12. LM AND TEM INVESTIGATIONS OF PARTIALLY DISSOLVED AND DEGRADED POLLEN GRAINS OF ELAEAGNUS ANGUSTIFOLIA L.

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Abstract

Partially dissolved pollen grains with 8 organic solvents were investigated with light microscope. Dissolution with diethylamine resulted in extremely altered pollen forms.

Based on the TEM data of the partial degradation with the 2-aminoethanol and further agents method, it resulted that the sporopollenin of *Elaeagnus angustifolia* is less resistant against degradation.

The 2-aminoethanol degraded the ultrastructure of the exine of the pollen grain. A similar extremely soluble sporopollenin was observed previously at the exospore of *Equisetum arvense* and at the exine of the genus *Quercus, Tilia*, and *Platanus*.

Key words: Experimental Palynology, *Elaeagnus angustifolia.*

Introduction

In a previous paper (KEDVES and PÁRDUTZ, 1982) the importance of the pollen grains of the genus *Elaeagnus* and its fossil forms was discussed. Our studies were carried out on two kinds of maturity of the pollen grains with different methods. LM, TEM and SEM methods were used, and the alterations in the slides, the Cushing effect was also investigated. The interesting ultrastructure of the foot layer in the apertural area and in general the ancient morphological characteristic features of this kind of pollen grains were the reason for the partial degradation of this pollen grains.

The aim of the first researches was to get information about the resistance of the sporopollenin of the pollen grains of *Elaeagnus angustifolia*, and to have TEM data for the partially degraded or dissolved pollen grains.

Materials and Methods

The pollen grains were collected by Miss Vanda KECSKEMÉTI on the 5th May 1997 in the Park of the Alsóváros Church in Szeged. Length of time of all experiments: 30 days at temperature of 30 °C.

Experiment No: 1/7-911. - 20 mg stamen + 5 ml distilled water + 0.2 ml diethylamine.

Experiment No: 1/7-912. - 20 mg stamen + 5 ml distilled water + 0.2 ml merkaptoethanol.

Experiment No: 1/7-913. - 20 mg stamen + 5 ml methanol.

Experiment No: 1/7-914. - 20 mg stamen + 5 ml ethanol.

Experiment No: 1/7-915. - 20 mg stamen + 5 ml n-propanol.

Experiment No: 1/7-916. - 20 mg stamen + 5 ml n-butanol.

Experiment No: 1/7-917. - 20 mg stamen + 5 ml i-amyl alcohol.

Experiment No: 1/7-918. - 20 mg stamen + 5 ml glycerine, 50%.

The dissolution experiments started on 9th June. Slides for LM investigations were mounted in glycerine-jelly hydrated of 39.6%.

For transmission electronmicroscopic investigations the following experiments were carried out:

Experiment No: 1/7-1350. - 20 mg stamen + 1 ml 2-aminoethanol, temperature 30 °C, length of time: 24 h.

Experiment No: 1/7-1351. - 20 mg stamen + 1 ml 2-aminoethanol, temperature 30 °C, length of time: 48 h.

Experiment No: 1/7-1352. - 20 mg stamen + 1 ml 2-aminoethanol, temperature 30 °C, length of time: 72 h.

Experiment No: 1/7-1353. - 20 mg stamen + 1 ml 2-aminoethanol, temperature 30 °C, length of time: 24 h + 10 ml $KMnO_4$ 0.01%, temperature 30 °C, length of time 24 h.

Experiment No: 1/7-1354. - 20 mg stamen + 1 ml 2-aminoethanol, temperature 30 °C, length of time: 48 h + 10 ml $KMnO_4$ 0.01%, temperature 30 °C, length of time 24 h.

Experiment No: 1/7-1355. - 20 mg stamen + 1 ml 2-aminoethanol, temperature 30 °C, length of time: 72 h + 10 ml $KMnO_4$ 0.01%, temperature 30 °C, length of time 24 h.

Experiment No: 1/7-1356. - 20 mg stamen + 1 ml 2-aminoethanol, temperature 30 °C, length of time: 24 h + 1 ml merkaptoethanol, temperature 30 °C, length of time 24 h.

Experiment No: 1/7-1357. - 20 mg stamen + 1 ml 2-aminoethanol, temperature 30 °C, length of time: 48 h + 1 ml merkaptoethanol, temperature 30 °C, length of time 24 h.

Experiment No: 1/7-1358. - 20 mg stamen + 1 ml 2-aminoethanol, temperature 30 °C, length of time: 72 h + 1 ml merkaptoethanol, temperature 30 °C, length of time 24 h.

After experiment the material was washed, postfixed with OsO_4 aq dil., and embedded in Araldite (Durcupan, Fluka). The ultrathin sections were made on a Porter Blum ultramicrotome in the EM Laboratory of the Institute of Biophysics of the Biological Research Center of the Hungarian Academy of Sciences. The pictures were taken on a Tesla BS-540 (resolution 6-7 Å).

Results

LM results (Plate 12.1., figs. 1-12)

Experiment No: 1/7-911. (Plate 12.1., figs. 1-5). - The diethylamine resulted in extremely altered forms. Most of the pollen grains lost its original morphological characteristic features. Sometimes the originally triangular symmetry altered into sexangular or polyangular. Two opposite triangular forms may be recognized at some altered pollen grains. At other forms (Plate 12.1., fig. 4) the characteristic apertural area may not be recognized, and plicae-like form appeared.

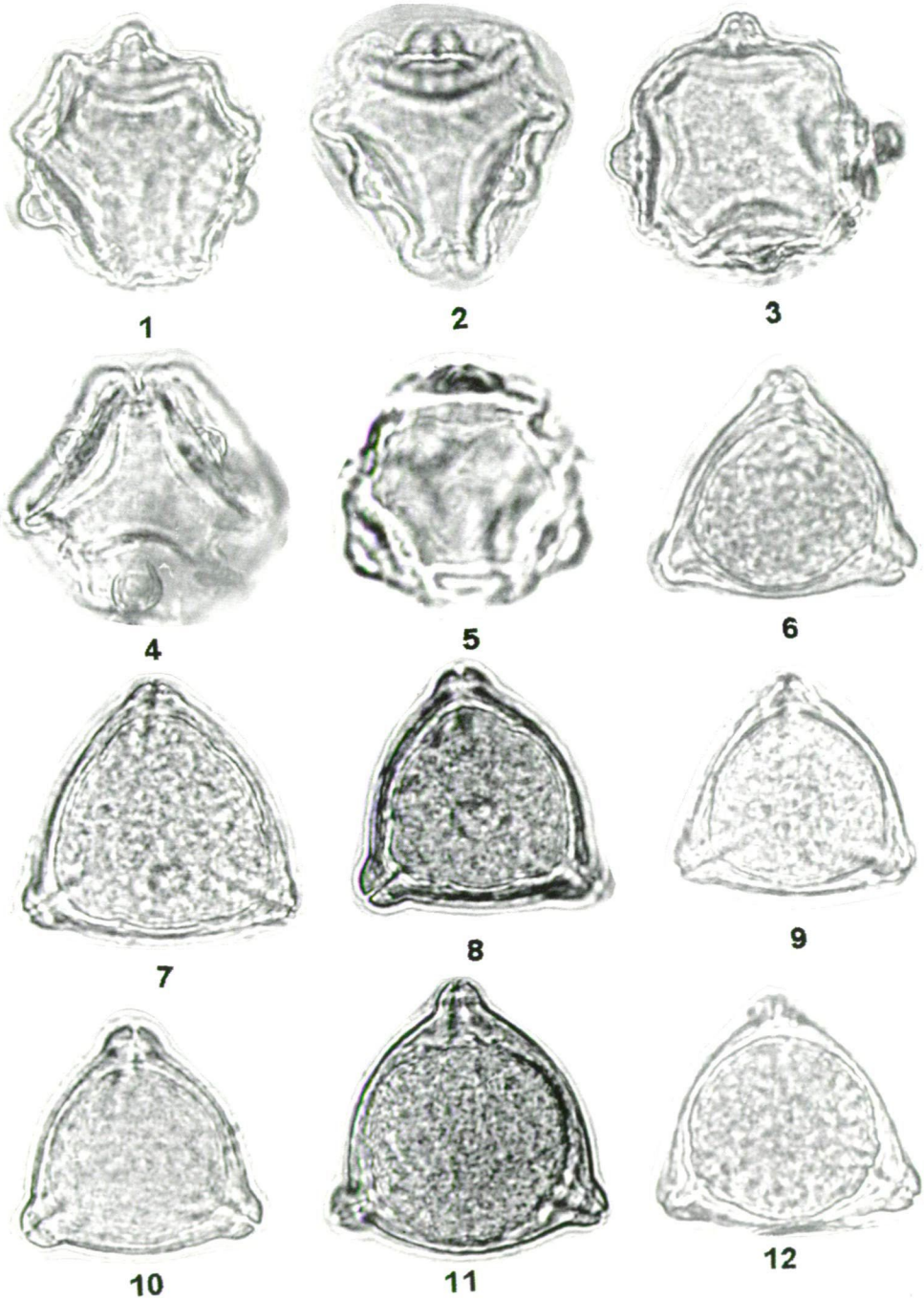

Plate 12.1.

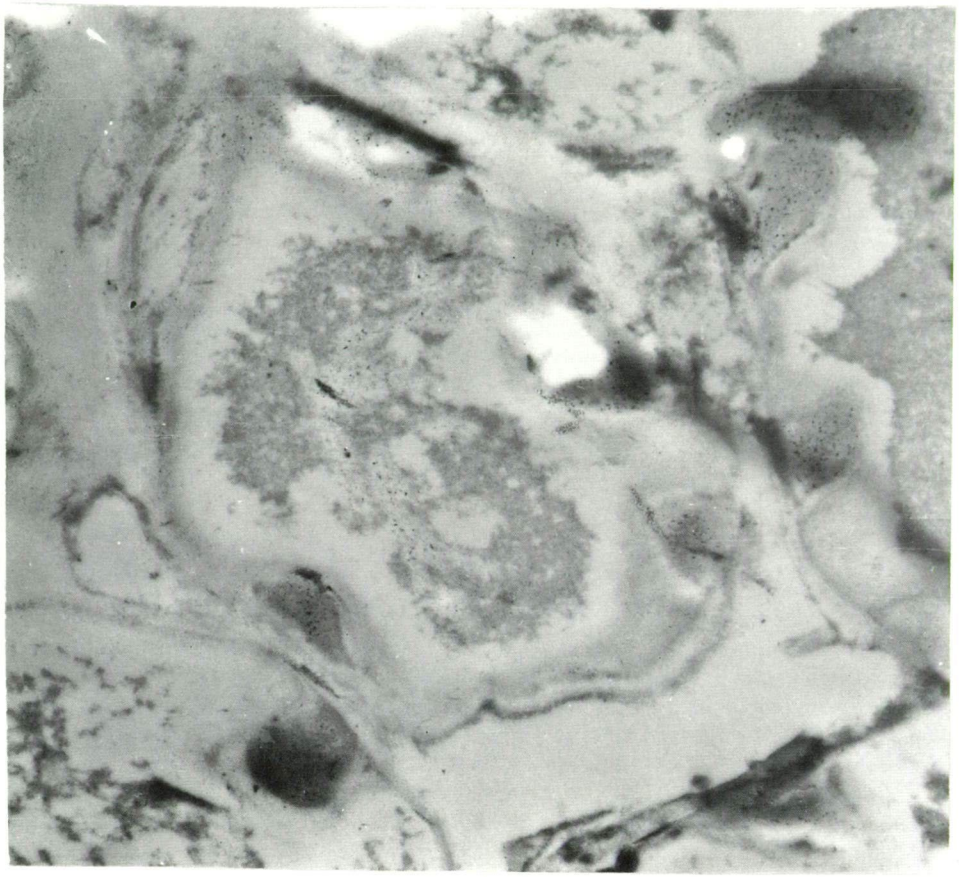

Plate 12.2.

Elaeagnus angustifolia L. TEM picture of partially degraded pollen grains, experiment No: 1/7-1350 Negative No: 7309, 7000x.

← Plate 12.1.

1-12. *Elaeagnus angustifolia* L. 1000x.
1-5. Experiment No: 1/7-911.
6. Experiment No: 1/7-912.
7. Experiment No: 1/7-913.
8. Experiment No: 1/7-914.
9. Experiment No: 1/7-915.
10. Experiment No: 1/7-916.
11. Experiment No: 1/7-917.
12. Experiment No: 1/7-918.

Experiments No: 1/7-912-918. (Plate 12.1., figs. 6-12). - It is well shown in the pictures, that the used alcohols have not altered the basic morphology of the pollen grains. The LM morphology of the protoplasm was not altered in contrast to the pervious experiment.

TEM results (Plate 12.2.)

Experiment No: 1/7-911. We tried to get exine ultrastructural data from the extremely altered pollen grains in consequence of the dissolution with diethylamine. After the investigation of the ultrathin sections of several blocks we have not observed any preserved ultrastructure of the pollen grain.

Experiment No: 1/7-1350. (Plate 12.2.).

It is worth of mentioning, that based on our previous experiments this partial degradation has not displayed the biopolymer system of the ectexine. In this way it is surprising, that the ectexine is extremely degraded. The infratectal layer is more or less completely destroyed. The tectum and the foot layer is a little electron dense. Beneath the remains of the ectexine a darker and a light layer surround the degraded protoplasm. Plasma membrane is not perceptible, but remnants of the nucleus were observed.

Discussion and Conclusions

The partial dissolution with diethylamine resulted in extremely important and interesting secondary forms. This is not so frequent after our experiments. The solubility was also remarkable and a little similar to the previously observed experiments at the exospore of *Equisetum arvense* L. (KEDVES and GÁSPÁR, 1994) and the pollen grains of the genus *Quercus* (KEDVES and GÁSPÁR, 1994, 1996), and *Platanus hybrida* BROT., and *Tilia platyphyllos* SCOP. (KEDVES et al., 1998).

It's interesting that the TEM investigations resulted after the most moderate partial degradation a very degraded ectexine. In general after the partial degradation with 2-aminoethanol the oxydation with $KMnO_4$ aq. dil. was also necessary to discover the biopolymer structure of the ectexine.

Taking into consideration the very interesting morphology to the pollen grains of the genus *Elaeagnus*, and the interesting fine structure of the exine of the non-experimental material, it seems that further experiments are necessary. But in this contribution we present our first experimental attempt, and the first few results.

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