noxes acted simultaneously.

The fluorine in the air was dosed colourimetrically, the $SO_2$ by oxidative methods and the dusts were collected in special vases. The fluorine in plants was dose by the glass electrode method, the soluble sulphates, by the Bardsley — Lancaster method and the total nitrogen, by the Kjeldahl method.

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The soil analyses were performed by the ICPA method, mainly two profiles being selected for this purpose : profile 1 in the nonpolluted area and profile 2 (to which some other surrounding locations were added) in the noxes affected area.

## RESULTS AND DISCUSSION

### a. THE CROP AND FLUORINE, SULPHUR AND TOTAL NITROGEN CONTENT OF THE PLANTS IN THE POLLUTED AREA

Maize, sorghum, wheat and soya beans plants were cropped in the maximum fluorine and sulphur dioxide interference zone, of an almost 40 hectares area.

Between kilometers 4.8 and 6 from the industrial platform, a control area was selected where there were the same cultures that existed in the neighbourhood of the works and where the soil had the same nature. The microclimate conditions were also extremely similar. The samples were taken in June and July and the obtained data — given in Table 1 — evidence, first, the fact that the plants in the polluted area have a high fluorine content. The fluorine quantities in the chemical analysis of the vegetation are almost 4 times increased compared to the witness area. The sulphur content increases approximately twice, while the total nitrogen content always decreases, at all noxes affected plants. The production in the considered zone decreases by approximately 40 per cent (Fig. 1).

*Table 1*

Fluorine, sulphur and total nitrogen determinations in plants

| Plant Portion | Zone | Flourine mg/g s.u. | | Sulphur mg/g s.u. | | $N_t$ % | |
|---|---|---|---|---|---|---|---|
| | | June | July | June | July | June | July |
| *Maize* | | | | | | | |
| — leaf | Witness | 1.6 | 1.65 | 2.9 | 2.7 | 1.31 | 1.36 |
| — whole plant | | 1.25 | 1.45 | 3.3 | 3.4 | 1.07 | 1.07 |
| — leaf | Polluted | 6.4 | 6.6 | 4.2 | 4.6 | 0.87 | 0.91 |
| — whole plant | | 6 | 6.8 | 3.8 | 4.2 | 0.9 | 0.9 |
| *Sorghum* | | | | | | | |
| — leaf | Witness | 1.9 | 1.9 | 2.2 | 2.3 | 1.27 | 1.01 |
| | Polluted | 4.9 | 5.7 | 4.7 | 4.7 | 1.16 | 1.08 |
| *Wheat* | | | | | | | |
| — leaf | Witness | 0.9 | 0.9 | 1.2 | 1.3 | 0.77 | 0.902 |
| — whole plant | | 0.8 | 0.8 | 0.9 | 0.9 | 1.11 | 1.2 |
| — leaf | Polluted | 3.2 | 2.7 | 2.7 | 2.7 | 0.66 | 0.68 |
| — whole plant | | 3.6 | 3.9 | 3.03 | 3 | 0.82 | 0.8 |



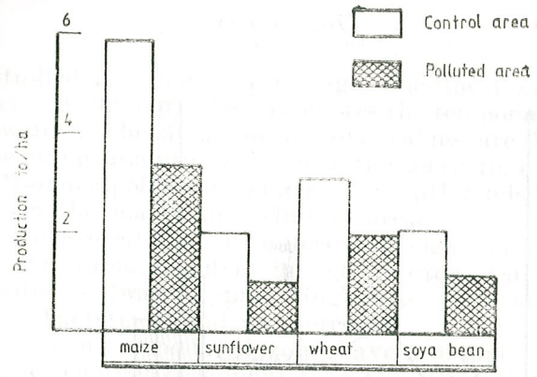


Fig. 1. — The yield obtained for various cultivated plants, in the control zone and in the polluted zone (0.026 — 0.135 mg/m³ fluorine).

The symptomatologic remarks indicated that the marrow, nonsucculent and sometimes maxy leaf species have the highest resistance; *Lolium perene*, *Agropyron repens* and, of the cultivated plants, *Ricinus communis* and *Soja hispida* proved a good resistance.

### b. ANALYSIS OF THE SOIL QUALITY AND EDAPHIC FLORA

The texture of the two profiles is of average fineness, clay — argillaceous at the surface and argillaceous in depth, the clay content (<0.002 mm) exceeding 40 per cent; this accounts for certain unfavourable soil qualities concerning the airhydro-regime, such as the creation of a periodic water excess, during the rainy seasons.

The apparent density exceeds 1.36 g/cm³, which indicates a strongly settled soil; the total porosity is very low (under 45 per cent), being completely unsatisfactory for most of depths.

The pH is neutral to low acid, its values being between and 6.92; 7.09 for the control profile and, respectively 6.01—6.57 for profile 2*

The humus has high values at profile 1, compared to profile 2, which indicates a degrading of the latter. As regards the sulphur content ($S-SO_4$ ppm), higher values were identified at the profile situated in the immediate neighbourhood of the industrial platform, compared to the control profile; at the same time, a washing tendency at soil depth is to be noticed, as deposits are formed at the surface.

In the eastern part of the platform (where there was a badly damaged wheat culture) and where the sulphur dioxide in the atmosphere exceeded 30 mg/m³, the determined sulphur content was much higher than at profile 2, exceeding the normally allowed average (Fig. 2).

* The pH analyses performed in 6 localities at 50—500 m. east of the work gave values of 5.09—5.61.

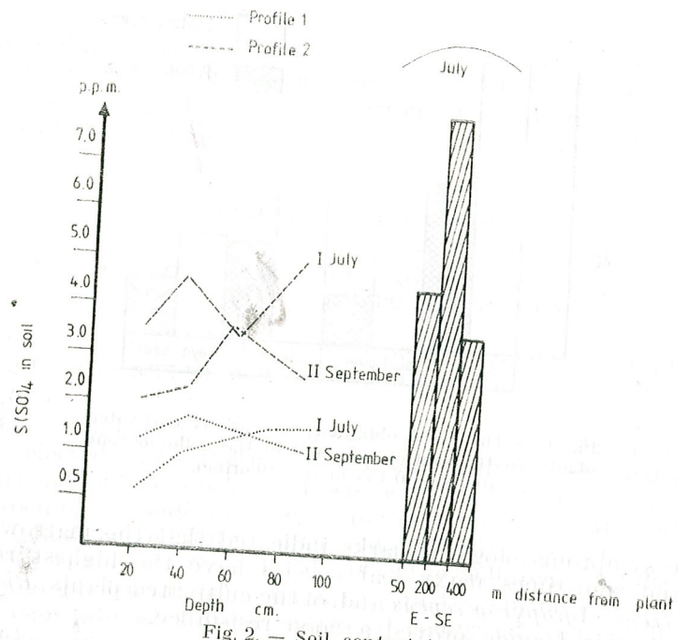


Fig. 2. — Soil content in S.

In order to check the way the sulphur and fluorine pollution affects the number of vegetal microorganisms in the soil and the enzymatic activity, in July and September the bacteria, fungi and algae, as well as the dehydrogenesis presence were determined. The latter presents insignificant variations in all the studied localities, and for this reason its values have not been included in this paper. But the number of fungi and especially of bacteria and algae /g s. u. is obviously higher at the control profile than at the one in the polluted area (Table 2).

*Table 2*

Soil characteristics and condition of edaphic flora

| Depth (cm) | Physical clay % 0.01 mm | Humus | pH | S — $SO_4$ ppm | No. of bacteria mil/g s.u. | No. of fungi mil/g s.u. | Algae no. of cells/g soil |
|---|---|---|---|---|---|---|---|
| Profile 1 witness | | | | | | | |
| 0—20 $A_0$ | 57.49 | 3.12 | 6.92 | 1.375 | 134.6591 | 304.375 | 17.316 |
| 20—30 A/B | 60.81 | 2.1 | 7.06 | 1.75 | 55.935 | 318.120 | 10.606 |
| 35—55 $Bt_1W$ | 64.87 | 1.62 | 6.93 | 1.375 | 251.042 | 252.730 | 520 |
| 80—100 $Bt_2W$ | 67.89 | 1.44 | 7.09 | 1.125 | 102.404 | 74.120 | — |
| Profile 2 polluted zone | | | | | | | |
| 0—20 A | 47.38 | 1.92 | 6.01 | 2.075 | 106.206 | 215.606 | 3.360 |
| 35—45 $Bt_1y$ | 63.68 | 1.14 | 1.45 | 2.375 | 53.700 | 326.842 | 4.898 |
| 55—75 $Bt_2$ | 64.47 | 0.96 | | 3.75 | 76.412 | 273.472 | — |
| 80—100 $Bt_2$ | 63.87 | 1.2 | 6.57 | 2.625 | 66.648 | 83.640 | — |



## CONCLUSIONS

1. The studied soils have an average fine-fine texture (more than 40 per cent clay <0.002 mm) which promotes the temporary stagnation of precipitation water . The apparent density values are higher than 0.4 cm/m³ and the humus quantity is lower in the zones that were (for more than 15 years !) under pollution influence ; the pH tends to turn acid (a clear tendency in the maximum pollution areas).

2. In the zones located in the immediate neighbourhood of the industrial platform, the sulphur content in soil increases, the dehydrogenasic activity fluctuates — always approaching zero — the number of fungi and especially of bacteria and algae decreases.

3. The plants in the polluted zone have a 4 times higher content and a twice higher content of sulphur than the vegetation in the control area. They present a typical symptomatology for the presence of the two mentioned substances.

4. The crops obtained in the impure atmosphere zones are diminished by approximately 40 per cent, mainly by the direct noxes action and, second, by soil pollution.

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# STRUCTURAL AND ENZYMATIC CHARACTERIZATION OF SOME WILD AND MUTANT STRAINS OF *ASPERGILLUS NIGER*

IOANA GOMOIU

The structural and enzymatic characterisation of enzyme producing strains of industrial importance is important both in controlling the process of the microorganism development and in biosynthesis processes.

The paper presents the investigations carried out on structural and enzymatic characterisation of wild and mutant strains of *Aspergillus niger*. Mutants strains present lower values as regards the diameter of conidian heads, the conidiophores length and vesicle diameter but these modifications are contained within the variability range accepted by *Aspergillus niger* (10).

Wild strain *A. niger* Cv in industrial medium produces amylases and some proteases, but mutant 3 only amylases.

Wild strain A. niger 150 and mutant 6 in industrial medium produce amylases and proteases.

The structural and enzymatic characterisation of enzyme producing strains of industrial importance is a necessary step in industrial microbiology since the biosynthesis processes, both quantitative and qualitative, depend on the development of the respective microorganism (4, 14, 18).

Moreover, the presence of several types of enzymes in the culture media may have positive implications on the processes of obtaining enzymatic preparates (7). We mention, therefore, here the positive influence of proteases, besides lipases and amylases, in the industrial preparate used by detergents industry and the negative one of these enzymes on the stability in time of amylases when obtaining a partly purified amylolytic preparate (1, 3, 12).

Aspergillus strains synthesize on suitable culture media a wide range of enzymes such as amylolytic and proteolytic complex (2, 8 — 11, 13).

The purpose of this paper is to give a morphological and enzymatical characterisation of wild and mutant strains of *A. niger*, cultivated in synthetical and natural media.

## MATERIAL AND METHODS

The investigations were carried out on two strains of *Aspergillus niger*, the wild type (*A. niger Cv*, *A. niger 150*) and two mutants (mutant 3 and 6) obtained after a mutagenic treatment with NTG.

The structural characterisation (macroscopical and microscopical) of strains mentioned above was performed in agarised Czapek medium malt extract. For macroscopical characterisation, the following characters were considered : the growth rate, aspect of micelle and fructification, the colony revert, the presence or absence of exudate and smell, etc. For microscopical characterisation, direct observations were performed on Petri plates, with an objective X 10 as regards the general aspect of the

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conidian ends and conidiophore, and on microscopical preparates (biometrical measurement sincluded) as regards the vegetative structures and fructification of taxonomical importance (conidiophores length and diameter, vesicles diameter, sterigma length and diameter, conidia diameter).

The enzymatic characterisation of the above mentioned strains was performed as follows :

— in synthetical medium Czapek with 1% starch, amylases production under stationary or stirred conditions (1UW = the quantity of amylase contained by 1 ml culture medium hydrolysing 1 mg starch in 30 min at 60'C)

— in industrial medium with sunflower bran and maize flour the production of the amylolytic and cellulasic, proteolytic complex (1 UE Cx = the enzyme quantity, which after an action the sol. CMC-Na 1%, at pH = 4.6, and 50°C ; IUE C1 is the quantity of enzyme that after 24 hours of action on filter paper at pH = 4 and 40°C produces 1 mg of glucose.

— in Na-caseinate 1% medium (proteases production, pH = 2.5 and 8.0).

The culture media 100 ml dispersed in Erlenmayer flasks of 750 ml were inoculated with spores suspensions and were incubated in stationary conditions. After 5 days incubation the medium was removed by filtering and culture medium was enzymatically characterized.

## RESULTS AND DISCUSSION

### STRUCTURAL CHARACTERISATION OF *A. NIGER* STRAINS AND MUTANTS 3 AND 6

In Czapek medium the colonies presented a reduced growth, with a diameter of 5.5 cm after 9 days of cultivation and 9 cm in malt extract medium. In Czapek medium, the basal micelle was white-yellow with thin and undulate edges ; mature fructifications showed that the reverse of the colony is white-yellow, it does not form an exudate and has a specific smell of mould. In medium with malt extract it is velvety, with thin and undulate edges, the fructifications appearing the third day, centrally localised, expanding in the 5th day on the whole surface of the colony, the reverse of the colony is brown-reddish ; the colony forms a fine exudate with typical smell of mould.

By direct examination of colonies grown in Czapek medium, globous conidia heads were noticed in incipient stages, becoming later radiary or columnary ; the diameter of conidian heads varies between 58 and 213 um as a function of age.

By examining the microscopical preparates the following were noticed :

— long conidiophores of 1.7—2.0 mm with a Φ of 9—25 μm, without colour and branching, with smooth walls, with the same thickness, but slightly thinner on the vesicle.

— vesicles with a diameter of 25—50 μm are globulous, hyaline and fertile on the whole surface ;



— the sterigma are hyaline and biseriated ; the primary with sizes of 21—25 μm 5—6μm and the secondary ones between 6—9 μm/3.0 — 3,3 μm. ;

— the conidia have a diameter of 2.3—4.7 μm, are globulous, of brown colour, with ornaments as bars.

Mutants 3 and 6 belong also to the group of *Aspergillus niger*. Their macroscopical characterisation is similar to the wild type. Slight differences were detected after the biometrical measurements as follows :

— the conidian head had a diameter between 77—194 μm ;

— the length of conidiosphores was of 1.9 μm and the diameter of 12—18 μm ;

— the vesicles diameter was between 18—43 μm ;

— the diameter of the conidia was of 3.6—4.7 μm.

By comparing these dimensions with those of wilde type we noticed lower values as regards the diameter of conidian heads, the conidiophores length and vesicle diameter. However, these modifications are contained within the variability range accepted by *A. niger*.

### THE ENZYMATIC CHARACTERISATION OF THE CULTURE LIQUIDS

The production of amylases by wild strains *A. niger Cv*, *A. niger* 150, mutants 3 and 6 is dynamically presented in table 1, in the synthetical and industrial media under the same conditions as shown in table 2.

We noticed therefore that the amylolytic activity on Czapek medium with starch, under stationary conditions is doubled under conditions of stirring. The amylolytic activities of mutants are higher than in case of wild strains.

On an industrial medium, under conditions of stirring, all strains present amylolytic activities and their values are comparable to those obtained under stationary conditions in a synthetical medium.

The amylolytic activity in the industrial medium, under stationary conditions, showed the highest values (90 UW/ml for *A. niger* Cv 118 UW/ml for mutant 3, 123 UW/ml for *A. niger* 150, 137 UW/ml for mutant 6) the 11th day, maintained also in the 13th day. These results confirm our previous data (11). Therefore we may appreciate that the highest values are obtained in an industrial medium as regards the amylolytic activity.

*Table 1*

Production of amylases by wild strains and mutants of *Aspergillus niger* in synthetical medium

| Strain | Amylolytic activity (UW/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Conditions of stirring | | | | | Stationary conditions | | |
| Days | 3 | 5 | 7 | 7 | | 9 | 11 | 13 |
| *A.niger* Cv | 0 | 0 | 0 | 0 | | 17 | 23 | 21 |
| Mutant 3 | 0 | 15 | 15 | 0 | | 17 | 30 | 20 |
| *A.niger* 150 | 0 | 17 | 0 | 0 | | 17 | 20 | 20 |
| Mutant 6 | 0 | 15 | 0 | 0 | | 17 | 27 | 17 |

*Table 2*

Production of amylases by wild strains and mutants of *Aspergillus niger* in industrial medium

| Strain | Amylolytic activity (UW/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Conditions of stirring | | | | Stationary conditions | | | |
| Day | 3 | 5 | 7 | 7 | 9 | 11 | 14 | |
| *A. niger* Cv | 70 | 91 | 52 | 70 | 85 | 90 | 90 | |
| Mutant 3 | 70 | 100 | 52 | 75 | 112 | 118 | 90 | |
| *A. niger* 150 | 52 | 91 | 70 | 75 | 123 | 123 | 123 | |
| Mutant 6 | 91 | 103 | 75 | 100 | 137 | 137 | 137 | |

## PRODUCTION OF PROTEASES

The production of proteolytic enzymes by the above-mentioned strains in synthetical medium with natrium caseolate and industrial medium, under stationary or stirred conditions is performed differently (table 3). Therefore, in the culture medium, obtained from strain *A. niger* Cv in

*Table 3*

Production of proteases by wild and mutant strains by *Aspergillus niger* in the synthetical and industrial medià

| Strain | | Proteolytic activity 6umol tyrosine/ml | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Conditions of stirring | | | | | | Stationary conditions | | | | | | | |
| Days | | | | | | | | | | | | | | | |
| pH | | 2.5 | 8.0 | 2.5 | 8.0 | 2.5 | 8.0 | 2.5 | 8.0 | 2.5 | 8.0 | 2.5 | 8.0 | 2.5 | 8.0 |
| *A. niger* Cv | Synthetical medium | 0 | 0.9 | 0 | 3.2 | 0 | 2.9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mutant 3 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. niger* 150 | | 1.2 | 0 | 2.7 | 0 | 1.9 | 0 | 0 | 0 | 4.7 | 0 | 5.1 | 0 | 4.7 | 0 |
| Mutant 6 | | 1.2 | 0 | 3.2 | 0 | 1.7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. niger* Cv | Industrial medium | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.2 | 0 | 3.2 | 0 | 1.2 | 0 |
| Mutant 3 | | 0 | 0 | 0 | 1.8 | 0 | 0 | 1.8 | 0 | 1.8 | 0 | 3.2 | 0 | 1.1 | 0 |
| *A. niger* 150 | | 0 | 0 | 0 | 0 | 0 | 0 | 2.1 | 0 | 3.6 | 0 | 4.7 | 0 | 2.7 | 0 |
| Mutant 6 | | 0 | 0 | 0 | 0 | 0 | 1.9 | 1.9 | 0 | 1.9 | 0 | 3.2 | 0 | 1.4 | 0 |

synthetical medium, no active proteases were pointed out, for a pH = = 2.5, neither in stationary nor stirring conditions.Only active proteases (pH=8.0) were pointed out under stirring condition. In the culture medium obtained from mutant 3, no active proteases were pointed out for pH= = 2.5.

In the culture media obtained from strain *A. niger* 150 and mutant 6 only active proteases at pH = 2.5 were detected; their activity was higher under conditions of stationary cultivation.

In the industrial medium, under stirring conditions, in the culture liquid obtained from *A. niger Cv* and *A. niger* 150 no proteolytic activities



were noticed at pH 2.5 or 8.0. Active proteases at pH = 8.0 were pointed out only for mutants 3 and 6, but their activities are however very low.

Under stationary conditions in industrial medium, no proteolytic activities were pointed out, at pH = 8.0 on none of culture liquids. However, some proteolytic activities were noticed at a pH = 2.5 in the culture liquids obtained from all strains. The analysis in table 3 in comparison to table 2 shows that in industrial medium, under stationary conditions (showing the highest amylolytic activities) the strains under study produce proteolytic enzymes at pH = 2.5 with high activities. These strains (except for mutant 3 in the synthetical medium) have the capacity to produce active proteolytic enzymes at pH = 8.0. The fact that they could not be pointed out may be due to the presence of specific inhibitors and the absence of adequate medium conditions for the synthesis of such enzymes. These conditions and those necessary to obtain enzymatic preparates rich in amylases may diminish the proteolytic activities if proteases were present in the culture media.

Literature shows that the strains *Aspergillus niger and Aspergillus oryzae* (19, 20) are good proteases producers but they cannot be pointed out in liquid media because of some polysaccharides existing in the medium.

## PRODUCTION OF CELLULASES

The strains under study produce cellulases in the industrial medium using cellulose from sunflower flour (table 4) but in low quantities.

*Table 4*

Production of cellulase by wild strains and mutants in synthetical medium

| Strain | Cellulosolytic activity | | | | | | |
|---|---|---|---|---|---|---|---|
| Days | 3 | | 5 | | 7 | |
| | $C_1$ | $C_x$ | $C_1$ | $C_x$ | $C_1$ | $C_x$ |
| *A. niger* Cv | 0 | 1,3 | 0 | 2,9 | 0 | 2,0 |
| Mutant 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. niger* 150 | 0 | 1,4 | 0 | 2,9 | 0 | 2,7 |
| Mutant 6 | 0 | 1,9 | 0 | 4,2 | 0 | 3,9 |

The strains under study do not produce enzyme $C_1$ which is able to degrade filter paper. Mutant 3 by mutagenic treatment lost the capacity to synthesize enzyme $C_x$ and mutant 6 received the cpacity to synthesize a higher quantity of $C_x$

## CONCLUSIONS

1. The morphological characteristics of wild strains permit us to ascribe them to the group *Aspergillus niger*.
2. *Aspergillus niger* mutant selected as being better amylase producers than wild strains does not present morphological modifications.

3. The maximum biosynthesis of proteases was pointed out in the culture medium from strain *A. niger* 150 under stationary conditions.

4. The maximum biosynthesis of amylolytic complex was pointed out in mutants 3 and 6 in the industrial medium

5. By mutagenic treatment, mutant 3 lost the capacity to synthesize enzyme $C_x$, and mutant 6 received it.

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# PROTOPLAST ISOLATION, FUSION AND REVERSION IN BACTERIA OF GENUS *BACILLUS*

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Taking into account the importance of the bacteria belonging to the genus *Bacillus* for enzyme production in industrial microbiology, a general and rapid method for protoplast isolation was developed.
The conventional reversion media used for *B. subtilis* protoplasts do not permit a direct selection of the transformants, because some antibiotics are inactivated by sodium succinate used as osmotic stabilising agent, making thus impossible an evident direct selection of the transformants on the reversion medium. Reversion dynamics on medium with sucrose was analyzed by using electron micrographs. The events taking place during the interspecific protoplast fusion were analyzed by electron microscopy, too.

The technique of protoplast fusion and transformation induced by polyethylenglycol (PEG) in bacteria inside the genus *Bacillus* became of an ever greater importance. That is because in comparison with the routinely used improvement methods, the protoplast fusion guarantees the obtaining of strains with new genetic characteristics at a high frequency (Hotchkiss and Gabor, 1983).

At the same time, the protoplast fusion allows a new rearrangement of the genetic traits in the strains obtained by mutagenesis, making possible even the crossing of the species barriers in some cases.

Alternatively, in comparison with the transformation based on cell competence, protoplast transformation induced by PEG (Chang and Cohen, 1979 ; Jandova and Tichy, 1982 ) allows the advantage of gaining it in strains where competence can hardly be induced or at all (Hopwood 1981, Jandova and Tichy, 1982).

It is also possible, using PEG induced transformation, to achieve the efficiency of the monomer plasmid forms, and not only of the oligomer ones, as in the case of competent cells (Chang and Cohen, 1979 ; Hopwood, 1981).

Likewise, the plasmid transfer can be performed by protoplast fusion, the plasmid purification being unnecessary in this case (Dancer, 1980 ; Vorobjeva, 1980).

Therefore, the technology mentioned above is extremely important for industrial microbiology, as most of the enzymes of biotechnological use are produced by bacteria of the genus *Bacillus* (Erickson, 1979).

For these reasons, we intended to establish a rapid and reproducible methodology for protoplast obtaining in species of biotechnological interest belonging to the genus *Bacillus*, in order to be used in fusion and transformation experiments. Considering the special importance of protoplast reversion and the possibility to obtain a first selection on a regeneration medium, we used an adequate one with sucrose as osmotic stabiliser, as on the usual regeneration medium, a first selection is not possible.

The isolation, the interspecific fusion and reversion stages were investigated by micrographs.

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## MATERIALS AND METHODS

The following bacterial strains were used :

1. *Bacillus subtilis* 168, from Dr. Goebel, F. R. G. (a strain frequently used as plasmid acceptor), in order to induce and transform protoplasts with DNA of pUB110 plasmid.

2. *Bacillus subtilis* (pSA0501) $Sm^R$, $trp^-$ $thr^-$ from Dr. G. Venema, Groningen, Univ., Holland, for interspecific fusion.

3. *Bacillus globigii*, $Rf^R$, from Dr. G. A. Wilson, U.S.A., for interspecific fusion, as the yellow colour of the colonies can be used as a marker.

4. *Bacillus licheniformis* $Em^R$, from Food Chemistry Institute Collection, for interspecific fusion, for the properties of releasing amylase.

5. *Bacillus subtillis* (pUB110) 268, $Km^R$, $thy^-$, $tyr^-$, from Dr. G. Venema, Groningen Univ., Holland, as DNA source of pUB110 plasmid.

*Methods* :

— In a variant, the protoplast isolation was performed by the method described by Chang and Cohen (1979) (bacteria cultivated in Penassay broth; the protoplasts were obtained during the exponential stage of growth by using lysozyme in concentration of 200 μg/ml in tris-maleat buffer, pH—6.5, mixed with Penassay broth).

— In another variant, the protoplasts were isolated according to our original method which consists in : bacteria were cultivated in nutrient broth for sixteen hours, the protoplasts being induced during the stationary period of bacteria growth, in order to obtain a larger quantity of cells. Before the treatment with lysozyme (200 μg/ml) in 0.2 M phosphate buffer with 0.5 M sucrose, a pretreatment was performed with $EDTANa_2$ in 0.02M tris—HCl buffer with 1M NaCl, pH—7.5.

— For transformation, the pUB110 plasmid DNA was purified according to the method of Ish-Horowitz (1981) and ascertained in 1% agarose gel. The mixture of plasmid DNA and *B. subtilis* protoplasts was exposed for one minute to the action of 40% $PEG_{4000}$.

— In the case of transformation, a direct selection on the regeneration medium was performed. The medium contained 0.5 M sucrose as osmotic stabiliser and 30 μg/ml km, for which the pUB110 plasmid has genetic determinants for resistance. Alternatively, a regeneration medium having Na succinate as osmotic stabiliser was used.

— For fusion induction, the protoplasts were washed and exposed for 1 minute at 40% $PEG_{4000}$, then rinsed and dispersed for regeneration.

— In order to supervise the regeneration, the protoplasts were maintained in the regeneration medium supplemented with 0.5 M sucrose for three and six hours, respectively.

— The electronmicroscopic study was carried out by fixing the material, consisting of protoplasts isolated, fused and reversed with 3% glutaraldehyde, followed by postfixation with 2% $OsO_4$ and inclusion in Epon. The ultrathin sections obtained with a Tesla ultramicrotom were stained by using uranyl acetate and lead citrate, according to RAYNOLDS (cit. by Hayat, 1972 ) and examined under a JEM—7 electronmicroscope.



## RESULTS AND DISCUSSIONS

### I. PROTOPLAST ISOLATION IN B. SUBTILIS, B. GLOBIGII AND B. LICHENIFORMIS

Generally, the protoplast induction in *B. subtilis* is performed by a simple treatment with lysozyme (Hopwood, 1981). There are, however, strains where the treatment with lysozyme induces a slow and inefficient release of protoplasts.

In Gram negative bacteria, where the presence of the outer membrane makes the penetration of lysozyme to the substrate difficult, the spheroplasts are obtained by a pretreatment with EDTA, before applying the lysozyme (Weiss, 1976). The pretreatment has the role to destabilise the outer membrane, facilitating the penetration of lysozyme to peptidoglycan (Witholdt, 1976).

Our preliminary studies showed a similar effect, in some cases the pretreatment with EDTA resulting in increasing the frequency of protoplast induction in strains relatively resistant to the treatment with lysozyme. The data are presented in Table 1 :

*Table 1*

Bacterial strains and the frequency of protoplast inducing

| No. | Bacterial strain | Genetic characters | Frequency of protoplast isolation | |
|---|---|---|---|---|
| | | | Pretreatment with EDTA | Without pretreatment with EDTA |
| 1 | *Bacillus subtilis* 168 | $Sm^R$ | 98% | 96% |
| 2 | *Bacillus subtilis* pSA0501 | $Sm^R$ $trp^-$ $thr^-$ | 90% | 52% |
| 3 | *Bacillus globigii* | $Rf^R$, prod. Bgl I | 95.2% | 92% |
| 4 | *Bacillus licheniformis* | $Em^R$ | 91.5% | 80.2% |

These studies also demonstrated the possibility to use cells in the stationary growth stage to isolate protoplasts by using the same method, with the advantage of producing a larger number of cells. That facilitates both the further manipulation in view of fusion and transformation, as well as the optic and electronmicroscopic examination, which implies important loss of cells by dilutions and repeated washings.

Another modification, in comparison with conventional methods, consists in using the phosphate buffer in concentration of 0.2 M, pH—6.5 for protoplast releasing, this buffer being also used during the subsequent stages of obtaining the preparates used in the electronomicroscopic studies ; in our opinion, the tris-maleat buffer recommended by some authors (Schaeffer, 1976 ; Chang and Cohen, 1979) for protoplast isolation, cannot be used under the circumstances described, because it inactivates the glutaraldehyde.

The micrographs (Figs 6—13) reveal the specific spherical shape of protoplasts, thus confirming the efficiency of our technique.

## II. REVERSION OF *B. SUBTILIS* PROTOPLASTS

### a. *The effect of sucrose used as osmotic stabiliser on the cell wall regeneration and on the selection of transformed cells*

The biological systems have the capacity to repair many lesions affecting their structures and functions. The protoplast reversion to the bacillary form belongs to this category of properties (Nečas, 1980). Although reversion does not mean only regeneration of cell wall, but implies a variety of regulatory mechanisms responsible for cell integrity, the central event of reversion is represented however by the regeneration of cell wall.

In the genus *Bacillus*, an eficient regeneration medium is of a special importance, as it may permit a first selection of genetically modified protoplasts by plasmid DNA transformation induced by PEG or by chromosomal and plasmidial gene transfer consequently to the fusion process.

Use of 0.5 M Na succinate as osmotic stabiliser in regeneration media (Landman, 1969 ; Wyrick, 1973 ; Chan and Cohen, 1979) is not adequate for studying the transfer of genetical determinants for resistance to some antibiotics (Km and Sm) (Jandova, 1987). The Na succinate inactivates the antibiotics (Km and Sm) contained by the regeneration medium in order to select strains resistant to these.

For these reasons, we used 0.5 M sucrose as osmotic stabiliser in the regeneration medium, supposing that, although *B. subtilis* produces the enzyme glycosidase that degrades sucrose (Jandova, 1987), the latter is supplied in such quantity to be efficient until the regeneration of the cell wall is finished.

Sucrose reduces by 30% the frequency of regeneration in comparison with that achieved by using succinate. This disadvantage is only apparent, because sucrose does not inactivate Km. That was demonstrated by pasing the cells transformed with pUB110 plasmid DNA from the regeneration medium supplied with sucrose as osmotic stabiliser and Km, on gelose with Km, the resistance being maintained in a ratio of 100%. If the transformed cells are transferred from the regeneration medium containing Na succinate as osmotic stabiliser, and Km, on gelose containing Km, the resistance is only 30%.

In conclusion, although the regeneration frequency on medium with sucrose is lower in comparison with the one achieved on the medium with succinate, sucrose permits an efficient direct selection of the transformed cells with pUB110 on the regeneration medium, which does not happen in the case of succinate.

The DNA of pUB110 plasmid extracted from the original strain (*B. subtilis* pUB110 268) and from the transformed cells (*B. subtilis* 168 in which protoplasts were isolated and transformed with pUB110), is pointed out by electrophoresis in 1% agarose gel (Fig. 14).

### b. *Dynamics of B. subtilis protoplast reversion*

There are contradictory points of view with regard to the morphological modifications accompanying the reversion, and their relationship with the initiation of cell division.



Therefore, Landman (1969), in a study on *B. subtilis* protoplast reversion induced by gelatine, considered that regeneration precedes division, although the often noticed among cells undergoing the reversion process some "branching forms", which might suggest the division.

Mc Quillen (1960) appreciates that during a longer incubation of *B. megaterium* protoplasts, division stages may be noticed. Some forms that can be related to the division process were also identified in variant defective of cell wall from *B. subtilis* and *B. licheniformis* (Wyrick and Rogers, 1973).

Our observations, performed both after three and six hours of maintaining the protoplasts in the regeneration medium, pointed out also forms which suggest the commencement of the division process (Fig. 1 and Fig. 5). "Branched forms" of the type identified by Landman, (1969) with a bipolar orientation of the nucleoid, are observed (Fig. 1 and Fig. 4). Fig. 5 displays the end of a division in a protoplast undergoing reversion, maintained for six hours in the regeneration medium.

The micrographs of the various stages of reversion point out an increased opacity at the periphery of the cells (Figs 1—5), which may be ascribed to the synthesis of the cell wall. We also noticed a great number of ribosomes related to the intense protein synthesis taking place during the regeneration process.

After maintaining the cells for three hours in the regeneration medium the cell shape becomes slightly long (Figs. 1 and 2), a modification that was more evident after six hours (Figs. 3 and 5).

However, the presence of cell wall is obvious in micrographs only after six hours of maintaining the protoplasts in the regeneration medium (Figs. 3, 4 and 5).

The various stages analysed support the idea of the existence of some preparative stages for the synthesis of the cell wall, not detectable in micrographs.

Our results suggest also the possibility that the division should start immediately after the initiation of the cell wall synthesis, the two processes having a simultaneous evolution.

## III. ASPECTS OF *B. SUBTILIS*, *B. GLOBIGII* AND *B. LICHENIFORMIS* PROTOPLAST FUSION

According to Frehel (1979), the protoplast fusion might be performed in two different stages : *1.* the initial stage, undetectable by micrographs, preparing the fusion, consisting in an activation of the plasma membrane, depending on high concentration of PEG, and *2.* the final stage, detectable by electronmicrographs.

The presence of the first stage was recently confirmed (Arnold, 1987) by demonstrating the indirect action of PEG on the cell membrane, determining the modification of its polarity. These processes would represent a prerequisite necessary for the beginning of the fusion.

The phenomenon can be clearly detected during the interspecific fusion, due to the differences in size between protoplasts belonging to different species.

In a first stage, it is evident the adherence of *B. subtilis* protoplasts with those of *B. globigii* (Fig. 6) or of *B. licheniformis* (Fig. 7).

In a next stage, the fused protoplasts have a "bispherical shape", being separated by an electronotransparent space, called "apparently empty vesicle" (Frehel, 1979) (Figs 8, 9, 10). Fig. 8 shows such a "bispherical shape" with an electronotransparent space between the fused protoplasts, having a continuous plasma membrane at their periphery.

Afterwards, it is supposed that the "vesicle" moves along the border of the fused protoplasts, altering the double membrane and loading itself with phospholipidic and proteic micelles; the space between the fused protoplasts becomes electrondense (Figs. 11 and 12).

In the final stage, the membranal boundaries between the fused protoplasts disappear, leading to the cytoplasm intermixing (Fig. 13).

These data prove that both the interspecific and intraspecific fusion processes are characterized by similar morphological modification at the cell level.

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G.I. GHIORGHIȚĂ, *Radiobiologie vegetală (Efecte ale radiațiilor nucleare la plante)*, Editura Academiei, Bucharest, 1987, 154 p.

The recent edition issued by Editura Academiei, *Plant Radiobiology* (*Effects of Nuclear Radiations on Plants*) (in Romanian) completes the Romanian specialty literature, being the first comprehensive work on plant radiobiology published in Romania and one of the few of this kind existing in scientific literature. Elaborated by a passionate scientist, dr. Gogu I. Ghiorghiță, the head of the department of Experimental Biology and Genetics from the Piatra Neamț "Stejarul" Research Station, a specialist that has worked for more than 15 years in this field, the work is a valuable synthesis regarding the effects of ionizing radiations on plants.

After a "Historical Review", enlisting a series of data referring to the stage of the investigations and contributions of some scientific centres in the field of radiobiology and plant radiogenetics, the author presents the main theories that try to explain "The Action Mechanism of Ionizing Radiations in Plants".

A chapter not too often met in the syntheses of plant radiobiology is "Some Effects Induced by Nuclear Radiations in Plants". The chapter presents — partly for the first time published — the physical-chemical modifications, the genetical effects, the morpho-hystoanatomical effects, the behaviour of some physiological and biochemical processes in plants when treated with radiations (plant growth and development; the photosynthesis and the assimilatory pigments; respiration, the oxido-reducing processes, the activity of enzymes; the oxidative phosphorylation; the water balance in plants; the nucleic acids; the proteins, aminoacids and nitrogen; the carbohydrates etc.). This chapter is a remarkable contribution of the author who, starting from his own results and from those of the scientific literature succeeded to make a good synthesis.

In a special chapter the author presents the phenomenon of "Radiostimulation", with profound applicative implications, its expressing modalities, the radiostimulation mechanism and the factors influencing its manifestation. A chapter, important for those studying the effects of ionizing radiations on plants, is "Factors Modifying Plant Radiosensitivity". It presents the physical (radiation type, dose and flow capacity of radiation, irradiation proceeding and time of irradiation, temperature, successive irradiation, magnetic treatments etc.), chemical (oxygen, water content, phytohormones, chemical mutagens etc.), and biological factors (specificity of the irradiated material, ploidy level, ontogenetic stage of development, cellular cycle phases, physiological and biochemical peculiarities etc.), which modify the plant radiosensitivity. Although the results obtained by different specialists investigating the factors that influence the irradiation plant resistance are rather heterogenous, often contradictory, using with a high professional competence the data of the scientific literature, the author presents in a unitary, concise and elegant manner the action of numerous factors, of different origins, on plant radiosensitivity.

The book we presented is a valuable Romanian contribution to the scientific world literature. The work is dense, elaborated in a sober and clear language, being extremely useful both to the specialist, for the wealth of data and syntheses, and to the reader interested to know the effects of ionizing radiations on plants.

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## AVIS AUX AUTEURS

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

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