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.

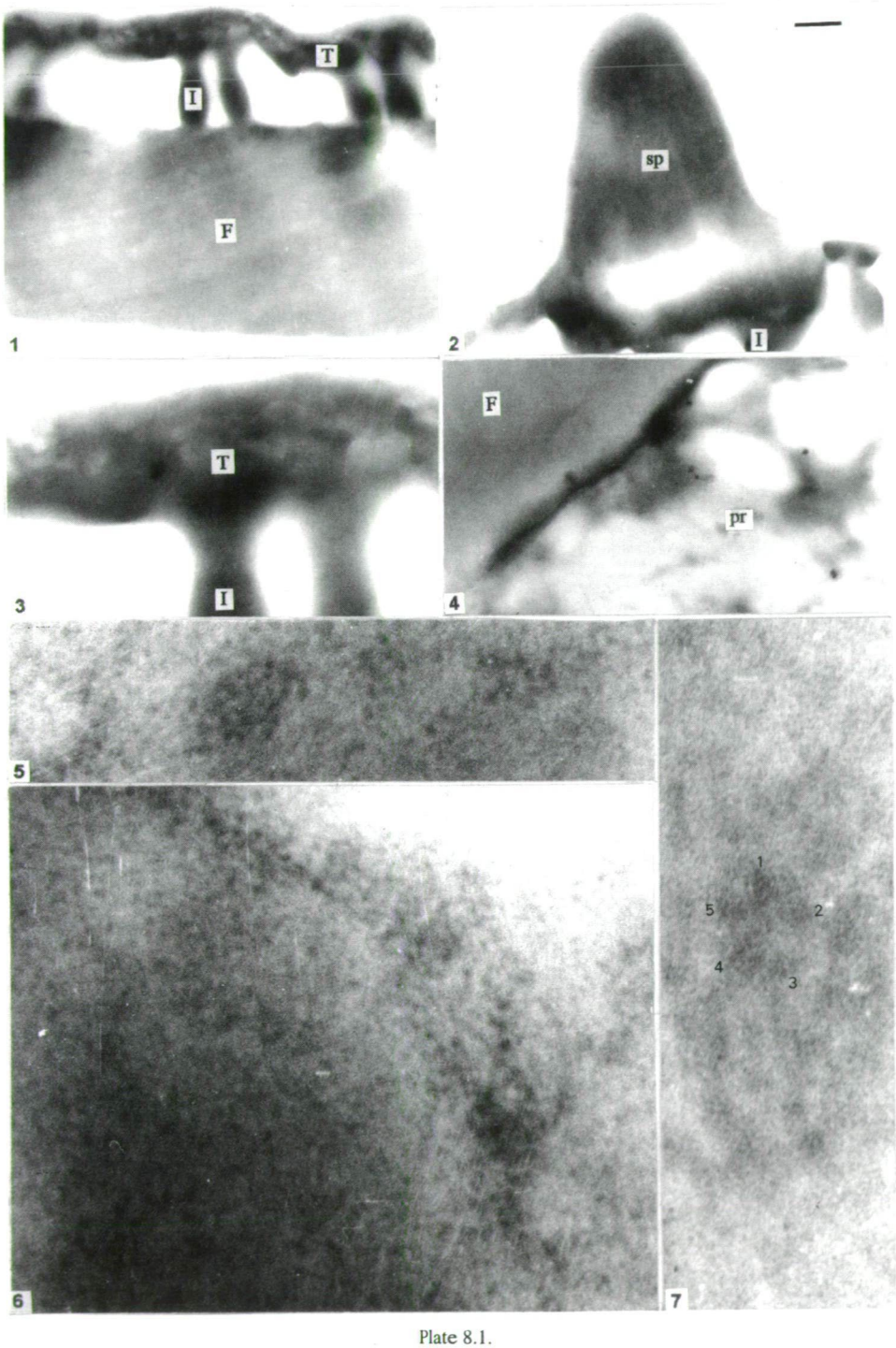

Plate 8.1.

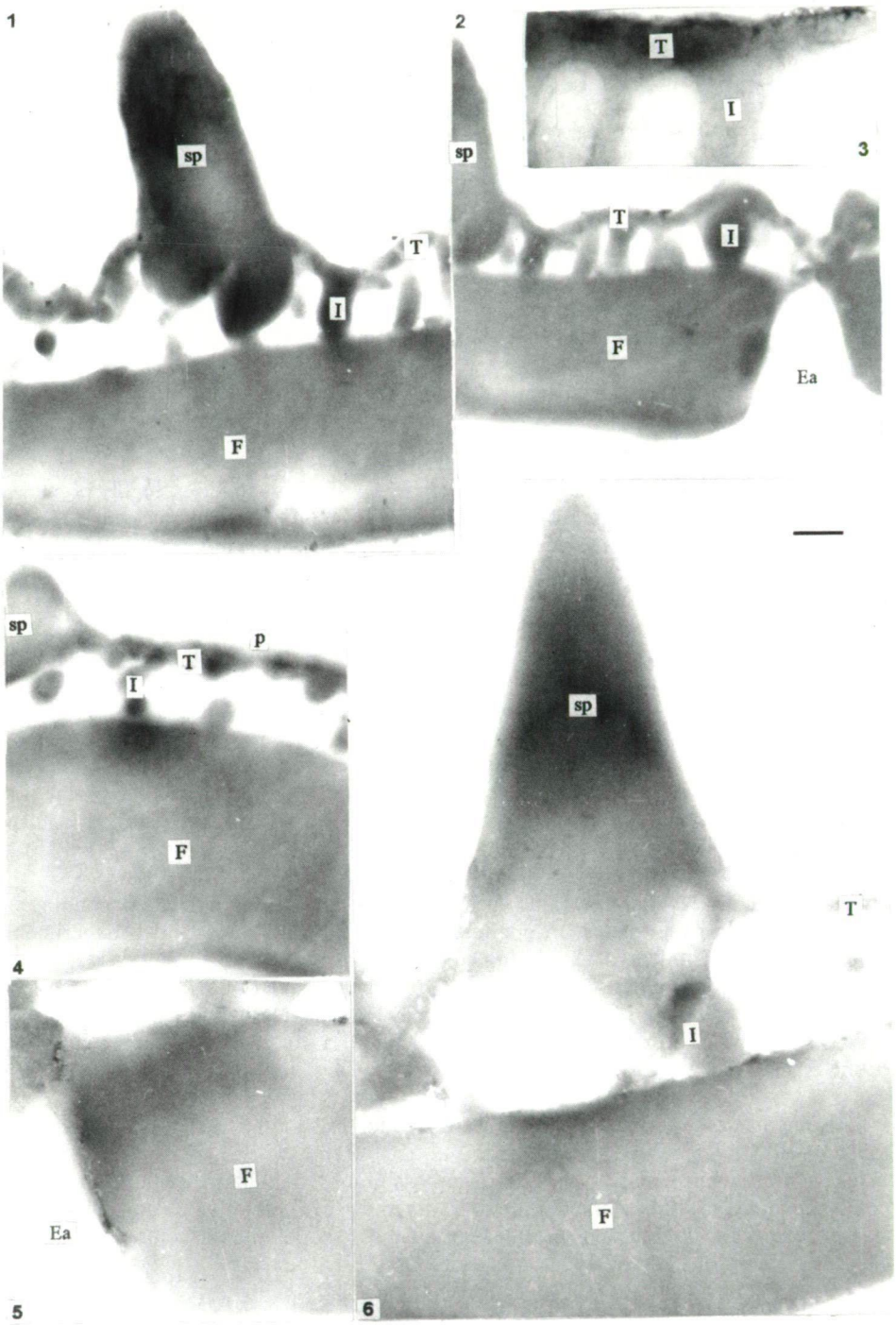

Plate 8.2.

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.

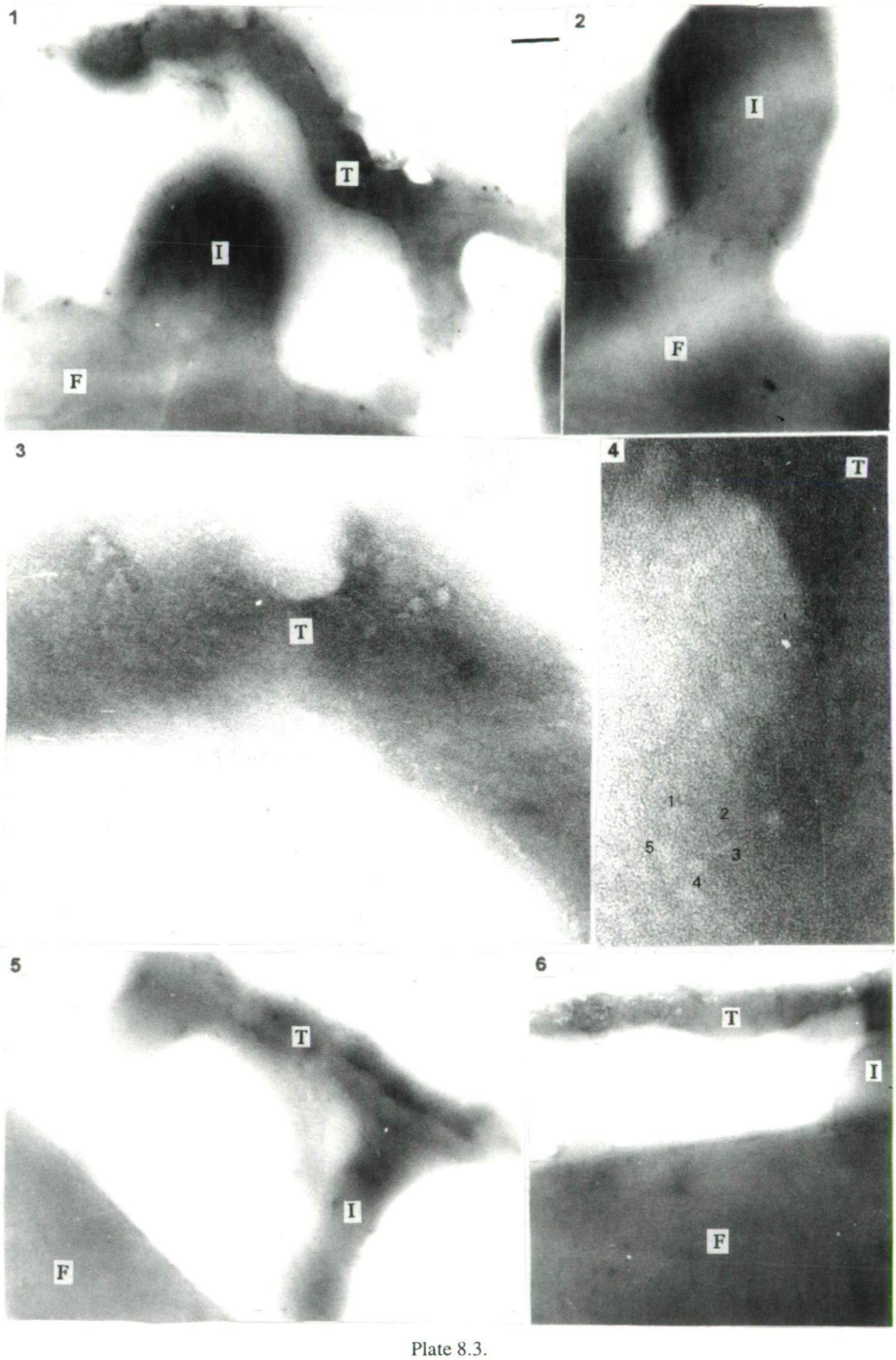

Plate 8.3.

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.

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