7. X-RAY EFFECT ON THE ULTRASTRUCTURE OF THE POLLEN GRAINS OF SALIX ALBA L.

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Abstract

Pollen grains of *Salix alba* L. were irradiated with CuKα X-ray at 35 KV, 20 mA during 5 s., 15 s., 35 s., and 60 s. and investigated with transmission electron microscope. The ectexine and the endexine are very resistant, but important alterations were observed in the ultrastructure of the intine and the protoplasm.

Key words: Palynology, recent, *Salix alba*, X-ray effect, TEM.

Introduction

The reticulate *Longaxones* pollen grains represent one of the early stages in the evolution of the *angiosperms*. In this respect the *Platanaceae* and *Hamamelidaceae* were pointed out concerning the evolution of the early *angiosperm* pollen grains. (KEDVES, 1998). The global regional distribution of the tricolpate, tricolporate reticulate pollen grains during the Lower Cretaceous was summarized by KREMP (1978).

The early pollen morphological characteristic features and the evolutionary significance was the reason that we have chosen a species of *Salix* for experimental studies.

Materials and Methods

The investigation material was collected by Mr. I. GÁSPÁR on the 20[th] April, 1993. Locality: The left bank of the river Tisza. The irradiations were made on the 04.08.1993 in the Radiological Laboratory of the Department of Mineralogy, Petrology and Geochemistry of the J. A. University, Szeged, with a BRON-OM1 apparatus. Radiation data: 35 KV, 20 mA, CuKα X-ray. Length of time and numbers of the experiments: 5 s., 1746, 15 s., 1747, 35 s., 1748, 60 s., 1749. The irradiated pollen material was postfixed with 1.0% OsO_4 aq. dil., and embedded in Araldite (Durcupan, Fluka). The ultrathin sections were made in the EM Laboratory of the Institute of Biophysics of the Hungarian Academy of Sciences, Biological Research Center on a Porter Blum ultramicrotome. The TEM photographs were made on an Opton EM-902 (resolution 2-3 Å), and on a Tesla BS-540 (resolution 6-7 Å). All pictures are unretouched.

Plate 7.1.

Salix alba L., Recent., Experiment No: 1746, general survey picture of the irradiated pollen grain. Negative no: 6074, 8.000x.

Plate 7.2.

1-5. *Salix alba* L., Recent. Experiment No: 1746.
1. Ultrastructure in the apertural area. Negative no: 6075, 15.000x.
2-4. Detail of the exine ultrastructure in the inter-apertural area.
2. Negative no: 6078, 50.000x.
3. Negative no: 6070, 50.000x.
4. Negative no: 6079, 50.000x.
5. Detail of the exine in the apertural area. Negative no: 6071, 50.000x.

Plate 7.3.

1-6. *Salix alba*. L. Recent. Experiment No: 1747.
1,2. General survey pictures of the irradiated pollen grain.
1. Negative no: 6110, 5.000x.
2. Negative no: 6095, 5.000x.
3,4. Ultrastructure in the apertural area.
3. Negative no: 6099, 50.000x.
4. Negative no: 6105, 15.000x.
5,6. Detail of the exine ultrastructure in the inter-apertural area.
5. Negative no: 6100, 50.000x.
6. Negative no: 6101, 50.000x.

Plate 7.4.

1-3. *Salix alba* L. Recent. Experiment No: 1748.
1. General survey picture of the irradiated pollen grain. Negative no: 6130, 5.000x.
2,3. Ultrastructure in the apertural area.
2. Negative no: 6131, 15.000x.
3. Negative no: 6129, 50.000x.

Plate 7.5.

1-3. *Salix alba* L. Recent.
1. Experiment No: 1748. Exine ultrastructure in the border of the apertural area. Negative no: 6127.
2,3. Experiment No: 1749.
2. Detail of the exine ultrastructure in the inter-apertural area. Negative no: 6086, 50.000x.
3. Exine ultrastructure in the apertural area. Negative no: 6084, 50.000x.

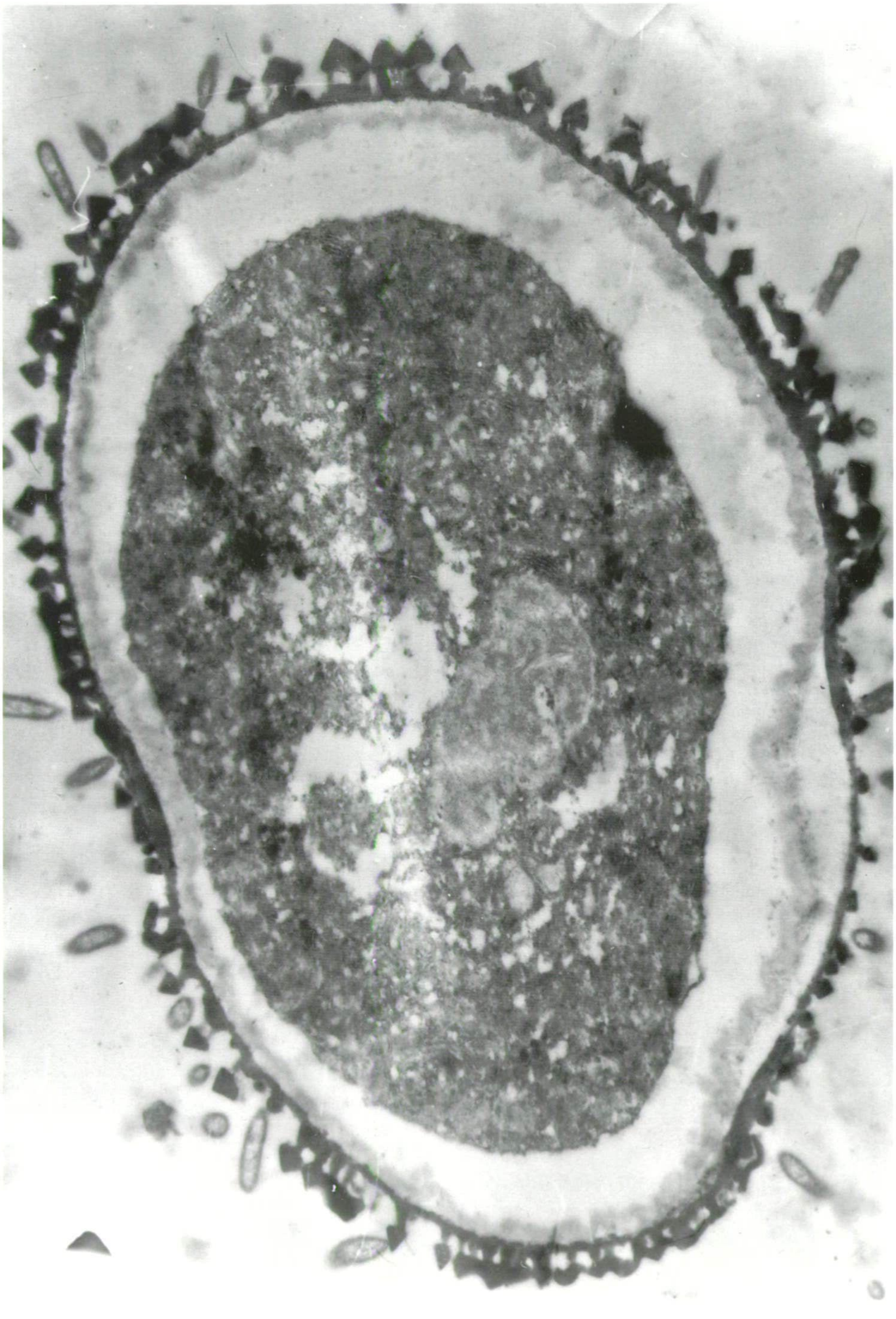

Plate 7.1.

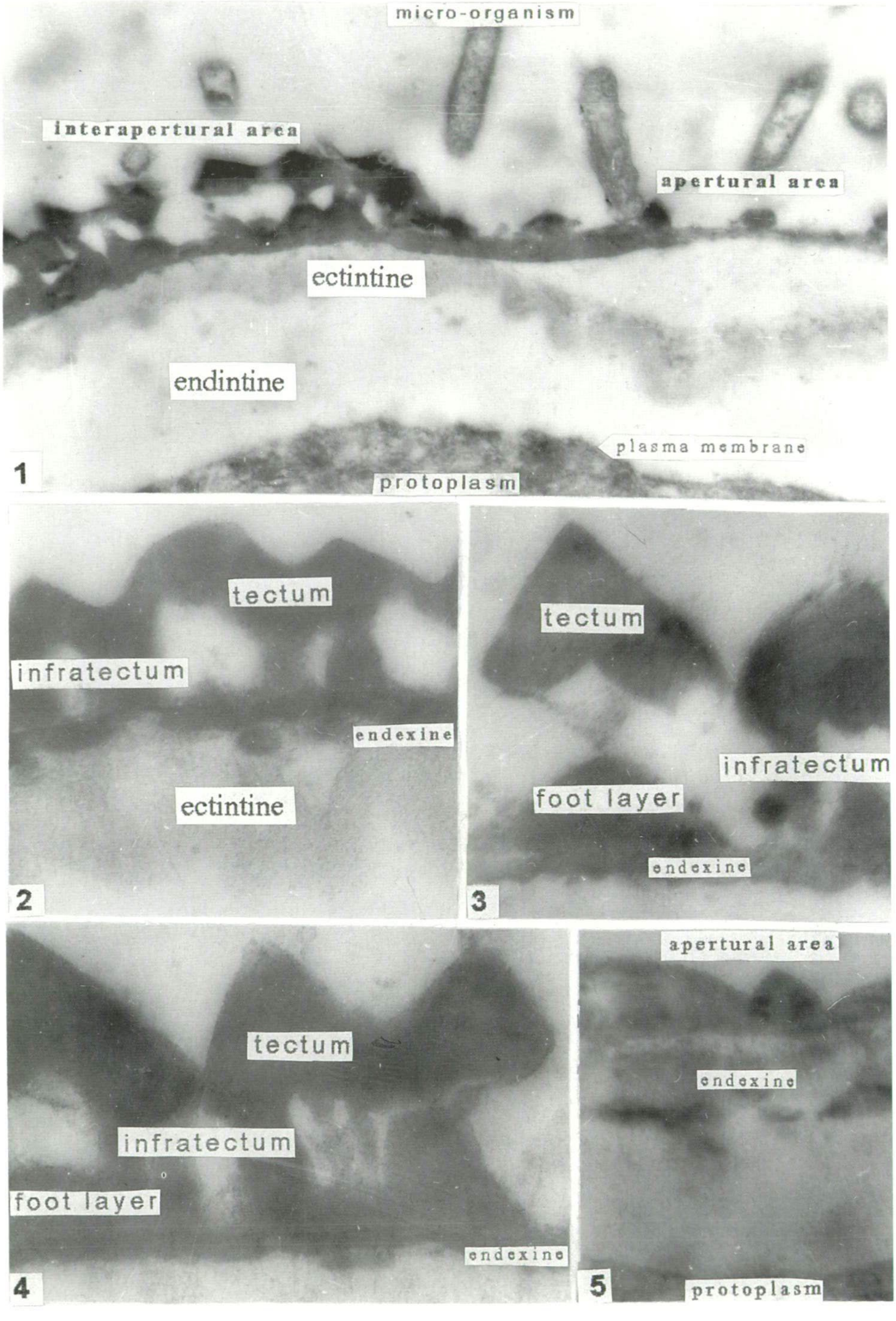
micro-organism
interapertural area
apertural area
ectintine
endintine
plasma membrane
1
protoplasm
tectum
infratectum
endexine
ectintine
2
tectum
infratectum
foot layer
endexine
3
tectum
infratectum
foot layer
endexine
4
apertural area
endexine
5
protoplasm

Plate 7.2.

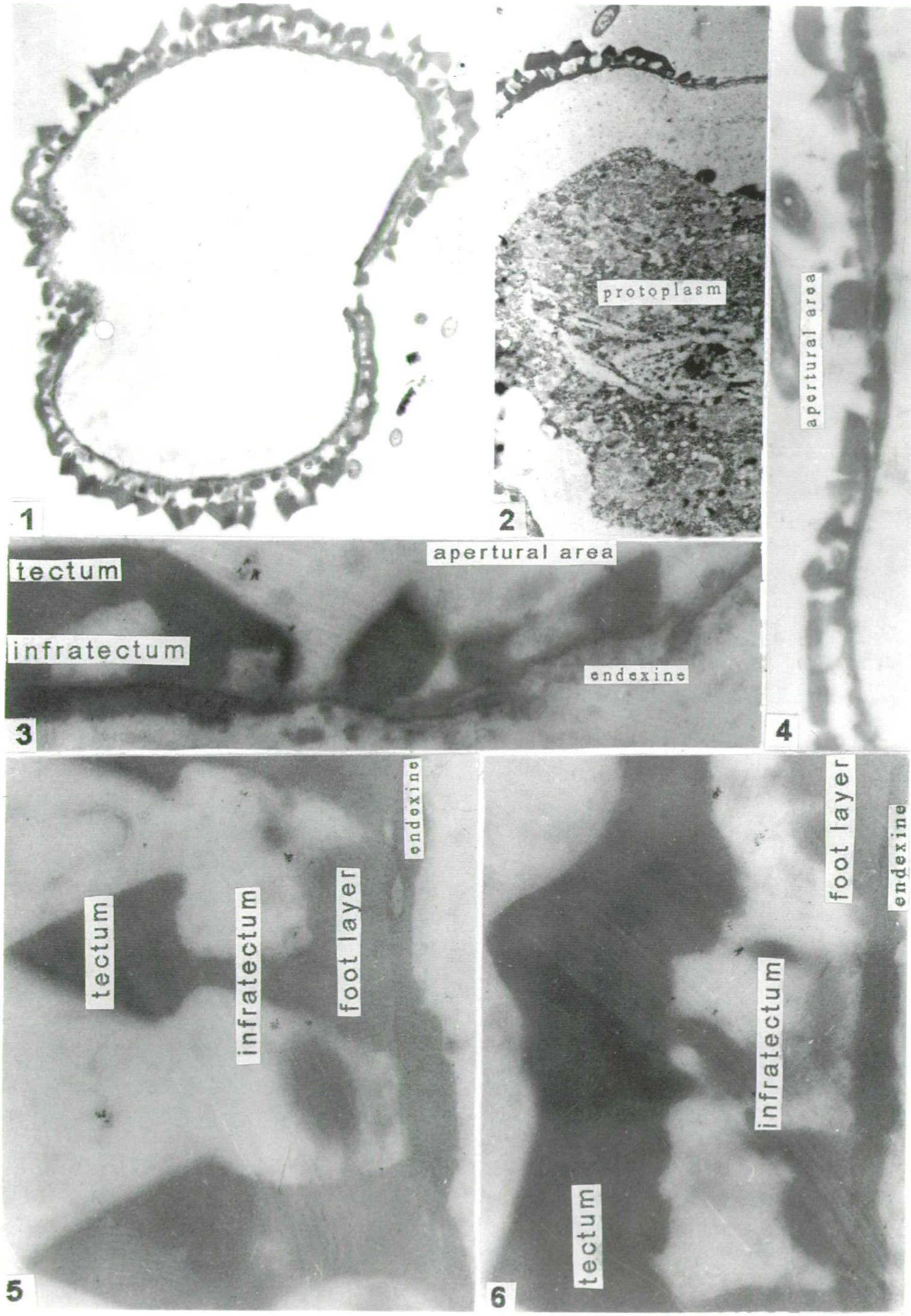
1
2
protoplasm
apertural area
tectum
infratectum
apertural area
endexine
3
4
endexine
tectum
infratectum
foot layer
5
foot layer
endexine
infratectum
tectum
6

Plate 7.3.

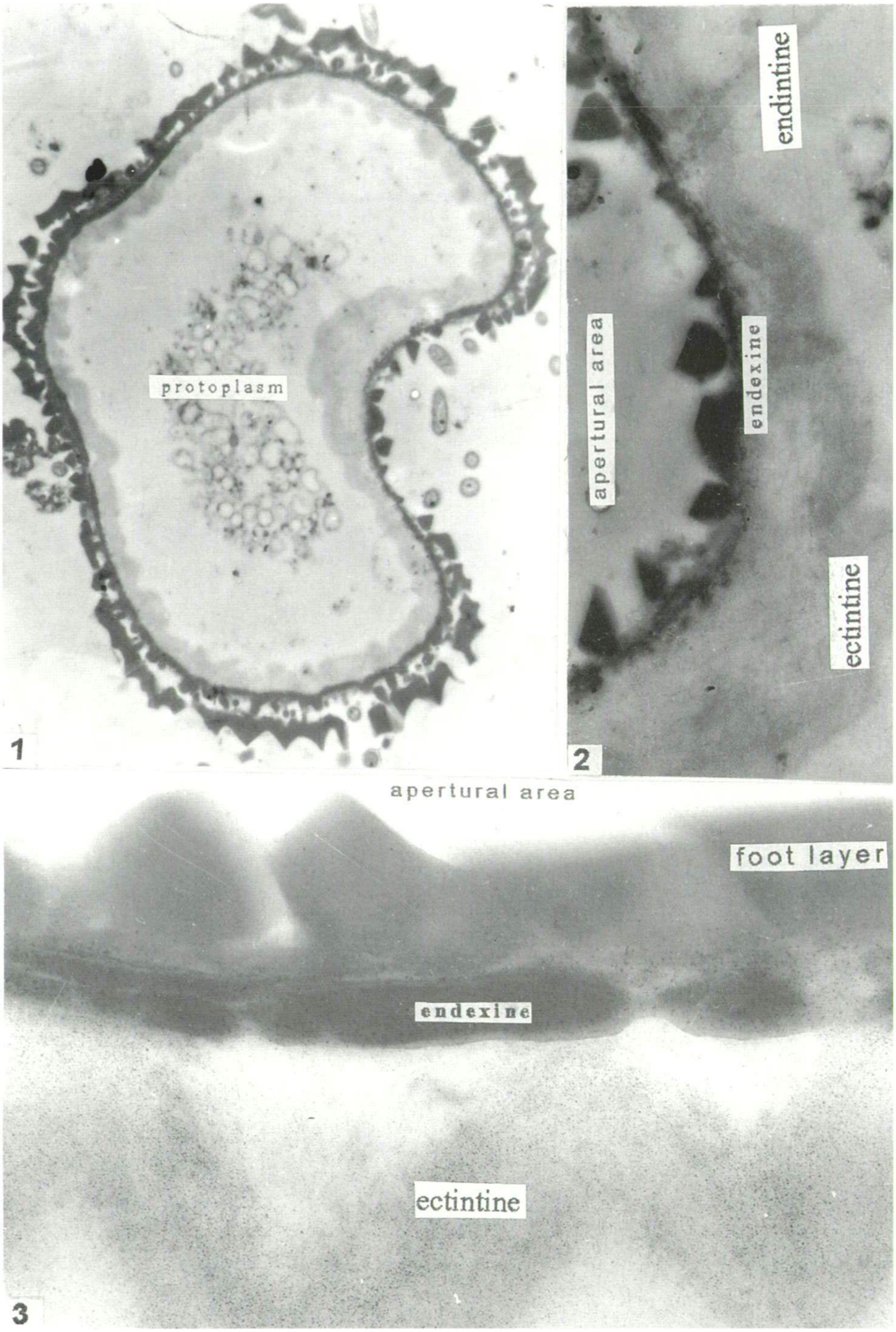
protoplasm
1
endintine
apertural area
endexine
ectintine
2
apertural area
foot layer
endexine
ectintine
3

Plate 7.4.

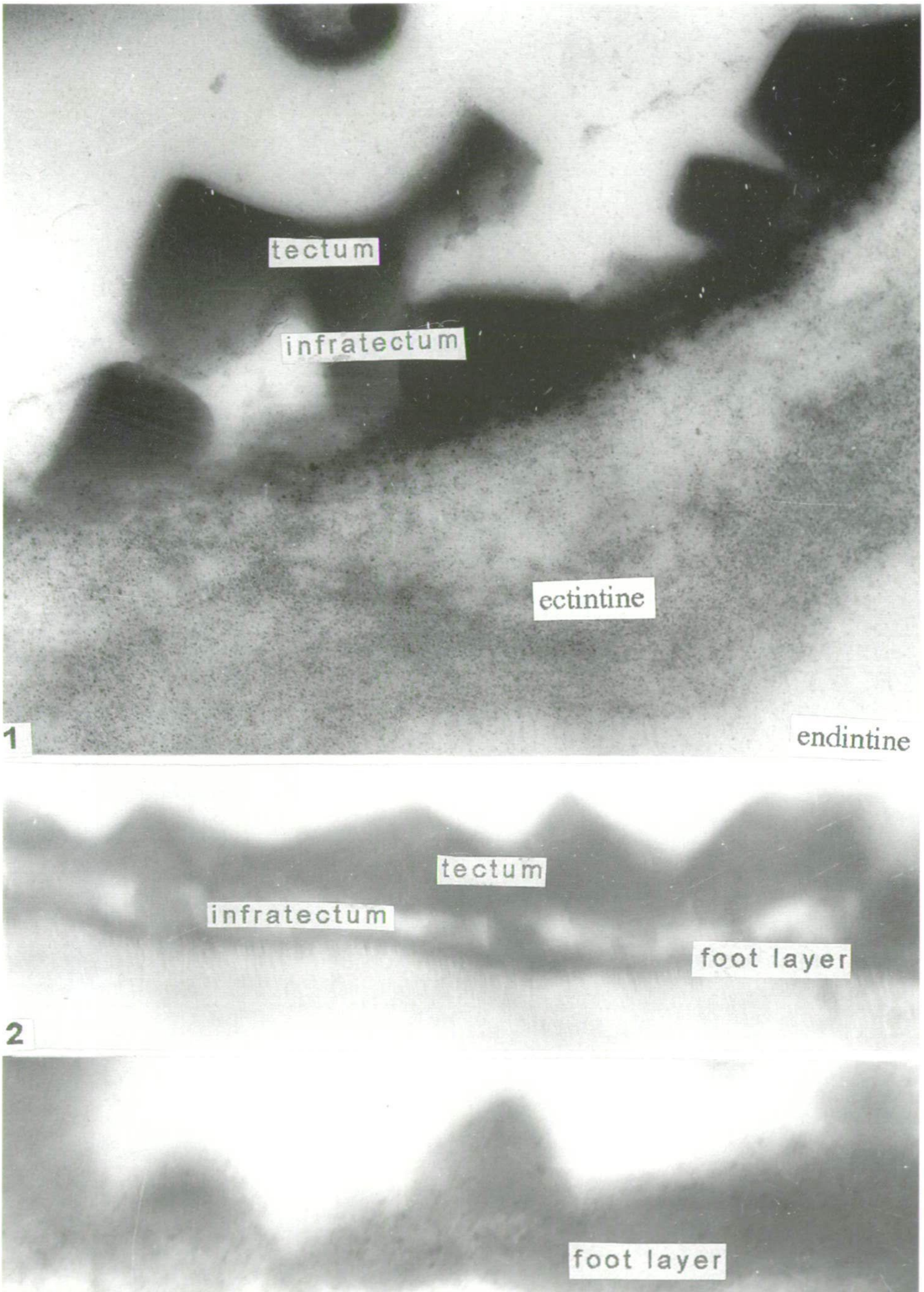
tectum
infratectum
ectintine
1
endintine
tectum
infratectum
foot layer
2
foot layer
3

Plate 7.5.

Results

Experiment No: 1746 (Plate 7.1., 7.2., figs. 1-5). – On the general survey TEM picture of the pollen grain (Plate 7.4) the following facts illustrated: 1. There are in all probability different kinds of micro-organisms on the surface. The ectexine is tectate perforate, infratectal layer columellar. At this, relatively low magnification, the endexine is not discernible. The intine is swelled, the two layers ectintine and endintine are characteristics. The electron density of the ectintine is stronger than of the endintine. The ectintine in consequence of the irradiation separate from the ectexine. This separation is more characteristic in the apertural area than in the non-apertural region. The plasma membrane is perceptible, sometimes separates also from the inner part of the protoplasm. At higher magnifications (Plate 7.2., figs. 1-5) the substance of the ectexine is homogeneous. The thin endexine is perceptible: in general this layer is more electron dense than the foot layer, sometimes discontinous (Plate 7.2., figs. 2-4). In the apertural area the ectexine is reduced to the foot layer and small remnants of the infratectal elements (Plate 7.2., figs. 1,5). The endexine is more fragmented here than in the inter-apertural area (Plate 7.1., fig. 5).

Experiment No: 1747 (Plate 7.3., figs. 1-6). – Essentially the TEM structure is identical to the previous experiment. The fine structure of the endexine is very characteristic in the inter-apertural (Plate 7.3., figs. 5,6) and in the apertural area (Plate 7.3., figs. 3,4). Worth of mentioning is that the ectintine is not well discernible as at the previous experiment, this is without doubt a methodical consequence.

Experiment No: 1748 (Plate 7.4., figs. 1-3, plate 7.5., fig. 1). – The intine swelled to a large measure, the ultrastructure of the protoplasm is disorganized (Plate 7.5., fig. 1). After this dose of irradiation some alterations were observed in the substance of the ectexine (Plate 7.5., fig. 1) namely large granules of stronger electron density appeared. Lamellar ultrastructure of the foot layer was observed in the apertural area (Plate 7.4., fig. 3). The measure of the fragmentation of the endexine is different in the apertural area to the inter-apertural region (Plate 7.4., figs. 2,3). The ectintine is well shown (Plate 7.4., figs. 1-3, plate 7.5., fig. 1), and finely granular, sometimes lamellar.

Experiment No: 1749 (Plate 7.5., figs. 2,3). – The substance of the ectexine is secondarily granular, particularly in the apertural area (Plate 7.5., fig. 3). Beneath the foot layer endexine was not observed. Probably in consequence of the degradation this is secondarily homogeneous. Degradation of the intine was also observed.

Discussion and Conclusions

At this species also the resistance of the ectexine to X-ray irradiation may be emphasized in comparison to the pollen grains of *Alnus glutionosa* (L.) GAERTN. (KEDVES and PÁRDUTZ, 1992). The fragmented endexine with its ultrastructural alterations by the irradiation dose is also interesting. The lamellar ultrastructure, which may together with the foot layer represent an early stage of the ontongeny, and in all respect of evolutionary point of view.

The ultrastructure of the intine and the alterations of this layer are also interesting. This layer was not investigated in detail at our experimental studies previously. The classical concepts of the stratification of the intine were summarized by TOMŠOVIC (1960) as follows:

Intina FRITZSCHE 1837 (the inner layer of the sporoderm which is composed of pectin and cellulose and is soluble by acids and alkalis).

Euintina KUPRIYANOVA 1955 = Endintine ELLIOTT 1951 (Where the intina is two-layered, there the inner layer consisting of cellulose fibrilla is impregnated with a pectinous matter).

Exintina FRITZSCHE 1837 (the outer layer of the two-layered intina is stronger than the euintina and is composed of pectin).

Recently IGERSHEIM (1997) investigated the different apertural intine protrusions in pollen grains of the *Rubiaceae*. Three variations have been distinguished by the different proportions of the two intine layers, the ect- and the endintine. Typically three-layered intine was described by LE THOMAS, SUÁREZ-CERVERA and GOLDBLATT (1997) from the pollen grains of the Afro-Madagascan genus *Aristea (Iridaceae, Nivenioideae)*. At two African species of the subtribe *Tragiinae (Euphorbiaceae)* also three-layered intine was established by the TEM investigations by SUÁREZ-CERVERA, GILLESPIE, FERNÁNDEZ-GONZÁLEZ and MÁRQUEZ (1997).

In conclusion it seems that the importance of the intine is greater than we believed earlier, and during our experimental studies it is important to investigate more circumstantially this layer, and the apertural protrusions.

Finally the pollen grains of *Salix* are allergenic, so in this respect the presence of the micro-organisms on the surface is important in this point of view. Combined allergenic effect may be presumed.

Acknowledgements

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