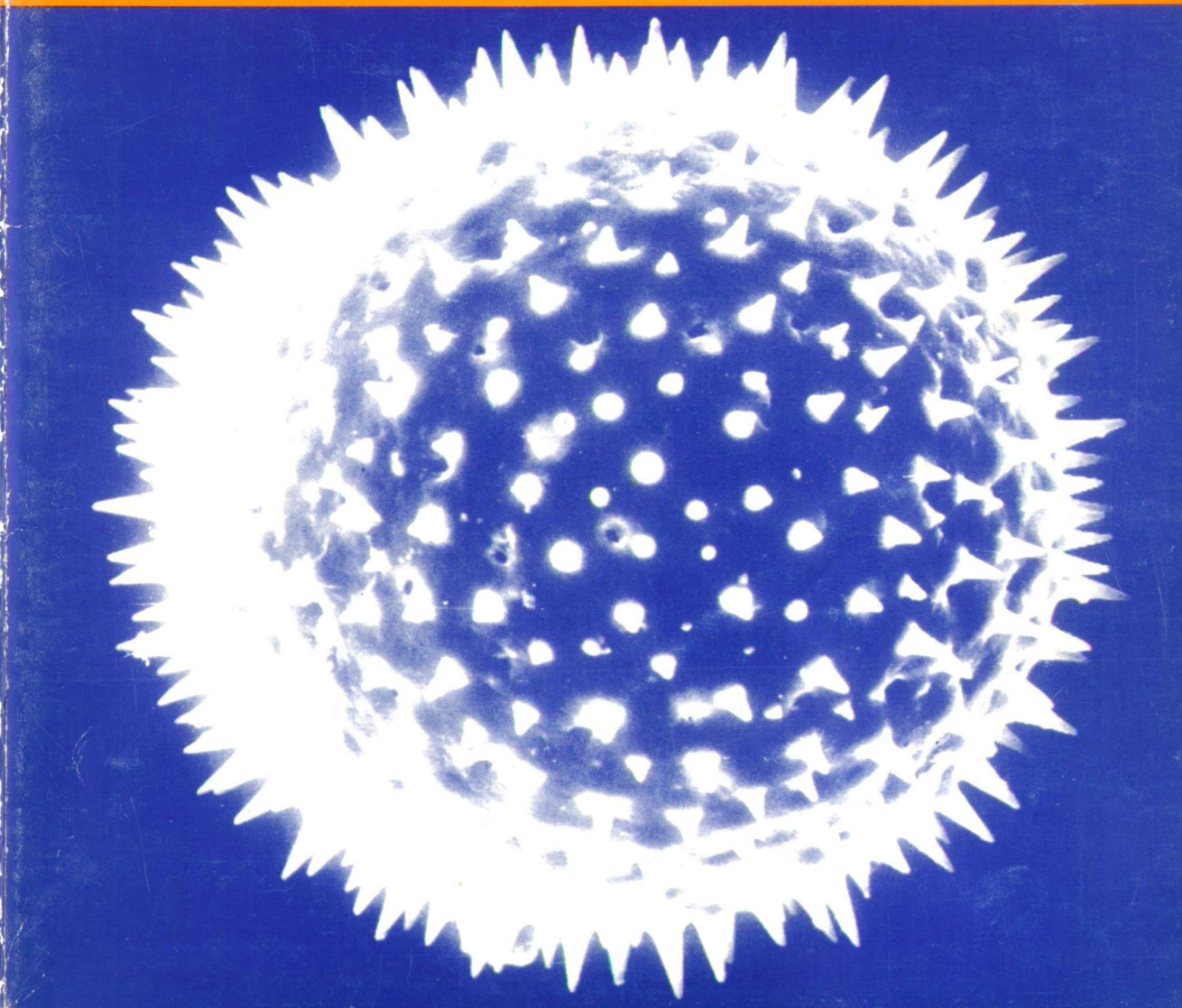
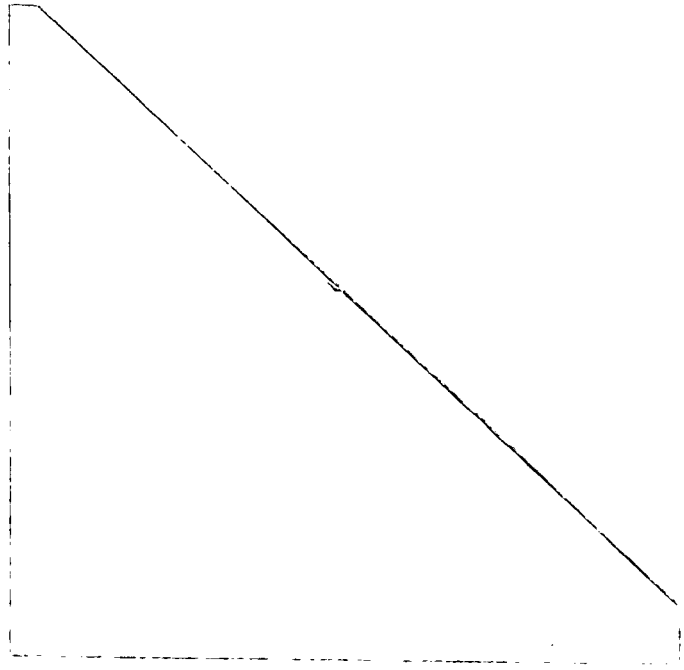
PLANT CELL BIOLOGY AND DEVELOPMENT 16





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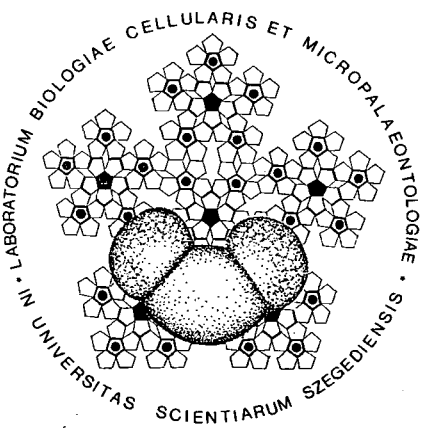

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Preface

We have received several sad news recently. Prof. Dr. M. PÉCSI, member of the Hungarian Acedemy of Sciences, passed away on 23 January, 2003. We have come a long way since we started a joint research program on the geography and anatomy of the Upper Tertiary lignites of Hungary. We planned to complete it together.

Last year we lost Dr. W.A. SARJEANT an excellent scientist of the research into fossil Dinoflagellatae.

The function of the Rector of the University of Szeged, Prof. Dr. R. MÉSZÁROS, who is the member of the Hungarian Academy of Sciences, has ended this summer. Previously he was the Dean of the Faculty of Sciences and the Rector of J.A. University and he has always helped our Laboratory. From now on he is going to concentrate his energy on his researches and on the education at the Department of Economic Geography. The staff of the Laboratory expressed their grateful thanks by giving him both the Commemorative and the Millenium Medal of the Laboratory in the same time.

The international joint research programs of the Laboratory has increased this year. In this moment we have active researches with the B.S.I.P. in India, the Ain Shams University in Egypt, El Salvador and Brasil.

For the financial support of the publication of this volume the staff of the Laboratory would like to express their grateful thanks:

to the Grant OTKA T 31715
to Prof. Dr. R. MÉSZÁROS, member of the Hungarian Academy of Sciences, Rector of the University of Szeged
to Prof. Dr. GY. TELEGDY, member of the Hungarian Academy of Sciences
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to Prof. Dr. G. MEZŐSI Dean of the Faculty of Science of the University of Szeged
to the Foundation for Szeged

Szeged, December 2003.

M. KEDVES
Head of the Laboratory

1. FRUIT MORPHOLOGY OF THE GENUS FIMBRISTYLIS (CYPERACEAE): SEM STUDY

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Abstract

Twelve species of the genus *Fimbristylis* (Cyperaceae) fruits have been studied under scanning electron microscope to know the detailed micromorphological features of the pericarp. It was found that morphological characters can be grouped into two main categories: i) fruits having reticulate ornamentation in the pericarp ii) ornamentation with combination of reticulate-tuberculate. It was found that 4 species having characters referable to category (i) are plants that usually grow in marshy/waste lands, bear only reticulate ornamentation of the pericarp, while 8 species having characters of category (ii) adaptable to both higher elevation as well as the wet places of the plains, have pericarp with robustly developed reticulate-tuberculate ornamentation.

Key words: Fimbristylis (Cyperaceae), fruits, morphology, SEM.

Introduction

The cosmopolitan family of Cyperaceae generally grow as perennial and annual herbs in the tropical regions of the world thriving along the wet/marshy lands as well as higher elevations up to 1828 meters. Some of the taxa are also adopted to dry desertic condition.

The family Cyperaceae includes 90 genera and 3200 species where *Carex* (1100 species), *Cyperus* (700 species), *Scirpus* (200 species), *Rhynchospora* (200 species), *Eleocharis* (150 species) and *Scleria* (100 species), whereas *Fimbristylis*, a large genus of warm region - name compounded 'fimbria', a fringe and 'stylus' (style), the latter being fringed with hairs, has 125 species. In the present study 12 species of the genus *Fimbristylis: F. albo-viridis, F. argentea, F. bisumbellata, F. complanata, F. cymosa, F. ferruginea, F. lawiana, F. miliacea, F. podocarpa, F. polytrichoides, F. quinquangularis, F. tenera* were subjected to scanning electron microscopy. The detailed morphological characters of the pericarp of the above species were recorded. Fruit morphology of the genus *Fimbristylis* has been studied under light microscope by a number of workers, BHANDARI (1990), DAHLGREN and CLIFFORD (1982), FERNALD (1950), HOOKER (1894), HUTCHINSON (1959), LAWRENCE (1969), SAMVATSAR (1996), TRIMEN and HOOKER (1900). The present study has been undertaken to know the detailed micromorphological features of taxonomical importance.

Materials and Methods

The material for the above studies was procured from the herbarium of Birbal Sahni Institute of Palaeobotany, Lucknow. The specimens were thoroughly cleaned by treating with absolute alcohol to avoid any alteration in the micromorphological features. They were further subjected to ultrasonic cleaning by changing the absolute alcohol repeatedly to remove any artifacts. For different views 3-4 specimens of each species were selected and mounted in the desired orientation on the double sided scotch tape. The specimens were conducted with silver dag and were subjected to sputter coating by using gold palladium (Au/Pd) target. The thickness of coating varied from 100-200 Å depending on the requirement of specimens. The coated specimens were then observed under scanning electron microscope (Phillips 505). To achieve better resolution the accelerating voltage varied up to 30 kV. The final observations were recorded for each specimen.

Results

F. miliacea (LINN.) VAHL. (Plate 1.1., figs. 1,2)

Fruits obovoid, trigonoid, reticulate with finger-like projections (tubercles) regularly dispersed all over the surface, colour pale yellow to brown, apex mucronate, base narrow, size 300-400 µm long and 200-250 µm broad. Perigynium thin, translucent, covering pericarp. Pericarp with ridges and furrows associated with tubercles, the size of tubercles 5-10 µm long, prominent or trigonoid ridges. The size of the cells of reticulum ranges from 5-20 µm. These cells become smaller at the apical and basal region.

Distribution: *F. miliacea* is found at the altitude up to 1828 meters abundantly, grows in all the warm regions of the world. Occasional in waste lands among grassland throughout India (HOOKER, 1894, SAMVATSAR, 1996).

F. podocarpa NESS (Plate 1.1., figs. 3,4)

Fruit ovoid, texture finely striated (ridges and furrows), colour brown apex with stylar base, base flattened, size 500-600 µm long and 300-400 µm broad. Perigynium thin, translucent, forming fine striations at the base. Pericarp consists of 18-20 ridges and furrows on each face, running parallel to each other converging towards base and apex. The cells of the pericarp form "honeybee comb" pattern. Patches of tubercles (20-30 µm) present on the apical part of the fruit.

Distribution: Frequently found in Western Himalaya to Upper Assam and Bangla Desh (Dacca), Chota Nagpur, Malaya and China (HOOKER, 1894).

F. cymosa R. BR. (Plate 1.1., figs. 5,6)

Fruit ovoid-trigonoid, texture reticulate-tuberculate, colour black, apex triangularly flattened, base narrow with stylar ring, size 300-400 µm long and 200-250 µm broad. Perigynium absent. Pericarp reticulate, tuberculate, tubercles mostly at the apical region covering half length of the fruit. The size of the reticular cells very small ranging up to 10 µm long and 5 µm broad, irregularly arranged with wavy (frill-like) margin.

Distribution: Common along the river banks and also in dry areas of the tropical regions of the Old Word (RAO and RAZI, 1981).

F. quinquangularis KUNTH (Plate 1.1., figs. 7,8)

Fruits elongated ovoid, trigonoid with prominent ridges, texture tuberculate, colour yellow to pale brown, apex flattened, base narrow, size 200-250 µm long and about 200 µm broad. Perigynium present, translucent. Pericarp tuberculate-reticulate, tubercles transversely flattened, run lineolate. Thin elongated reticulate cell present throughout the pericarp surface. Size of the tubercles ranges from 10-30 µm long.

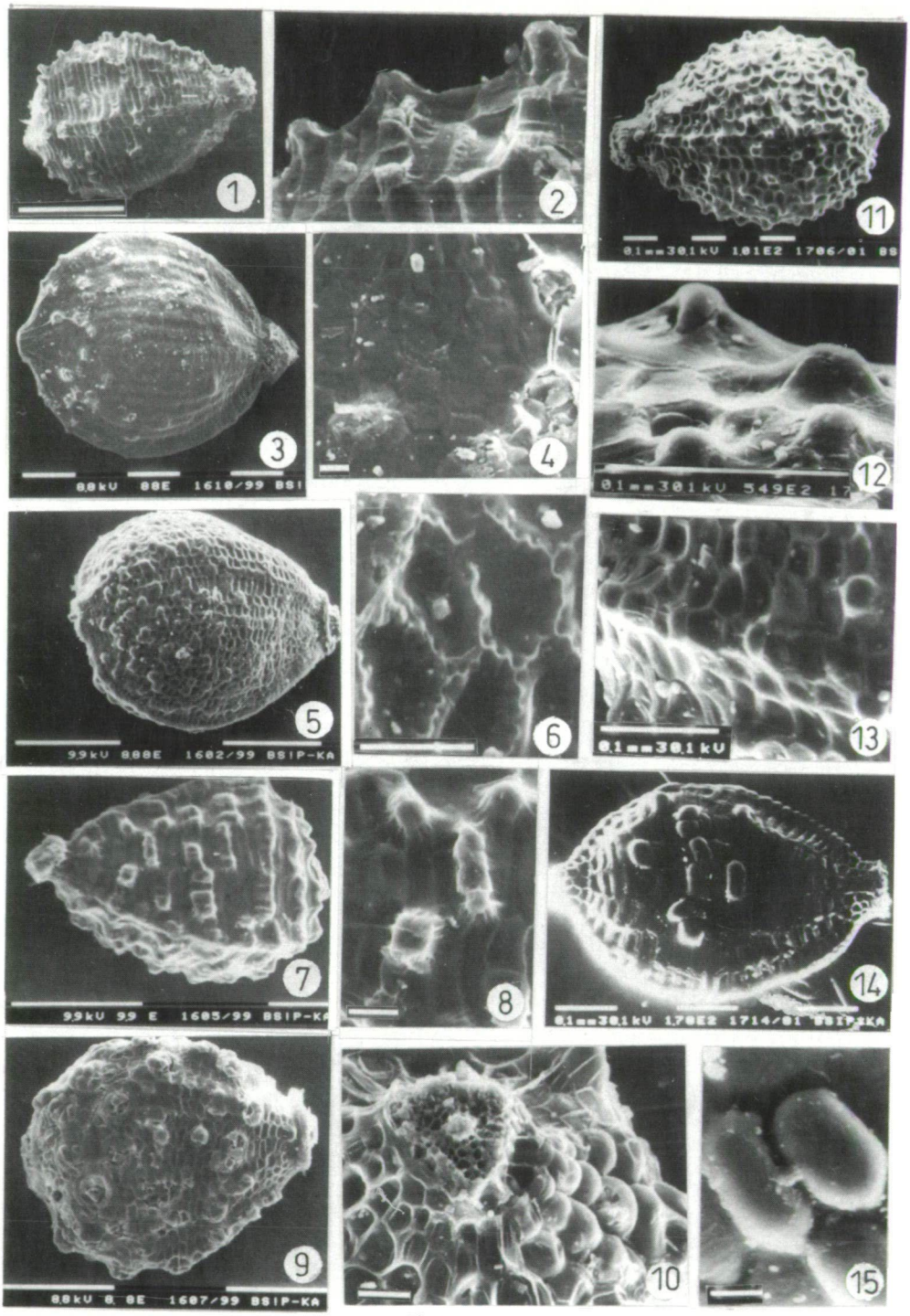
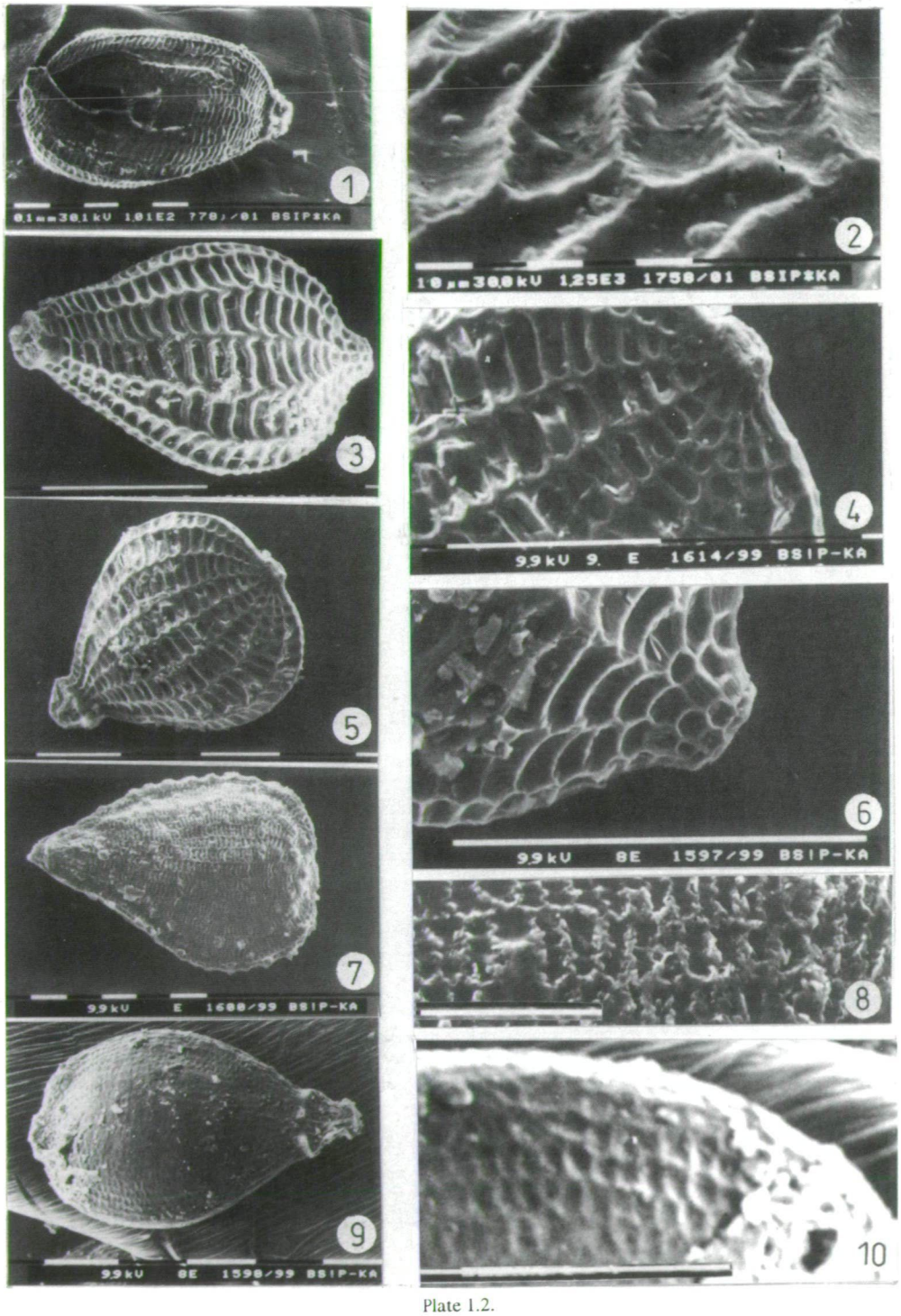


Plate 1.1.


1
0.1mm 30.1kV 1.01E2 770µ/01 BSIP*KA
2
10µm 30.0kV 1.25E3 1758/01 BSIP*KA
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7
9.9kV E 1608/99 BSIP-KA
8
9
9.9kV 8E 1598/99 BSIP-KA
10

Plate 1.2.

Distribution: The species commonly grow in marshlands throughout India, Sri Lanka, Malaysia, China, Australia. It also grows at the height of 1220 meters (HOOKER, 1894, BHANDARI, 1990, SAMVATSAR, 1996).

F. complanata (RETZ.) LINK. (Plate 1.1., figs. 9,10)

Fruit obovoid, texture tuberculate - reticulate, colour brown, apex rounded, base narrow, attachment scar triangular with distinct vascular supply, size 250-300 µm long and 200 µm broad. Perigynium present, translucent, forming striations at the bases. Pericarp reticulate with sparsely arranged tubercles, each tubercle, consists of 3-7 bulbous cells mostly located along the ridges. The size of the tubercles ranges from 15-20 µm. The size of the pericarp cells is generally smaller and ranges from 10-15 µm. They become squarish at the base and apex of the fruit.

Distribution: Found throughout the warm regions of India growing commonly on wet places and fields (HOOKER, 1894, SAMVATSAR, 1996).

F. tenera SCHULT (Plate 1.1., figs. 11-13)

Fruit globose - ovoid, trigonoid, texture reticulate - tuberculate with distinct ridges, colour brownish, apex rounded slightly projected with flat tip, base slightly narrow. Size of fruit 700-800 µm long and 500-600 µm broad. Perigynium absent. Pericarp reticulate, cells more or less rectangular to squarish becoming smaller towards base and apex (20-50 µm), cells of the ridge area form prominent tubercles throughout the pericarp.

Distribution: Commonly found in wet grounds particularly on the margins of ponds, also commonly occurs as weed in the fields and waste lands, throughout India (HOOKER, 1894, SAMVATSAR, 1996).

Plate 1.1.

SEM micrographs of *Fimbristylis* fruit

1,2. *F. miliacea* - showing trigonoid ridges bearing prominent tubercles.
3,4. *F. podocarpa* - parallel ridges and furrows and sparse tubercles near apical region.
5,6. *F. cymosa* - pericarp showing reticulate and tuberculate ornamentation with frilled cell margins.
7,8. *F. quinquangularis* - showing distinct trigonoid ridges, pericarp reticulate with lineolate tubercles.
9,10. *F. complanata* - pericarp showing reticulate pattern with tubercles on the ridges.
11,12,13. *F. tenera* - showing reticulate pericarp and uniformly distributed tubercles on the surface.
14,15. *F. argentea* - fruit showing reticulate, sparsely tuberculate pericarp, cells in linear rows.
Figs.: 1,3,5,7, 9,11 and 14. bar = 0.1 mm
Figs.: 2,4,6,8,10,12,13 and 15. bar = 10 µm

Plate 1.2.

SEM micrographs of *Fimbristylis* fruit

1,2. *F. lawiana* - ladder-like arranged reticulate cells, cell margin wavy.
3,4. *F. bisumbellata* - pericarp cells horizontally elongated between the ridges.
5,6. *F. albo-viridis* - pericarp showing scalariform arrangement of the cells.
7,8. *F. polytrichoides* - pericarp reticulate, cells with wavy margin.
9,10. *F. ferruginea* - pericarp showing squarish arrangement of cells.
Figs.: 1,3,5,7 and 9. bar = 0.1 mm
Figs.: 2,4,6,8 and 10. bar = 10 µm

F. argentea VAHL (Plate 1.1., figs. 14,15)

Fruit obovoid - elongated, texture reticulate, ridges and furrows absent, colour yellow, apex with remnant of style, base narrow, size of the fruit 500-600 µm long and 250-300 µm broad. Perigynium very thin, translucent, covering pericarp, forming striations at apical and basal parts. Cells of the pericarp rectangularly arranged in linear rows, 30-50 µm long and 10-20 µm broad, at some places the cells are prominently projected.

Distribution: Occurring from Bengal to Central India, Sri Lanka and Mauritius. Very common in paddy fields (SAMVATSAR, 1996).

F. lawiana LINN. (Plate 1.2., figs. 1,2)

Fruit obovate, texture reticulate with ridges and furrows, colour dark brown, apex rounded, base narrow having ring-like structure. Size ranges up to 1 mm long and 500-600 µm broad. Very thin, translucent perigynium present. Pericarp with reticulate cells arranged ladder-like in between ridges. Cells more or less sinuate with wavy margin, rectangular, 40-50 µm long and 15-20 µm broad.

Distribution: Generally found along the moist places and paddy fields, it profusely grows in the waste lands of India, Sri Lanka (HOOKER, 1894).

F. bisumbellata (FORST.) BUB. (Plate 1.2., figs. 3,4)

Fruit obovoid - elongated, texture prominently reticulate with ridges and furrows converging towards base, colour white - light yellow, apex with remnant of style base, acuminate, base narrow, size 200-250 µm long and 150-200 µm broad. Pericarp covered with very thin perigynium, the cells of pericarp arranged in rows giving ladder-like appearance. The cells are longer than broad, 5-15 µm, smaller cells are present at the apical and basal area of the fruit.

Distribution: Mostly found in the warm areas of the world, prominently grows throughout India forming dense rosette-like structure, common in marshy places associated with other sedges. (BHANDARI, 1990, RAO and RAZI, 1981, SAMVATSAR, 1996).

F. albo-viridis CLARKE (Plate 1.2., figs. 5,6)

Fruit obovate, texture striated, reticulate (ridges and furrows distinct), colour brown apex rounded with stylar base, remnant of stalk present at the base. Size ranges from 300-400 µm long and 250-300 µm broad. Perigynium absent. Pericarp reticulate, cells vary from 10-30 µm long 5-15 µm broad, the smaller cells are prominently seen at the base and apical region. The cells of reticulum between two ridges form ladder-like pattern (scalariform), 8-9 ridges present on each face of the fruit converging towards base and apex.

Distribution: Chiefly found in East Bengal and Upper Assam (HOOKER, 1894).

F. polytrichoides VAHL (Plate 1.2., figs. 7,8)

Fruits obovoid, biconvex, tapering towards apex, texture reticulate with tuberculate projections prominently seen along the lateral ridges, colour pale yellow, apex flattened, base narrow. Size 700-800 µm long 400-500 µm broad. Perigynium not seen. Pericarp cells reticulate, dispersed in between fine ridges, cells rectangular 15-30 µm with thick, wavy margin.

Distribution: Chiefly found in Bengal to Sri Lanka along the sea banks. Common in the tropics of the Old World (HOOKER, 1894).

F. ferruginea (LINN.) VAHL (Plate 1.2., figs. 9,10)

Fruit obovoid - elongated, texture obscurely reticulate, ridges and furrows not seen, colour yellow, apex blunt, obtuse, base narrow with stylar ring, size of the fruit ranges 500-600 µm long, 300-400 µm broad. Perigynium absent. Pericarp cells uniformly arranged in vertical rows very small in size, more or less squarish in shape.

Distribution: Largely distributed in Australia, Polynesia, Malaysia and India, a gregarious sedge found in marshy places throughout these regions. It is also found abundantly near the sea-shores of the warmer regions (HOOKER, 1894, SAMVATSAR, 1996).

Species	Pericarp	Distribution
F. miliacea	Ridges and furrows associated with prominent tubercles (5-10 µm)	Found up to 1828 meters growing in warm regions and waste lands, grass lands of the world
F. podocarpa	Ridges and furrows (16-20 on each face), parallel, tuberculate patches at apical part	Frequent in Western Himalaya to upper Assam, Bangla Desh, Malaya, China
F. cymosa	Reticulate, tubercles (restricted apically), cell margin frilled	Common along the river banks and dry lands of tropical regions
F. quinquangularis	Reticulate-tuberculate (lineolate) throughout the pericarp	Common throughout marshy lands also grows up to 1220 meters in tropical regions
F. complanata	Reticulate with sparse tubercles, tubercles prominent on ridges (10-15 µm)	Common in warm regions, wet places
F. tenera	Reticulate, ridges and furrows prominent, fine tubercles throughout (20-50 µm)	Common in wet grounds along the margin of ponds as weed
F. argentea	Reticulate-sparsely tuberculate, cells in linear rows, rectangular (30-50 µm)	Commonly occur in paddy fields of warm regions especially India, Sri Lanka, Mauritius
F. lawiana	Reticulate cells arranged like 'ladder' in between ridges, sinuate, wavy (40-50 µm) margin	Commonly occur in paddy fields and waste lands in India and Sri Lanka
F. bisumbellata	Reticulate ridges and furrows, cells arranged ladder-like, horizontally elongated (5-15 µm)	Mostly in warm areas of the world forming dense rosettes throughout India and other parts of the world
F. albo-viridis	Cells smaller, arranged in between ridges, scalari form (10-30 µm)	Chiefly found in East Bengal and Upper Assam
F. polytrichoides	Reticulate with fine ridges, cells rectangular with wavy margins (15-30 µm)	Chiefly found in Bengal, Sri Lanka, along the sea banks, tropics of the Old World
F. ferruginea	Cells uniformly arranged in vertical rows small squarish (15-20 µm)	A gregarious sedge of marshlands throughout warmer parts of the world

Table 1.1.

Comparative characters of pericarp and distribution of different species of *Fimbristylis*.

Discussion and Conclusions

On the basis of micromorphological observations of different species of *Fimbristylis* the character can be used to understand the differences and relationship between different species of this genus. It can also be helpful in systematic classification of the family Cyperaceae to use the distinct micro-morphological characters of the fruits.

The different ornamentation pattern of the pericarp of 12 species studied under scanning electron microscope can be categorized in two groups i) reticulate type - including *F. albo-viridis, F. bisumbellata, F. ferruginea, F. lawiana,* ii) reticulate - tuberculate type including *F. argentea, F. complanata, F. cymosa, F. miliacea, F. polytrichoides, F. podocarpa, F. quinquangularis* and *F. tenera.* The cells of the pericarp in *F. albo-viridis* are smaller, arranged in scalariform pattern between ridges and measures 10-30 μm in size. However, in *F. bisumbellata* the pericarp cells though arranged ladder-like but are horizontally elongated, usually smaller, 5-15 μm in size. In *F. ferruginea* the cells of the pericarp are uniformly arranged in vertical rows, they are squarish in shape measuring 15-20 μm in size. It was observed that the reticulate ornamentation of the pericarp in *F. lawiana* shows that the cells are arranged ladder-like between the ridges. The cells are sinuate and show wavy margin, they are larger in size ranging between 40-50 μm (Table 1.1.).

The species with reticulate-tuberculate pericarp show difference in the distribution of tubercles. The tubercles in *F. argentea* are sparse, arranged in linear rows, in *F. complanata* the tubercles are prominent whereas in *F. cymosa* and *F. podocarpa* the tubercles can only be seen at the apical part of the fruit. In *F. miliacea* the tubercles are quite prominent.

HOOKER (1894) considered *F. podocarpa* and *F. albo-viridis* similar in pericarp ornamentation. However, the SEM studies reveal that though *F. podocarpa* bears ridges and furrows they are not as prominent as in *F. albo-viridis*. He also indicated the resemblance between *F. quinquangularis* and *F. miliacea*. Both can be distinguished from each others on the basis of pericarp characters: *F. miliacea* bears large number of tubercles mainly along the trigonoid ridges while *F. quinquangularis* has sparse and flattened tubercles in between the ridges as seen under SEM.

The genus *Fimbristylis* is adopted to various habitats ranging from sea level up to 1828 meters. Some species can also grow in cooler climate of the Himalayan region indicating a wide range of adoptable habitat. Sometimes they grow to form dense rosettes and also form gregarious sedge helping against soil erosion.

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2. AN UNUSUAL TRIASSIC SEED FROM INDIA

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Abstract

Under the name *Kedvesospermum montidorsum* a new seed is described from Middle Triassic sediments of Nidpur, India. The seed is distinguished by its asymmetric micropylar opening - an unusual feature not generally common among the gymnosperms.

Key words: Palaeobotany, fossil seed, Triassic, n.gen. et sp.

Introduction

Recent studies by PANT and BASU (1977), MANIK (1988), SRIVASTAVA and MANIK (1990, 1993a,b, 1996, 1999), SRIVASTAVA et al. (1998, 2001) have contributed significantly to the systematics of Triassic seeds from India. Continued investigations further on seed remains from Nidpur have revealed the presence of distinctive grade of evolution. The record from pre-Cretaceous beds of plant fossils displaying characters which, according to our present knowledge, are indicative of angiospermid traits. So far the known pre-Cretaceous plant remains, which do not demonstrate the total complement of modern angiospermous characters but share one or more characteristics could reasonably be thought to be the angiospermid plant remain are: *Fraxinopsis major-alata* seeds of fruits from the Rhaetic of Argentina (WIELAND, 1929), *Furcula granulifer* – a dicotyledonous type of leaf impressions from Rhaetic of Greenland (HARRIS, 1932), *Sueviоxylon zonatum* from Jurassic of Germany (KRÄUSEL, 1928), *Sanmiguelia* a monocot type of leaf from the Upper Triassic of Colorado, U.S.A. (BROWN, 1956, TIDWELL et al., 1977), pollen grains cf. *Tricolpites (Eucommiidites) troedssonii* ERDTMAN 1948, juglandoid grains from Upper Jurassic of Isle of Wight (BORGE and ERDTMAN, 1954), *Sporojuglandoidites jurassicus* – from Lower Cretaceous of Rajmahal Hills, India (VISHNU-MITTRE, 1955) and *Problematospermum ovale* – an ovoid elongate seed with a pappus on tube at one end resembling to the achens and pappus of Compositae from Late Jurassic of Kazakhstan (TURUTANOVA and KETOVA, 1930, KRASSILOV, 1973). This finding adds an other evidence to the occurrence of a seed displaying angiospermoid feature prior to Cretaceous period.

Results

Genus: *Kedvesospermum* nov. gen.

Diagnosis:
Seed oblong ellipsoidal, micropylar opening lateral or asymmetrical, chalazal end

Plate 2.1.

globose, centrally recessed spatulate structure located surrounded by radiating fine creases, epidermal cells isodiametric, nucellar membrane distinct.

Derivatio nominis: In honour of Professor Dr. M. KEDVES, Szeged, Hungary whose monumental contribution in the field of Palaeobotany-Palynology is a widely known fact.

Differential diagnosis: In its asymmetric or lateral position of micropylar opening, the present seed-taxon can be distinguished from the other gymnospermous seed genera, however, in the same particular character it compares with the seed designated under *Spermatites* MINER, 1935 which was found in association of angiospermous remains from Cretaceous coals of Western Greenland.

Species: *K. montidorsum* n. sp.

Diagnosis:

Seed ellipsoidal, oblong to sub-globose, measuring 2 mm in length, 1.5 mm in breadth, micropylar opening asymmetric or lateral, chalazal end thickened and rounded, centrally spatulate recessed structure with radiating markings distinct, outer integument thin bearing fine irregular creases over the surface, sides slightly rounded, outlines with fine undulations, outer integument composed of elongated-rectangular cells, irregularly arranged surface, wall striated, lateral- and end-walls straight, nucellar cells exceptionally of bigger size having thickly cutinized anticlinal walls, surface uneven, cells encircling the micropylar opening forming a sort of thickened rim around the pore, chalazal end consists of dense mass of compact cells.

Isotype: Nos. 37279, 37303, 37307, 37419, 37420, 37421, 37422, 37423, 37424.

Holotype: No. 37305 (Fig. 2A, Plate 2.1., figs. 1-7 in Birbal Sahni Institute of Palaeobotany.

Locus typicus: Nidpur, Sidhi District, Madhya Pradesh, India.

Age: Middle Triassic (Tiki-Formation).

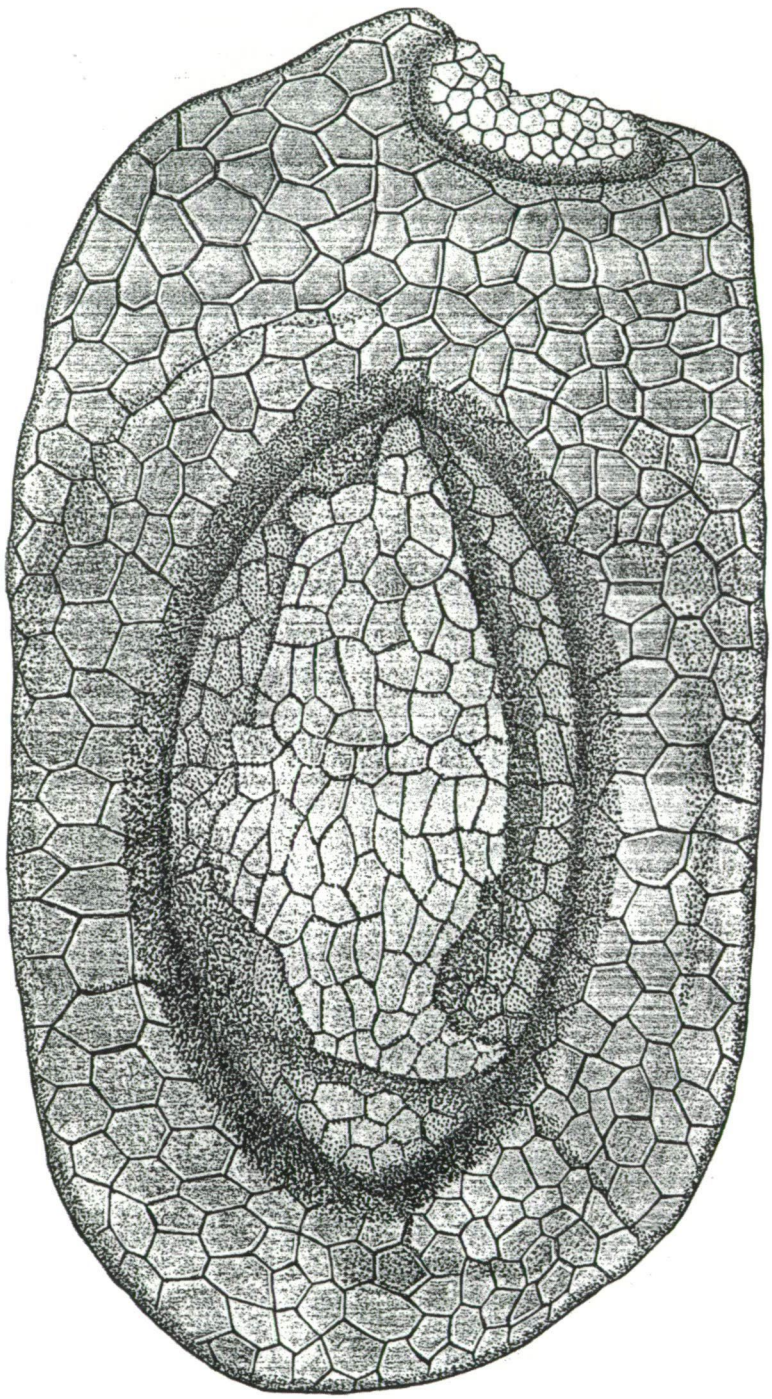
Plate 2.1.

Kedvesospermum montidorsum n. gen. et sp.

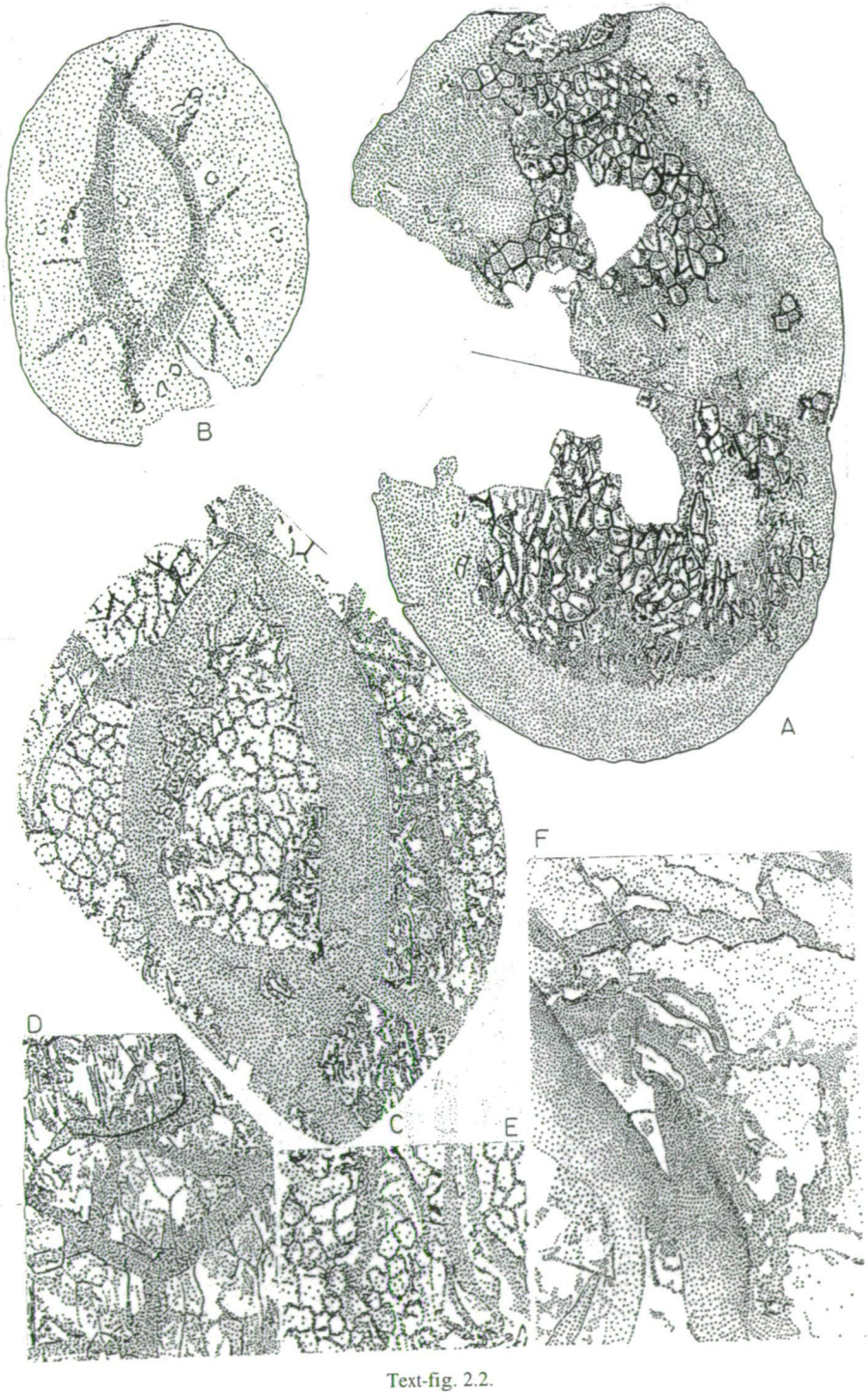
1. Unmacerated seed dipped in glycerine. Holotype BSIP No. 37305 - S/14 7.0x,
2. Seed after processing showing asymmetrical micropylar end. BSIP No. 37305 - S/14 19.5x.
3. Upper half of seed enlarged to show the micropylar hole associated with outer integument. BSIP No. 37305 - S/14 39.0x.
4. Chalazal portion of seed with cellular details and thickened end. BSIP No. 37305 - S/14 19.5x.

5-10. Seed exposed in glycerine. 5. BSIP No. 37419 - S/490 7.8x, 6. BSIP No. 37303 - S/12 7.8x, 7. BSIP No. 37279 - S/471 7.8x, 8. BSIP No. 37420 - S/685 7.8x, 9. BSIP No. 37421 - S/305 7.8x, 10. BSIP No. 37426 - S/272. 7.8x.

11. Specimen figured in No. 8 after treatment. BSIP No. 37420 - S/685 39.0x.
12. Seed after maceration with distinct creases over outer integument. BSIP No. 37421 - S/305 16.3x.
13. Seed showing highly thickened outline and chalazal end. BSIP No. 37420 - S/685 3.9x.
14. Seed after acid treatment showing nucellar membrane. BSIP No. 37422 - S/703 3.9x.
15. Seed on dorsal face showing moderately recessed, centrally located, elongate ellipsoid, spoon-shaped structure surrounded by radiating markings up to the margin. BSIP No. 37307 39.0x.
16. A part of nucellar membrane depicting cellular thickenings and interspersed with minor folds. BSIP No. 37423 - S/164 39.0x.
17. A part of micropylar region of seed with distinct nucellar beak. BSIP No. 37423 - S/164 117.0x.
18. Cellular details exhibiting imprints of epidermal cells probably representing inner integument adherent intimately to nucellar membrane composed of fairly big sized rhombic cells. BSIP No. 37424 - S/470 39.0x.
19. Outer integument - epidermal structure. BSIP No. 37426 - S/164 39.0x.
20. A portion of outer integument on both faces showing cellular details. BSIP No. 37425 - S/164 39.0x.



Text-fig. 2.1.



Text-fig. 2.2.

Text-fig. 2.1. .

Kedvesospermum montidorsum n. gen. et sp., a reconstruction of seed exhibiting overall shaped, cutinized membranes, centrally elongate, ellipsoid, spoon-shaped, moderately recessed structure associated with asymmetrical micropylar opening.

Text-fig. 2.2.

Kedvesospermum montidorsum n. gen. et sp. A. Seed after treatment showing structural details of cutinized membranes. Asymmetrical micropylar hole and chalazal end BSIP No. 37305 - S/14 78x. B. A complete seed after maceration showing overall outline of nucellus associated with centrally located, recessed, ± spatulate structure surrounded by radiating folds. BSIP No. 37303 - S/12 19.5x. C. Central portion of seed with spoon-shaped structure associated with membrane consisting of fairly big-sized rhombic cells, (D) imprints of epidermal cells adherent usually over the nucellus. BSIP No. 37 - S/470 39.0x. E. Cells of outer integument intermingled with radiating folds. BSIP No. 37425 - S/164 19.5x. F. Cellular details between radiate folds BSIP No. 37425 - S/164 117x.

Comparison and Discussion

As already mentioned the most distinctive character of the present seed is the possession of asymmetric micropylar opening which, according to our current knowledge, has only been marked in the fossil seeds *Spermatites* MINER 1935 (represented by seven species) in the association of angiospermous fossil plant from Upper Cretaceous coals of Western Greenland.

The present seeds assignated of *Kedvesospermum montidorsum* while comparing in its overall configuration with *Spermatites pylophorus* MINER 1935 depicts close identity with the later in the feature of lateral position of micropylar opening. Further in the presence of recessed spatulate-shaped structure associated with radiating creases, the seed *K. montidorsum* approaches to the seed *Vitis pseudorotundifolia* of the family Vitaceae described by TIFFNEY and BARGHOORN (1976) from Tertiary of North-Eastern U.S.A. As a consequence, these seed which share the angiospermid traits could reasonably be thought from angiosperm-like plant form. Obviously, the earliest forms would not demonstrate the total complement of modern angiospermid trend. However, it could be also speculated that in the beginning not a fully formed angiosperm must have evolved but their subsequent diversification should have been occurred during late Jurassic-Cretaceous time in a relatively short period. Additionally, the angiospermid traits marked in a few Triassic plant around the globe reflect towards the belief that angiosperms may conceviably have arisen during the Triassic period. To identify pre-Cretaceous flowering plants, the criteria from modern organisms, which have had millions of years to evolve, have been taken to use as to depict the differentiation of angiospermid character. In this reference MELVILLE's (1960) opinion appears to be quite reasonable that the earliest angiosperms may have looked so different from the ideas about them that in fossil state they probably would not be recognized.

However, there is the possibility of recognizing the flowering plants among gymnosperms, in *K. montidorsum* the asymmetrical micropylar opening is suggestive of these seeds to be borne laterally aggregated into infructesences. Such earliest forms must have been of small population size and quite delicate in nature. The pollen recorded in pre-Cretaceous strata are quite similar to monosulcate grains of angiosperms (HUGHES, 1976). In this context, it would be worth to mention here that the palynoassemblage of Nidpur Triassic sediments have also yielded *Praecolpatites* BHARADWAJ and SRIVASTAVA (1969) which is quite similar to monocolpate grains of primitive angio-

sperms. In case *Praecolpatites* is found in situ it may provide clue for earliest angiosperms.

Further, during preceding years upsurge in research by DOYLE (1977) and HICKEY and DOYLE (1977) have shown that the oldest angiospermid forms were not advanced.

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3. FOSSIL GYMNOSPERM WOOD FROM ASWAN AREA

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Abstract

Fossil woods were collected from two localities of Late Cretaceous Age to the west of Lake Nasser (Aswan area). In this contribution the anatomy of two samples are described. The preservation is not so well but the remnants are without doubt of gymnospermous origin. Based on some wood anatomical data earlier characteristics are observed, similar to the recent Podocarpaceae taxa.

Key words: Xylotomy, fossil, gymnosperm, Upper Cretaceous, Egypt.

Introduction

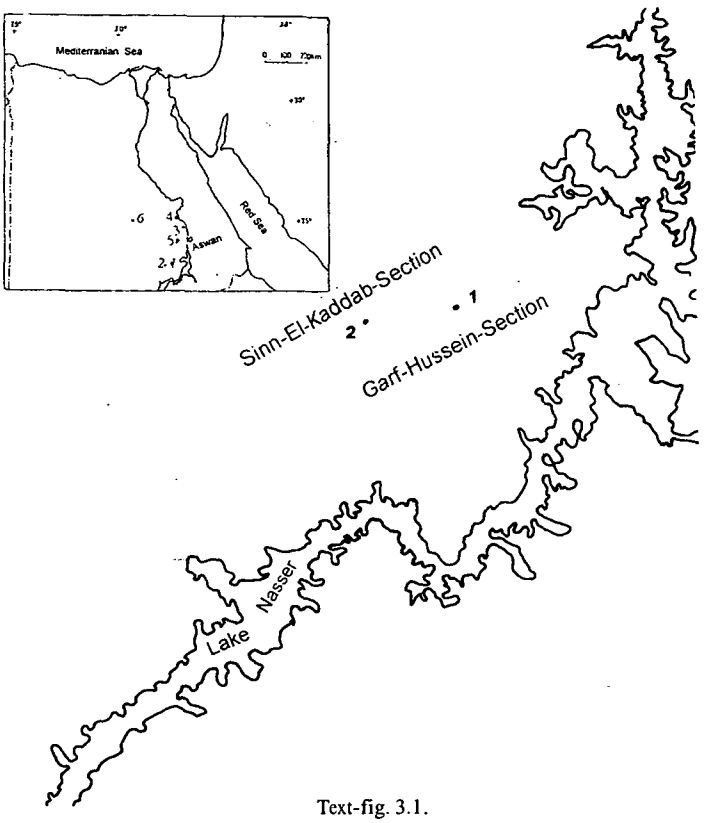
The present work deals with the study of fossil woods collected from two localities of Late Cretaceous Age to the west of Lake Nasser within Aswan area. These localities are Sinn-El-Kaddab and Garf Hussein (Text-fig. 3.1.). Fossil woods of gymnosperms had been reported earlier (e.g.: UNGER, 1858, 1859, KRÄUSEL, 1939, YOUSSEF et al., 2000) from localities not far away from the present study area, namely from the road between Esna and Wadi Halfa, Gebel Garra and Kharga Oasis (Text-fig. 3.1.).

The Study Area

The study area lies 120 km to the southwest of Aswan city on the western side of Lake Nasser. The area is covered by strata which belong to Nubia Sandstone Formation (Late Cretaceous, OSMAN, 1992). The area includes two fossiliferous localities, south of Gebel Sinn-El-Kaddab and Garf Hussein (Text-fig. 3.1.). In these two localities numerous fragmented tree trunks occur scattered on the surface (Plate 3.1., fig. 1), most of them are 1.5–3 m long and 20-30 cm in diameter. Many of these trunks or wood logs are highly silicified and variously colored.

Materials and Methods

One specimen was collected from each locality. The specimen of Sinn-El-Kaddab locality belongs to the lower part of the section (Text-fig. 3.2.,A). This part is composed of white to yellowish white, hard, ill-sorted sandstone beds. The specimen of Garf Hussein locality also belongs to the lower part of the section (Text-fig. 3.2.,B). This part is composed of conglumeratic sandstone and kaolinitic clay beds. It is a pity that after the preparation of cross thin ground section (LACEY, 1963) from the specimen of Sinn-El-Kaddab, R.L.S, T.L.S., and Garf Hussein two specimens were accidentally lost, however, the 3 slides were rescued.



Text-fig. 3.1.

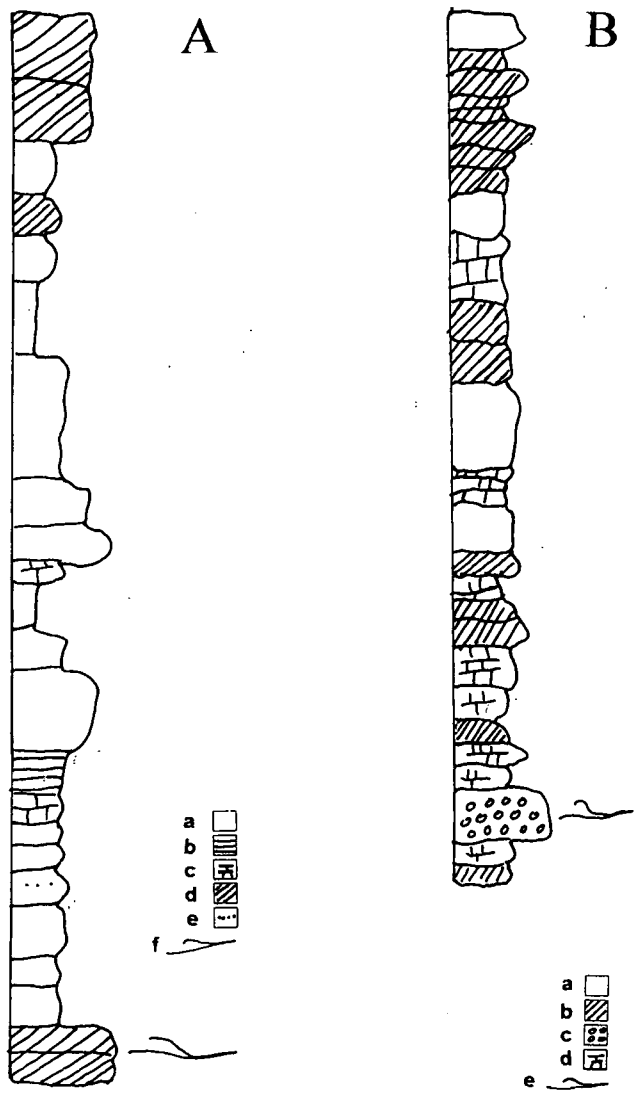
Map showing the situation of the two fossiliferous localities Sinn-El-Kaddab (1) and Garf Hussein (2) and other nearby localities namely: Road between Esna and Wadi Halfa (3,4), Gebel Garra (5) and Kharga Oasis (6).

Results

Careful study of the three sections prepared from the two specimens (Plate 3.2., figs. 1-4) proved that they belong to only one species of gymnosperms which is described as follows:

A coniferous wood made of tracheids, axial parenchyma, ray parenchyma and sometimes traumatic tissues. Annual rings distinct, quite narrow, 5-6 mm. Transition from early to late wood is abrupt. The late wood is very narrow with one to three cells in width.

Tracheids in the early wood quadrate to rectangular in cross section: 15-18 μm in tangential and radial diameters respectively, thin walled, 2-3 μm. Late wood tracheids, smaller than early wood tracheids, 12-16 μm x 11-16 μm in tangential and radial diameters respectively, thick walled, 3-5 μm. Bordered pits, in uniseriate rows, circular-quadrate 5-10μm in diameter and with rounded apertures. Cross field pits, one large window like per a field, 10-15 μm in diameter.



Text-fig. 3.2.

A. - Sinn-El-Kaddab Section. Supplied by Dr. Rifaat Osman, Geology Department, Faculty of Science, Benha Branch of Zagazig University. 1 cm = 4 m. a. - structureless, b. - laminated, c. - calcareous, d. - cross bedding, e. - clay, f. - trunks.

B. - Garf Hussein Section. Supplied by Dr. Rifaat Osman, Geology Department, Faculty of Science, Benha Branch of Zagazig University. 1 cm = 4 m. a. - structureless, b. - cross bedding, c. - conglomerate, d. - calcareous, e. - trunks.

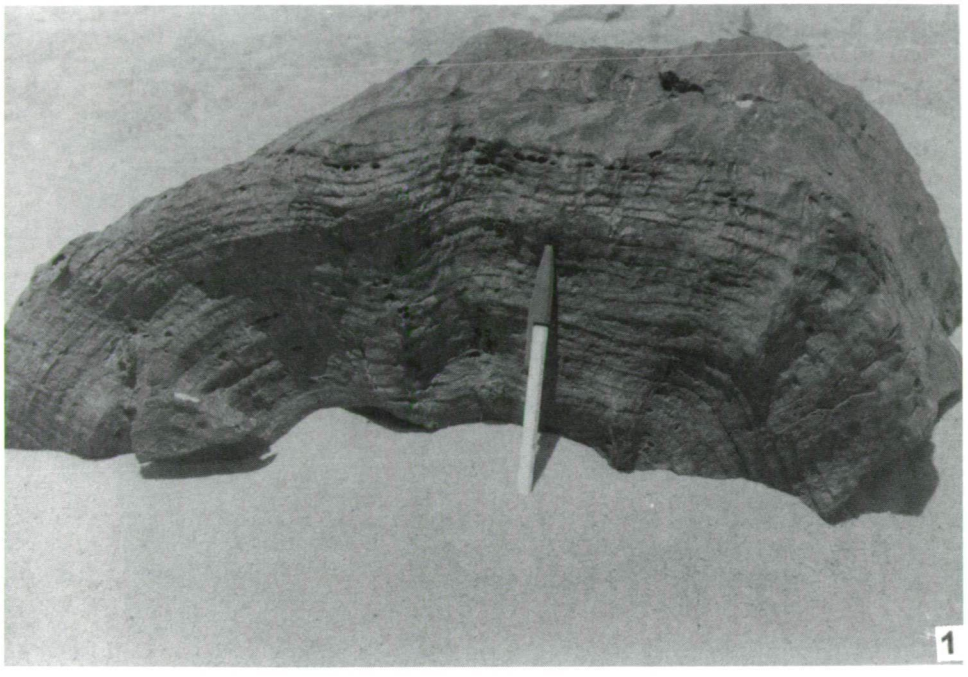


Plate 3.1.

1. One of the two specimens of Aswan Area. Photograph taken at the site of collection.

Axial parenchyma in strands and abundant single cells diffused within the annual rings: 5 x 10 µm in size. Horizontal walls of the axial parenchyma are thick and smooth, 3-4 µm.

Rays uniseriate and constituted from parenchymatous cells, 1-5 cells high, rectangular in radial section.

Discussion and Conclusions

The narrow annual rings indicate moderate alterations in the yearly clima. A relatively short drier period may be supposed. The observed wood anatomical characteristic features in comparison to other Senonian data refer to a tropical gymnosperm taxon. The cross field pits in the radial section are observed by GREGUSS (1949) from Senonian fusit collected from the brown coal basin of Ajka, Hungary. In the monograph of the fossil

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Plate 3.2.

Cf. *Podocarpoxylon* sp.
1. Part of cross-section showing a row of late tracheids (arrows), 55x.
2. Part of the same section at a higher magnification showing a traumatic resin canal (arrow), 120x.
3. Part of a tangential section showing resinous xylem parenchyma (arrows) and uniseriate rays (R), 130x.
4. Part of a tangential section magnified to show one row of pits on tracheids (arrow), 750x.

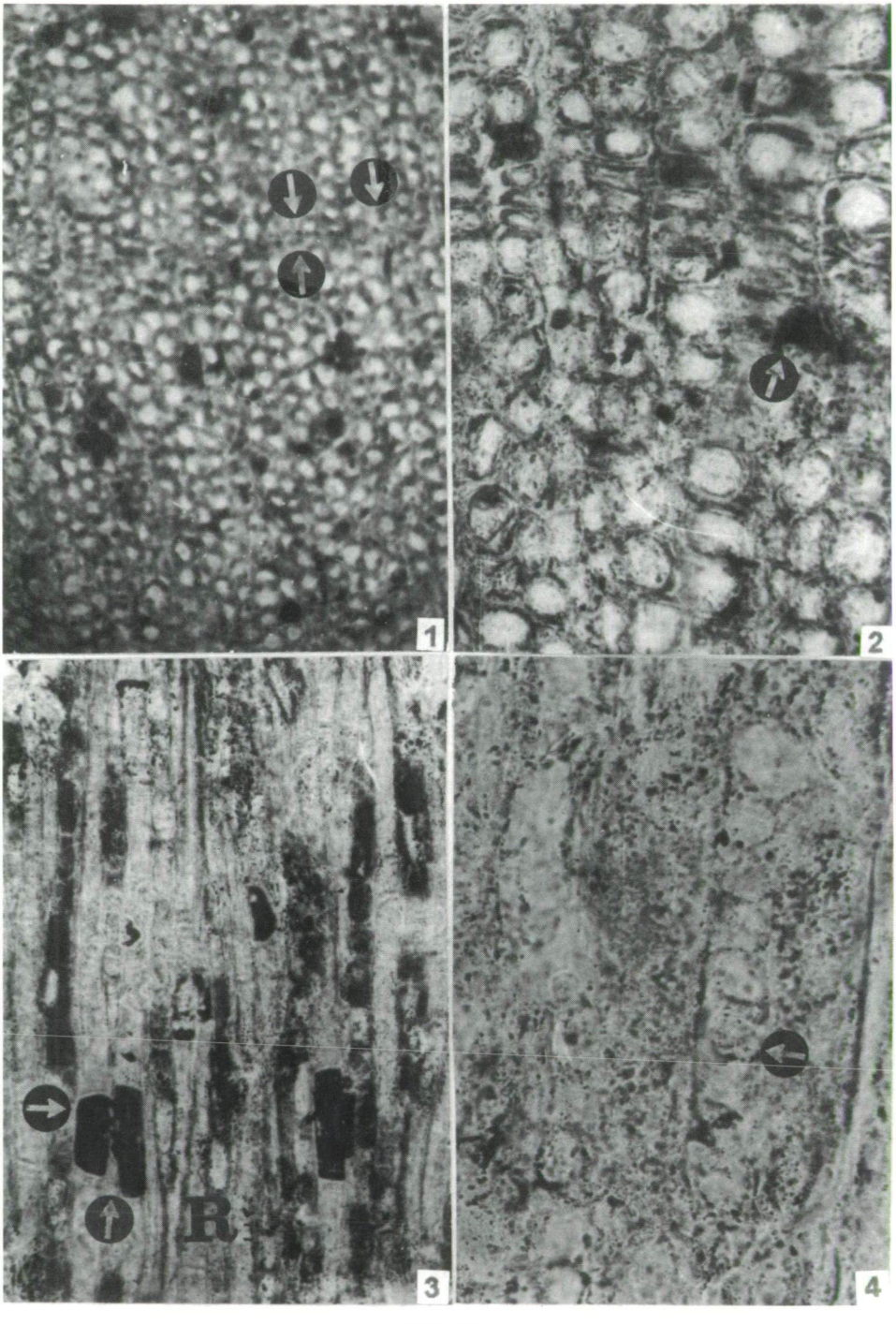

Plate 3.2.

gymnosperm woods of Hungary GREGUSS (1967) published the wood anatomy of several tropical taxa *(Araucarioxylon, Agathoxylon, Podocarpoxylon).* The recently described remains may not be identical with the *Podocarpoxylon ajkaense* GREGUSS 1949, because of the much shorter rays, but in all probability may be related to the family of Podocarpaceae.

In resumé because of the not so well preservation of the fossil wood it is the best to remain to the description and for designation cf. *Podocarpoxylon* sp. seems the most reasonable in this moment. In this context it may be worth to mention that a species of *Podocarpoxylon* (*P. wekitii* LEMOIGNE and BEAUCHAMP) is known to exist in Ethiopia which lies not far from the southeast of Egypt (cf. DUPÉRON-LAUDOUENEIX and DUPÉRON, 1995). Podocarpaceous fossil wood was published by BAMFORD et al. (2002) from the Mesozoic of Southern Sahara (Mali).

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4. INVESTIGACIONES SOBRE EL EFECTO DE LA SOLUCION DE C60 FULLERENO/BENZOL EN LAS ESPORAS DE ALSOPHILA SALVINII HOOK. REALIZADAS CON LOS MICROSCOPIOS OPTICO Y ELECTRONICO DE TRANSMISION

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Resumen

Esporas de *Alsophila salvinii* fueron tratadas con una solucion de C60 fullereno/benzol durante 1 a 6 días y, con mercaptoetanol, por 2 dias, para luego ser investigadas con los microscopios óptico y electrónico de transmisión. Por medio del microscopio óptico se estudiaron las variedades de formas y el diámetro de las esporas. La ultraestructura de la pared consiste en una ectexina muy gruesa y una perípora delgada. Se observó acumulación de fullereno, especialemente, en la parte externa de la pared.

Palabras clave: Palinología Experimental, reciente, *Alsophila salvinii*, MO, MET.

Introduccion

Nuestros primeros resultados experimentales en torno a las esporas de *Alsophila salvinii* ya fueron publicadas (LAGOS et al., 2003). Previamente se estableció la estructura biopolímera altamente organizada que puede ser modelada con fullereno (KEDVES, ROJIK y VÉR, 1991, KEDVES y ROJIK, 1994, etc.). La importancia de los estudios con la solución de fullereno/benzol, en las investigaciones de la organización biopolímera de la pared de las esporas y de los pólenes fue enfatizada en 1996 (KEDVES). Los primeros datos obtenidos con el MET, en paredes parcialmente degradadas de *Botryococcus braunii* KÜTZ., aisladas de Húngara Alginito, fueron publicadas por KEDVES y FREY (2002). A continuación se estudiaron los granos de polen de *Taxus baccata*, así como operaciones de simetría en las estructuras biopolímeras que se describieron con la aplicación del nuevo método (KEDVES et al., 2003).

Existen diferentes tipos de estudios experimentales sobre la resistencia y estructura biopolímera de la pared de las esporas (KEDVES, 1990, COLLINSON, HEMSLEY y TAYLOR, 1993) y ya se han publicado los resultados. Hay numerosas publicaciones con respecto a la ultraestructura de las esporas de las Pteridófitas: LUGARDON (1965, 1972, 1974, 1975, 1976, 1980, 1981, 1986, 1992a,b), LUGARDON y HUSSON, (1982), LUGARDON y PIQUEMAL (1993), GIUDICE y MORBELLI (1988), DETTMANN y CLIFFORD (1991), VAN KONIJNENBURG-VAN CITTERT y KURMANN, (1994), etc.

TRYON y LUGARDON (1991) han publicado, conjuntamente, monografías de las esporas de las pteridófitas incluyendo su ultraestructura. En la pared de las esporas de *A. bryophila* TRYON, fue descrita una perispora triestratificada. En lo referente a las observaciones de los elementos ornamentales de la parte externa de la pared, se usaron los siguientes trabajos: VAN CAMPO (1957), KRUTZSCH (1959), KREMP (1965), RUEDA-GAXIOLA (1969), PUNT, BLACKMORE, NILSSON y LE THOMAS (1994).

El objetivo de nuestros estudios combinados, es obtener los primeros resultados relacionados con el efecto de la degradación parcial, incluyendo la solución de C60 fullereno/benzol, sobre la morfología y ultraestructura de la pared de las esporas.

Materiales y Metodos

El material de experimentación fueron 2 mg de esporas secas; para cada experimento fueron agregados 6 ml de mercaptoetanol, por 48 horas. Después de lavar las esporas con agua destilada, fueron secadas. Se agregaron 5 ml de una solución de C60 fullereno/benzol y 5 ml de benzol. Se sometieron a una temperatura de 30° C durante un espacio de tiempo de 1, 2, 3, 4, 5 y 6 días (Experimento No.: T-12-312-317). Las esporas, después del tratamiento con la solución de C60 fullereno/benzol, fueron lavadas con benzol y, luego, secadas. Para los estudios con el microscopio óptico, las esporas fueron montadas en gel de glicerina hidratada al 39.6% (cf. LOBREAU, 1966). Para las investigaciones de la ultraestructura, las esporas secas fueron embebidas en Araldita. Los cortes ultrafinos fueron hechos con un ultramicrótomo Porter Blum con cuchillas de cristal, en el Laboratorio de Biología Celular y Micropaleontología Evolutiva de la Universidad de Szeged. Las microfotografías con el MET, fueron tomadas en el Laboratorio de Microscopía Electrónica del Instituto de Biofísica del Centro de Investigaciones Biológicas de la Academia de Ciencias de Hungría, con un equipo Tesla BS 540, con una resolución aproximada de 6 - 7 Å y un Zeiss Opton EM - 902, con una resolución de 2-3 Å. Ninguna fotografía fue retocada.

Resultados

Resultados con el microscopio óptico (Lámina 4.1., figs. 1-12)

Los datos cualitativos con el microscopio óptico son, aproximadamente, idénticos. No se observaron alteraciones características en la estructura de la pared y en los organelos del protoplasma. Se distinguen muy bien los característicos elementos granulares del protoplasma.

Los datos cuantitativos se resumen como sigue:

A - Diametro de las esporas en posición polar

Numero del experimento	Tamaño (µm %)								Tamaño dominante (µm %)	Promedio (µm %)
	22.5	25.0	27.5	30.0	32.5	35.0	37.5	40.0		
T-12-312		6.0	17.5	28.0	38.0	10.0	0.5		38.0	21.70
T-12-313	0.5	7.0	18.0	28.0	35.5	9.5	1.5		35.5	30.64
T-12-314		5.5	33.5	34.0	19.5	7.5			34.0	29.75
T-12-315	2.0	6.5	26.5	31.0	23.0	10.5	0.5		31.0	28.39
T-12-316		5.5	12.5	14.5	22.0	24.5	20.0	1.0	24.5	32.79
T-12-317			0.5	3.5	16.0	38.0	35.5	6.5	38.0	35.60

En el diámetro de las esporas se pueden observar interesantes alteraciones. Existe un aumento importante entre el primero y segundo experimento, con respecto al que hubo entre el quinto(5th) y el último(6th) experimento. Es muy significativa la diferencia de los valores promedios del primer experimento: 21.7µm y del ultimo: 35.6µm.

B - Variedad de formas

	triangular	convexa	concava	polar triplanoide	triplana	ecuatorial	monoleta	
T-12-312	4.0	22.5	5.0	52.5	1.0	15.0		%
T-12-313	2.5	10.0	12.0	70.0		5.5		
T-12-314	1.5	2.5	19.0	66.5	10.0	0.5		
T-12-315	1.0	2.0	19.0	51.5	25.5	1.0		
T-12-316	2.5		11.5	50.0	34.0	1.5	0.5	
T-12-317	2.0		17.0	44.0	35.5	1.5		

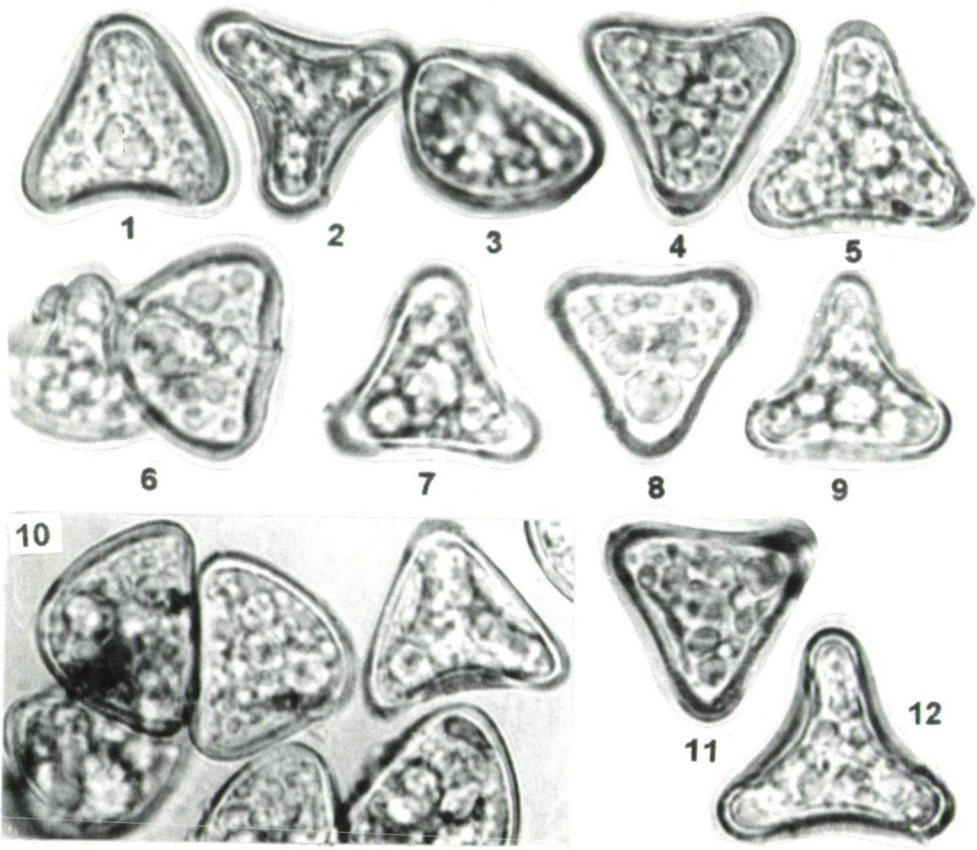

Lámina 4.1.

Alsophila salvinii HOOKER: microfotográfía de las esporas tomadas con microscopio óptico, después de los experimentos.
Aumento:1.000x.
1-3. Experimento No.: T-12-312.
4,5. Experimento No.: T-12-313.
6,7. Experimento No.: T-12-314.
8,9. Experimento No.: T-12-315.
10. Experimento No.: T-12-316.
11,12. Experimento No.: T-12-317.

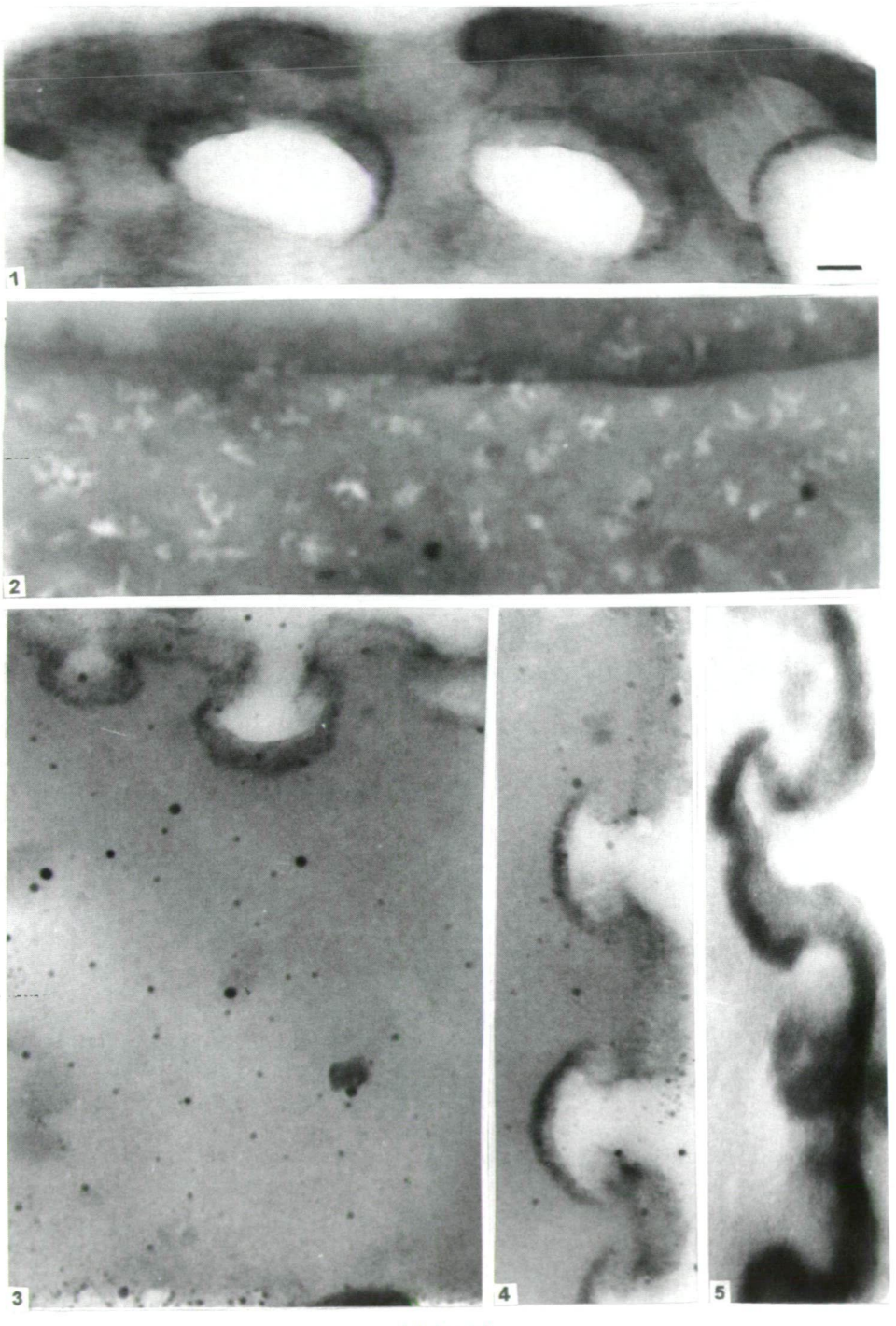

Lámina 4.2.

Lámina 4.2.

Alsophila salvinii HOOKER: utraestructura de la pared de las esporas, después del experimento.
1,2. Experimento No.: T-12-312. 1. Negativo No.: 10198. 2. Negativo No.: 10201.
3,4. Experimento No.: T-12-313. 3. Negativo No.: 10184. 4. Negativo No.: 10184.
5. Experimento No.: T-12-314. Negativo No.: 10174.
Escala: 0.1 µm.

Basados en los datos cuantitativos de las diferentes formas, podemos enfatizar lo siguiente:

1 - En el primer experimento la forma convexa ocurre en considerable cantidad y un número significativo de esporas se encuentran en posición ecuatorial. Esa característica disminuye en el segundo experimento y, en los últimos, son nulas o se observan en pequeños porcentajes.

La forma dominante es la triplanoide, con gran número de formas triplanas y cóncavas.

Resultados con el microscopio electrónico de transmisión (Lámina 4.2., figs. 1-5, Lámina 4.3., figs. 1-8, Lámina 4.4., figs. 1-5)

En general se pueden confirmar los siguientes resultados:

1 - La gruesa exóspora es resistente; en la parte más externa de la oscura y pequeña pared de la espora, con toda probabilidad, está presente una perispora.
2 - La perispora y la región marginal de las fóveas son electrónicamente densas. Esta densidad electrónica puede ser a consecuencia de la impregnación de fullereno.

De acuerdo con los detalles observados, es importante mencionar los siguientes resultados:

1 - En el primer experimento (Lámina 4.2., figs. 1,2) se advierte muy bien la característica diferencial por la impregnación del fullereno, fig. 1. En la pared compacta original de la exóspora existen, más o menos, agujeros luminosos algo irregulares (fig. 2). Esto puede ser secundario, probablemente una parte del fullereno no actuó durante el tiempo, relativamente corto, del experimento y esta reacción ocurrió durante las investigaciones con el MET.
2 - En el segundo experimento se observaron gránulos oscuros globulares en la exóspora (Lámina 4.2., figs. 3,4). En este experimento está bien ilustrada la ultraestructura característica de la fóvea. La parte interna de la fóvea es más oscura que la región externa y, particularmente, en la fotografia 4, se pueden observar unidades biopolímeras altamente organizadas.
3 - En los últimos experimentos, usando el instrumental del MET, no se observaron más alteraciones importantes (Lámina 4.2., fig. 5, lámina 4.3., figs. 1-8, lámina 4.4., figs. 1-5). La homogénea exóspora está bien mostrada en la fotografia 8 de la lámina 4.3 y en las otras fotos se muestran los elementos ornamentales en diferente posición.

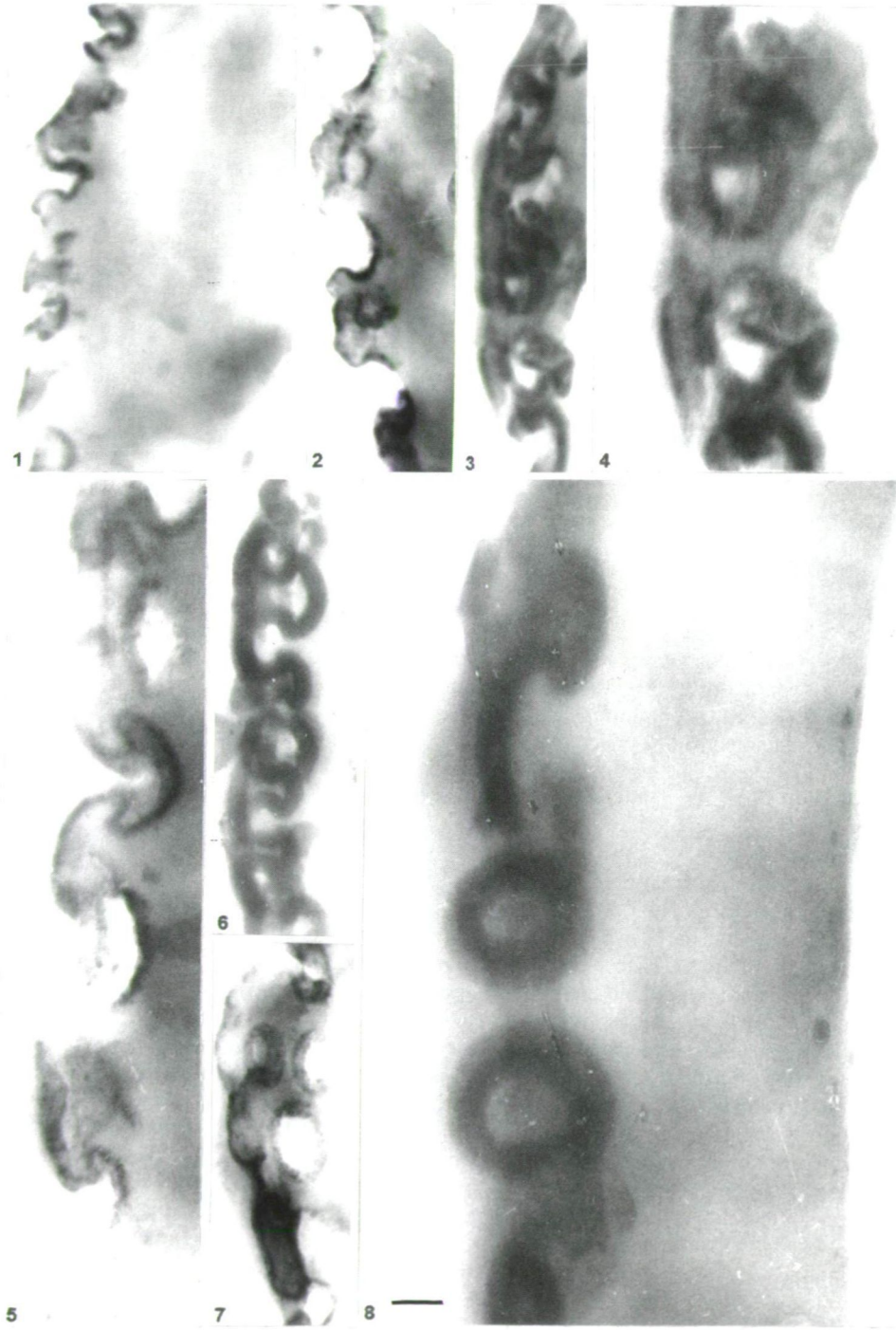

Lámina 4.3.

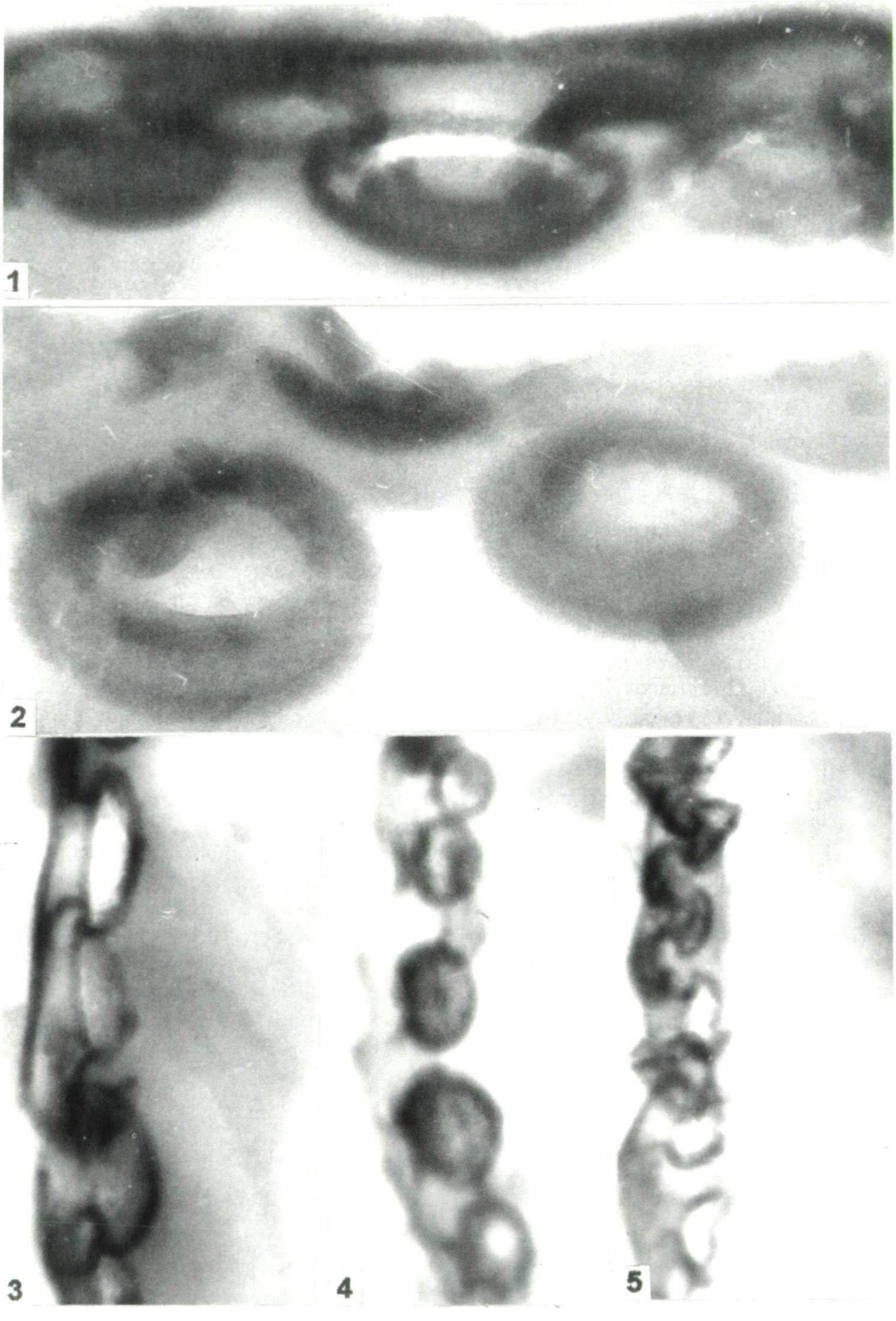

Lámina 4.4.

Lámina 4.3.

Alsophila salvinii HOOKER: ultraestructura de la pared de las esporas, después del experimento.
1,2. Experimento No.: T-12-314. 1. Negativo No.: 10173. 2. Negativo No.: 10177.
3,4. Experimento No.: T-12-314. 3. Negativo No.: 10231. 4. Negativo No.: 10231.
5-7. Experimento No.: T-12-316 5. Negativo No.: 10265. 6. Negativo No.: 10266. 7. Negativo No.: 10264.
8. Experimento No.: T-12-317. Negativo No.: 10272.
Escala: figs. 1-3, 6,7: 0.2 µm, figs. 4,8: 0.1 µm.

Lámina 4.4.

Alsophila salvinii HOOKER: ultraestructura de la pared de las esporas, después del experimento.
1-5. Experimento No.: T-12-317. 1. Negativo No.: 10268. 2. Negativo No.: 10275. 3. Negativo No.: 10269.
4. Negativo No.: 10769. 5. Negativo No.: 10270.
Aumento: figs. 1. y 2.: 100.000x, figs. 3-5. 50.000x.

Discusion y Conclusiones

1 - En nuestros primeros experimentos realizados con esporas de pteridófitas, se varificó que se puede user la solución de C60 fullereno/benzol, para la degradación parcial de la pared de las esporas. Obtuvimos resultados satisfactorios sin el uso del OsO_4 en la postfijación. La aceptación diferenciada del fullereno en la parte más externa de la pared de la espora, confirmó que este método puede ser útil para establecer diferencias en el sistema molecular.

2 - Los instrumentos del MET, de alta resolución, se userán para la ultraestructura de la parte más externa de la pared, con la esperanza de encontrar estructuras biopolímeras.

3 - Con base en los resultados del microscopio óptico, será interesante estudiar, también, la ultraestructura del protoplasma de las esporas.

4 - Las monográfias de TRYON y LUGARDON (1991) son de mucha utilidad e importantes para la interpretación de los datos del microscopio electrónico de las esporas recientes y fósiles.

4.1 - La ultraestructura de *Alsophila bryophila* TRYON, es diferente de nuestra especie porque su perísporа es triestratificada.

4.2 - TRYON y LUGARDON (1991) publicaron similares resultados con el MET, de las siguientes especies: *Sphaeropteris lockwoodiana, S. elongata, S. myosuroides, Trichipteris costaricensis* y *T. schiedeana.*

En resumen, los primeros datos experimentales de las esporas de *Alsophila salvinii* fueron exitosos. En el futuro trataremos de obtener datos ultraestructuraless del sistema biopolímero de la pared de las esporas.

Es probable que los resultados con la perísporа sean similares a los nuestros obtenidos con las esporas de *Selaginella bellula* MOORE (KEDVES, 1990).

Agradecimiento

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5. EXPERIMENTAL INVESTIGATIONS ON THE POLLEN GRAINS OF ELAEAGNUS ANGUSTIFOLIA L. AND JUGLANS REGIA L.

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Abstract

Pollen grains of *Elaeagnus angustifolia* L. and *Juglans regia* L. were partially degraded with 2-aminoethanol for 30 minutes, 1, 5, 10 and 24 hours. Unstained and stained pollen grains were investigated with the LM method. The weak resistance of the sporopollenin of the ectexine of the pollen grains of *Elaeagnus angustifolia* was established during these experiments. Based on the LM data the pollen grains of *Juglans regia* are relatively resistant. Alterations in the diameter of the pollen grains were established depending on the length of time of the partial degradation. The trend of the alterations was different in the unstained and stained pollen grains. After 24 hours of treatment using the TEM method, moderate disintegrations were observed in the ultrastructure of the ectexine.

Key words: Experimental Palynology, recent, partial degradation

Introduction

Our investigations were carried out on the partially degraded allergenic pollen grains of *Elaeagnus angustifolia* L. and *Juglans regia* L. Based on our previous investigations on the pollen grains of *Elaeagnus angustifolia*, it was established that the sporopollenin of the ectexine of this pollen grain is less resistant to organic solvents (KEDVES and HORVÁTH, 2000). For example, after treatment with diethylamine important alterations were observed in the basic morphology of the pollen grains. No significant alterations were observed after X-ray irradiation of the pollen grains of *Elaeagnus angustifolia* (KEDVES and KÁROSSY, 1998). Later (KEDVES and MADARÁSZ, 2001) investigated the alterations caused by high temperature and 2-aminoethanol in this species.

Based on our previous different experiments, it was established that the ectexine of the pollen grains of the *Juglans* genus is resistant (KEDVES and KINCSEK, 1989, KEDVES and KÁROSSY, 1997, KEDVES, KÁROSSY and BORBOLA, 1997).

Both pollen types are important in the evolution of angiosperm pollen grains, so we hope that the new data presented here will be useful additions to the investigations of allergenic pollen grains and to the investigations of the fossil organic material, primarily with regard to the problems of the preservation and fossilisation of this kind of pollen grains.

The aim of this contribution was to get new, comparative data for the two species, which are different from the point of view of ectexine resistance.

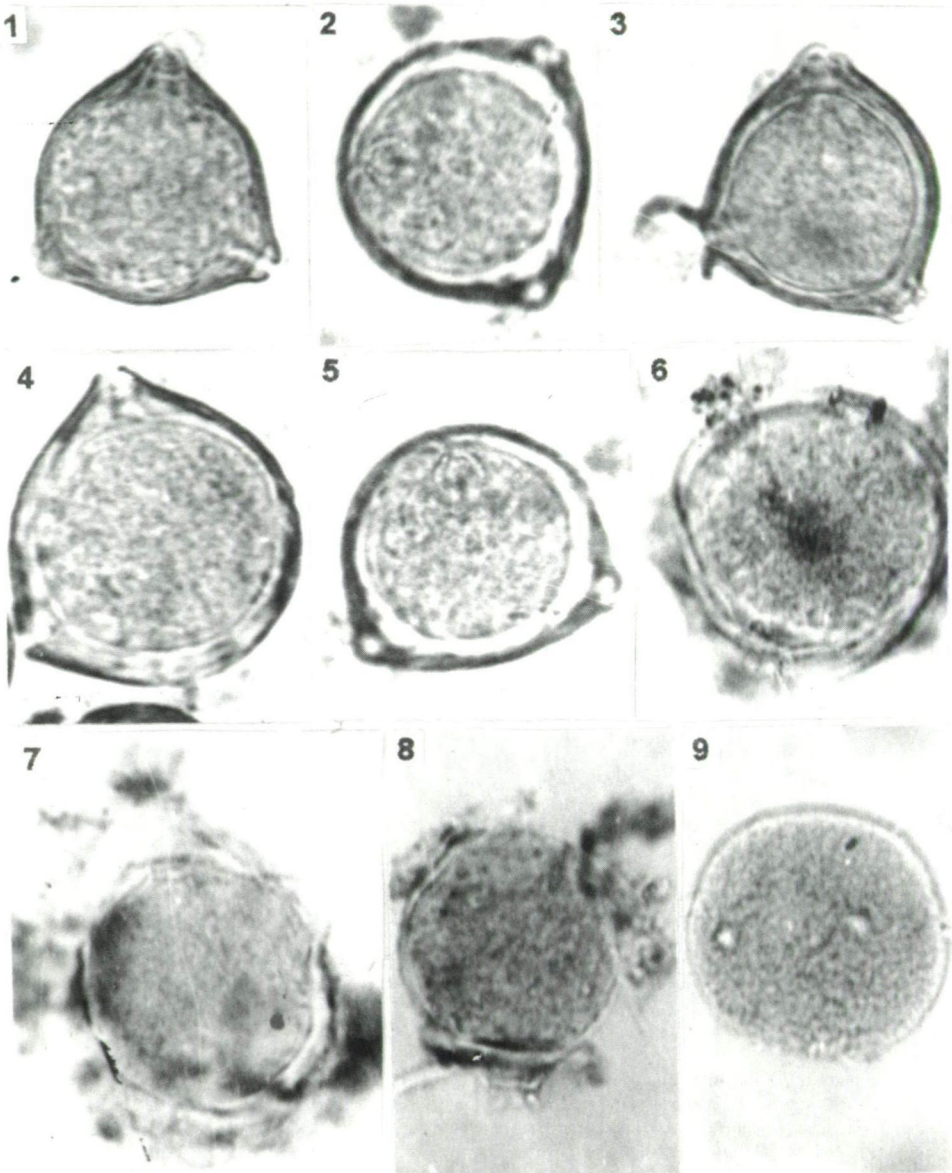

Plate 5.1.

1-9. *Elaeagnus angustifolia* L. LM pictures of the partially degraded pollen grains with 2-aminoethanol for different lengths of time
1-3. Experiment No.: T-12-378, length of time: 30 minutes.
4-6. Experiment No.: T-12-379, length of time: 1 hour.
7-9. Experiment No.: T-12-380, length of time: 5 hours.
Magnification: 1000x.

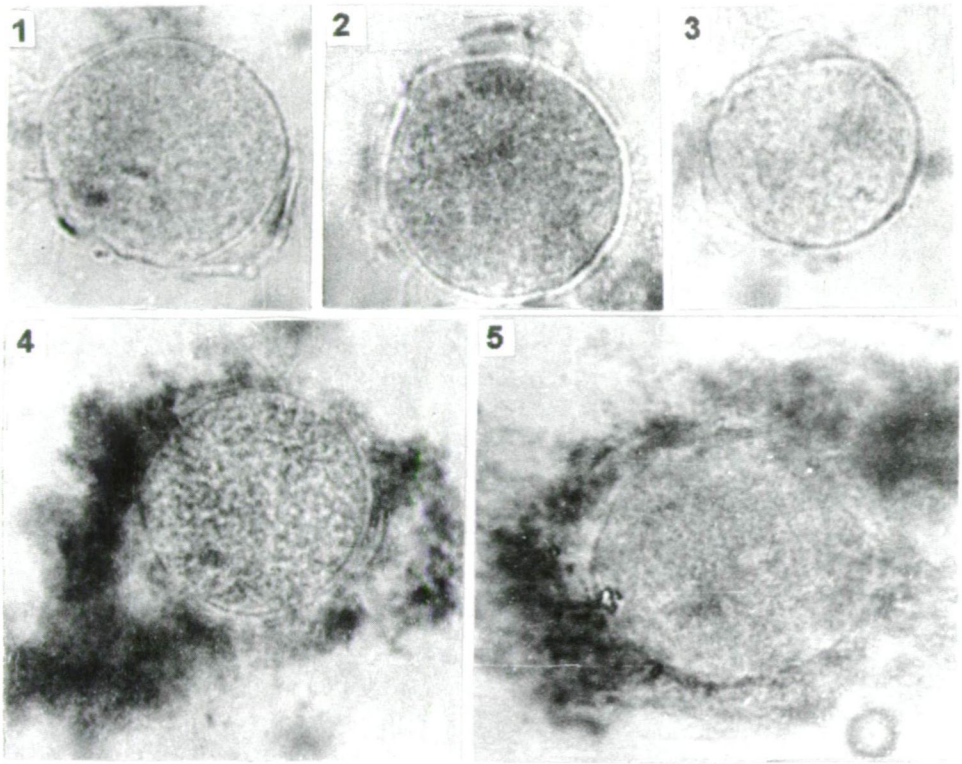

Plate 5.2.

1-5. *Elaeagnus angustifolia* L. LM pictures of the partially degraded pollen grains with 2-aminoethanol for different lengths of time.
1-3. Experiment No.: T-12-381, length of time: 10 hours.
4,5. Experiment No.: T-12-382, length of time: 24 hours.
Magnification: 1000x.

Materials and Methods

Pollen material for our investigations of *Elaeagnus angustifolia* L. and *Juglans regia* L. was collected by Zs. Szászvári. Locality: Szeged, cultivated land. Date of collection: 02.04.03. for *Juglans regia* L. and 02.05.10. for *Elaeagnus angustifolia* L. The experiments were carried out as follows.

Temperature: 30 °C. Quantity of the experimental pure pollen material: 5 mg 2 ml 2-aminoethanol were added for all of the experiments.

Length of time: 30 minutes, 1, 5, 10 and 24 hours. Experiment numbers for *Elaeagnus angustifolia* L.: T-12-378 - 382 and for *Juglans regia* L.: T-12-361 - 365. For LM investigations unstained (A) and stained (B) pollen grains with methylviolet were mounted in glycerine-jelly hydrated at 39.6%. From *Juglans regia* L. some experimental material was prepared for TEM studies and mounted for LM investigations (Ar) also. One experimental material was ultrathin sectioned and investigated with transmission electron microscope. The ultrathin sections were made on a Porter Blum ultramicrotome. The TEM pictures were taken in the EM Laboratory of the Institute of Biophysics of the Biological Recearch Center of the Hungarian Academy of Sciences, Szeged on a Tesla BS 540 (resolution 6-7 Å) and a Zeiss Opton EM-902, resolution 2-3 Å. All pictures are unretouched.

Results

Elaeagnus angustifolia L. (Plate 5.1., figs. 1-9, plate 5.2., figs. 1-5)

Pollen grains treated for 30 minutes (Plate 5.1., figs. 1-3). The greatest part of the pollen grains seems to be non-altered. In polar view triangular amb with convex sides. Sometimes not so characteristic thickening of the intine and in one specimen, the protrusion of the protoplasm was observed. After 1 hour of treatment (Plate 5.1., figs. 4-6), characteristic alterations were observed in some specimens. The sides of the pollen grains are more convex and sometimes circular. The thickening of the intine is characteristic (Plate 5.1., fig. 5). Protrusions of the protoplasm and the degradation of the ectexine was also observed (Plate 5.1., fig. 6). After 5 hours (Plate 5.1., figs. 7-9) the circular amb of the pollen grains is characteristic. At the greatest part of the pollen grains, around the "inner body", remnants of the ectexine are present (Plate 5.1., figs. 7,8). The quantity of the pollen grains without a wall is remarkable (Plate 5.1., fig. 9). The treatment for 10 hours resulted in the same alteration (Plate 5.2., figs. 1-3). Finally, in the last experiment, the greatest part of the pollen grains is represented by the "inner body" and around it, in some specimens, there are remnants of the wall. (Plate 5.2., figs. 4,5).

Juglans regia L. (Plate 5.3., figs. 1-13, plate 5.4., figs. 1-4)

LM results (Plate 5.3., figs. 1-13)

Microphotos on Plate 5.3. illustrate well the resistance of the molecular system of the sporopollenin of the ectexine of this species. In contrast with the previous species, the ectexine was not completely destroyed during the degradation processes. However, in photo 3 and 6 in Plate 5.3. the effect of the fixation and embedding processes is well shown by the LM method. The alteration of the diameter of the pollen grains, dependant on the length of time of the treatment, resulted in the following:

1. The average value of the diameter increased gradually in the unstained pollen grains.
2. The stain effect in this case is unusual, no regularity was observed based on statistical analysis.

TEM results (Plate 5.4., figs. 1-4)

The general survey picture (Plate 5.4., fig. 1) illustrates well the relatively thick tectum with spinae, the characteristic granular or irregular elements of the infratectal layer and the foot layer. The intine seems to be degraded, but before degradation it was swollen. In highly magnified pictures (Plate 5.4., figs. 2-4), degradations in the submicroscopical level were observed. The characteristic channels of the tectum sometimes disappeared. Light parts indicating the place of the channels are marked with an arrow in picture 2. Pictures taken with a high resolution TEM instrument (Plate 5.4., figs. 3,4) illustrate different kinds of alteration in the biomacromolecular system of the sporopollenin. Light and electron dense globular units of 10-15-20 Å were observed (Fig. 4). The 2-aminoethanol revealed some biopolymer units, but a quasi-periodic or quasi-equivalent pattern of the globular biopolymer structures was not observed.

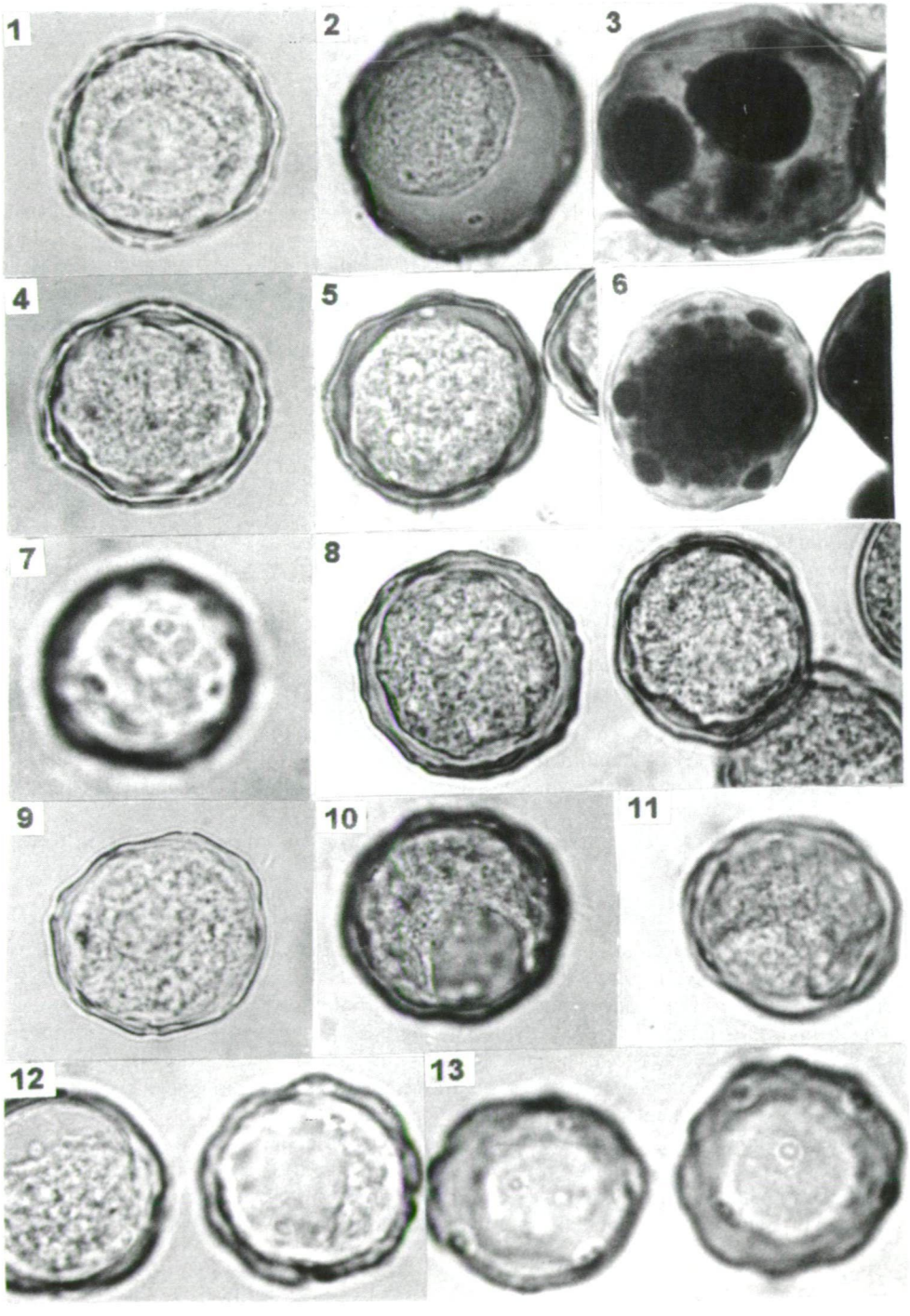

Plate 5.3.

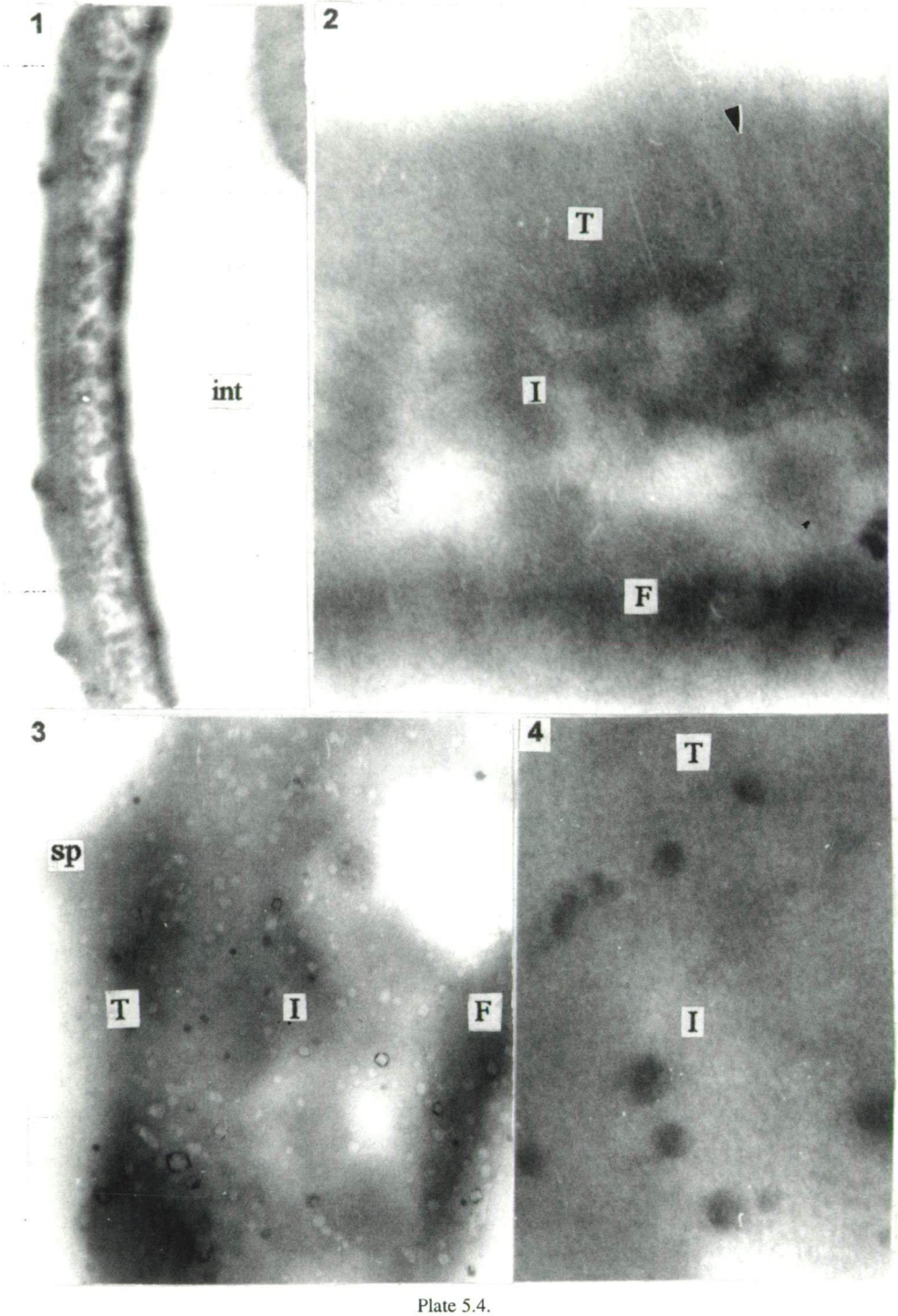

Plate 5.4.

Plate 5.3.

1-13. *Juglans regia* L. LM pictures of partially degraded pollen grains with 2-aminoethanol for different lengths of time.
1-3. Experiment No.: T-12-361, length of time: 30 minutes, 1: A, 2: B, 3: Ar.
4-6. Experiment No:: T-12-362, length of time: 1 hour, 4: A, 5: B, 6: Ar.
7,8. Experiment No.: T-12-363, length of time: 5 hours, 7: A, 8: B.
9-11. Experiment No.: T-12-364, length of time: 10 hours, 9: A, 10,11: B.
12,13. Experiment No.: T-12-365, length of time: 24 hours, 12: A, 13: B.
Magnification: 1000x. Explanation: A = unstained, B = stained pollen grains, mounted in glycerine-jelly. Ar = Pollen grains mounted in Araldite after fixation and embedding processes.

Plate 5.4.

1-4. *Juglans regia* L. TEM pictures of the partially degraded pollen grains with 2-aminoethanol after 24 hours.
1. General survey picture of the ultrastructure of the pollen grain. Magnification: 15.000x. Negative No.: 10190.
2,3. Detail of the ultrastructure of the partially degraded ectexine. Magnification: 100.000x. 2. Negative No.: 10191, 3. Negative No.: 13891.
4. Detail of the partially degraded tectum and infratectal layer. Magnification: 500.000x. Negative No.: 13897.
Explanation: T = tectum, I = infratectum, F = foot layer, int = intine.

Discussion and Conclusions

1. In one of our previous papers (KEDVES and PÁRDUTZ, 1982) we reviewed the most important publications concerning the pollen type of *Elaeagnus angustifolia* both recent (THANIKAIMONI, 1972, etc.) and fossil (KRUTZSCH, 1962, GRAY, 1964, GRUAS-CAVAGNETTO, 1978, *Elaeagnacites* KE et SHI 1978 in SUNG TZE CHEN and TSAO LIU, *Elaeagnuspollenites* HUANG 1980). Based on previous LM, TEM and SEM investigations, it was established that p. 82: "Each of the complex methods, but principally the TEM one suggests that the pollen grains of *Elaeagnus angustifolia* L. may be regarded the morphological analogue of *Complexiopollis* W. KR. 1959 emend. TSCHUDY 1973, without supposing direct botanical relationship between the two." The lamellar foot layer in the apertural area may be one of the early ultrastructural characteristic features of the pollen grain.

2. These new data support again the peculiarities of this kind of pollen type at the molecular level of the ectexine's sporopollenin. The weak resistance aganist the effect of 2-aminoethanol and the relatively good preservation of the protoplasm.

3. Pollen grains of the genus *Juglans* are important stratigraphic elements in the spore-pollen assemblages of Tertiary sediments in the Northern Hemisphere. LM, SEM and TEM morphology of recent *Juglans* pollen grains was investigated by several authors (ERDTMAN, 1952, STONE, REICH and WHITFIELD, 1964, KUPRIYANOVA, 1965, WHITEHEAD, 1965, STONE and BROOME, 1971, VAN CAMPO and LUGARDON, 1973, RYABKOVA, 1987, TARNAVSCHI et al., 1987, BOS and PUNT, 1991). KUTLUK and AYTUG (1999) investigated the fossil fruit from Turkey and, as concerns the dissemination of the pollen grains, emphasized the importance of temperature, relative humidity, precipitation and wind.

4. In general, we have established that the biomacromolecules of the pollen grains of *Juglans* are suitable for the preservation of these pollen grains during sedimentation. The degradations which we have observed in the ultrastructure of the ectexine, namely the disappearance of the tectum channels, may be advantageous for preservation.

5. In summary, the solubility of the biomacromolecules of the ectexine are different in the different taxa and, as we have established previously, (KEDVES and GÁSPÁR, 1996) may also be altered by ecological conditions within one species. This characteristic feature may be taken into consideration in the interpretation of different kinds of palynological investigations.

Acknowledgements

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6. TRANSMISSION ELECTRON MICROSCOPY OF PARTIALLY DEGRADED POLLEN GRAINS OF ACER PLATANOIDES L.

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Abstract

Pollen grains of *Acer platanoides* L. were partially degraded with 2-aminoethanol for different durations. For second degradation agent $KMnO_4$ aq. dil. and merkaptoethanol was used. Partial dissolution with diluted (50%) glycerine was made for 30 days. Based on the new results we concluded that the biopolymer system of the ectexine of this species is of weak resistance.

Key words: Experimental Palynology, recent, *Acer platanoides*, TEM.

Introduction

There are several publications concerning the allergenic character of the pollen grains of the genus *Acer* and of *A. platanoides* also. MOLNÁR (1999) summarized this problem in detail. Aeropalynological data were published for example by CHEN S.-H. and HUANG, T.-C. (1980), ADO, GUBANKOVA and POROSHINA (1986), DE LEONARDIS et al. (1986), TYCZKA (1986), WANG XIAN-ZENG (1986), LEBBE, VIGNES and HIDEUX (1988), GUPTA et al. (1991), MAJUMDAR and CHANDA (1991), JÁRAI-KOMLÓDI and MEDZIHRADSZKY (1993). It is important to mention that PEHLIVAN (1995) in her atlas for allergenic pollen grains of Turkey enumerated a large number of species of this genus - *A. campestre* L. subsp. *campestre, A. cappadocicum* GLEDT., *A. divergens* PAX var. *divergens, A. hyrcanum* F. et MEY subsp. *hyrcanum, A. monspessulanum* L., *A. negundo* L., *A. platanoides* L., *A. pseudoplatanus* L., *A. sempervirens* L., *A. tataricum* L. and *A. trautvetteri* MEDW. Combined investigations (LM, TEM and SEM) on the pollen grains of *Acer platanoides* L. were carried out by NILSSON, PRAGLOWSKI and NILSSON (1977).

The aim of this contribution is to get experimental data about the solubility of the biomacromolecules of the ectexine and in general, cytological data for this kind of allergenic pollen grain.

Materials and Methods

The investigation material was collected by Miss B. VARGA in the Botanical Garden of the University of Szeged. The quantity for all experiments was 5 mg dry pollen material. Temperature: 30 °C.

1. Treatment with 2 ml 2-aminoethanol for 24, 48 and 72 hours, experiment numbers: T-12-325, 326, 327.

2. Treatment with 2 ml 2-aminoethanol as previously, after this $KMnO_4$ (aq. dil. 1%) was added for 24 hours, experiment numbers: T-12-328, 329, 330.

3. Treatment with 2 ml 2-aminoethanol as previously (1), after this 2 ml merkaptoethanol was added for 24 hours, experiment numbers: T-12-331, 332, 333.

4. Partial dissolution with glycerine aq. dil. (50%) for 30 days, experiment number: T-12-334.

After washing, the pollen grains were postfixed with OsO_4 (aq. dil.), dehydrated with ascending alcohol series, after with alcohol/propylenoxide - propylenoxide and embedding in Araldite (Durcupan, Fluka). The ultrathin sections were made with glass knives on a Porter Blum ultramicrotome. The pictures were taken in the EM Laboratory of the Institute of Biophysics of the Biological Research Center of the Hungarian Academy of Sciences on a Tesla BS 540 instrument. All pictures are unretouched.

General Problems

Basic work for the SEM and TEM characteristic features of these pollen grains is the monograph of NILSSON, PRAGLOWSKI and NILSSON (1977). By the SEM data the surface is striate, but the colpus membrane is granular. TEM data: relatively thin tecum supratectate ornamental elements, short columellar infratectal and foot layer. Between the columella there are finely granular elements. Essentially this is a heterogeneous infratectal layer. Ultrastructure of the heterogeneous infratectal layer was published from fossil pollen grains of *Granotricolporites miniverrucatus* (ROCHE 1968) KEDVES 1978 by KEDVES and PÁRDUTZ (1973) from the Lower Eocene (Sparnatian) layers of the Paris Basin Concerning ultrastructure of the intine important establishments were published by NILSSON, PRAGLOWSKI and NILSSON (1977) namely the differentiated ultrastructure of the intine which might be called ectintine and endintine. Concerning the ultrastructure and the pollenkitt of several species of the genus *Acer* on account was published by HESSE (1979a). From the point of view of our species, we cite as follows, p. 277: "*A. platanoides* contains a great deal of granular and homogeneous pollenkitt; it not only fills up the exine cavities but also extends as a thick ± homogeneous nongranular layer of pollenkitt over the tectum surface; therefore the pollen is very sticky."

With the ultrastructure of the intine SUÁREZ-CERVERA and SEAONE-CAMBA (2001) distingshed the following three different layers: 1. Exintine, pectinic, 2. Intine media pectinic with proteide inclusions, 3. Endintine cellulosic. Accumulation of the antigens in the intine was previously supposed by several authors, e.g.: KNOX and HESLOP-HARRISON (1970, 1971), KNOX, HESLOP-HARRISON and REED (1970), KNOX, WILLING and ASHFORD (1972). This is the reason why we also investigated the intine during our researches.

Results

1. Partial degradation with 2-aminoethanol (Plate 6.1., figs. 1-4, plate 6.2., fig. 1-4)

In general, the ectexine was degraded. Characteristic layers of ectexine (tectum, infratectum, foot layer) cannot be recognized. Only one layer of the ectexine, the tectum, is well shown. Along the tectum there are globular electron dense units. After 24 hours of treatment (Plate 6.1., figs. 1,2) small vacuoles occurred in the protoplasm. In Plate 6.1., figs. 3,4 larger vacuoles appeared in the degraded protoplasm. Remnants of the in-

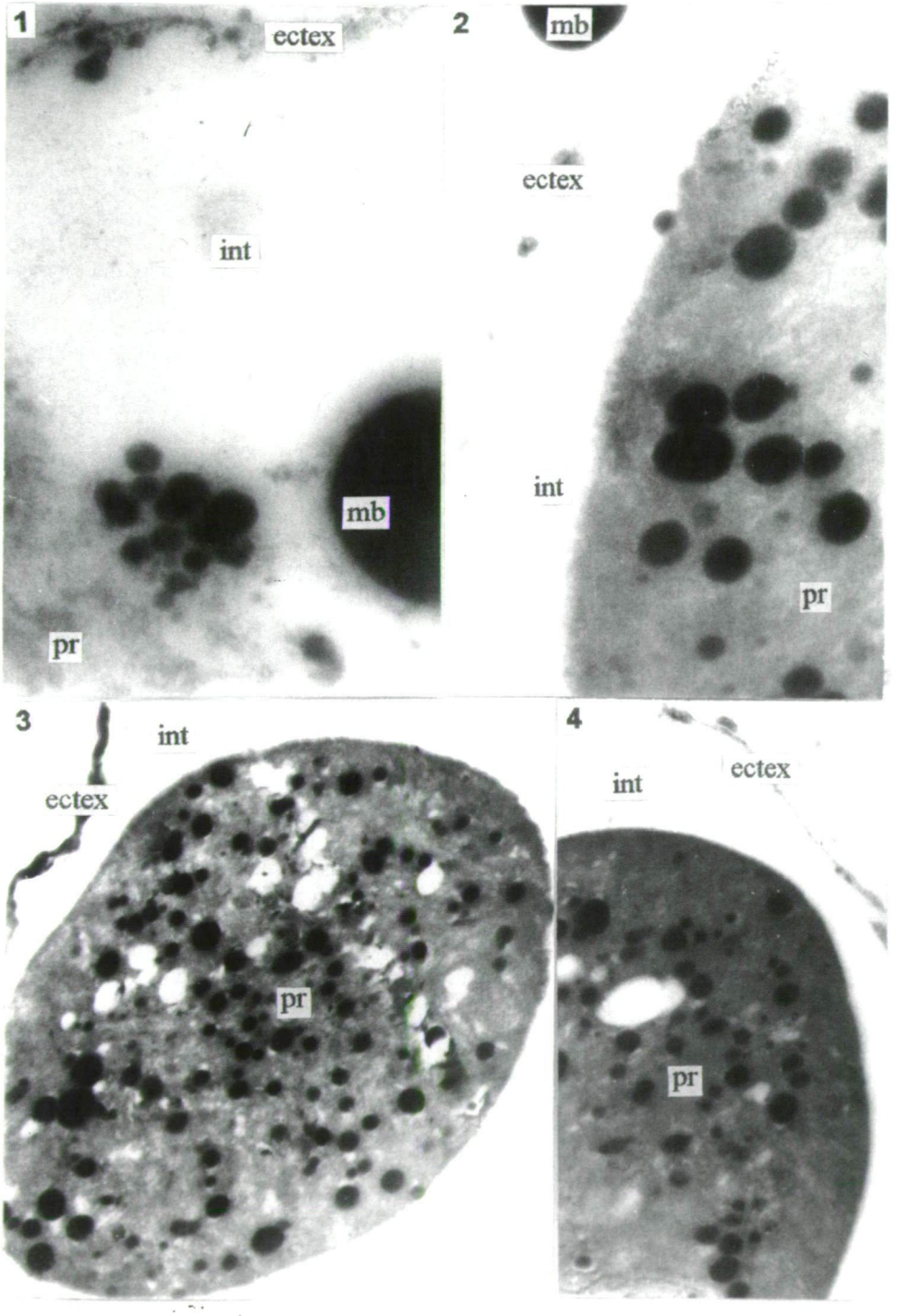

Plate 6.1.

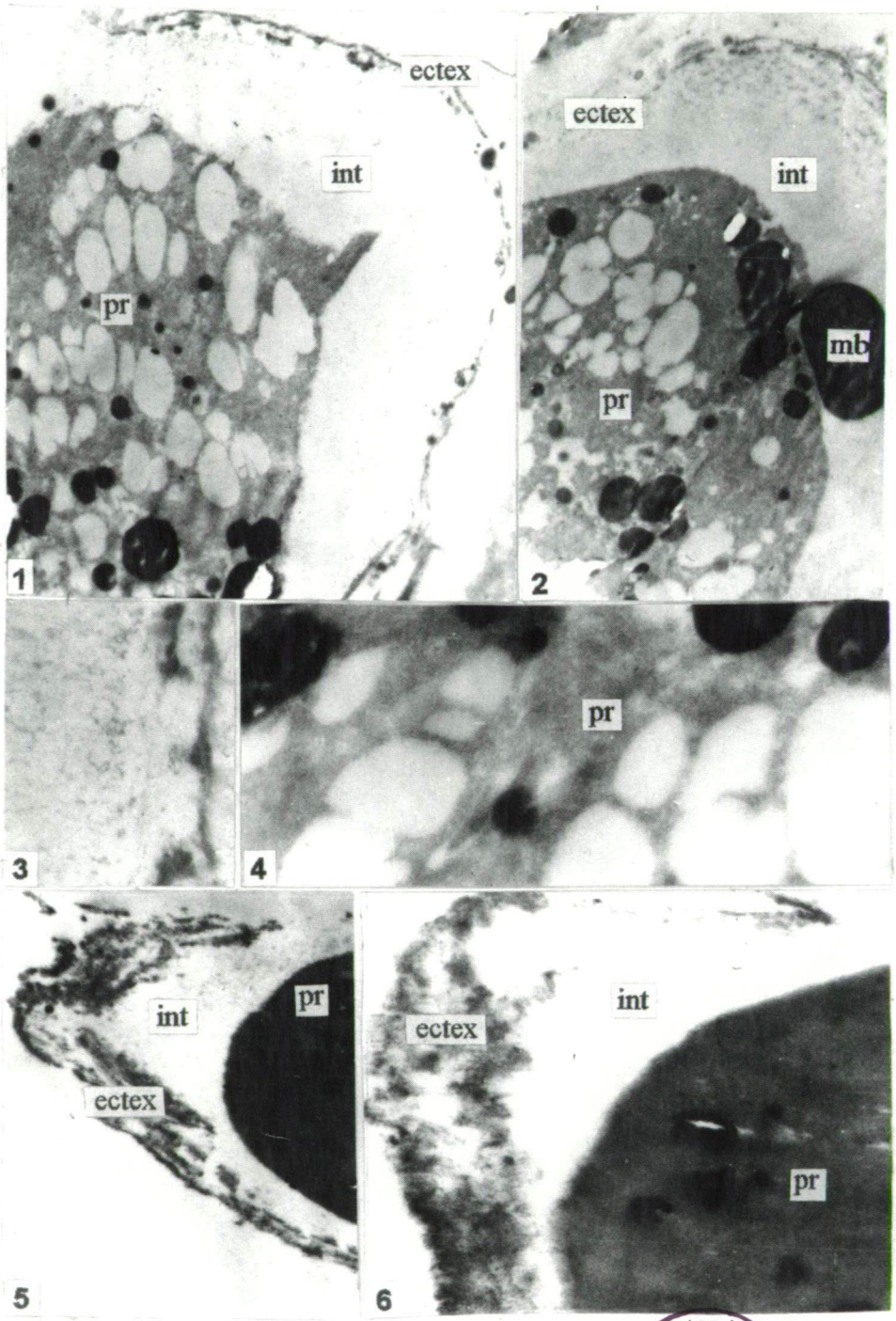

Plate 6.2.

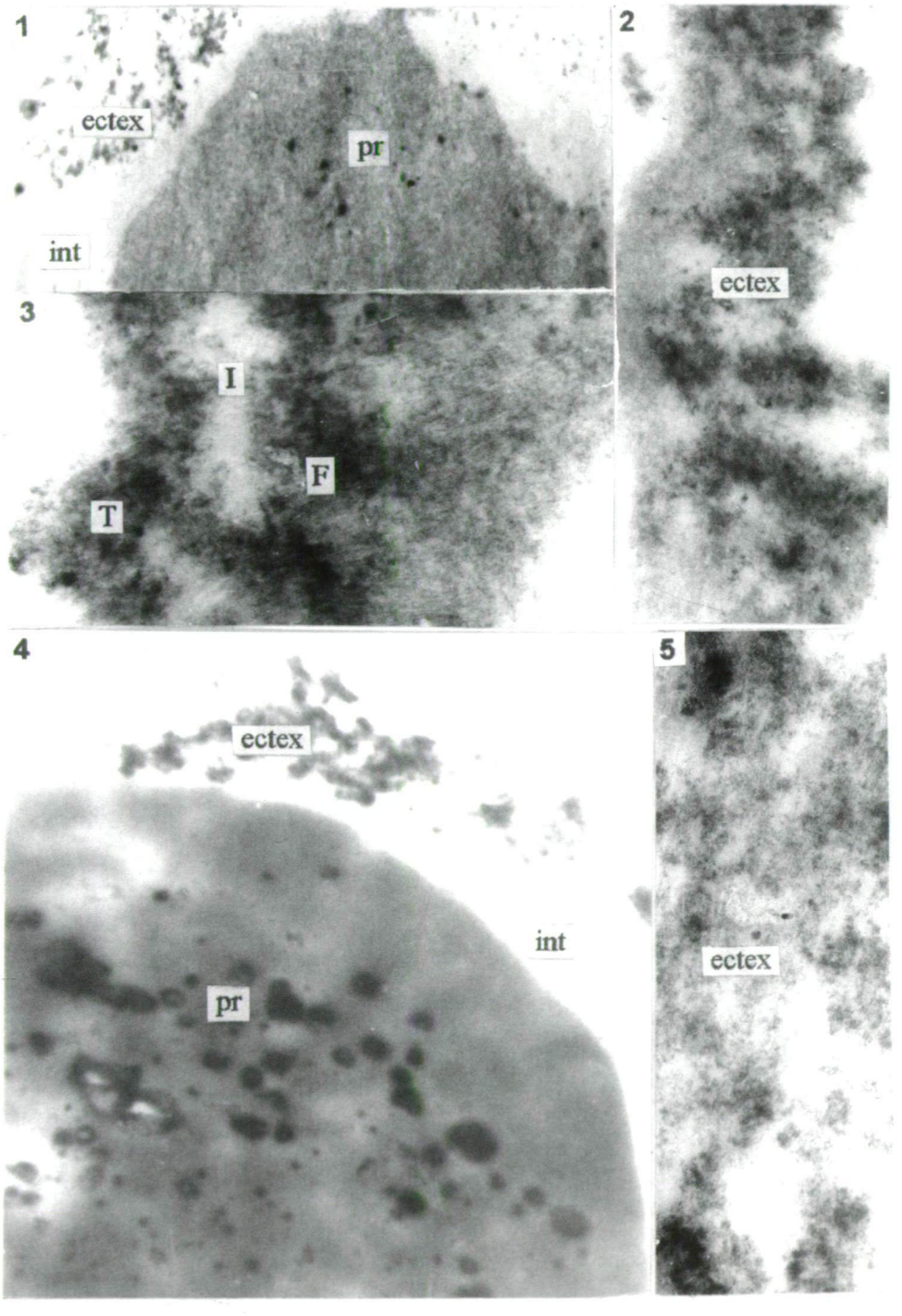

Plate 6.3.

tine was not perceptible in these experiments, but the larger light holes probably indicate the swelling of the intine before the degradation. In Plate 6.2., figs. 2,3 some remains of endexine can be seen after 72 hours of treatment. Large vacuoles and microbodies of different size are present in the protoplasm in the place of the intine. Beneath the remnants of the ectexine layers the microbodies are smaller. The protoplasm is relatively resistant to degradation.

2. Partial degradation with 2-aminoethanol and $KMnO_4$ (Plate 6.2., figs. 3-6, Plate 6.3., figs. 1-5).

Degradation of the protoplasm organelles is characteristic in all three kinds of experiments with these degradation agents. The consistency of the protoplasm is more or less homogeneous or finely granular. Vacuoles are only sometimes perceptible, the electron dense microbodies are smaller and were probably partially degraded. But the remnants of the ectexine are better contrasted than in the previous experiments in all probability in consequence of the effect of the potassium permanganate (Plate 6.2., figs. 5,6, Plate 6.3., figs. 2,3,5). In pictures 2,3 in Plate 6.3., the ectexine layers may be recognized, but the sporopollenin biopolymers are hardly damaged. Sometimes there are remains of the ectexine, particularly in the most destructive experiment, see pictures 4,5 in Plate 6.3.

3. Partial degradation with 2-aminoethanol and merkaptoethanol (Plate 6.4., figs. 1-4, Plate 6.5., figs. 1,2)

The results of the three kinds of experiments are nearly identical. The wall layers, including the exine and the intine, completely disappeared during the experiments. The protoplasm and sometimes the plasma membrane is relatively well preserved (Plate 6.5., fig.1.). There are several microbodies of different size and relatively tiny vacuoles in the protoplasm. Sometimes dark microbodies are outside of the protoplasm in the colpus area (Plate 6.4., figs. 1,2) and outside of the plasma membrane probably in the intine (Plate 6.4., figs. 3,4).

4. Partial dissolution with diluted (50%) glycerine (Plate 6.5., fig. 3)

Unusual granular ultrastructure was observed. The original ultrastructural elements of the wall and the organelles of the protoplasm are not in a suitable preservation. A peculiar dissolution or an infection may be presumed at this experiment, but microorganisms were not observed.

Plate 6.1.

Acer platanoides L., partially degraded pollen grains with 2-aminoethanol.
1,2. Length of time: 24 hours, 1. Negative No.: 10071, 15.000x, 2. Negative No.: 10078, 15.000x.
3,4. Length of time: 48 hours, 3. Negative No.: 10082, 5.000x, 4. Negative No.: 10084, 5.000x

Plate 6.2.

Acer platanoides L.
1-4. Partially degraded pollen grains with 2-aminoethanol (72 hours)
1. Negative No.: 9983, 5.000x, 2. Negative No.: 9978, 5.000x, 3. Negative No.: 9979, 15.000x, 4. Negative No.: 9988, 15.000x
5,6. Partially degraded pollen grains with 2-aminoethanol (24 hours) and with $KMnO_4$ (24 hours)
5. Negative No.: 9963, 5.000x, 6. Negative No.: 9964, 15.000x

Plate 6.3.

Acer platanoides L.
1-3. Partially degraded pollen grains with 2-aminoethanol (48 hours) and with $KMnO_4$ (24 hours)
1. Negative No.: 10090, 5.000x, 2. Negative No.: 10001, 15.000x, 3. Negative No.: 10002, 50.000x
4-5. Partially degraded pollen grains with 2-aminoethanol (72 hours) and with $KMnO_4$ (24 hours)
4. Negative No.: 10095, 5.000x, 5. Negative No.: 10005, 50.000x

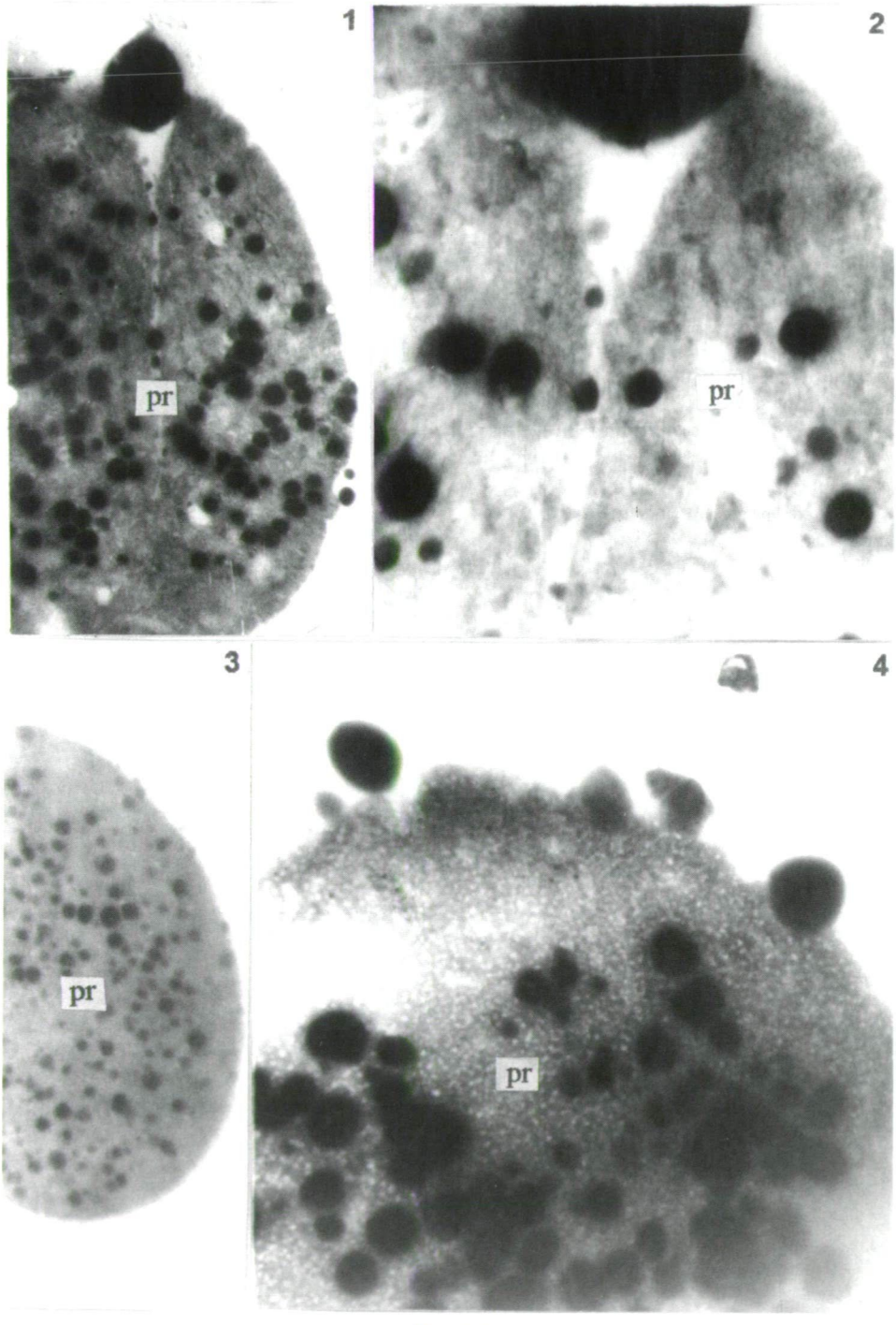

Plate 6.4.

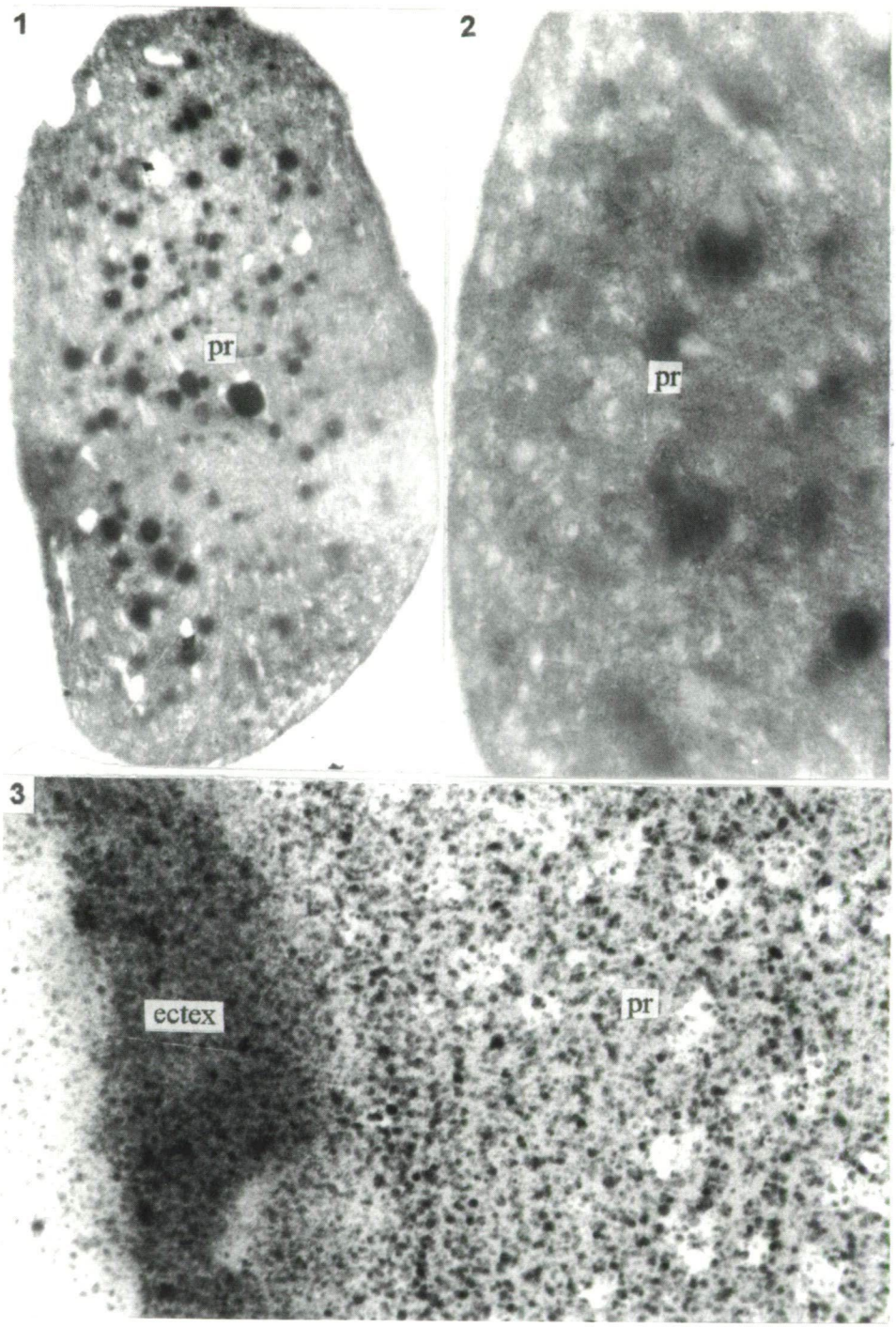

Plate 6.5.

Plate 6.4.

Acer platanoides L.
1-2. Partially degraded pollen grains with 2-aminoethanol (24 hours) and with merkaptoethanol (24 hours)
1. Negative No.:10066, 5.000x, 2. Negative No.: 10067, 15.000x
3-4. Partially degraded pollen grains with 2-aminoethanol (48 hours) and with merkaptoethanol (24 hours)
3. Negative No.:10055, 5.000x, 4. Negative No.:10064, 15.000x

Plate 6.5.

Acer platanoides L.
1-2. Partially degraded pollen grains with 2-aminoethanol (72 hours) and with merkaptoethanol (24 hours)
1. Negative No.:10046, 5.000x, 2. Negative No.:10048, 15.000x
3. Partially dissolved pollen grains with glycerine (50%) for 30 days
Negative No.:10039, 100.000x

Discussion and Conclusions

1. The extremely weak resistance of the biomacromolecular system of the ectexine may be pointed out in the first place. It is also worth mentioning that the degradation processes with 2-aminoethanol and merkaptoethanol destroyed the wall completely. Remnants of the ectexine were observed after partial degradation with 2-aminoethanol and with the combination of the partial degradation with $KMnO_4$ (aq. dil.). It is interesting that after the last mentioned strongest degradation processes, the layers of the ectexine were perceptible.

2. The pollenkitt was investigated by several authors e.g.: HESLOP-HARRISON (1976), HESSE (1978, 1979a,b,c, 1980). We refer to the paper of CASTELS et al. (1999): hydrated pollen grains are completely coated with pollenkitt. Droplets of pollenkitt occur in the cavities of the tectum. It is possible that the microbodies under the tectum remnants (e.g.: Plate 6.1., figs. 1,2, plate 6.2., fig. 1) are droplets of pollenkitt in the cavities of the infratectal layer. The droplets in the intine or in the colpal area may also be of this origin. The microbodies in the protoplasm are questionable.

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7. SEM INVESTIGATIONS ON THE PARTIALLY DEGRADED POLLEN GRAINS OF FAMILY MALVACEAE

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Abstract

Pollen grains of *Malva sylvestris* and *Hibiscus syriacus* were partially degraded with 2-aminoethanol for durations of 30 minutes, 1, 5, 10 and 24 hours. Results of the light microscopic observations on these pollen grains have been published by KEDVES et al. (2003). The present study is based on scanning electron microscopic observations and provides new data on the morphological alterations and the superficial degradation of these pollen grains at fine structure level. The differences in degree of alterations on sculpture, ectexine, spines and thick foot layer with respect to resistance and susceptibility of the sporopollenin have also been discussed.

Based on these data, it is concluded that the pollen grains of *Malva sylvestris* are comparatively more sensitive than that of *Hibiscus syriacus*, as these show less response to 2-aminoethanol treatment.

Key words: Experimental Palynology, recent, Malvaceae, partial degradation, SEM.

Introduction

During earlier studies (KEDVES et al., 2003) the results on light microscopic observations of the partially degraded pollen grains of *Malva sylvestris* and *Althaea officinalis* were explained. Using the data of such studies, more detailed observations based on scanning electron microscopy of the superficial alterations caused by 2-aminoethanol for the periods of 30 minutes, 1, 5, 10 and 24 hours are illustrated here.

ROWLEY and PRIJANTO (1977) published TEM data on experimentally altered exine of Malvaceae pollen. DENIZOT (1978) studied effect of ethanolamine on the exine and sculpture under various durations on the pollen grains of *Malva sylvestris*. She observed a little alteration in the exinal sculpture of these pollen grains.

Morphological changes caused by 2-aminoethanol illustrated with SEM enable us to ascertain the differences in alterations and also the comparison of morphological features of both species. Comparison of non-altered, semi-altered and altered characters in these pollen ascertains degree of suceptibility of different ectexinal layers and differential behaviour of sporopollenin with 2-aminoethanol. These phenomena may vary with respect to the morphological structures as well as the pollen types.

Materials and Methods

The pollen material for these studies was collected in Szeged, September, 2002. The experiments with 2-aminoethanol were conducted in the Cell Biological and Evolutionary Micropaleontological Laboratory of the University of Szeged and SEM observations were made at Birbal Sahni Institute of Palaeobotany

Lucknow by using standard dehydration and preparation techniques. The present investigation is based on the earlier descriptions and methods used by KEDVES et al. (2003). The present study allows generation of more detailed data on changes in shape, size as well as the exinal features including spines and collumellae. In addition to these, the response on ectexinal characters, partial or severe alterations in pollen grains by 2-aminoethanol at 30°C for 30 minutes, 1, 5, 10 and 24 hours are described.

Results

Malva sylvestris LINN. (Plate 7.1., figs. 1-7, plate 7.2. and plate 7.3., figs. 1-6)

1. Fresh pollen grains (Plate 7.1., figs. 1,2). Amb circular, radially symmetrical, spheroidal. Size 80-95 μm. Exine ± smooth-echinate, spines 6-8 μm long, smooth, conical with pointed tips.

2. After 30 minutes of dissolution with 2-aminoethanol deformation in morphological features of the pollen grains are clearly distinct (Plate 7.1., figs. 3-5) and depressions at several places are common. The exoapertures (colpi) are clearly visible. In some pollen grains tectum gets completely dissolved and fibrillar structures attached with few spines at certain places forming net or exoskeleton are clearly visible (Plate 7.1., fig. 4). The exinal stratification showing distinct columellar elements with minor corrosion in outer spinal layer is noticed (Plate 7.1., fig. 5 and magnified photo, plate 7.2.). It is observed that the outer and inner part of the endoaperture is not identical. This kind of endopore was observed earlier by DENIZOT (1978) in *Malva sylvestris* LINN., CULHANE and BLACKMORE (1988), *Alcea rosea* LINN., *Althaea officinalis* LINN., LA SERNA RAMOS and DOMINGUEZ SANTANA (1991) and in two varieties of *Lavatera acerifolia*. In *Malva alcea* CULHANE and BLACKMORE (1988) observed that the outer and inner perforations of the foot layer are of equal diameter.

3. After one hour of treatment (Plate 7.1., figs. 6,7) more alterations were observed on the exinal surfaces with dissolutions and depressions in the tectum. The dissolution of tectum resulted in the uncovering of infratectal elements and free columellar ends at their upper surface are noticed. The endoaperture is unaltered and distinct between intercolumellar structures. The spines get detached at several places and infratectal layer also gets affected (Plate 7.1., fig. 7).

4. Five hours of treatment resulted in rupturing of pollen grains with detachment of maximum number of spines as well as tectal layer (Plate 7.3., figs. 1,2). Dissolution of tectum caused liberation of columellae at upper surface as observed in the previous experiment. Endoapertures clearly visible, partial degradation in infratectal layer also noticed.

5. The effect of 2-aminoethanol after 10 hours (Plate 7.3., figs. 3,4) resulted in deformation in exinal sculpture with dissolution of ectexinal surface to some extent, e.g. tectum is completely dissolved, upper part of columellae is also severely affected, maximum number of columellae are dissolved at their upper surfaces, thinning of spines and rupturing of the grain is usual.

6. Maximum degradation in morphology of the pollen grains is clearly visible after 24 hours of treatment with 2-aminoethanol (Plate 7.3., figs. 5,6). The infrasculptural elements are coalesced with each other due to their severe dissolution. The columellae and spines dissolved and appeared like gemmae or bulging structures or sometimes completely dissolved, coalesced and melted over the deformed columellar layer (Plate 7.3., fig. 5).

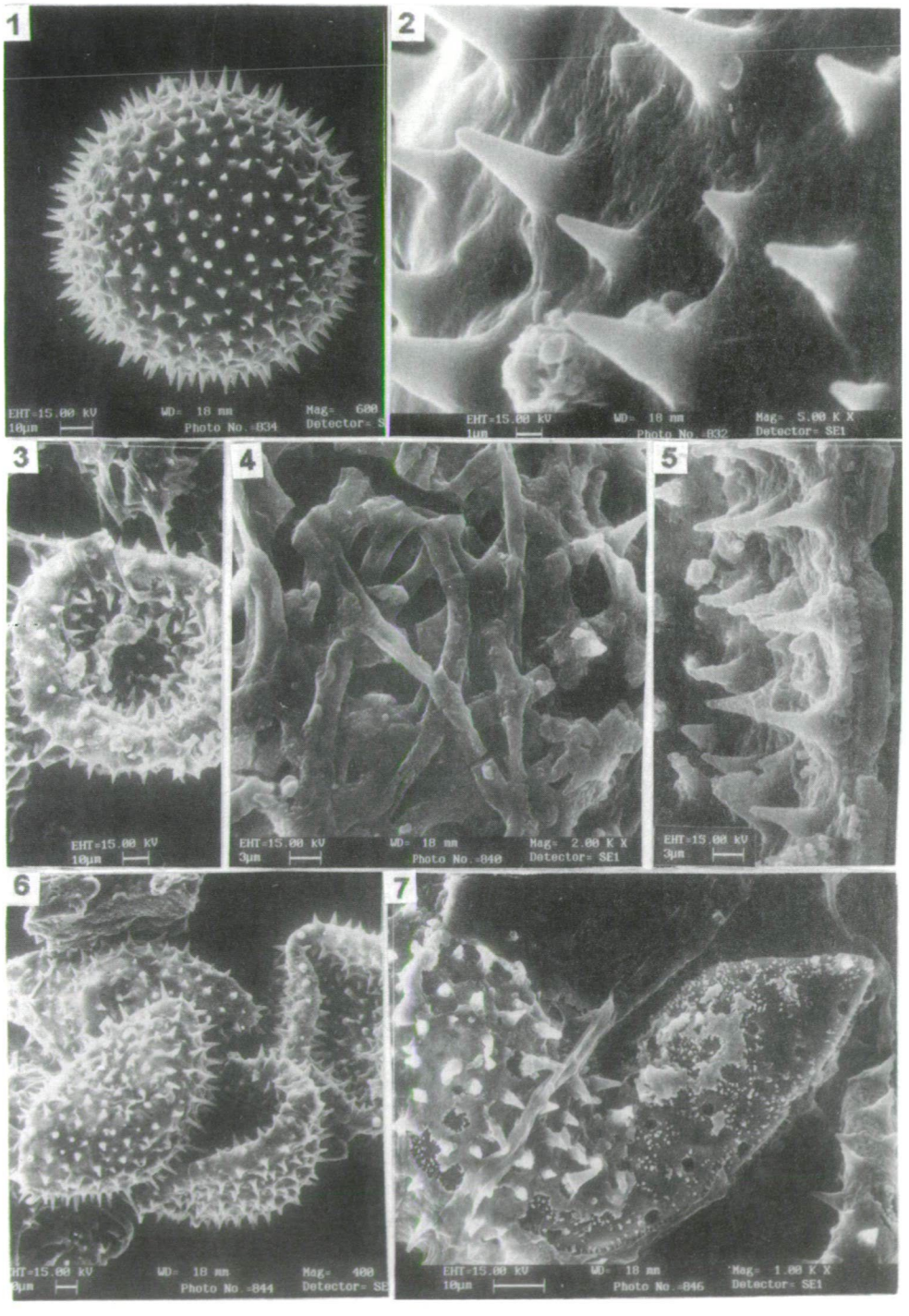
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Plate 7.1.

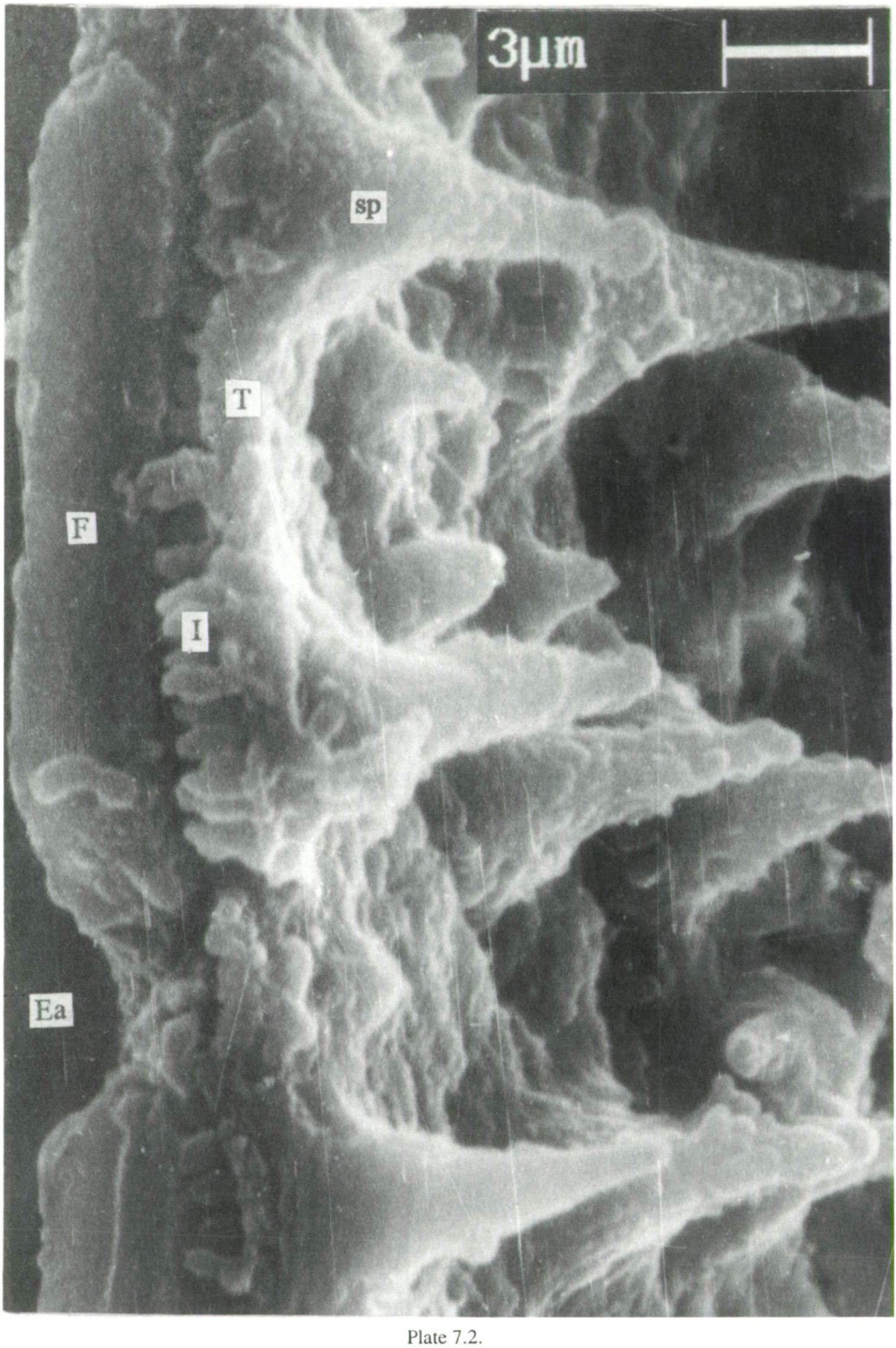

Plate 7.2.

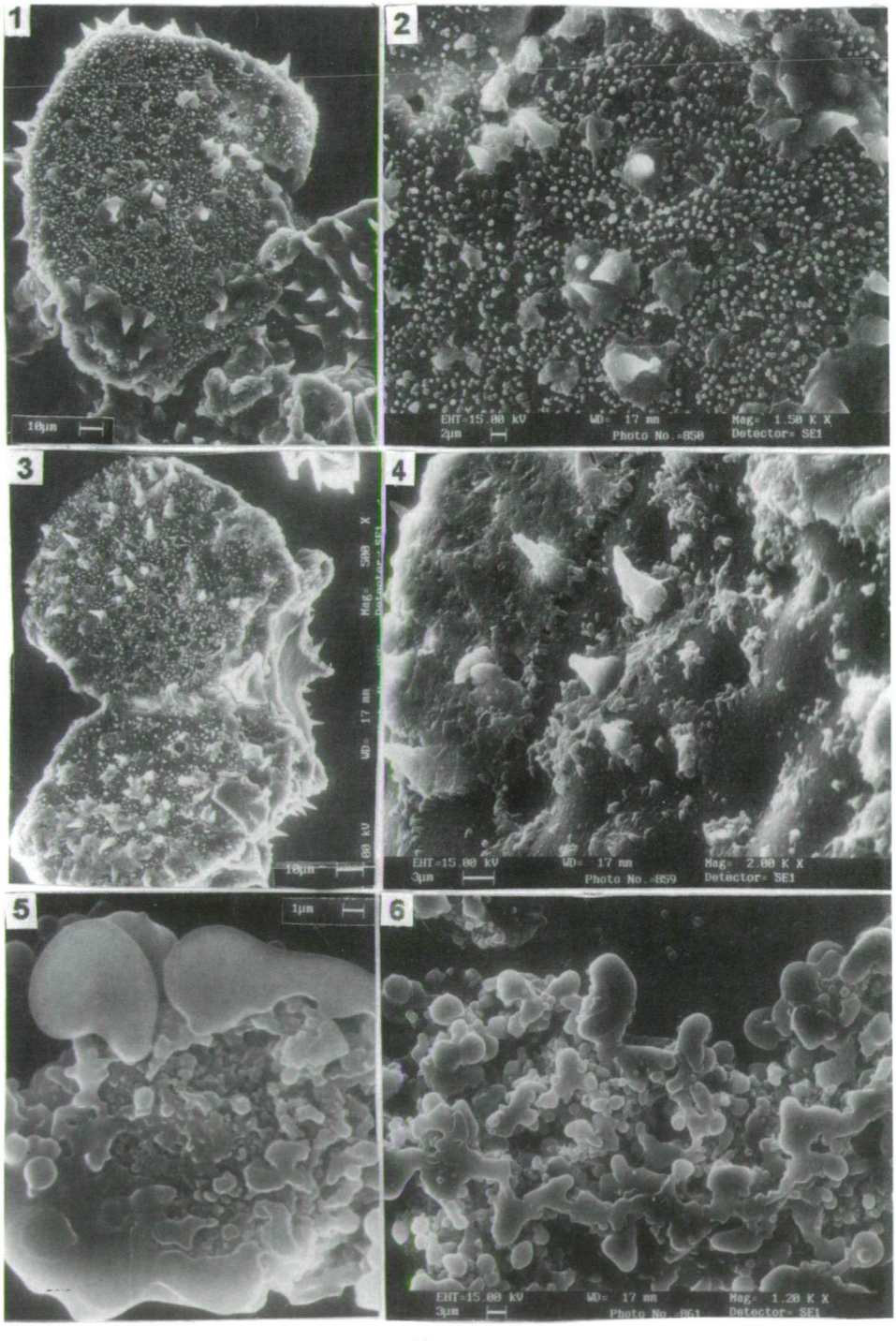
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Plate 7.3.

Hibiscus syriacus LINN. (Plate 7.4., figs. 1-6 and plate 7.5., figs. 1-6)

1. Fresh pollen grains: amb circular, radially symmetrical, spheroidal in shape. Size 80-110 μm. Spinose, spines 15-20 μm long, conical with blunt tips, polypontoporoidate. Exine granulate at interspinal and interapertural regions. Tectum perforate, mucilage around the apertures are visible (Plate 7.4., figs. 1,2).

2. After treatment with 2-aminoethanol for 30 minutes the deformation in the entire grain is noticed, depressions or shrinkage at several places are common. The apertures become distinct and mucilage disappeared (Plate 7.4., figs. 3,4).

3. The degradation after one hour caused further depressions and changes in the basic morphology of the pollen grains. Surface of tectum is same as in the previous one, but sometimes more bluntness in the tip of the spines is noticed (Plate 7.4., figs. 5,6).

4. Different kind of characteristic deformations are caused by five hours of treatment. Severe depressions at several places in pollen grains are common in highly shrunk grains. Spines are sometimes embedded or maybe detach from their base, the perforation of the tectum is very characteristic and exoapertures are also deformed or depressed (Plate 7.5., figs. 1,2).

5. After ten hours more deformations were observed as various secondary features, outer morphology appears like triradiate forms, some spines become sinuosus. The tectum shows differential depressions (Plate 7.5., figs. 3,4).

6. Treatment with 2-aminoethanol for 24 hours caused different kind of deformations in secondary features, such as depressions in tectal regions, detachment of spines and enlargement of sculptural structures at interspinal spaces and around the base of the spines (Plate 7.5., figs. 5,6).

Plate 7.1.

1-7. *Malva sylvestris* LINN.
1-2. Fresh pollen grains.
3-5. Pollen grains partially degraded with 2-aminoethanol for 30 minutes. 4. Please note the fibrillar network.
5. Change in ectexinal layers and thinning of spines.
6-7. Pollen grains partially degraded with 2-aminoethanol for 1 hour. 6. Shrinking of pollen grains. 7. Dissolution of tectum and detachment of spines.

Plate 7.2.

Malva sylvestris LINN. Highly magnified picture of Plate 7.1., fig. 5. sp = spine, T = tectum, I = infratectum, F = foot layer, En = endoaperture.

Plate 7.3.

1-6. *Malva sylvestris* LINN.
1-2. Pollen grains degraded with 2-aminoethanol for 5 hours. 1. Tectal layer completely dissolved. 2. Magnified view of fig. 1, showing free columellae and few spines.
3-4. Pollen grains degraded with 2-aminoethanol for 10 hours. 3. Ruptured pollen grains with free columellae.
4. Columellae dissolved to reduce their sizes and few thin spines attached to it.
5-6. Pollen grains degraded with 2-aminoethanol for 24 hours. 5. Severe alteration on columellae which are melted and fused with each other. 6. Coalescence of columellae, the effect of severe alteration by 2-aminoethanol.

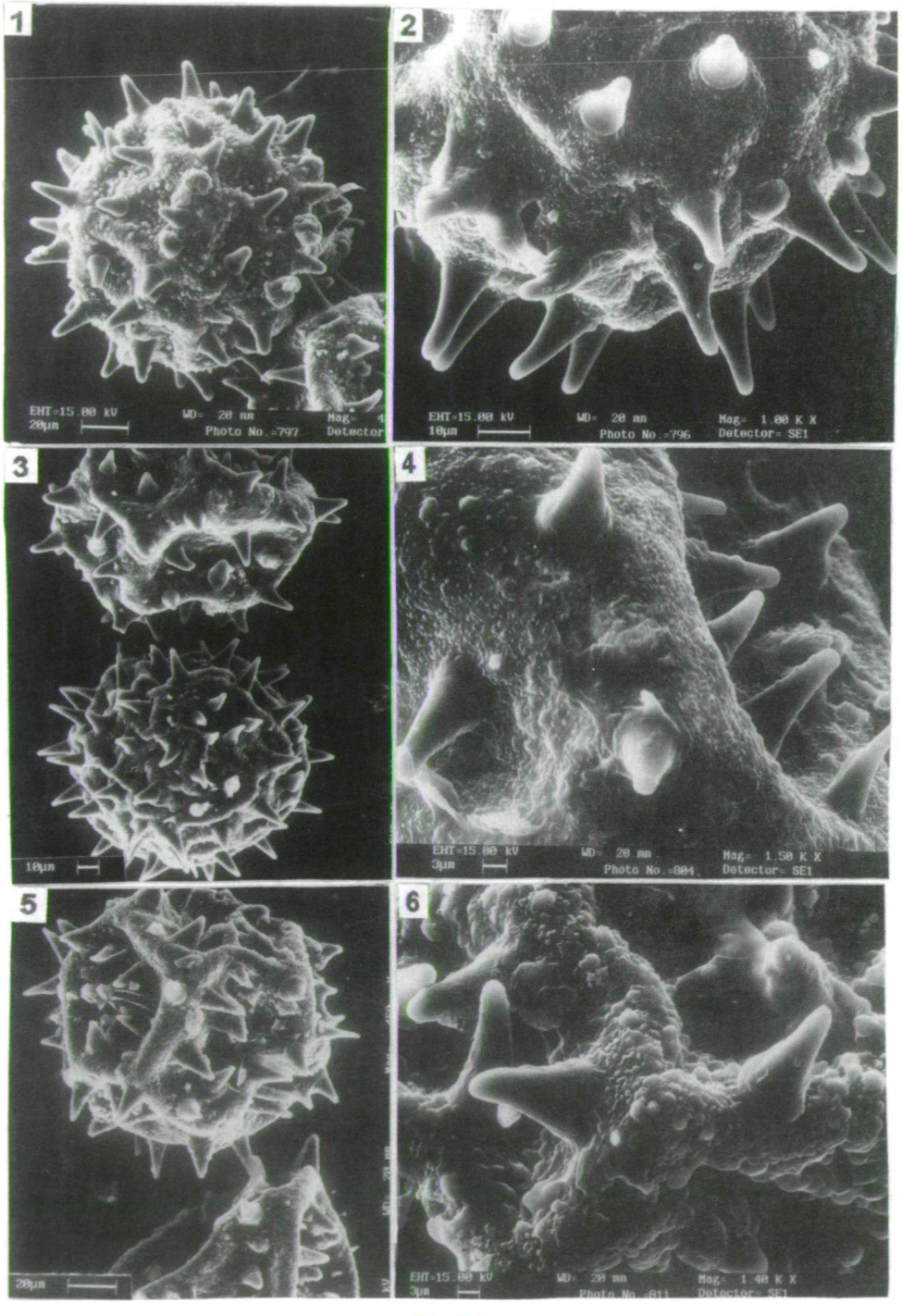
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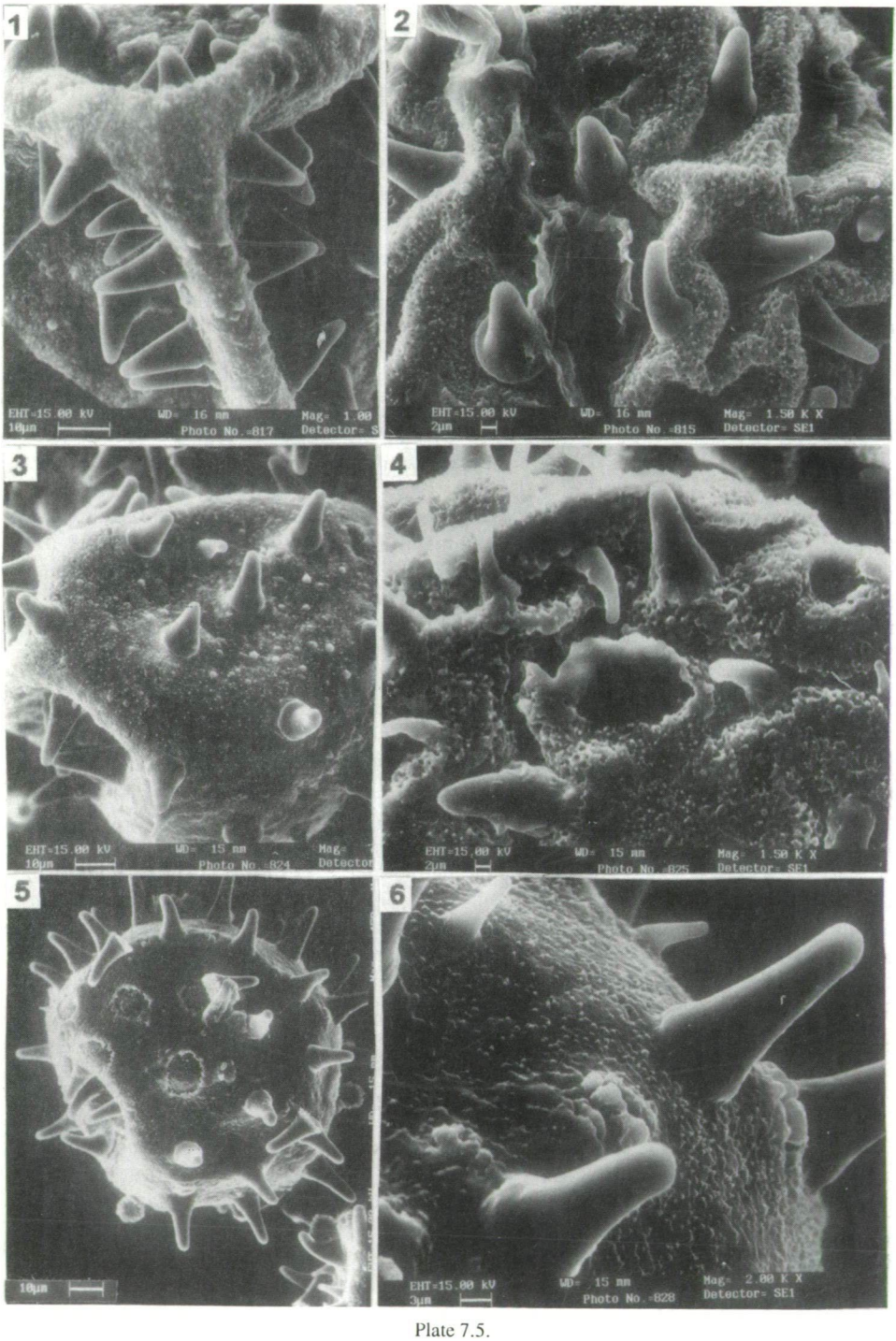
Plate 7.4.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Mag= 1.50 K X
2µm
Photo No.=815
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10µm
Photo No.=824
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WD= 15 mm
Mag= 1.50 K X
2µm
Photo No.=825
Detector= SE1
5
10µm
6
EHT=15.00 kV
WD= 15 mm
Mag= 2.00 K X
3µm
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Detector= SE1

Plate 7.5.

Plate 7.4.

1-6. *Hibiscus syriacus* LINN.
1-2. Fresh pollen grains
3-4. Pollen grains partially degraded with 2-aminoethanol for 30 minutes, a little depression is noticed.
5-6. Pollen grains partially degraded with 2-aminoethanol for 1 hour. Further depressions causing shrinking of the grains.

Plate 7.5.

1-6. *Hibiscus syriacus* LINN.
1-2. Pollen grains partially degraded with 2-aminoethanol for 5 hours. 1. Alteration caused to appear like triradiate forms. 2. Magnified view of fig.1, showing shrinking of tectum layer.
3-4. Pollen grains partially degraded with 2-aminoethanol for 10 hours, showing dissolution of mucilage, resulting in the appearance of aperture and partial detachment of some spines from their base.
5-6. Pollen grains partially degraded with 2-aminoethanol for 24 hours, showing not much alterations than the treatment for 10 hours. The granulate interspinal spaces and apertures are clearly visible.

Discussion and Conclusions

Treatment with 2-aminoethanol for various durations causes differential alterations and deformation of the morphological features in pollen grains of Malvaceae. Its effect under certain durations is very specific to different structures which are clearly visible in our experiments and results in pollen grains of *Hibiscus syriacus* and *Malva sylvestris.* These results provide a new concept to the differential behaviour of sporopollenin with 2-aminoethanol. These data enable us to understand that pollen grains of various species of a family constitute specific exinal structural patterns, which may or may not be altered after certain duration of treatment with 2-aminoethanol or other chemicals. In earlier stages of the treatment (after 30 minutes and 1 hour) pollen grains of *Hibiscus syriacus* show little alteration, whereas, at this stage almost maximum exinal characters are mostly deformed in *Malva sylvestris.* It shows that different taxa have a peculiar resistance system and secondary alterations are limited up to a certain level only. Likewise, some characters are severely affected or deformed in pollen grains of *Malva sylvestris* at the first stage of the treatment and at later stages (after 24 hours) almost all pollen characters are severly altered. But some pollen grains have resistant features which are not easily altered as observed in *Hibiscus syriacus.* The resistance in these pollen grains may be due to the presence of mucilage on the ectexine, which might serve as a protecting layer to the tectum and other superficial characters. In other words, the mucilaginous coating does not allow 2-aminoethanol to react with sporopollenin.

SOUTHWORTH (1974) and DENIZOT (1978) also observed differential solubility of pollen exine by various chemicals at certain durations, which modify their ultrastructures. KNOX and HESLOP-HARRISON (1969) opined that the sporopollenin of the pollen exhibits differential cytochemical localization of enzymes. Sometimes these enzymes are rapidly diffused by various chemicals. The present study (Plate 7.1., fig. 4) also supports the view of STANLEY and LINSKENS (1965) and KNOX and HESLOP-HARRISON (1969) regarding diffusion of pollen proteins by various chemicals. In our study the fibrillar structure is also seen on the pollen grains of *Malva sylvestris* after 30 minutes of treatment with 2-aminoethanol. The ectexinal characters of the pollens were rapidly degraded and a barren fibrillar skeleton network on the amb is clearly visible, representing a character of allergenic pollen (STANLEY and LINSKENS, 1965). This phenomenon is

very important for understanding the differential behaviour of sporopollenin during preservation or fossilization of pollen grains under the influence of various ecological, chemical and edaphic factors.

Acknowledgements

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8. TRANSMISSION ELECTRON MICROSCOPY OF THE EXINE OF MALVA SYLVESTRIS L. TREATED WITH C60 FULLERENE/BENZOL SOLUTION AND MERKAPTOETHANOL

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Abstract

Pollen grains of *Malva sylvestris* L. were treated with C60 fullerene/benzol solution for 1 - 6 days (Experiment No.: T-12- 294-299) and with merkaptoethanol for 24 hours. The cleaned and dried pollen material was embedded in Araldite and was ultrathin sectioned. The transmissiom electron microscopical results are as follows: 1. The protoplasm organells were disintegrated easily. 2. The sporopollenin of the ectexine is relatively resistant. 3. The fullerene accumulated differentially in the ectexine layers and at present object the ultrastructure was suitable for TEM study without any postfixation treatment. 4. In general the thick foot layer accepted less fullerene than the thin tectum and the infratectal layer. 5. The treatment in some cases degraded partially the molecular system of the sporopollenin of the ectexine. 6. Large holes and rarely electron dense globular biopolymer units were observed in linear and irregular arrangement. 7. Occasionally regular pentagon and hexagon units occurred which indicated the presence of a quasi-periodic and/or quasi-equivalent biopolymer organization of the ectexine. 8. The diameter of the negative holes is much larger than those of the quasi-crystalloid dimensions.

Key words: Palynology, recent, *Malva sylvestris,* partial degradation, C60 fullerene/benzol solution, merkaptoethanol.

Introduction

Pollen grains of *Malva sylvestris* L. were included in our research program in the experimental study of the allergenic pollen grains. LM data of partially degraded Malvaceae pollen grains *(Malva sylvestris, Hibiscus syriacus)* were published earlier (KEDVES et al., 2003). In this volume the SEM results of the above mentioned experiments are presented TRIPATHI, MADHAV KUMAR, KEDVES and JACSÓ (2004). Regarding the importance of the C60 fullerene/benzol solution in the partial degradation of the biopolymer structures of the plant cell wall was emphasized earlier (KEDVES, 1996) and a short review of the first results in this new field of experimental studies was published later (KEDVES 2001/2002).

Regarding the ultrastructure of the Malvaceae pollen grains the first data were published by BRONCKERS and HORVAT (1963) on the exine of *Gossypium hirsutum* L. Basic ultrastructure morphology is identical with the further TEM data on the pollen grains of this family, e.g.: *Hibiscus syriacus* L. by TAKAHASHI and KOICHI (1988)

Several experiments were carried out using the LM method. Ultrastructure after partial degradation was investigated by ROWLEY and PRIJANTO (1977) and by DENIZOT (1978).

Considering the previous publications we may conclude that the molecular system of the investigated Malvaceae pollen grains are relatively resistant. Concerning the nomenclature of the exine we used the work of KREMP (1965) and PUNT et al. (1994).

The aim of this paper is to obtain ultrastructure information about the importance of the C60 fullerene/benzol solution in the partial degradation of the pollen grains of *Malva sylvestris*. To this the interesting ultrastructure of the Malvaceae ectexines (characteristic spinae, thin tectum, very thick foot layer) is another argument for our present researches.

Materials and Methods

The investigation material was collected by Miss D. JACSÓ in Szeged, 7th September, 2001. The experiments were started on the same day. 5 ml C60 fullerene/benzol solution were added to 30 stamina, for 1-6 days (Experiment Nos.: T-12-294, 295, 296, 297, 298, 299).Temperature: 30 °C. After washing with benzol and drying, 4 ml merkaptoethanol were added to the pollen grains for 24 hours. Washing with distilled water and drying again and the dry material were embedded in Araldite (Durcupan, Fluka).

The ultrathin section was made on a Porter Blum ultramicrotome with glass knives. The TEM pictures were taken in the EM Laboratory of the Institute of Biophysics of the Biological Research Center of the Hungarian Academy of Sciences on a Tesla BS 540 and Zeiss Opton EM-902 instrument. All pictures are unretouched.

Results

Experiment No.: T-12-294 (Plate 8.1., figs. 1-4)

The spine, tectum and the columellar infratectal layer accepted the fullerene better than the thick foot layer (Plate 8.1., figs. 1-3). Sometimes the outer part of the foot layer and below the infratectal elements of the foot layer were partially dark coloured. The protoplasm was disintegrated and the innermost part of the ectexine was extremely dark. Partial degradation with light globular holes was observed in the tectum (Plate 8.1., figs. 1,3). The arrangement of these holes was irregular.

Experiment No.: T-12-295 (Plate 8.1., figs. 5-7)

This experiment revealed well the highly organized biopolymer units of the ectexine. Dark globular units of about 3-6 Å in diameter were observed. Regular pentagons and hexagons occurred (Plate 8.1., fig. 7), which may be useful for further symmetry operations. Further different kinds of arrangement of the dark globular units were observed such as linear irregular or cluster-like biomacromolecular systems.

Experiment No.: T-12-296 (Plate 8.2., figs. 1-4)

In contrast with the previous experiment this treatment resulted in no characteristic biopolymer structures. Illustrated are the dark spine, tectum and pro parte the foot layer (Plate 8.2., figs. 1-4). A relatively thin innermost part of the foot layer accepted the fullerene differentially. Sometimes the elements of the columellar infratectal layer were partially degraded (Plate 8.2., fig. 2). The characteristic endoaperture is illustrated in Plate 8.2., fig. 2.

Experiment No.: T-12-297 (Plate 8.2., figs. 5,6)

The results were nearly the same as the previous one, but sometimes dark and light globular units were observed (Plate 8.2., figs. 5,6). This experiment revealed globular biopolymer units, but in the same time, degraded similar biopolymer units. Fig. 6 illustrated well the characteristic spine of this pollen grain, including the peculiar acceptance of the fullerene.

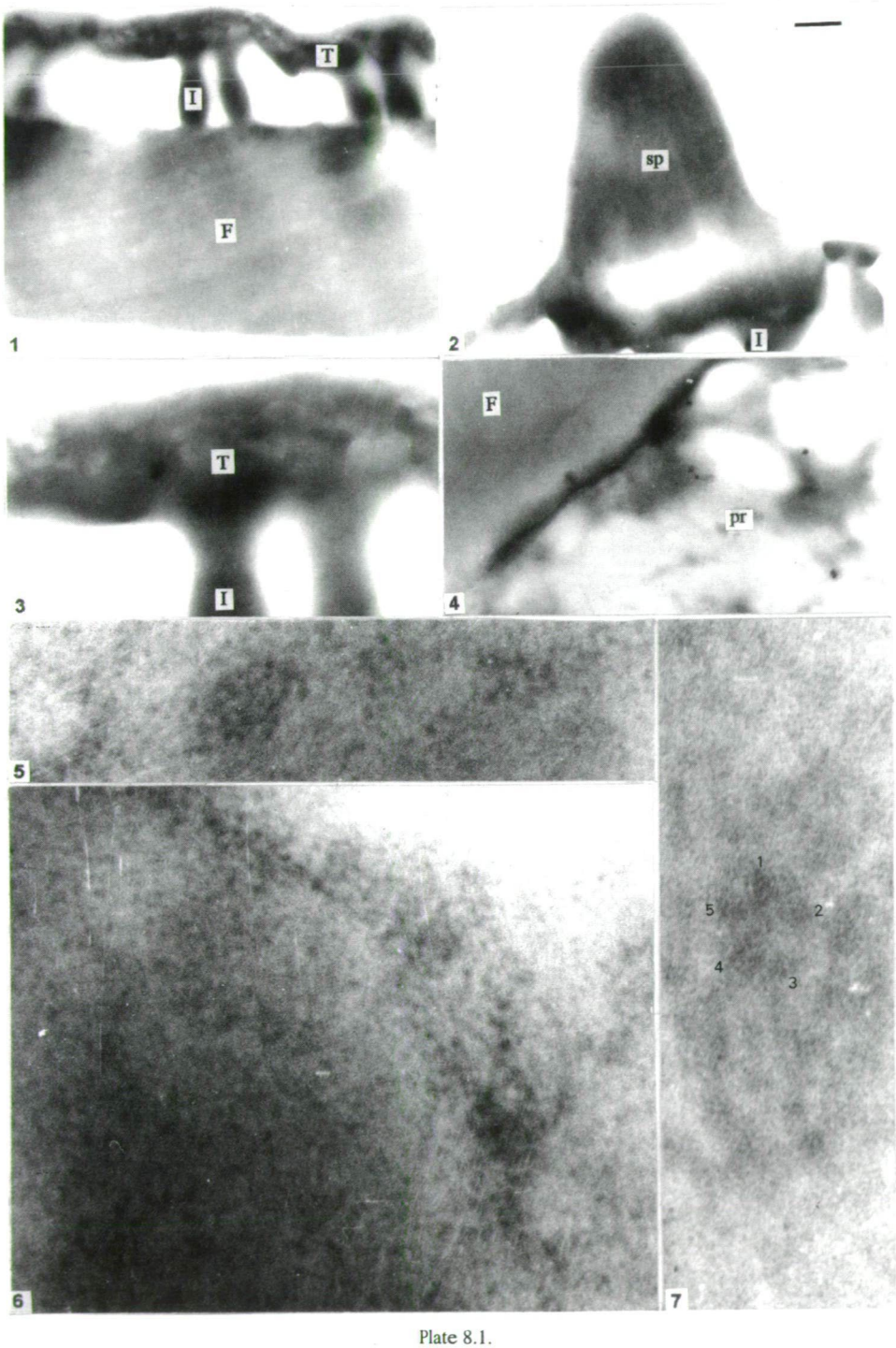

Plate 8.1.

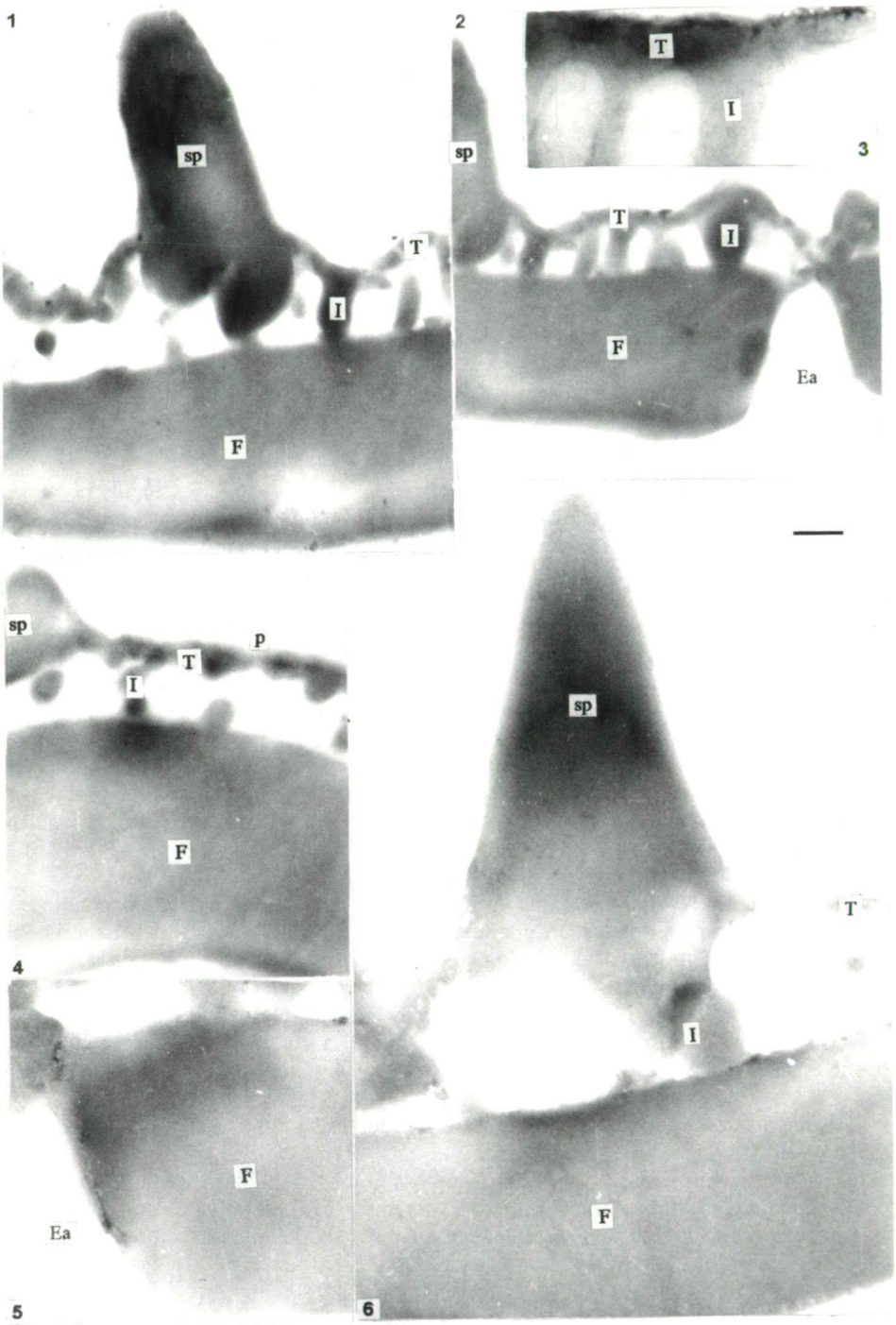

Plate 8.2.

Plate 8.1.

1-7. Ultrastructure of the pollen grains of *Malva sylvestris* L. after experiment.
1-4. Experiment No.: T-12-294. 1. Negative No.: 9603, 2. Negative No.: 9599, 3. Negative No.: 9604, 4. Negative No.: 9600.
5-7. Experiment No.: T-12-295. 5. Negative No.: 12759, 6. Negative No.: 12759, 7. Negative No.: 12760.
Bar scale: figs. 1,2,4: 0.67 μm, fig. 3: 0.2 μm, figs. 5,6: 0.02 μm, fig. 7: 0.0067 μm.
T = tectum, I = infratectum, F = foot layer, sp = spine, pr = protoplasm.

Plate 8.2.

1-6. Ultrastructure of the pollen grains of *Malva sylvestris* L. after experiment.
1-4. Experiment No.: T-12-296. 1. Negative No.: 9996, 2. Negative No.: 9994, 3. Negative No.: 9883, 4. Negative No.: 9998.
5,6. Experiment No.: T-12-297. 5. Negative No.: 9871, 6. Negative No.: 9869.
Bar scale: figs. 1,4: 0.67 μm, fig. 2: 1.0 μm, fig. 3: 0.2 μm, figs. 5,6: 0.4 μm.
T = tectum, I = infratectum, F = foot layer, sp = spine, Ea = endoaperture, p = pore.

Experiment No.: T-12-298 (Plate 8.3., figs. 1-4)

Results of this experiment may be characterized by the degradation of highly organized biopolymer units (Plate 8.3., figs. 1,3,4). The arrangement of these light holes varied.

Among these there are some that might be investigated with symmetry operation.

The layers of ectexine accepted fullerene in an equal amount.

Experiment No.: T-12-299 (Plate 8.3., figs. 5,6)

No major difference can be revealed between this one and the previous experiment.

Discussion and Conclusions

1. These experiments revealed that the biopolymer structure of the ectexine is relatively resistant. DENIZOT (1978) emphasized that the action of ethanolamine on the pollen grains of *Malva sylvestris* varies according to the duration of the treatment and the preliminary treatment with acetolysis or boiling ethanol.

2. The different layers of ectexine accept fullerene on a different scale depending on time used for the experiments. In contrast with these results the last two experiments ended in the layers of ectexine accepting an equal amount of fullerene. In this respect the transmission electron microscopical results of ROWLEY and PRIJANTO (1977) are important. The degradation of the different parts of the ectexine including the spinae was not the same, well illustrated in picture 21. in Plate 11. of the paper of ROWLEY and PRIJANTO (1977).

3. Some of the experimental results were planned to be objected to symmetry operations.

In case of regular pentagon there are two possibilities: may be either the element of a metastable quasi-crystalloid skeleton, or the fragment of a biopolymer unit which may be modelled with fullerenes. The dimension of these biopolymer structures must be of angstrom dimensions (circa 8-28 Å).

4. Light holes of larger dimension indicate more higly organized biomacromolecular systems. The regular pentagon may also be subjected to symmetry operations.

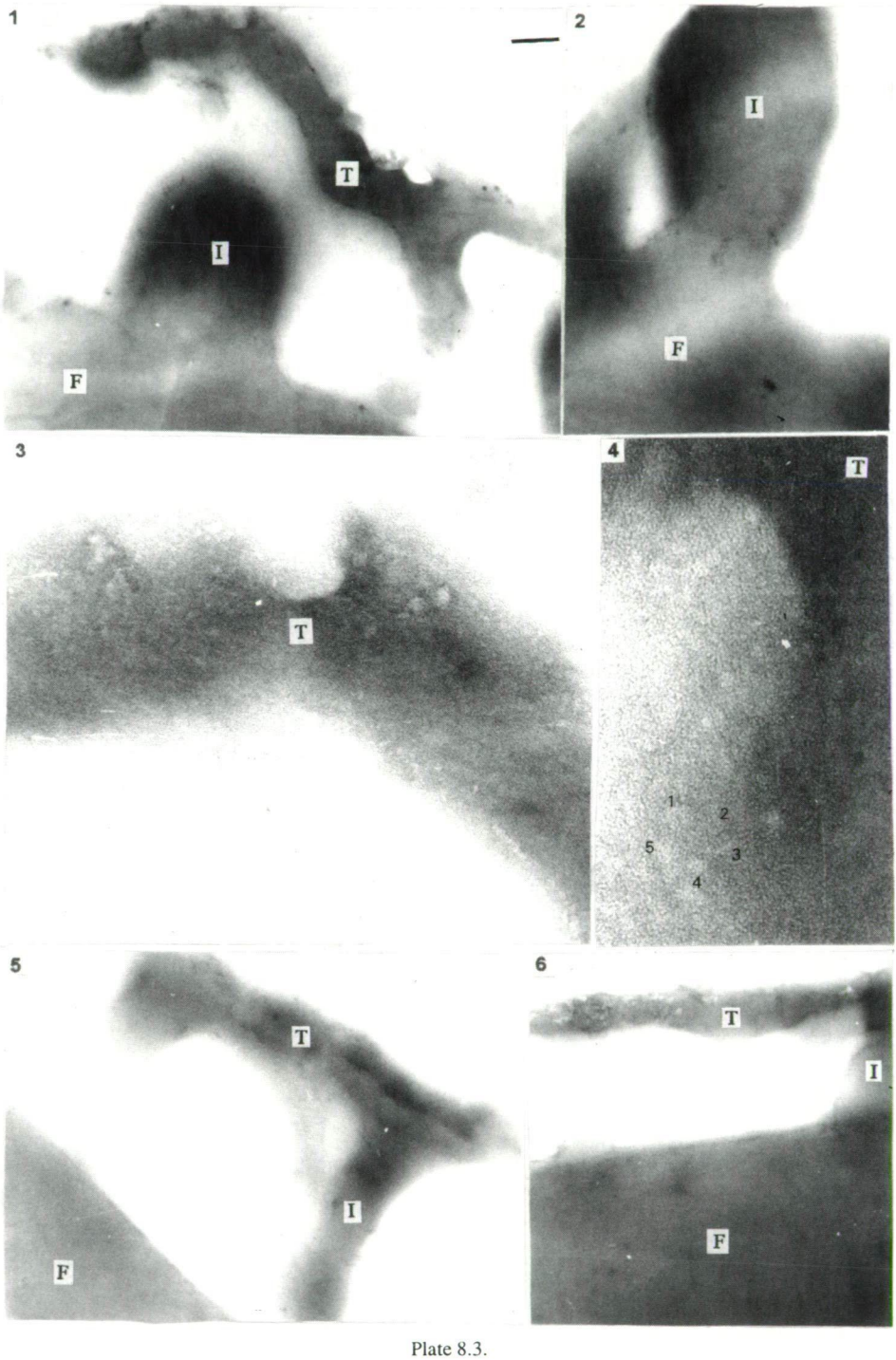

Plate 8.3.

Plate 8.3.

1-6. Ultrastructure of the pollen grains of *Malva sylvestris* L. after experiment.
1-4. Experiment No.: T-12-298. 1. Negative No.: 9764, 2. Negative No.: 9763, 3. Negative No.: 11747, 4. Negative No.: 11748.
5,6. Experiment No.: T-12-299. 5. Negative No.: 9756, 6. Negative No.: 9760
Bar scale: figs. 1,2,5,6: 0.2 μm, fig. 3: 0.05 μm, fig. 4: 0.02 μm.
T = tectum, I = infratectum, F = foot layer.

Acknowledgements

This work was supported by Grant OTKA T 31715. The writers are very thankful to Eric CAULTON Director (Scottish Centre for Pollen Studies, Edinburgh, Scotland, UK) for critically reading the manuscript and for his valuable suggestions.

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9. ÉTUDES EXPÉRIMENTALES SUR QUELQUES GRAINS DE POLLEN DES AMENTIFÈRES

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Sommaire

Les altérations morphologiques dues à une température élevée (200 °C) maintenue pendant 10 minutes, 1 heure, 5 heures et 10 heures ont été étudiées sur les grains de pollen de *Quercus robur, Quercus cerris, Salix alba, Alnus glutinosa, Corylus avellana, Corylus colurna* et *Betula verrucosa*. Les grains de pollen de trois échantillons d'une espèce *(Quercus robur)* ont fait l'objet des prélèvements suivants: 1. Grains de pollen récoltés le même jour dans des localités différentes (2,3). 2. Grains de pollen d'une seule localité mais à des dates différentes (1,2). Des altérations qualitatives et quantitatives ont été observées. Le rapport P/E est un caractère important pour les Longiaxes, étant donné qu'au cours de la sédimentation ce caractère peut changer de façon remarquable et causer des problèmes pour la détermination des grains de pollen fossiles. En général, les altérations qui produisent les formes secondaires concernent: 1. La morphologie fondamentale des grains de pollen. 2. Le système moléculaire de la sporopollénine et de l'endexine. 3. La maturité du grain de pollen y compris le degré de polymérisation des systèmes moléculaires. 4. Les conditions écologiques, et en premier le climat, pendant l'ontogénèse des grains de pollen. 5. La distance entre les dates de prélèvement et celles des expériences.

Mots clés: Palynologie, actuel, Amentiferae, effet d'une haute température.

Introduction

Les variations morphologiques des spores et des grains de pollen ainsi que les altérations secondaires sont importantes de plusieurs points de vue, par exemple celui de la taxonomie, de la phylogénie ou de l'étude des associations sporopolliniques trouvées dans des sédiments d'âges géologiques différents. C'est chez les sporomorphes fossiles que ce problème est le plus compliqué car il faut tenir compte de plusieurs facteurs, parmi lesquels les plus importants sont:

1. Les variations morphologiques infra-spécifiques: par exemple la forme du contour concave, triplanoïde, triplane des spores trilètes (DEÁK, 1959, KEDVES, 1960,1961). Le nombre des apertures et la taille (CLAUSEN, 1960) des grains de pollen des Angiospermes, ou quelquefois le caractère (tricolpé, tricolporoïdé, tricolporé) peut changer.

2. Les variations de la taille et autres caractères qui peuvent changer suivant les conditions écologiques (cf. PESTOVA et MARTYNIUK, 1998).

3. Les altérations au cours de la sédimentation (cf. PRAGLOWSKI, 1966).

3.1. Effet de l'hydratation (cf.: WODEHOUSE, 1935, SOUTHWORTH, 1986, DUHOUX, 1972, 1982, KEDVES et al., 1999, KEDVES et al., 2000, etc.). Ici il y a lieu de mentionner à nouveau l'importance de l'étude des grains de pollen à sec sans aucune préparation.

3.2. Altérations dues à une température élevée: KIRCHHEIMER (1933a,b) a souligné les différents facteurs au cours de la sédimentation (haute température, pression activité enzymatique - microbiologique). WILSON (1961) a établi la relation entre la conservation des sporomorphes et le charbon fixé des couches houillères. MCINTYRE (1972) a souligné l'importance du charbon fixé par des études expérimentales. La maturité de la matière organique a été étudiée pour les recherches pétrolières. STAPLIN (1969) a récapitulé l'histoire du métamorphisme. Selon HUGHES, HARLAND et SMITH (1976) l'abondance et l'état de conservation des sporomorphes est en relation avec l'effet géothermique. MANUM et al. (1977) pense que les caractères diagénétiques des sporomorphes et des autres débris végétaux sont un indicateur excellent de la température pendant la maturation de la matière organique. WANG KAIFA, LI YIYIN et ZHANG HUIZHI (1991) ont établi que la dégradation thermique de la sporopollénine est importante dans la génération des hydrocarbures. POTONIÉ et REHNELT (1971) ont supposé une aromatisation de la sporopollénine au cours de la fossilisation. Selon PIÉRART (1978) une polymérisation supplémentaire se déroule au cours de la diagenèse. La couleur des sporomorphes (index de IAT) peut donner des indications pour la température de la sédimentation (cf. GUTJAHR, 1966, CORREIA, 1967, 1971, WILSON, 1971, SALAS et SEILER, 1980, STAPLIN 1977, TSCHUDY, 1969, GRAY et BOUCOT, 1975, MANUM et al. 1977, FREDERIKSEN, 1983, UTTING 1989, UTTING et al. 1989, CONWAY, 1994, etc.).

3.3. Des traitements différents peuvent également changer la morphologie des grains de pollen (FAEGRI et DEUSE, 1960, ANDERSEN, 1978).

Pour mieux comprendre les caractères morphologiques des sporomorphes fossiles il est souhaitable, de continuer des études expérimentales Depuis 1989 (KEDVES et KINCSEK) au cours de nos recherches sur les grains de pollen et spores actuels, nous avons fait un effort pour mieux documenter les altérations secondaires qui peuvent se produire au cours de la sédimentation.

Matériel et Méthode

Nous avons choisi les types polliniques qui sont importants du point de vue de l'évolution des grains de pollen des Angiospermes dans la région des Normapolles. Des Longiaxes: *Quercus robur* L., avec trois échantillons: 1. Localité: Újszeged et date de récolte 1992, 2. Localité: Újszeged et date de récolte 1996, 3. Jardin Botanique de l'Université et date de récolte 1996. *Quercus cerris* L. Localité: Jardin Botanique de l'Université et date de récolte 1995, *Salix alba* L. Localité: Jardin Botanique de l'Université et date de récolte 1992.

Des Bréviaxes: *Alnus glutinosa* (L.) GAERTN. Localité: Jardin Botanique et date de récolte 2000, *Betula verrucosa* EHRH. Localité: Jardin Botanique et date de récolte 1989, *Corylus colurna* L., Localité: Szeged et date de récolte: 2000, *Corylus avellana* L. Localité: Szeged et date de récolte 2000.

Grains de pollen frais et portés à la température de 200 degrés pendant 10 minutes, 1 heure, 5 et 10 heures, montés dans la glycérine-gélatine hydratée à 39,6% (LOBREAU, 1966), et étudiés au microscope optique. On a étudié les altérations qualitatives et quantitatives

Résultats

Longiaxes

Quercus robur L., *Qu. cerris* L., *Salix alba* L. Fig. 9.1,2, Planche 9.1, 1-24, planche 9.2, 1-20, planche 9.3, 1-14)

La distribution des pourcentages de grains de pollen en position polaire et équatoriale est présentée sur la figure 9.1. Les pourcentages des grains de pollen frais en position polaire (P) et en position équatoriale (E) sont à peu près identiques.

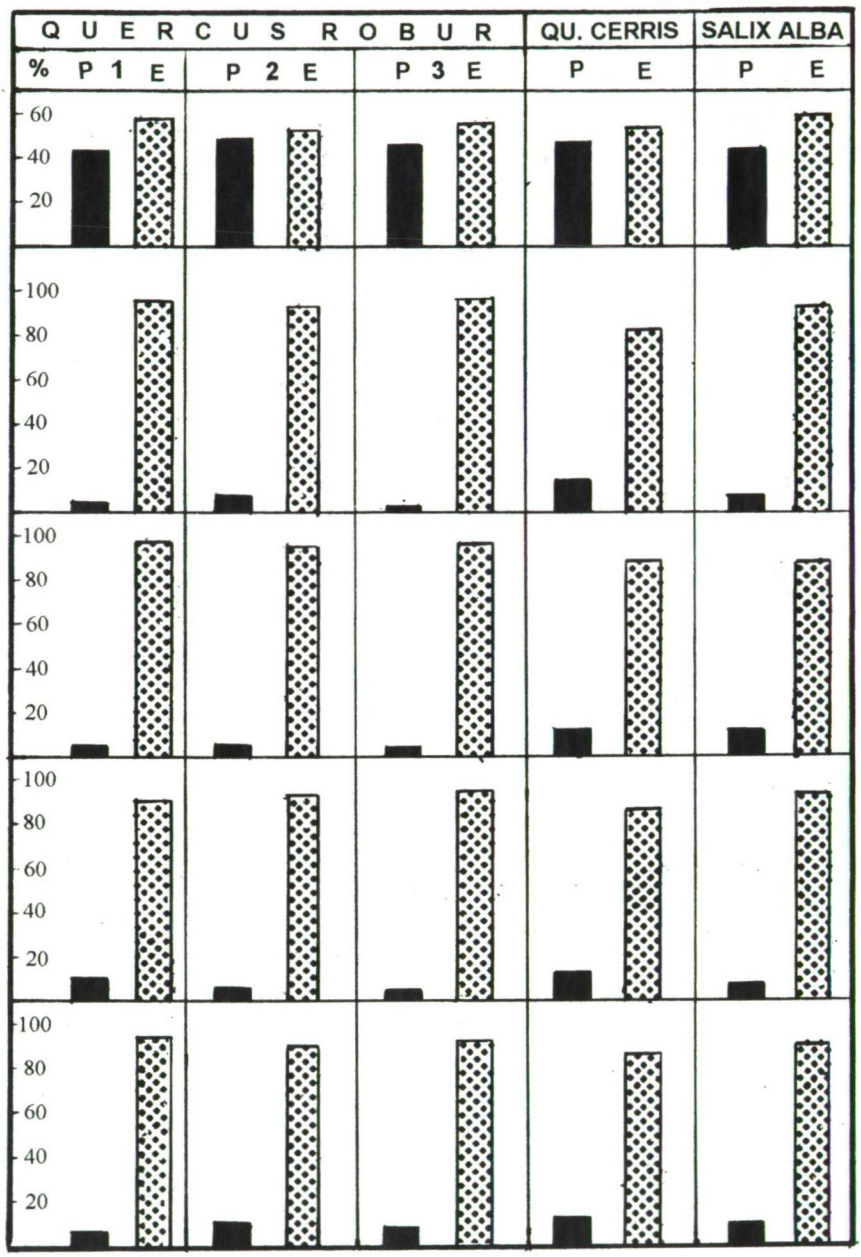

Fig. 9.1.

La distribution des grains de pollen en vue polaire et équatoriale des Longiaxes étudiés. En haut la distribution des pourcentages des grains de pollen frais, en bas ceux des grains de pollen chauffés pendant 10 minutes, 1, 5 et 10 heures.

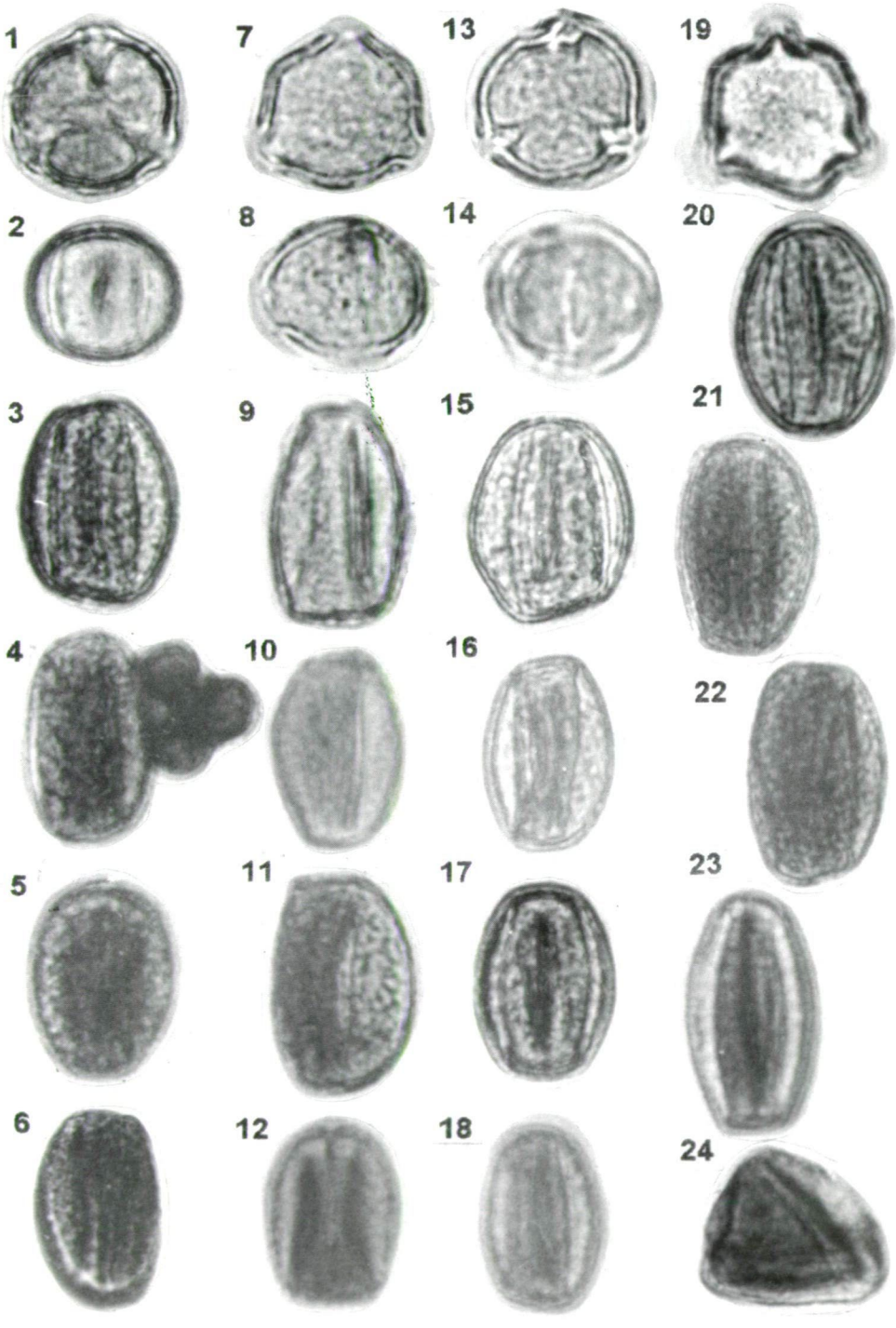

Planche 9.1.

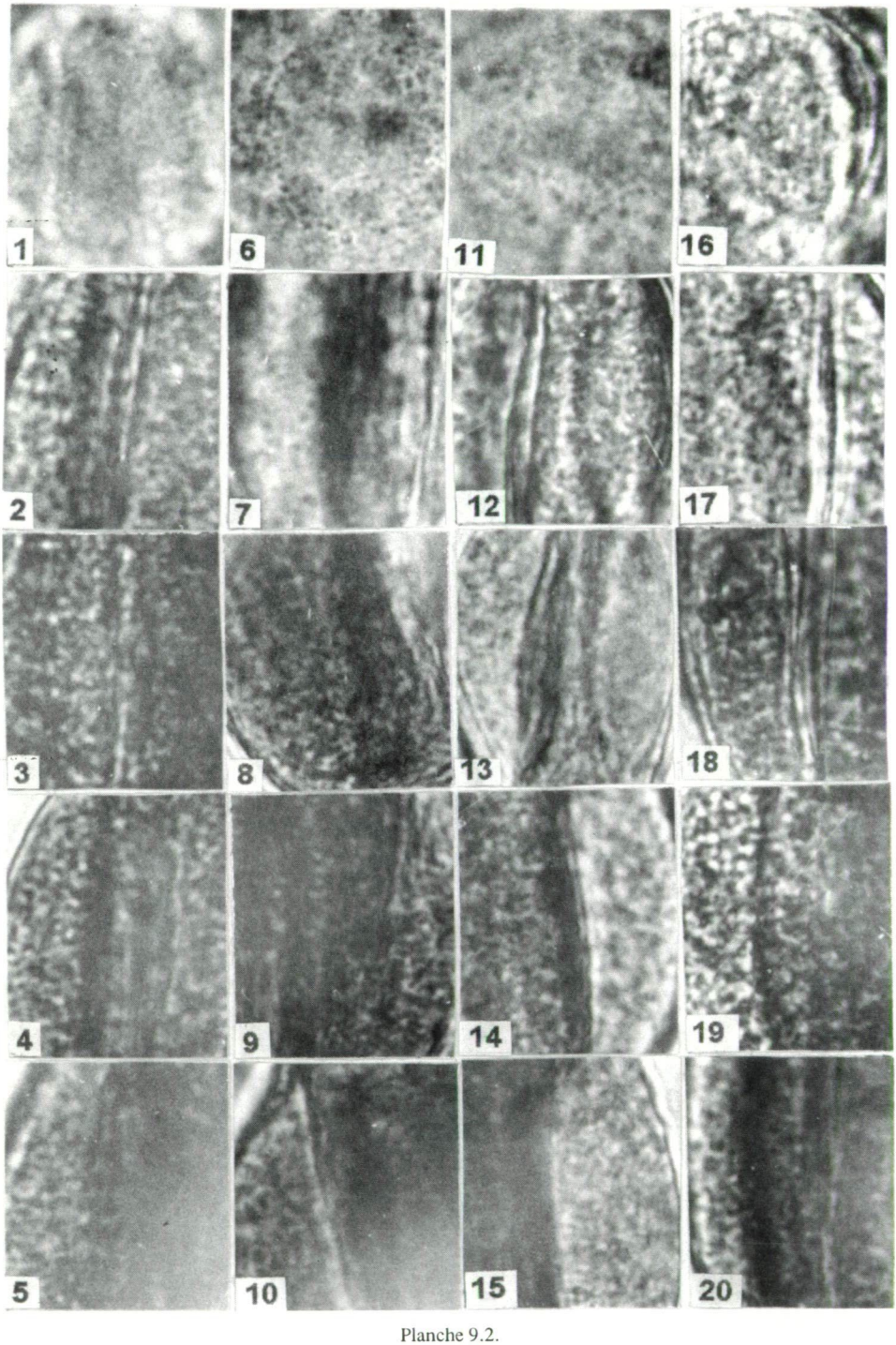

Planche 9.2.

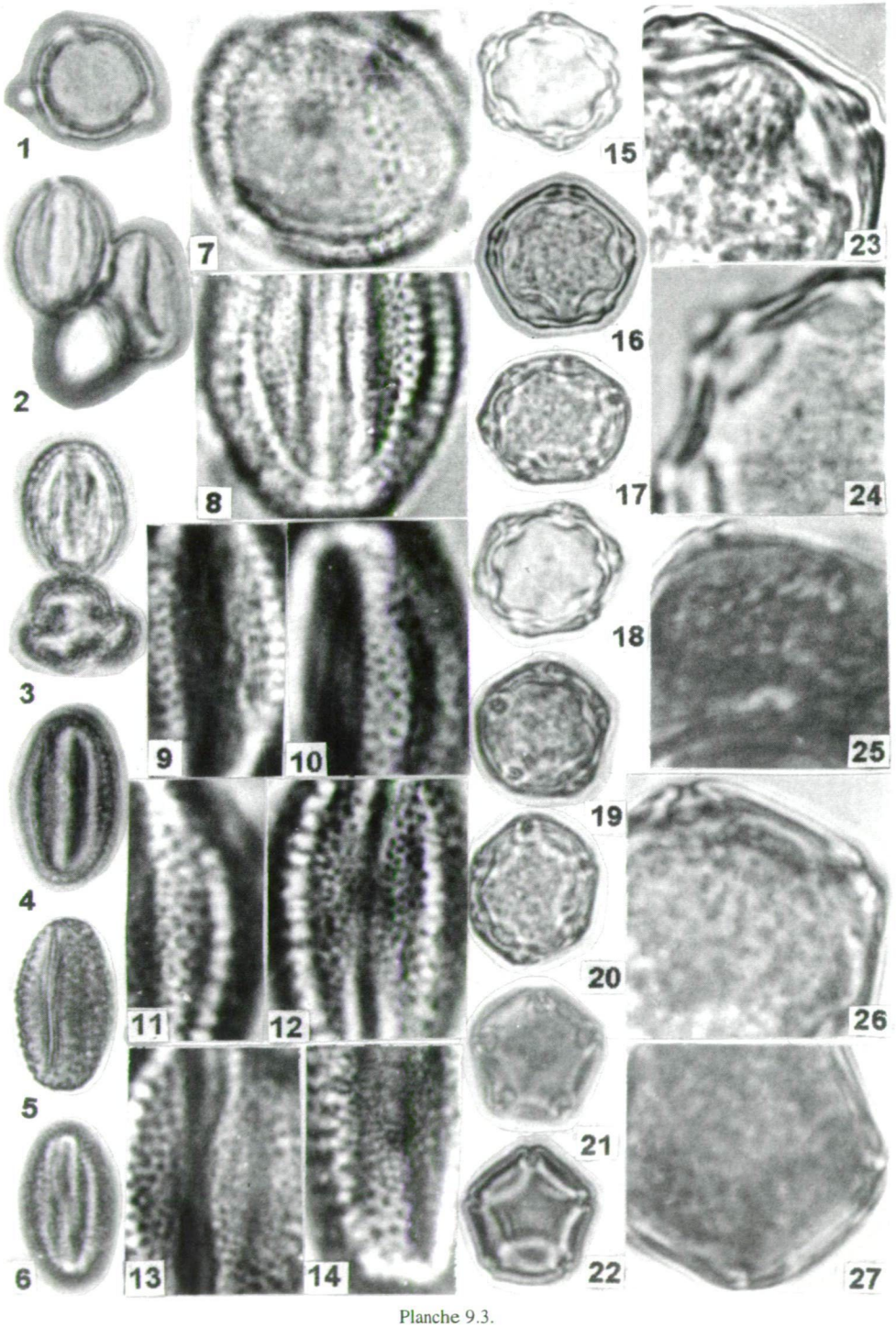

Planche 9.3.

Après un chauffage court de 10 mn, il y a un changement rapide dont le résultat est que les positons équatoriales sont dominantes quantitativement. Après un chauffage de 1, 5 et 10 heures la plupart des grains sont en vue équatoriale et les pourcentages sont plus ou moins constants. Les photos des pollens des échantillons du genre *Quercus* sont présentés sur la planche 9.1, fig. 1-24, celles de *Salix alba* sur la planche 9.3, fig. 1-6. Il est clair, qu'après un chauffage, ce qui change essentiellement est la symétrie du grain de pollen. Mais en ce qui concerne l'ornementation des grains de pollen étudiés nous n'avons pas trouvé d'altérations remarquables causées par une température élevée (Planche 9.2, fig. 1-20, planche 9.3, fig. 7-14). Nous avons calculé les valeurs des axes polaire/équatorial (P/E) et figuré les distributions et la quantité dominante de ces valeurs (Figure 9.2). Nous avons trouvé des différences parmi les différents échantillons de *Quercus robur*, de plus les échantillons de *Quercus cerris* diffèrent de ceux de *Quercus robur*. Mais les grains de pollen frais de *Salix alba* sont ressemblants de ce point de vue à l'échantillon 3 de *Quercus robur*. Les courbes statistiques de variation des diamères sont représentées sur la fig. 9.3. Les valeurs des grains de pollen frais sont totalement différentes de celles des grains de pollen soumis aux expériences.. Les échantillons 2 et 3 sont semblables des localitées différentes, mais pour la même date de prélévement. *Salix alba* ressemble un peu à *Quercus robur* 2 et 3, c'est curieux étant donné que la date du prélévement est la même que celle de l'échantillon 1.

Légende de la Planche 9.1.

Fig. 1-18. *Quercus robur* L., 1-6. Échantillon 1, 7-12. Échantillon 2, 13-16. Échantillon 3. 1, 2, 7, 8, 13, 14. grains de pollen frais, 3,9,15. durée du chauffage: 10 minutes, 4,10,16. durée du chauffage: 1 heure, 5,11,17. durée du chauffage: 5 heures, 6, 12, 18. durée du chauffage: 10 heures.

Fig. 19-24. *Quercus cerris* L., 19. grain de pollen frais, 20. grain de pollen chauffé pendant 10 minutes, 21. grain de pollen chauffé pendant 1 heure, 22. grain de pollen chauffé pendant 5 heures, 23,24. grain de pollen chauffé pendant 10 heures. Toutes les photos sont au grossissement 1000.

Légende de la Planche 9.2.

Fig. 1-15. *Quercus robur* L., 1-5. Échantillon 1, 6-10. Échantillon 2, 11-15. Échantillon 3.

Fig. 16-20. *Quercus cerris* L.

1,6,11,16. Grains de pollen frais, 2,7,12,17. Grains de pollen chauffés pendant 10 minutes, 3,8,13,18. Grains de pollen chauffés pendant 1 heure, 4,9,14,19. Grains de pollen chauffés pendant 5 heures, 5,10,15,20. Grains de pollen chauffés pendant 10 heures. Toutes les photos sont au grossissement 2.500.

Légende de la Planche 9.3.

Fig. 1-14. *Salix alba* L., 1,2,7. Grains de pollen frais, 3,8. Grains de pollen chauffés pendant 10 minutes, 4,9,10. Grains de pollen chauffés pendant 1 heure, 5,11,12. Grains de pollen chauffés pendant 5 heures, 6, 13,14. Grains de pollen chauffés pendant 10 heures.

Fig. 15-27. *Alnus glutinosa* (L.) GAERTN., 15,16,23. Grains de pollen frais, 17,18.24. Grains de pollen chauffés pendant 10 minutes, 19,20,25. Grains de pollen chauffés pendant 1 heure, 21,26. Grains de pollen chauffés pendant 5 heures, 22,27. Grains de pollen chauffés pendant 10 heures. Fig. 1-6,15-22 sont au grossissement 1000, 7-14, 23-27 sont au grossissement 2.500.

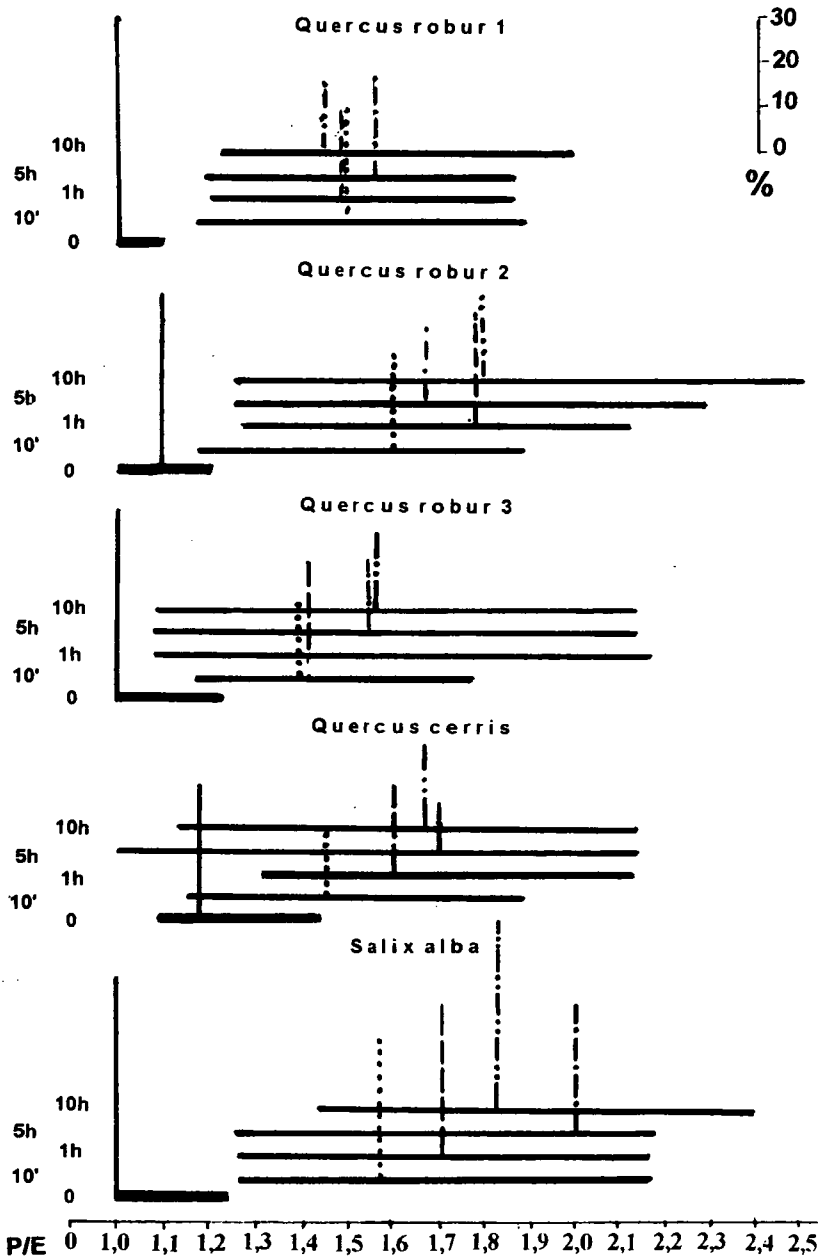

Fig. 9.2.

Les valeurs du rapport des axes polaire/équatorial, des grains de pollen des Longiaxes étudiés, les distributions des valeurs et les pourcentages maximaux sont représentés.

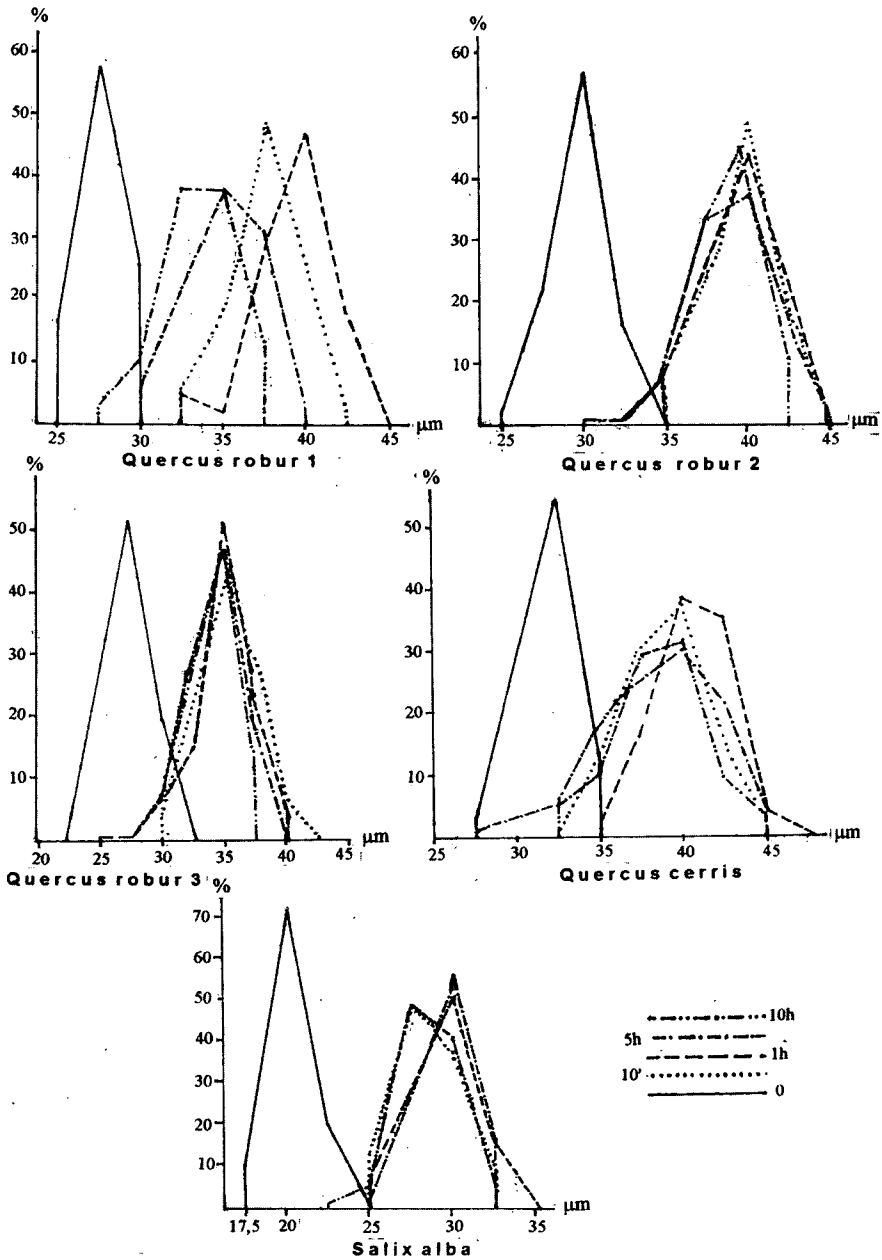

Fig. 9.3.

Courbes statistiques de l'axe polaire des grains de pollen Longiaxes étudiés.

Bréviaxes

Alnus glutinosa (L.) GAERTN. (Planche 9.3, fig. 15-27, fig. 9.4)

Dans les caractères qualitatifs, nous n'avons pas trouvé de différences importantes.

Les variations du diamètre sont les suivantes. après chauffage court, presque pas de changement, ensuite les courbes statistiques sont différentes. Il est à noter que 2 courbes (1 et 10 h) ont une certaine ressemblance. La courbe statistique des grains de pollen chauffés pendant 5 heures, ressemble aux courbes de grains de pollen frais, et chauffés pendant 10 mn. Il y a une diminution de taille.

Betula verrucosa EHRH. (Planche 9.4, fig. 1-5, fig. 9.4)

On a pu observer des altérations qualitatives qui sont sont des caractères anciens (Planche 9.4, fig. 3-5). Les plicae secondaires sont caractéristiques sans doute des grains de pollen du Crétacé supérieur et du Tertiaire inférieur dans la région des Normapolles. Les altérations du diamètre des grains de pollen sont intéressantes (Fig. 9.4). La courbe des grains de pollen frais a un maximum remarquable, et les dimensions minimale et maximale ne sont pas trop éloignées. Particulièrement intéressant est le changement après 10 mn, il n'y a pas un maximum frappant. Les valeurs maximales des courbes de grains de pollen chauffés 5 à10 heures sont faibles. Une heure de chauffage a causé une certaine augmentation du diamètre.

Corylus avellana L. (Planche 9.4, fig. 6-10, fig. 9.4)

Les altérations qualitatives, les formes secondaires avec plicae sont apparues après une heure de chauffage (Planche 9.4, fig 8), mais remarquables après 5 heures du traitement (Planche 9.4, fig. 8). Nous avons observé des formes secondaires avec oculi (Planche 9.4, fig. 10), ce caractère est aussi ancien, voir les espèces du genre de forme *Oculopollis* du Crétacé supérieur et du Tertiaire inférieur de la région des Normapolles. En ce qui concerne le diamètre de ces pollen grains, il n'y a pas de grande différence entre les courbes statistiques (Fig. 9.4).

Corylus colurna L. (Planche 9.4, fig. 11-15, fig. 9.4)

Les altérations qualitatives ne sont pas si frappantes que chez l'espèce précédente. Il faut cependant remarquer qu'il y a des différences notables d'altération de diamètre entre les deux espèces de *Corylus* (Fig. 9.4). Une diminution de taille plus ou moins régulière a été observée au cours de nos études.

Conclusions

Les expériences à haute température de 200 °C sont convenables pour modéliser les altérations des sporomorphes des sédiments nettement métamorphiques. Suivant les résultats obtenus jusqu'ici, on peut tirer les conclusions suivantes:

Légende de la Planche 9.4.

Fig. 1-5. *Betula verrucosa* EHRH., 6-10. *Corylus avellana* L., 11-15. *Corylus colurna* L.
1,6,11. Grains de pollen frais, 2,7,12. Grains de pollen chauffés pendant 10 minutes, 3,8,13. Grains de pollen chauffés pendant 1 heure, 4,9,14. Grains de pollen chauffés pendant 5 heures, 5,10,15. Grains de pollen chauffés pendant 10 heures. Toutes les photos sont au grossisement 2.500.

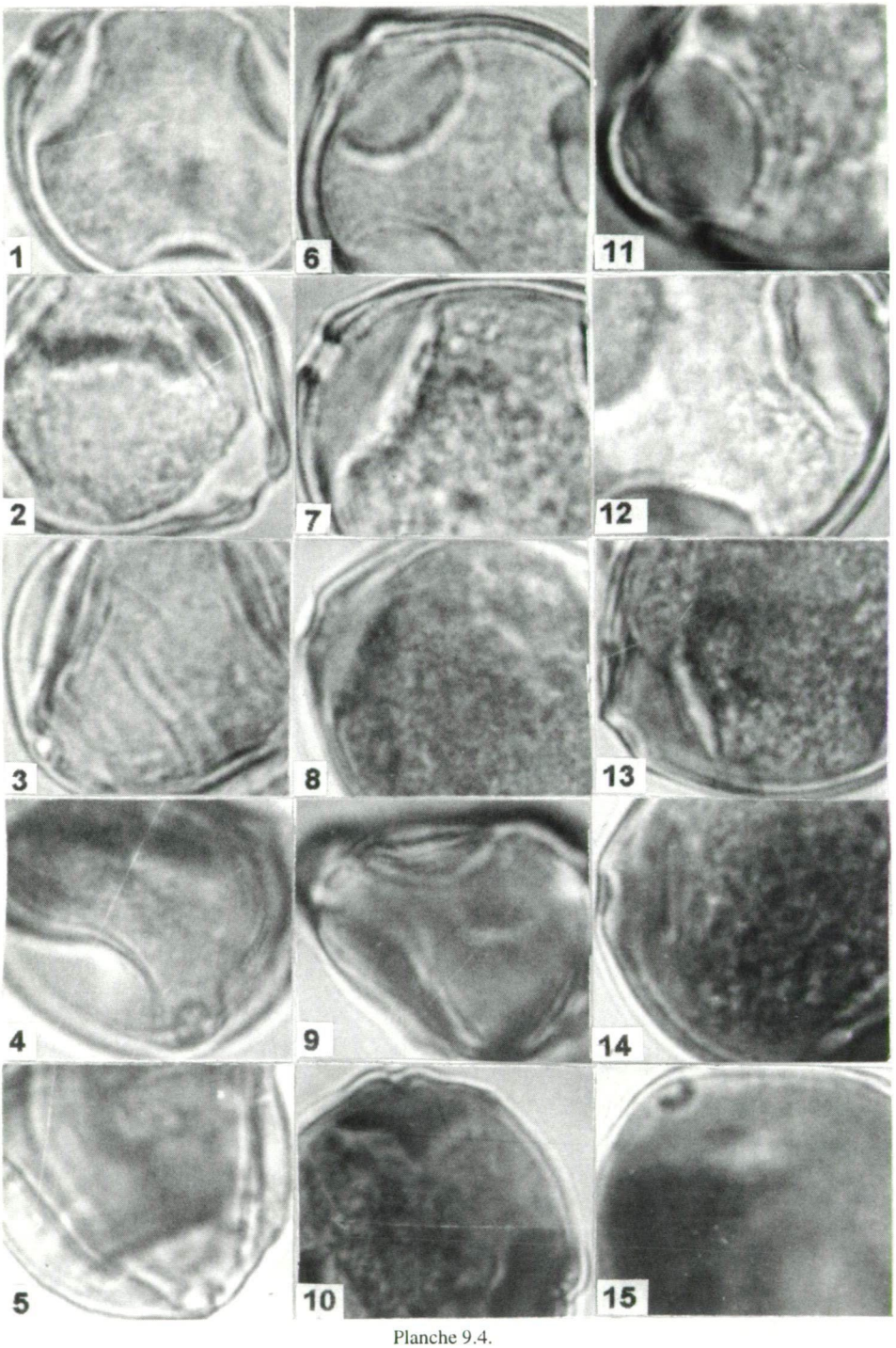

Planche 9.4.

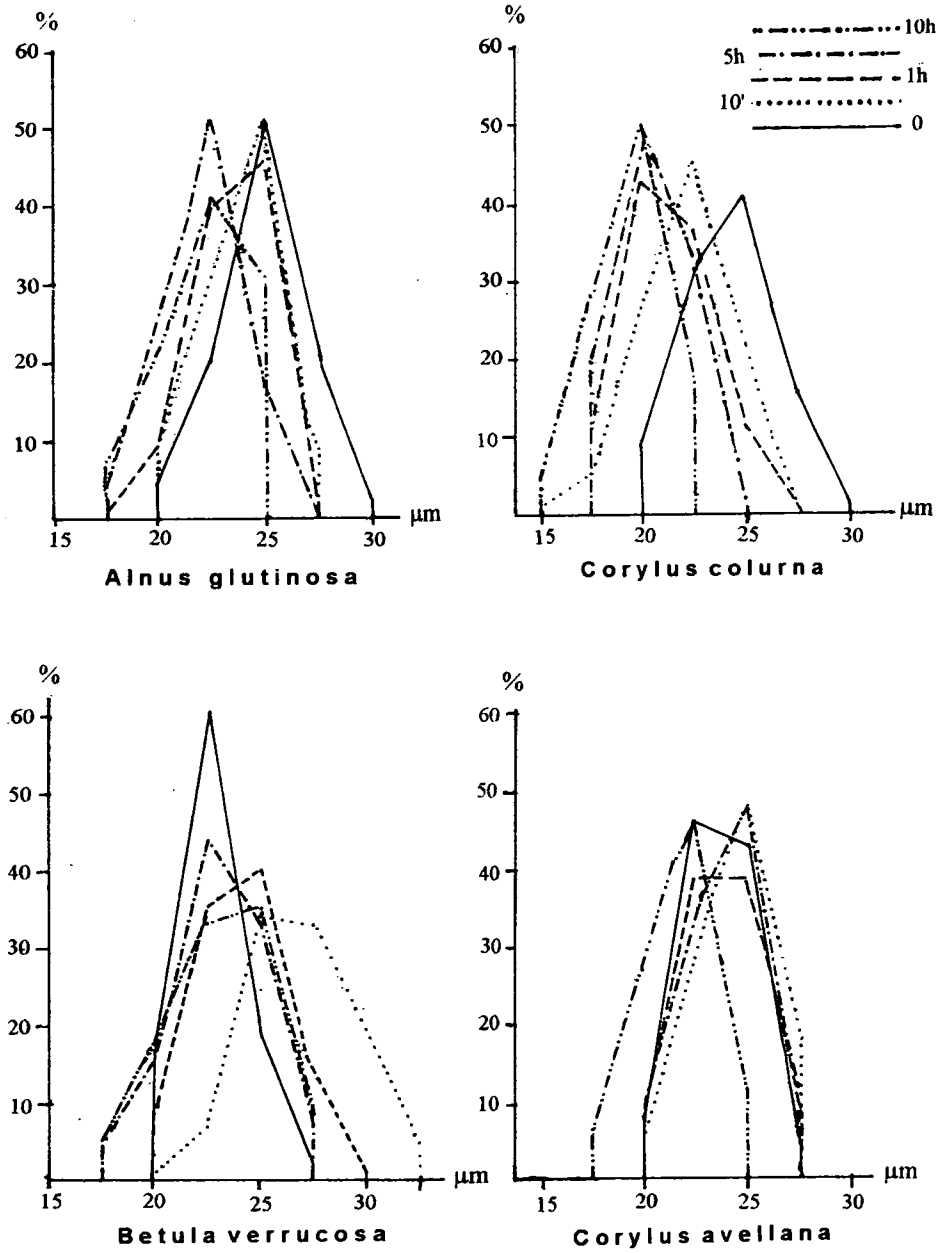

Fig. 9.4.

Les courbes statistiques du diamètre des grains de pollen des Bréviaxes étudiés.

1. Le rapport de l'axe polaire et équatorial change rapidement chez les grains de pollen Longiaxes étudiés en fonction de la hausse de température. Ceci vaut peut-être pour tous les grains de pollen tricolpés - tricolporoïdés ou tricolporés.

2. Les conditions écologiques sont importantes, mais les altérations qui se produisent après le prélèvement sont également à prendre en compte Ceci se répercute sur le système moléculaire de la sporopollénine Nous avons continué plusieurs expérimences dans ce domaine, la plus récente concernant les grains de pollen du *Quercus robur* a été publiée par KEDVES, PÁRDUTZ et VARGA (2002).

3. Nos nouveaux documents soulignent toujours la complexité du système moléculaire de la paroi pollinique. L'évaluation des résultats de différentes expériences sur les grains de pollen d'une même espèce ammènera à des conclusions valables sur le sujet. On pourra, par exemple, établir que la sporopollenine des grains de pollen de *Quercus* est peu résistante. Une résistance importante peut par contre être établie chez les grains de pollen de *Juglans.*

4. Les altérations secondaires dans la morphologie générale des sporomorphes et la résistance du système moléculaire de la paroi pollinqiue doivent être prises en compte au cours des études des associations sporopolliniques fossiles (fossilisation sélective). En étudiant les spores et les grains de pollen allergènes nous pouvons également déduire que la solubilité de la paroi externe est un facteur de l'efficacité allergénique.

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10. ULTRASTRUCTURE OF THE PARTIALLY DEGRADED POLLEN GRAINS OF CORYLUS AVELLANA L. WITH 2-AMINOETHANOL AND C60 FULLERENE/BENZOL SOLUTION

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Abstract

Pollen grains of *Corylus avellana* were partially degraded with 2-aminoethanol for 30 minutes, 1, 5, 10 and 24 hours and the treatment was continued with 5 ml C60 fullerene/benzol solution and 5 ml pure benzol for 5 days. The transmission electronmicroscopical results are as follows: 1. The ectexine and the damaged protoplasm was contrasted by the C60 fullerene without any usual fixation and postfixation processes. 2. The accumulation of the fullerene is not the same on the different parts of the ectexine. 3. Some experiments revealed the molecular system of the ectexine. Mostly cyclic molecules were observed. Clusters of cyclic molecules but sometimes peculiar linear arrangement was observed. 4. Degradation or dissolution of large biomacromolecules were also established.

Key words: Experimental Palynology, recent, *Corylus avellana*, TEM.

Introduction

Pollen grains of the genus *Corylus* are important in several points of view, such as evolutionary vegetation history (cf. RICH, 1988, MAGYARI, 2002, FOMBELLA BLANCO et al., 2003) and as allergenic elements. Based on the work of MOLNÁR (1999) the following papers are important in the allergenic character of the pollen grains of the genus *Corylus:* SAUMANDE et al. (1980), ERIKSSON (1978), DALEN and VOORSHORST (1981) and CROSTA et al. (1996). Pollen grains of the genus *Corylus* or *C. avellana* are published in a number of aeropalynological papers, some selected ones are: SAUMANDE et al. (1980), RICHARD et al. (1986), BOREL and BRIOUDE (1986), DE LEONARDIS et al. (1986), TYCZKA (1986), SPIEKSMA et al. (1986), ADO et al. (1986), BOEHM and LEUSCHNER (1989, 1991), LEUSCHNER (1989), NILSSON (1990), BOEHM (1991), JÁRAI-KOMLÓDI (1991), JÁRAI-KOMLÓDI and MEDZIHRADSZKY (1993), PEHLIVAN (1995), CAULTON et al. (2001), SUÁREZ PEREZ et al. (2002).

KEDVES and PÁRDUTZ (1973) published the ultrastructure of the pollen grains of three species of the genus *Corylus* (*C. avellana* L., *C. colurna* L. and *C. sieboldiana* BLUME). Scheme of the ultrastructure in the apertural area of *C. colurna* was published. Thick, channeled tectum, surface with tiny spinae (coni), granular and columellar infratectal layer, relatively thin foot layer and characteristic lamellar endexine in the apertural area. Different kind of experimental studies were carried out on the pollen grains of *C. avellana* L., KEDVES (1986, 1987, 1988), KEDVES and KINCSEK (1989). Using the TEM method in partially degraded exine biomacromolecular structures were published.

The previous attempts in which the C60 fullerene/benzol solution was used in the partial degradation of the biopolymer systems of the plant cell wall were succesful. The first results were summarized by KEDVES (2001/2002) and several new results are included in the present volume also.

The aim of this paper is to obtain new data on the partially degraded exine of the pollen grains of *C. avellana* in comparison with the previous experimental results.

Materials and Methods

The pollen material was collected by Dr. É. SIPOS-KEDVES on April 3, 2002, in her garden. 2 ml 2-aminoethanol were added to 5 mg dry pollen grain. Temperature: 30 °C. Lengths of time: 30 minutes (T-12-439), 1 hour (T-12-440), 5 hours (T-12-441), 10 hours (T-12-442), 24 hours (T-12-443). After washing and drying, 5 ml C60 fullerene/benzol solution were added to the degraded pollen material for 5 days. Washing with pure benzol and drying was followed by embedding in Araldite (Durcupan, Fluka). The ultrathin sections were made with glass knives on a Porter Blum ultramicrotome. The pictures were taken in the EM Laboratory of the Institute of Biophysics of the Biological Center of the Hungarian Academy of Sciences on a Tesla BS 540 (resoluion 6-7 Å) and a Zeiss Opton EM-902 instrument, resolution 2-3 Å. All pictures are unretouched.

Results

Experiment: T-12-439 (Plate 10.1., figs. 1-3)

The characteristic channels of the tectum disappeared. The tiny spinae (coni) of the tectum and sometimes the outermost part of the tectum accepted the fullerene better than the outer part of the ectexine (Plate 10.1., fig. 1). The highly magnified pictures of the infratectal layer illustrate well the peculiar ultrastructure of this layer and the differential acceptance of the fullerene by the different parts within this layer.

Experiment: T-12-440 (Plate 10.1., figs. 4-7)

The general survey picture of the ultrastructure of the ectexine is more or less identical with the previous experiment (Plate 10.1., fig. 4). The highly magnified pictures taken with high resolution power instrument (Plate 10.1., figs. 5-7) resulted in the following: 1. Differential accumulation of the fullerene in different parts of the infratectal layer (Plate 10.1., fig. 5) is similar to the previous experiment (Plate 10.1, fig. 3). 2. Pictures of high magnification (Plate 10.1., figs. 6,7) illustrate the biomacromolecular system of the ectexine near to the infratectal layer. Based on these results, the molecular system is composed of cyclic molecules of different arrangements. Linear arrangement of the cyclic molecular system is illustrated in picture 7 of Plate 10.1.

Experiment: T-12-441 (Plate 10.1., figs. 8-12)

The general survey picture of the ultrastructure of the pollen grain illustrates the accumulation of the fullerene in the degraded protoplasm (Plate 10.1., fig. 8). In picture 9 of Plate 10.1. the highly organized globular macromolecules are shown. These dark globular units are in the infratectal and the foot layer. In the highly magnified pictures (Plate 10.1., figs. 10-12) the details are much clearer. There are globular or anastomosing irregular light holes particularly in the foot layer (Plate 10.1., fig. 11). In the highly magnified picture (Plate 10.1., fig. 12) the molecular system can also be observed.

Experiment: T-12-442 (Plate 10.2., figs. 1-3)

In general, the degradation of the ectexine is well shown. In some parts of the ultrathin section beneath the foot layer a light layer, the endexine, without any structure is perceptable (Plate 10.2., fig. 1). The molecular system without any highly organized unit is shown in picture 2 of Plate 10.2.

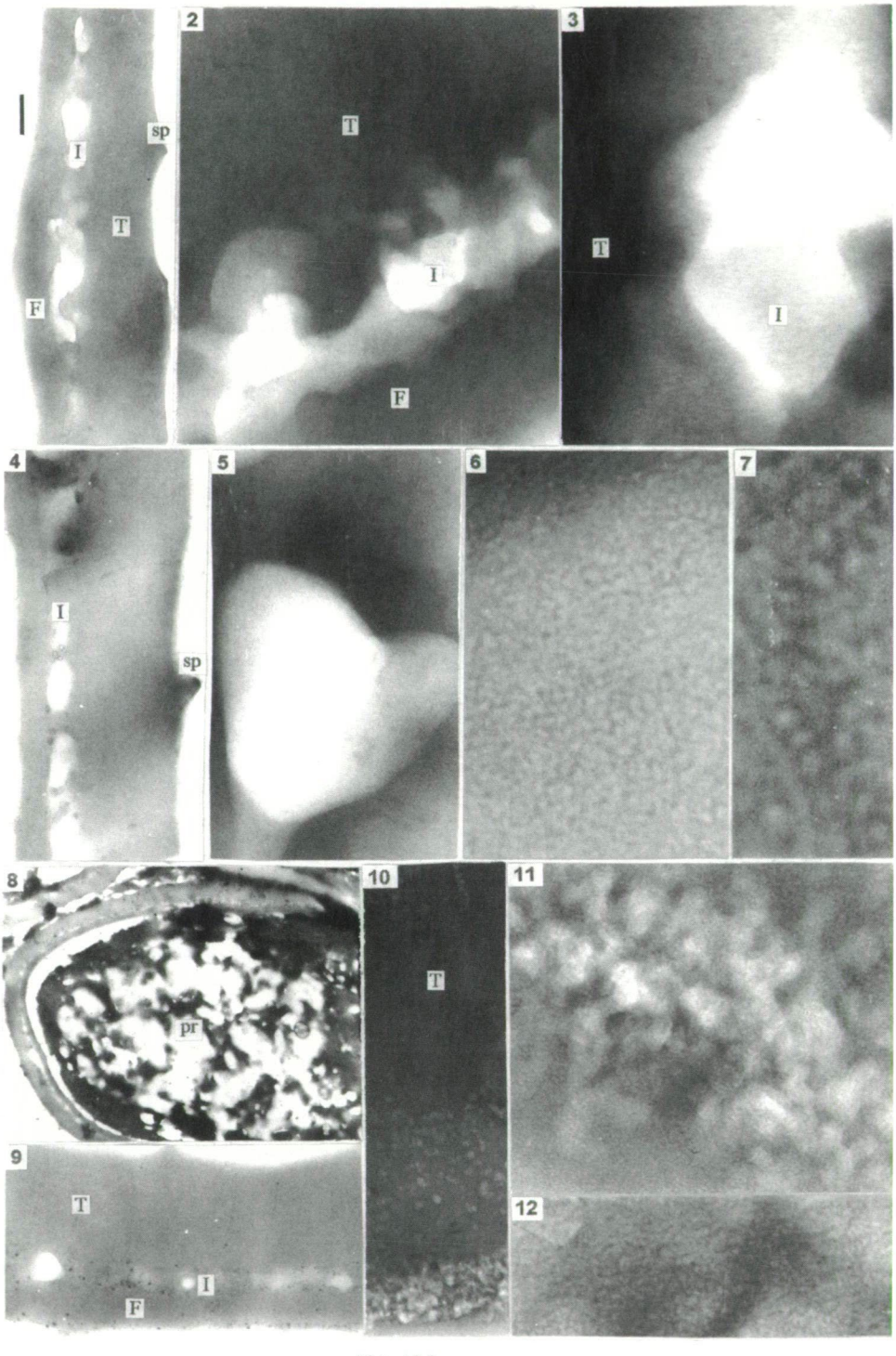

Plate 10.1.

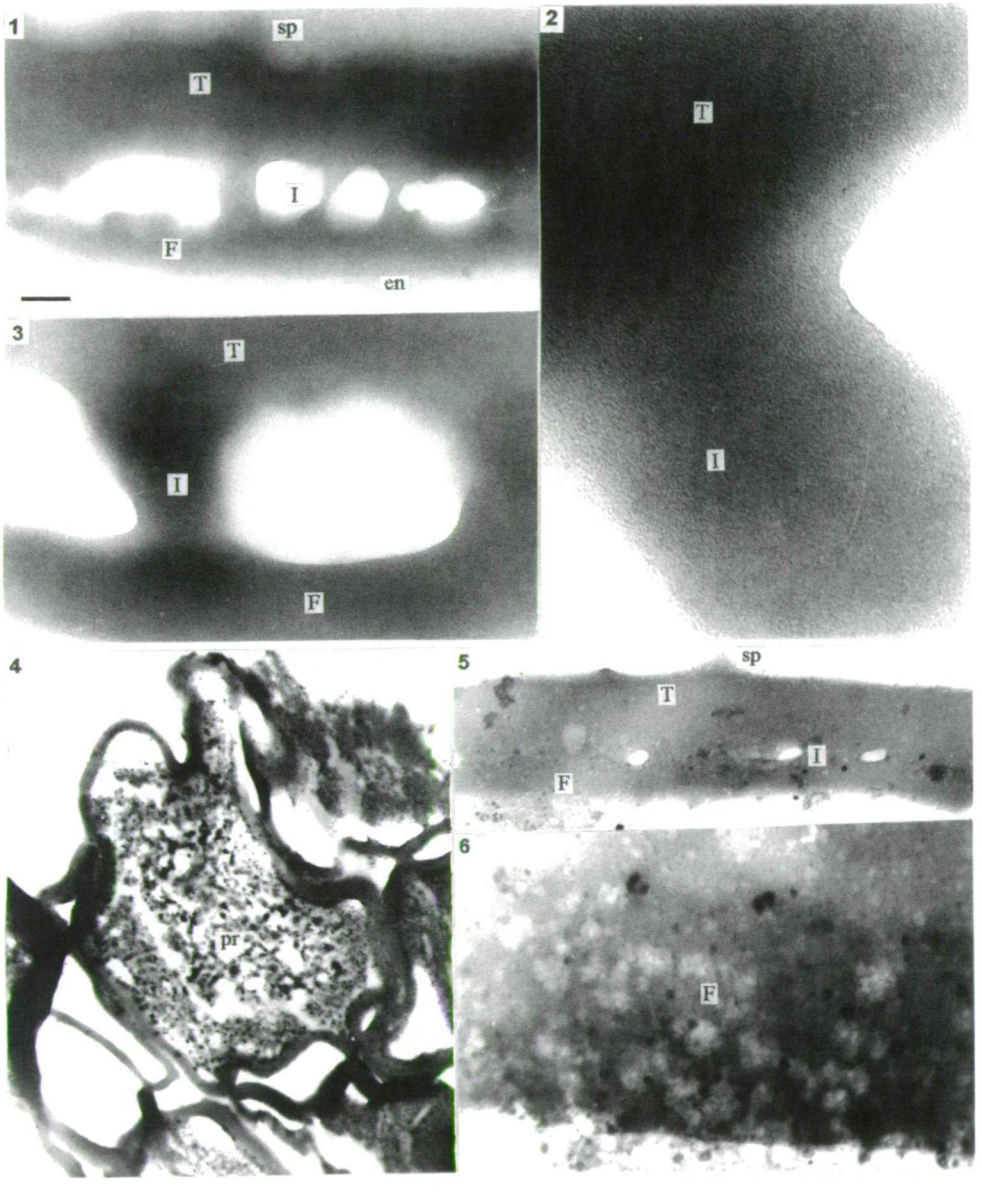

Plate 10.2.

Plate 10.1.

1-12. *Corylus avellana* L. ultrastructure of partially degraded pollen grains with 2-aminoethanol and with C60 fullerene/benzol solution. Duration of the treatment with C60 fullerene/benzol solution was always 5 days.
1-3. Experiment No.: T-12-439, treatment with 2-aminoethanol for 30 minutes. 1. Negative No.: 10200, 2. Negative No.: 14123, 3. Negative No.: 14125.
4-7. Experiment No.: T-12-440, treatment with 2-aminoethanol for 1 hour. 4. Negative No.: 10347, 5. Negative No.: 14176, 6,7. Negative No.: 14179.
8-12. Experiment No.: T-12-441, treatment with 2-aminoethanol for 5 hours. 8. Negative No.: 10348, 9. Negative No.: 10378, 10. Negative No.: 10180, 11. Negative No.: 14182, 12. Negative No.: 14183.
Bar scale: figs. 1,4,9: 0.2 μm, figs. 2,5,10: 0.06 μm, figs. 3,11: 0.02 μm, figs. 6,12: 0.01 μm, fig. 7: 0.004 μm, fig. 8: 2 μm.
T = tectum, I = infratectum, F = foot layer, sp = spine, pr = protoplasm.

Plate 10.2.

1-6. *Corylus avellana* L. ulrastructure of partially degraded pollen grains with 2-aminoethanol and with C60 fullerene/benzol solution. Duration of the treatment with C60 fullerene/benzol solution was always 5 days.
1-3. Experiment No.: T-12-442, treatment with 2-aminoethanol for 10 hours. 1. Negative No.: 10381, 2. Negative No.: 14204, 3. Negative No.: 14202.
4-6. Experiment No.: T-12-443, treatment with 2-aminoethanol for 24 hours. 4. Negative No.: 10357, 5. Negative No.: 10361, 6. Negative No.: 14172.
Bar scale: figs. 1,5: 0.2 μm, fig. 2: 0.02μm, figs. 3,6: 0.06 μm, fig. 4: 2 μm.
T = tectum, I = infratectum, F = foot layer, sp = spine, en = endexine, pr = protoplasm.

Experiment: T-12-443 (Plate 10.2., figs. 4-6)

The general survey picture illustrates well the degraded protoplasm with dark microbodies and the dark pollen wall, the ectexine (Plate 10.2., fig. 4). The degradation of the ectexine, particularly of the foot layer, is well shown (Plate 10.2., fig. 6).

Discussion and Conclusions

Considering the previous partial degradation experimental results we can point out the following:

1. During these experiments the C60 fullerene/benzol solution was enough to contrast the ectexine and the degraded protoplasm also.

2. The differential acceptance of the fullerene in the tiny spinae (coni) and the outer part of the tectum was observed at the first two experiment (T-12-439 and 440). It is worth noting the disappearance of the tectum channels.

3. The trend of the partial degradation is not linear. Similarities between the results of T-12-439 and T-12-442 may be emphasized. The degradation of the ectexine was nearly the same as in experiment T-12-441 and T-12-443.

4. The experiment T-12-440 was the most suitable in the discovery of the molecular system of the sporopollenin of the ectexine. The different kind of moleular patterns are important to understand the complexity of this biomacromolecular structure.

5. There are some similarities between these results and those obtained in the ectexine of *Malva sylvestris* L., (KEDVES et al., 2004) published in this volume.

Finally the experimental investigation of the plant biomacromolecular systems have several perspectives and opportunities in the future.

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11. SYMMETRY OPERATIONS ON THE BIOPOLYMER UNITS OF THE PARTIALLY DEGRADED EXINE OF PHOENIX DACTYLIFERA L.

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Abstract

Symmetry operations were carried out on two regular pentagonal and a hexagonal biomacromolecular units of the partially degraded ectexine of *Phoenix dactylifera* L. At one of the regular pentagons the tenfold rotation resulted in unusual and completely new results. In two circles two times ten regular pentagons appeared around the rotation center. This phenomenon may be modelled with a three dimensional quasicrystalloid skeleton. The inner globular units of the outer regular pentagonal circle has six connections with the other globular biopolymer units. At the hexagon the incomplete rotation methods were also used and some methodical alterations were introduced.

Key words: Biopolymer symmetry operations, *Phoenix dactylifera*, ectexine.

Introduction

In a previous paper (KEDVES, PÁRDUTZ et al., 2003) the ultrastructure of the partially degraded pollen grains of *Phoenix dactylifera* L. was published. Experiment with 2-aminoethanol for 48 hours and with $KMnO_4$ for 24 hours revealed regular pentagonal biopolymer units. In this paper it is pointed out that these regular pentagons are smaller than those observed in the partially degraded ectexine of *Phoenix sylvestris* L. (KEDVES et al., 2001). We investigated again the TEM negatives taken with high resolution power and we planned to carry out symmetry operations on the two regular pentagons. During our new investigations we observed a regular hexagon also. This kind of biomacromolecular unit observed and discussed previously may be:

1. TICOS polyhedra established by BURSILL and PENG JU LIN (1985) which was demonstrated previously in the partially degraded ectexine of *Pinus griffithii* (KEDVES, 1991b).
2. Regular hexagon without any connections (cf. KEDVES, 1990a, 1991a).
3. Hexagon connected to a regular pentagon as a fragment of a biopolymer structure which may be modelled with fullerenes (KEDVES, BÉRES et al., 2003).

The aim of this paper is to investigate these biopolymer units with the two dimensional rotation method and to obtain new data on this field of research.

Materials and Methods

The Negative No.: 10529 was used for these symmetry operations which were previously published in P.C.B.D. vol.15, p. 87. For the regular pentagon we started with fivefold and tenfold rotation methods. For

the hexagon sixfold, twelvefold and two kinds of incomplete rotation were used. At the incomplete rotations, in contrast with our previous publications, the number of the biopolymer units are indicated in index.

The most important methods for the investigations of the quasi-crystalloid skeleton are as follows: 1. Two dimensional, the modified Markham (MARKHAM et al., 1963) rotation method 1.1. Rotation of a regular pentagon in nm dimension, ROWLEY (1967) 1.2. Rotation at the intermediate diameter of the nm and Å dimension, FLYNN and ROWLEY (1971) 1.3. Rotation in Å dimension (KEDVES, 1988a,b, 1989a,b, 1990a,b, KEDVES et al., 1991, KEDVES and FARKAS, 1991, KEDVES et al., 1992, KEDVES and PÁRDUTZ, 1993, KEDVES, TÓTH and GOTTL, 1994, KEDVES and TÓTH, 1994, KEDVES, TÓTH and VÉR, 1995, KEDVES et al., 1998, KEDVES and BORBOLA, 1998, KEDVES, PÁRDUTZ and MADARÁSZ, 2000, KEDVES et al., 2001, KEDVES, SASHALMI and SZÉCSÉNYI, 2002, KEDVES, BÉRES et al., 2003, KEDVES and JACSÓ 2003, KEDVES, PÁRDUTZ et al., 2003, KEDVES, PRISKIN et al., 2003). 2. Three dimensional modelling (KEDVES, 1991c, 1992, KEDVES, TÓTH and FARKAS, 1993). 3. Computer modelling (KEDVES, M. and KEDVES, L., 1995, 1996, 1997, 1999).

Results

Plate 11.1., fig. 5 illustrates the biopolymer units of the partially degraded ectexine. Well shown are a number of biopolymer structures, we chose two regular pentagons (A and B) and a hexagon (H) for symmetry operarations.

Biopolymer A (Plate 11.1., figs. 1,2, plate 11.2.)

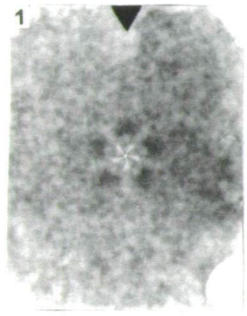
The fivefold rotation reinforced the globular biopolymer units of the regular pentagon and in the same time varified the regularity of the biopolymer structure. A not so characteristic rotation area appeared. The tenfold rotation resulted in a large, well defined rotation area and several points of symmetry. In picture magnified for 5 million the peculiarities of this kind of rotation are well illustrated. Around the rotation center there are several dark points of symmetry which are arranged to form regular pentagons. Two circles of ten regular pentagons were established. These may be the components of the quasi-crystalloid skeleton. The inner globular biopolymer units of the outer pentagons are connected with six other units making connection with both the inner and the outer pentagonal units.

Biopolymer B (Plate 11.1., figs. 3,6)

The fivefold rotation reinforced the regular pentagon and resulted in a characteristic rotation area of pentagonal form. Several secondary points of symmetry appeared at this method also. Five relatively large biopolymer units form a large pentagon around the original biopolymer structure. At the border of the rotation area there are five groups of biopolymer units which may be a pentagon-dodecahedrane unit. After tenfold rotation (Plate 11.1., fig. 6) around the rotation center, a characteristic circle of dark globular biopolymer units appeared which may be useful for further secondary rotations. The border of the rotation area is characteristic.

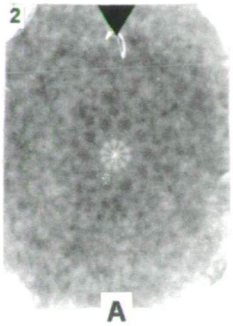
Biopolymer H (Plate 11.1., figs. 4, 7-9)

The sixfold rotation resulted in characteristic rotation area composed of two borders which may be the components of a bordering three dimensional pattern (Plate 11.1., fig. 4). There are six characteristic globular biopolymer units at the inner bordering area which may be useful for further secondary symmetry operations. The twelvefold rotations (Plate 11.1., fig. 7) resulted in several secondary points of symmetry around the rotation center and a characteristic rotation area. The dimensions of the dark globular biopolymer units are different. The twelve globular biopolymer units of the outermost circle are the largest. Different biopolymer networks are shown around the rotation area (Plate 11.1., fig. 7). Incomplete rotations (Plate 11.1., figs. 8,9) show several globular biopolymer units without characteristic arrangement. There are some differences between the two incomplete rotations, namely the rotation in fig. 8 shows regular arrangement of globular units forming a hexagon.



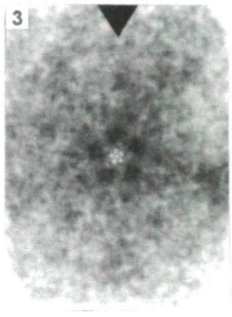
A

C.P.5.A.5.5.



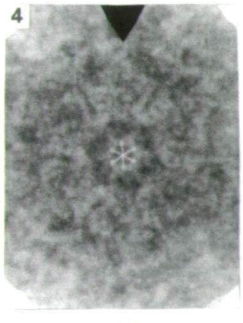
A

C.P.5.A.5.10.



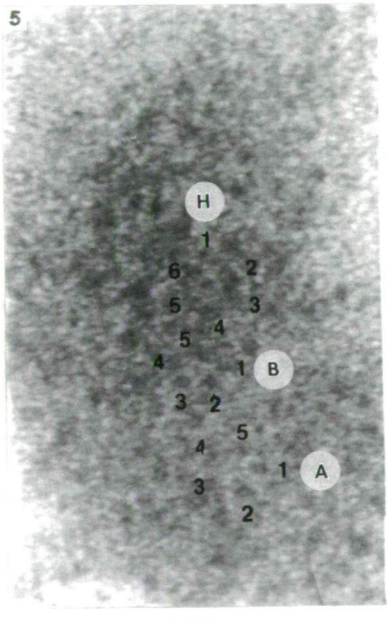
B

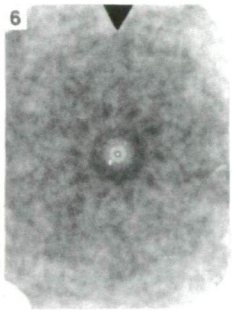
C.P.5.A.5.5.



H

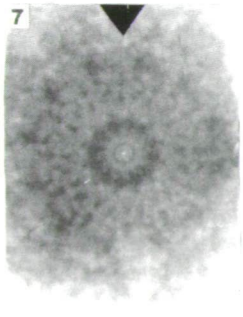
C.P.6.A.6.6.





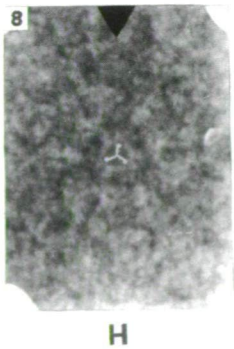
B

C.P.5.A.5.10.



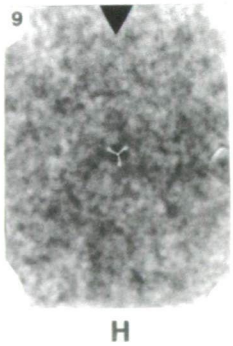
H

C.P.6.A.6.12.



H

I.P.6.A.6.3$_{1,3,5}$



H

I.P.6.A.6.3$_{2,4,6}$

Plate 11.1.

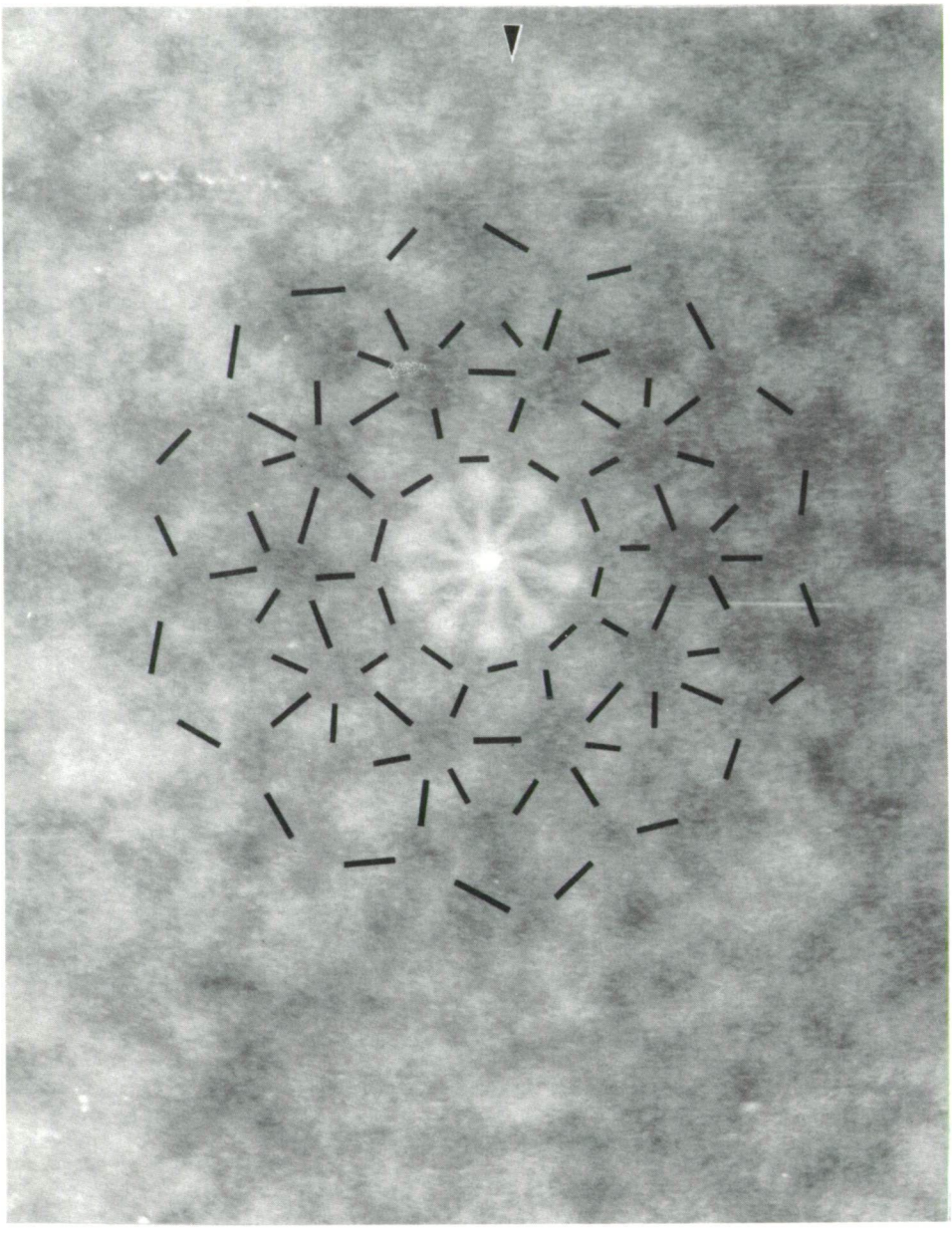


Plate 11.2.

Discussion and Conclusions

The discovery of the quasicrystals was made by SHECHTMAN, BLECH, GRATIAS and CAHN (1984), but the nomination was introduced by LEVINE and STEINHARDT (1984). Four years later, in the partially degraded ectexines of the pollen grains of *Pinus griffithii*, the quasi-crystalloid biomacromolecular system was discovered (KEDVES, 1988). This biopolymer organization was investigated extensively in our laboratory. Several results and multidisciplinary contacts of the quasicrystals were shortly reviewed for the 10th anniversary of the discovery of the quasicrystals by KEDVES (1994). We cite again the most important establishments concerning our investigations: MACKAY (1976, 1981), PENROSE (1979), BURSILL and PENG JU LIN (1985), AUDIER and GUYOT (1986), NELSON (1986), SCHNEER (1988) and HARGTITAI (1990).

In the Materials and Methods part we reviewed the data referring to the different experimental methods. It is interesting that recently we have had to turn back to the two dimensional symmetry operations and, based on our recent investigations, we obtained several unexpected new results. During these symmetry operations the biopolymer network of the tenfold rotation of the biopolymer "A" is interesting. The surrounding ten regular pentagons may be modelled with the three dimensional Penrose unit of KEDVES (1992), p. 74, fig. 1.

Recently we have started to include C60 fullerene/benzol solution also in the partial degradation of the plant cell wall. For the symmetry operations of the sixfold biomacromolecular structures the incomplete rotation method may be useful. In this way the recently introduced index system seems to be important.

Finally, these new data support again the concept that the biomacromolecular system of the plant cell wall, especially of the sporoderm, is much more complicated than we believed earlier.

Acknowledgements

This work was supported by Grant OTKA T 031715.

Plate 11.1.

Phoenix dactylifera L. Biopolymer structure of the partially degraded ectexine and symmetry operations of two regular pentagons and a hexagon.

1,2. Regular pentagon biomacromolecule "A", 1. fivefold, 2. tenfold rotation pictures.
3,6. Regular pentagon biomacromolecule "B", 3. fivefold, 6. tenfold rotation pictures.
4. Sixfold rotation picture of the hexagonal biomacromolecule.
5. Ultrastructure of the partially degraded ectexine. The investigated biomacromolecular units are marked. A and B are regular pentagons, H is a regular hexagon.
7. Twelvefold rotation picture of the hexagonal biomacromolecular system.
8,9. Incomplete rotation picture of the regular hexagonal biolpolymer unit. Bar scale: 0.01.

Plate 11.2.

Phoenix dactylifera L. Magnified picture of the tenfold rotation picture, illustrated in Plate 11.1., fig. 2. Ten regular pentagons appeared around the rotation center in consequence of the symmetry operation. Globular biomacromolecular units in the center of the pentagons may be the components of the pentagon-dodecahedrane bipolymer structures.
Bar scale: 0.002.

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In memoriam

SIWERT NILSSON

1933–2002

TO THE MEMORY OF PROF. DR. SIWERT NILSSON

Professor Siwert NILSSON was born in Bräcke, Sweden, on July 30, 1933. He began his university studies at Uppsala University and obtained MSc (Botany, Geography, Zoology) in 1959 and 1965, the licentiate degree in the Institute of Systematic Botany. In 1959 as an assistant he started his research work at the Swedish Council of Natural Sciences in the world-famous Palynological Laboratory headed by Professor Gunnar Erdtman in Stockholm.

Professor NILSSON began his pollen morphological studies on the family of Apocynaceae and with the Gentianaceae. In 1970 he defended his PhD thesis entitled "Pollen morphological studies in the Gentianaceae". After the transfer of the Palynological Laboratory into the Swedish Museum of Natural History in Stockholm Dr. NILSSON took over the leadership and remained there as director until his retirement on July 1, 1998.

Concerning the pollen morphological studies he made a great effort on international organization level to solve the problems in the terminology of the spores and pollen grains including the nomenclature of the different layers of the wall of the sporoderm.

In 1973 he initiated a research program on Aerobiology. He was a pioneer in this field of science in the Stockholm Palynological Laboratory and he recognized the necessity of the multidisciplinary cooperation with meteorological and medical institutions in Stockholm. The handbook "Atlas of Airborne Pollen Grains and Spores in Northern Europe" published by S. NILSSON, J. PRAGLOWSKI and L. NILSSON has always been a basic comprehensive work in the investigations on allergenic pollen grains.

In 1992, in recognition of his scientific achievements, Dr. NILSSON was nominated as Professor in Palynology. He was not only a great scientist in Palynology but an excellent teacher as well. He led several postgraduate students from both Sweden and foreign countries to PhD degree.

Six books and about 100 papers were published by Professor NILSSON. He was in his sixties when he became assistant editor of Grana Palynologica, from 1978 co-editor of Grana and in 1985 he became editor-in-chief. After his retirement he continued his scientific activity at the Palynological Laboratory as Professor Emeritus. Until his death he remained the editor-in-chief of Grana.

He was honoured with awards and medals by many scientific organizations. His sudden and early death was a terrible shock not only to his family and the Palynological Laboratory of Stockholm, but to palynologists all over the world.

Every member of the Cell Biological Laboratory of the University of Szeged received in the greatest sadness the unexpected death of Professor S. NILSSON.

By his death we have lost a great man, a prominent teacher and an excellent scientist in one.

His memory will always remains with us.

The staff of the Cell Biological and Evolutionary
Micropaleontological Laboratory
of the University of Szeged

13. LIST OF PUBLICATIONS OF THE LABORATORY UNTIL DECEMBER 2003

Compiled by

G. HALÁSZ

Cell Biological and Evolutionary Micropaleontological Laboratory of the University of Szeged, H-6701, P.O.Box 993, Szeged, Hungary

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Chronicle

compiled by

ZS. THURZÓ

Visiting Scientist

14.07.2003. - Dr. Vivi VAJDA (Department of Geology, Lund University, Tornavägen 13, SE-223 63 Lund, Sweden) visited the laboratory. A joint rese
arch program was discussed on the combined study of Hungarian Upper Cretaceous sediments. Palynological researches were projected for the future

Photo 1.

Dr. Vivi VAJDA in the office of Prof. Dr. M. KEDVES. Picture was taken by Dr. É. SIPOS-KEDVES.

January 16 - February 5, 2003, Birbal Sahni Institute of Palaeobotany, Lucknow,Uttar Pradesh, India.

Prof. Dr. M. KEDVES visited the Birbal Sahni Institute of Palaeobotany within the scientific exchange program between the Indian (INSA) and Hungarian (MTA) Academies. During his stay he worked with Dr. S.K.M. TRIPATHI and Dr. Madhav KUMAR on joint research progams. The following papers were finalized or are before completion:

1. SEM investigations on the partially degraded pollen grains of Family Malvaceae.
2. Effect of the C60 fullerene/benzol solution on the ultrastructure of the wall of *Cycas rumphii* pollen grains.
3. SEM and TEM investigations on partially degraded cuticles of *Cycas rumphii* MIQ.
4. Molecular system and symmetry operations on the highly organized biopolymer structures of the cuticles of *Cycas rumphii.*

Further joint programs were discussed and elaborated.

Photo 2.

From left to right: Dr. S.K.M. TRIPATHI, Scientist E, Prof. Dr. M. KEDVES, Dr. Madhav KUMAR, Scientist D in front of the Birbal Sahni Institute of Palaeobotany (Lucknow, Uttar Pradesh, India).

B.S.I.P. celebrated the 57th Anniversary of Republic Day on January 26, 2003. Director Prof. A.K. Sinha hosted national flag on the institute building. Dr. Jyotsna ROY delivered a speech to the memory of soldiers who sacrificed their lives for India's independence. Dr. Prof. A. K. SINHA highlighted the significance of this day and briefly talked about the diversity of culture in the Indian subcontinent. This small meeting was followed by a tea party in the garden of the institute.

September 1-4, Bordeaux, France XVIIIth Symposium of APLF. Organizing Committee: J.-L. TURON, F. EYNAUD, M.-F. SANCHEZ-GONI, L. LONDEIX, ST. DESPRAT and M.-H. CASTERA. On the 2nd September M. KEDVES delivered the following paper:

KEDVES, M., PÁRDUTZ, Á., HALÁSZ, G., KOVÁCS, J. and THURZÓ, ZS.: L'importance de la solution de C60 fullerene/benzénique dans la dégradation partielle de l'ectexine de *Malva sylvestris.*

October 6-12. Madrid, Departmento de Paleontología, Universidad Complutense de Madrid, Facultad de Ciencias Geológicas, Ciudad Universitaria, Spain.

Prof. Dr. M. KEDVES as visiting scientist worked in the Department of Paleontology of the U.C.M. on the new joint research programs with Prof. Dr. C. ALVAREZ RAMIS and Dr. M.T. FERNÁNDEZ MARRÓN.

Hungarian Scientific Activities

January 3, 2003 the 15th volume of Plant Cell Biology and Development was published. July 25, 2003 the monograph of the „Geonómia az ezredforduló után" was published by the Geonomical Subcommittee of the Hungarian Academy of Sciences. Responsible for publication: Béla NAGY, Editor: Endre DUDICH. Preface by György PANTÓ, member of the HUAC, President of the Section of Earth Sciences. Contributors: Ferenc BENKŐ, Szaniszló BÉRCZI, László CSEREPES, Zuárd DITRÓI PUSKÁS, Endre DUDICH, Erzsébet ILLÉS, Miklós KEDVES, Béla LUKÁCS, Teréz PÓKA and Gyula SZÖŐR. This volume was dedicated to the 100th Birth Anniversary of Prof. Dr. Elemér SZÁDECZKY-KARDOS who established Geonomy, an extremely multidisciplinary field of science and published several monographs concerning this subject.

August 16-22, 2003 Symmetry Festival was held in Budapest. Prof. Dr. M. KEDVES was the member of the Hungarian Advisory Board. His contribution "Symmetry of the biomacromolecules of the plant cell wall" was reported. For some unexpected reasons he could not present his contributionn there. The abstract will be completed and published in the next volume of P.C.B.D.

Laboratory Meetings and News

04.01.03. Presentation of the new (16th) volume of Plant Cell Biology and Development. Papers of the joint research programs with the Birbal Sahni Institute of Palaeobotany were discussed. Organization of the new volume of P.C.B.D.

18.02.03. Report from the achievements in the Birbal Sahni Institute of Palaeobotany. Organization of the research programs of the Laboratory. Projection of diapositives of spores, gymnosperm pollen grains and Lucknow (Cathedral Church, Hazrat Gunj).

01.03.03. Discussion and the redaction of the papers which are included in the 16th volume of P.C.B.D. Other actual businesses.

05.04.03. Problems of the Laboratory because of the new economic situation of our financial supporters. Redaction questions of the 16th volume of P.C.B.D.

03.05.03. Review the research programs of the Laboratory for this year considering the fact that the Grant OTKA T 31715 will be ended in 31 December of 2003. A new application was completed and presented to the Foundation of OTKA. The decision will be made probably in January or February, 2004. The research programs for the summer of this year.

Plate 1.

Plate 2.

07.06.03. Projection of the volume 2005 of P.C.B.D. The conract with the Printer Office was concluded and the cover was also settled and printed. Manuscripts for this volume started to be collected.

21.08.03. The traditional exclusive reception was held in the Laboratory on the occasion that on the same day in 1990 the Rector of the University signed the establishment of our research unity.

25.08.03. Prof. Dr. R. MÉSZÁROS member of the Hungarian Academy of Sciences was awarded the Commemorative and the Centennary Medal of the Laboratory. As Dean of the Faculty of Science, later as Rector of the University he has always helped our Laboratory in every respect.

23.08.03. Overview of the current researches of the Laboratory, international joint research programs.

27.09.03. Report from the participation at the XVIIIth Symposium APLF held in Bordeaux. The current state of the 16th volume of PCBD. Actual businesses.

Projections: LM morphology and ultrastructure of the spores, recent and fossil.

25.10.03. About the final redaction of the 16th volume of P.C.B.D. Planning of the compilation of the next volume.

Projections: LM morphology and ultrastructure of the gymnosperm pollen grains. Actual problems.

29.11.03. Discussion and redaction of papers for the 17th volume of P.C.B.D. Discussion and international scientific contributions.

Projections: LM morphology and ultrastructure of the angiosperm pollen grains.

13.12.03. The scientific achievements of the Laboratory during this year. Presentation of the first (not bounded) copy of the 16th volume of Plant Cell Biology and Development. Discussion of the joint research programs with the B.S.I.P., Lucknow, India and the next ICP held in Granada, Spain.

Projections: Quasi-crystalloid organization in the plant cell wall.

Teaching Program of the Laboratory

In the year 2003 the following lectures were delivered:

1. Applied Palynology, 2 + 2. 2. Biopolymer organization and symmetry, 2 + 0. 3. Theory of evolution and Natural Philosophy, 2 + 0. 4. Theory of the Supernova 2 + 0. 5. Basic Palynology, 2 + 2. 6. Quasi-crystalloid biopolymer structures 2 + 2.

Plate 1.

1. J. KOVÁCS, 2. ZS. THURZÓ, 3. From left to right: J. KOVÁCS, ZS. THURZÓ, Prof. Dr. M. KEDVES and A. BORBOLA. 4. From left to right: A. BORBOLA, J. KOVÁCS and ZS. THURZÓ. 5. A. BORBOLA. Pictures were taken by Dr. É. SIPOS-KEDVES.

Plate 2.

1,2. From left to right Prof. Dr. R. MÉSZÁROS member of the Hungarian Academy of Sciences and Prof. Dr. M. KEDVES in the office of the laboratory.

Award

Prof. Dr. M. KEDVES was awarded the medal of the International Scientist of the Year 2002 by the International Biographical Centre Cambridge, United Kingdom.

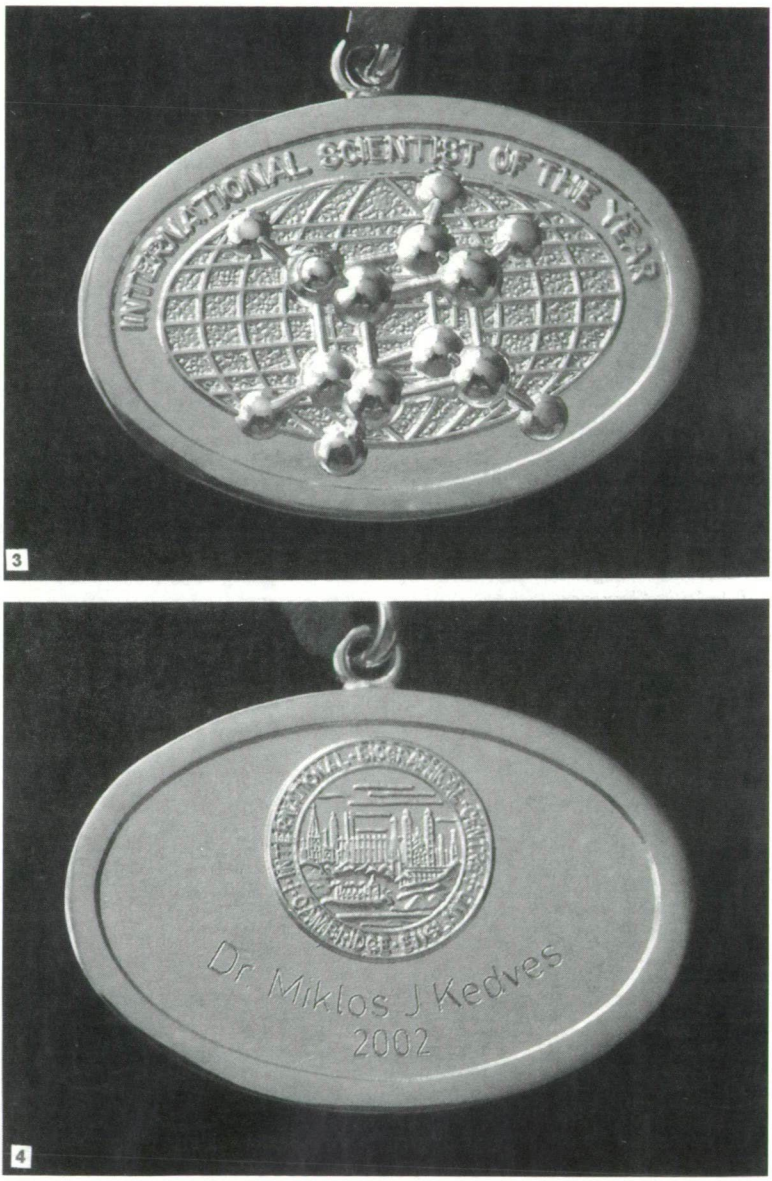

Photo 3,4.

Front and back picture of the medal. Pictures were taken by Dr. É. SIPOS-KEDVES.

EGY – kh

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