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.

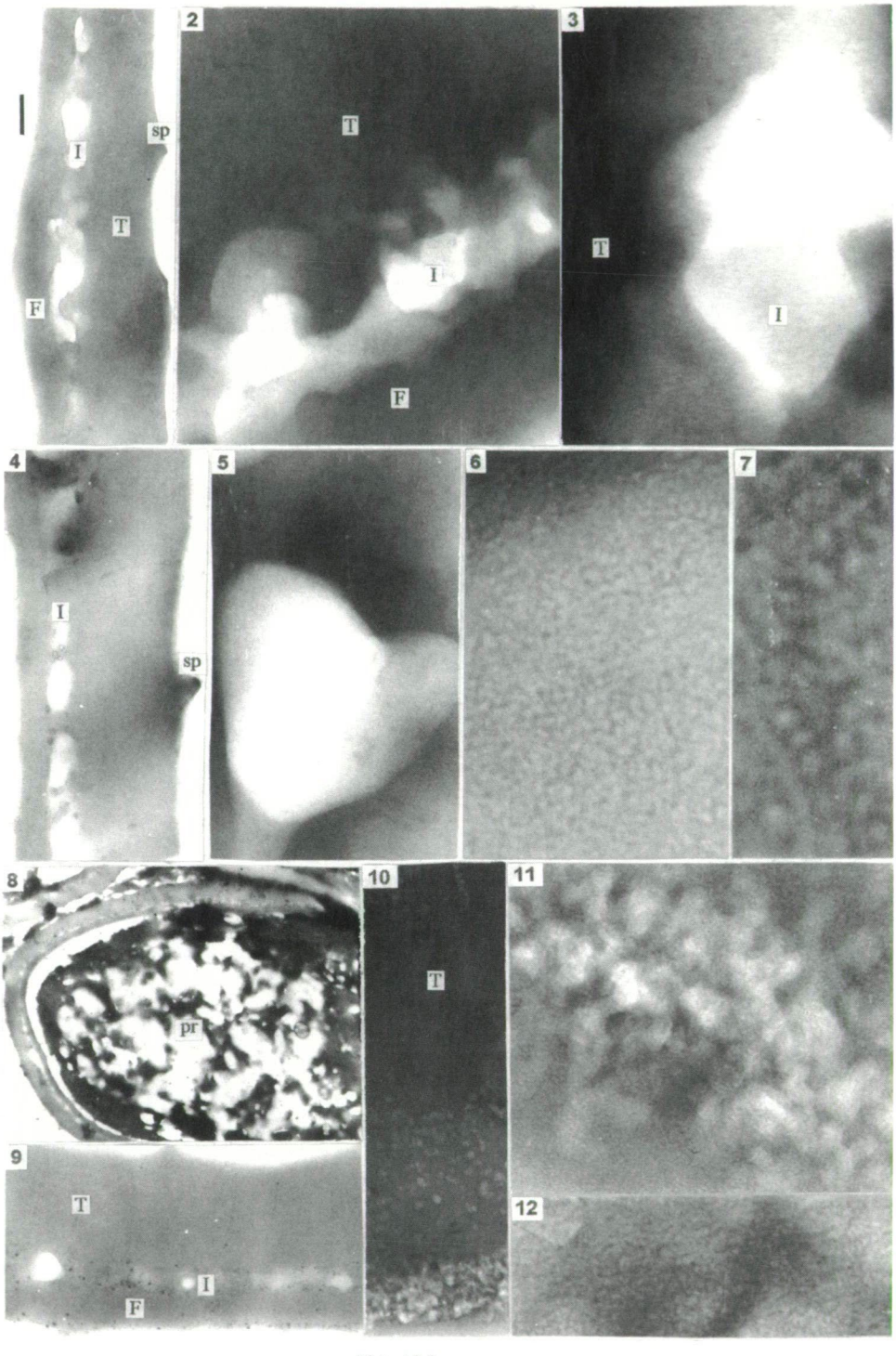

Plate 10.1.

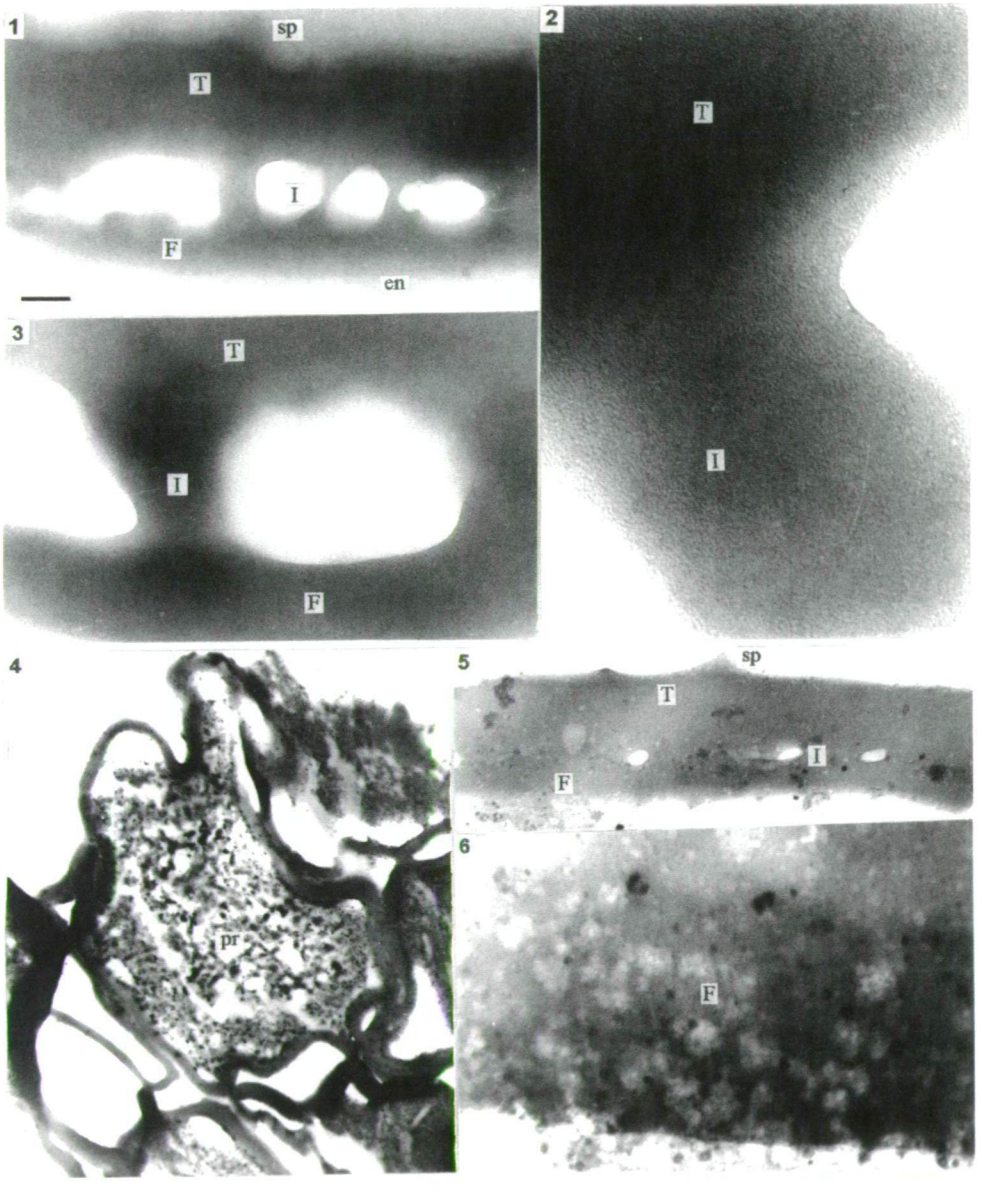

Plate 10.2.

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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