7. TRANSMISSION ELECTRON MICROSCOPY OF PARTIALLY DEGRADED POLLEN GRAINS OF AMBROSIA ARTEMISIIFOLIA (RAGWEED)

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Abstract

Pollen grains of *Ambrosia artemisiifolia* L. were partially degraded with 2-aminoethanol, potassium permanganate and merkaptoethanol. The partial degradation with 2-aminoethanol and potassium permanganate revealed the biopolymer structure of the ectexine. Several pecularities were established after partial degradation, such as: 1. The very resistant pollenkitt in the holes of the infratectal layer. 2. Rarely micro-organisms were observed on the surface of the tectum. 3. The partial degradation resulted in different layers in the molecular structure of both tectal and infratectal surfaces. 4. The outer part has strong electron density and probably with radially oriented helical structures. 5. The lamellar structure of the foot layer and in particular of the ectexine was also discovered by the combined partial degradation with 2-aminoethanol and potassium permanganate. 6. Network of mostly cyclic molecules were observed at the ultrathin sections of the partially degraded ectexine. 7. The merkaptoethanol at the end revealed the organelles of the protoplasm.

Key words: Palynology, recent, *Ambrosia artemisiifolia*, experimentally degraded, TEM.

Introduction

Pollen grains of *Ambrosia artemisiifolia* are extremely allergenic. This is the reason why a great number of papers are dealing with the presence of the pollen grains of *Ambrosia* in the air. Some selected papers are as follows: GRATER and STEMEN (1967) emphasized, that among the aeroallergenic pollen grains the ragweed have been the most intensively investigated among the aeroallergen palynomorphs. According to O'ROURKE (1996) the *Ambrosia* with 45 species is the most important aeroallergen in North America. Two species (*A. trifida, A. elatior*) was discussed in the first place with the two acidic proteins as major antigens (E and K). LEUSCHNER (1985) pointed, that the *Ambrosia* was discovered in Switzerland in 1970. Later, LEUSCHNER, BOEHM and MARI (1990) discussed the spreading of this plant. It is worth mentioning, that the pollen grains of *Ambrosia* were not included in the monograph of NILSSON, PRAGLOWSKI and NILSSON (1977) and in the atlas of PEHLIVAN (1995). The localisation of the antigenic and allergenic proteins in the intine was established by several authors: KNOX and HESLOP-HARRISON (1970, 1971), KNOX, HESLOP-HARRISON and REED (1970), KNOX, WILLING and ASHFORD (1972). During our TEM studies of partially dissolved pollen grains of ragweed pollen grains chloroplasts were observed in the intine (KEDVES and PÁRDUTZ, 2000). On the surface of the thylakoid membranes different kinds of molecular systems were observed which may be important in the biosynthesis of the pollen grains. In another paper (KEDVES, PÁRDUTZ and MADARÁSZ, 2000) a poorly preserved regular pentagon of the partially degraded exine was studied with the symmetry operation, and for the first time the molecular structure of the globular units of the metastable biopolymer system was published. These units are composed of a cluster of cyclic molecules. The occurrence of ragweed pollen grains in Hungary was pointed out by JÁRAI-KOMLÓDI (1991), MEZEI et al. (1991, 1992), JÁRAI-KOMLÓDI and JUHÁSZ (1993), JÁRAI-KOMLÓDI and MEDZIHRADSZKY (1993) and MOLNÁR (1999).

In our experimental studies on recent pollen grains allergenic pollen grains were also investigated. Several times we noticed the presence of micro-organisms on the surface or in the holes of the infratectal layer ectexine which may be factors of combined allergenic effect. The importance of the exine ultrastructure in the allergenic effect was pointed out in several papers. NILSSON, PRAGLOWSKI and NILSSON (1977) published several important TEM data from allergenic pollen grains and spores from Northern Europe. CERCEAU-LARRIVAL (1986), ABADIE et al. (1986, 1988), CERCEAU-LARRIVAL and DEROUET (1988), CERCEAU et al. (1991) emphasized that the channels in the tectum of the pollen grains promote the diffusion of the water soluble allergenic proteins. Ultrastructure of acetolyzed pollen grains of the genus *Ambrosia* were published by PAYNE and SKVARLA (1970).

The aim of this paper is to present in detail the results of the partially degraded pollen grains of *Ambrosia artemisiifolia*, to get further ultrastructural on biopolymer characteristic features, which may be connected to the allergenic character of the pollen grain.

Materials and Methods

The material for this investigation was collected by M. KEDVES on the 1998. The partial degradation was as follows:

Temperature 30°C, 5 mg dry pollen grains.

Experiment No.:1/7-1391. - 1 ml 2-aminoethanol, length of time 24h.

Experiment No.: 1/7-1392. - 1 ml 2-aminoethanol, length of time 48h.

Experiment No.: 1/7-1393. - 1 ml 2-aminoethanol, length of time 72h.

Experiment No.: 1/7-1394. - 1 ml 2-aminoethanol, length of time 24h, washing, + 10 ml 0.01% $KMnO_4$, length of time 24h.

Experiment No.: 1/7-1395. - 1 ml 2-aminoethanol, length of time 48h, washing, + 10 ml 0.01% $KMnO_4$, length of time 24h.

Experiment No.: 1/7-1396. - 1 ml 2-aminoethanol, length of time 72h, washing, + 10 ml 0.01% $KMnO_4$, length of time 24h.

Experiment No.: 1/7-1397. - 1 ml 2-aminoethanol, length of time 24h, washing, + 1 ml merkaptoethanol, length of time 24h.

Experiment No.: 1/7-1398. - 1 ml 2-aminoethanol, length of time 48h, washing, + 1 ml merkaptoethanol, length of time 24h.

Experiment No.: 1/7-1399. - 1 ml 2-aminoethanol, length of time 72h, washing, + 1 ml merkaptoethanol, length of time 24h.

After washing, the pollen material was postfixed with OsO_4 aq. dil. 1% and embedded in Araldite. The ultrathin sections were made in the EM Laboratory of the Institute of Biophysics of the Biological Research Center of the Hungarian Academy of Sciences on a Porter Blum ultramicrotome. The pictures were taken in a Zeiss EM-902 by resolution 2-3 Å as in Plate 7.2, figs. 1-7, Plate 7.3., figs. 1-4 and a Tesla BS-540, resolution of 6-7 Å, in Plate 7.1., figs. 1-6, and Plate 7.4., figs. 1-6. All pictures are unretouched.

Results

Partial degradation with 2-aminoethanol

Experiment No.: 1/7-1391 (Plate 7.1., figs. 1,2). - The endexine is well separated from the foot layer, its ultrastructure is more or less finely lamellar. Essentially no important alterations were observed. Plasma membrane and the organelles of the protoplasm are relatively well preserved.

Experiment No.: 1/7-1392 (Plate 7.1., fig. 3). - The ultrastructure is similar to the previous sample, differences in the electron density of the ectexine and endexine are observed, namely the ectexine is more electron dense than the endexine.

Experiment No.: 1/7-1393 (Plate 7.1., figs. 4-6). - One microorganism probably of bacterial origin was observed on the surface (Plate 7.1., fig. 4). Characteristic electron dense material of pollenkitt are in the holes of the infratectal layer. The intine separates from the foot layer by its electron density. The degradation of the plasma membrane and the organelles of the protoplasm are characteristic.

Partial degradation with 2-aminoethanol and $KMnO_4$ aq. dil.

Experiment No.: 1/7-1394 (Plate 7.2., figs. 1-7). - This experiment resulted in important alterations in the fine structure of the exine of the pollen grains. Three layers can be distinguished at the originally homogeneous ectexine. The outer and the inner surfaces are characteristic. The outermost electron dense layer (Plate 7.2., figs. 2,3) is composed of more or less globular units. These units are arranged in radially oriented linear structures or their disposition is irregular. A light zone of 28-36 Å follow this layer. The inner part of the ectexine is finely granular (Plate 7.2., figs. 1,2,4,5). These granular structures represent the molecular structures of the ectexine (Plate 7.2., figs. 6,7). The ultrastructure of the inner surfaces of the infratectal layer is identical with the outermost part of the tectum. The partially degraded foot layer is lamellar, with some electron dense particles or layers. The endexine is also lamellar (Plate 7.2., fig. 1). The biopolymer system is well documented with this experiment. The diameter of the globular units are 4-5 Å. There are different kinds of arrangements. Linear, pentagonal (cyclic) and irregular structures were observed (Plate 7.2., fig. 6). The molecular structure sensu strictu (Plate 7.2., fig. 7) represents a network composed of cyclic molecular structures.

Experiment No.: 1/7-1395 (Plate 7.3., figs. 1-4). - The stratification of the ectexine is similar to the previous experiment, but the degradation of the outermost electron dense layer of the ectexine in some parts of the surface is characteristic (Plate 7.3., fig. 1). The ultrastructure of the inner walls of the ectexine is not always the same, in all probability in consequence of the differences in the experimental effect. The lamellar ultrastructure of the foot layer is not so characteristic (Plate 7.3., figs. 1,3), but very characteristic finely lamellar ultrastructure was observed in some parts of the intine (Plate 7.3., fig. 1). The biopolymer and the molecular system of the foot layer is illustrated in fig. 4, Plate 7.3. The molecular structure revealed is also mostly composed of cyclic units. The diameters of the higly organized biopolymer structures are 6-8 Å. Several linear arrangements of these units were observed in more or less radial orientation.

Experiment No.: 1/7-1396 (Plate 7.4., fig. 1). - A general survey picture illustrated the ultrastructural alterations of the ectexine. But the electron density of the endexine is stronger than those of the ectexine including the foot layer too. Fine structure of the intine was not observed. The characteristic degradation of the plasma membrane is illustrated. The organelles of the protoplasm are also partially degraded.

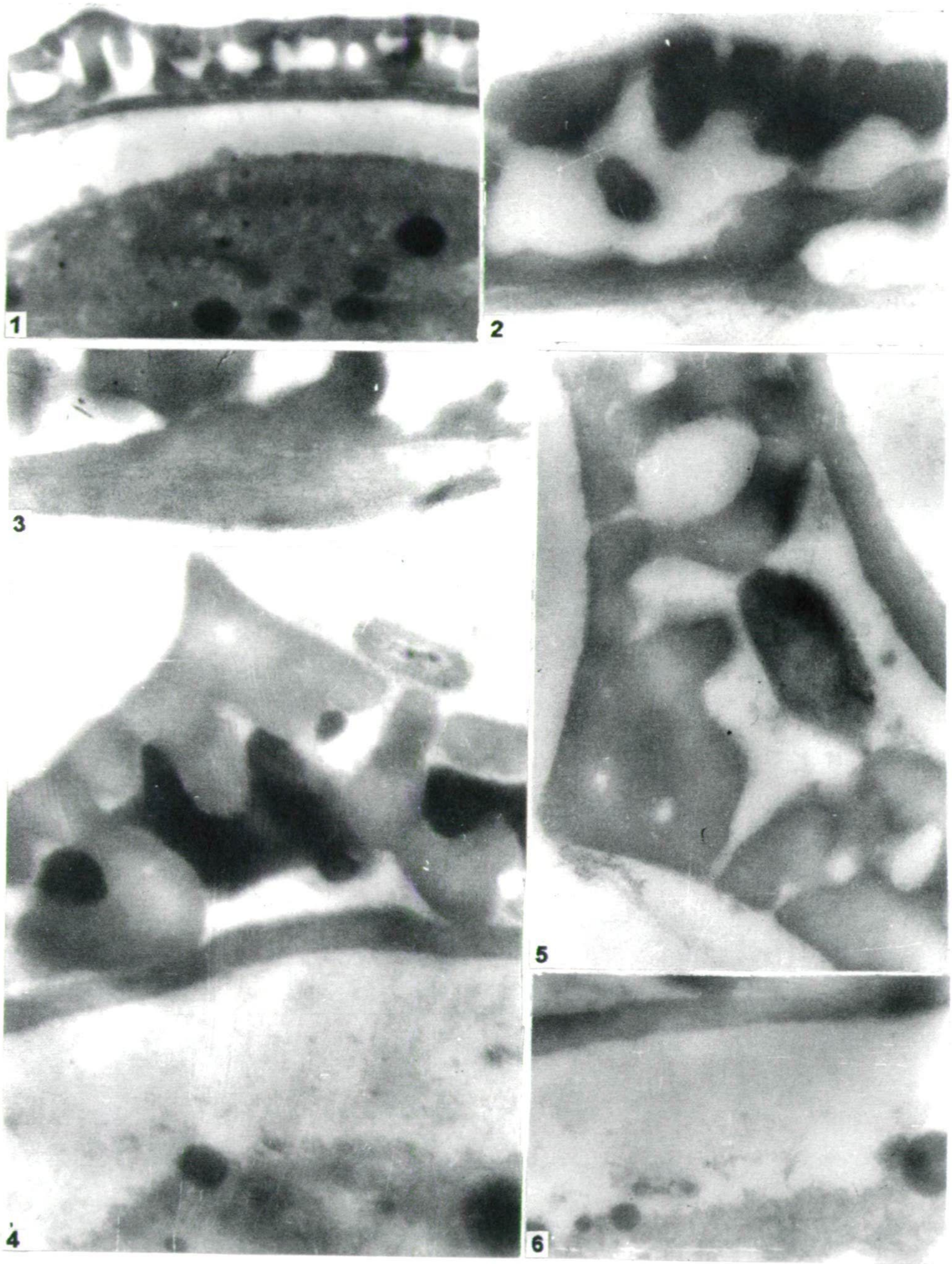

Plate 7.1.

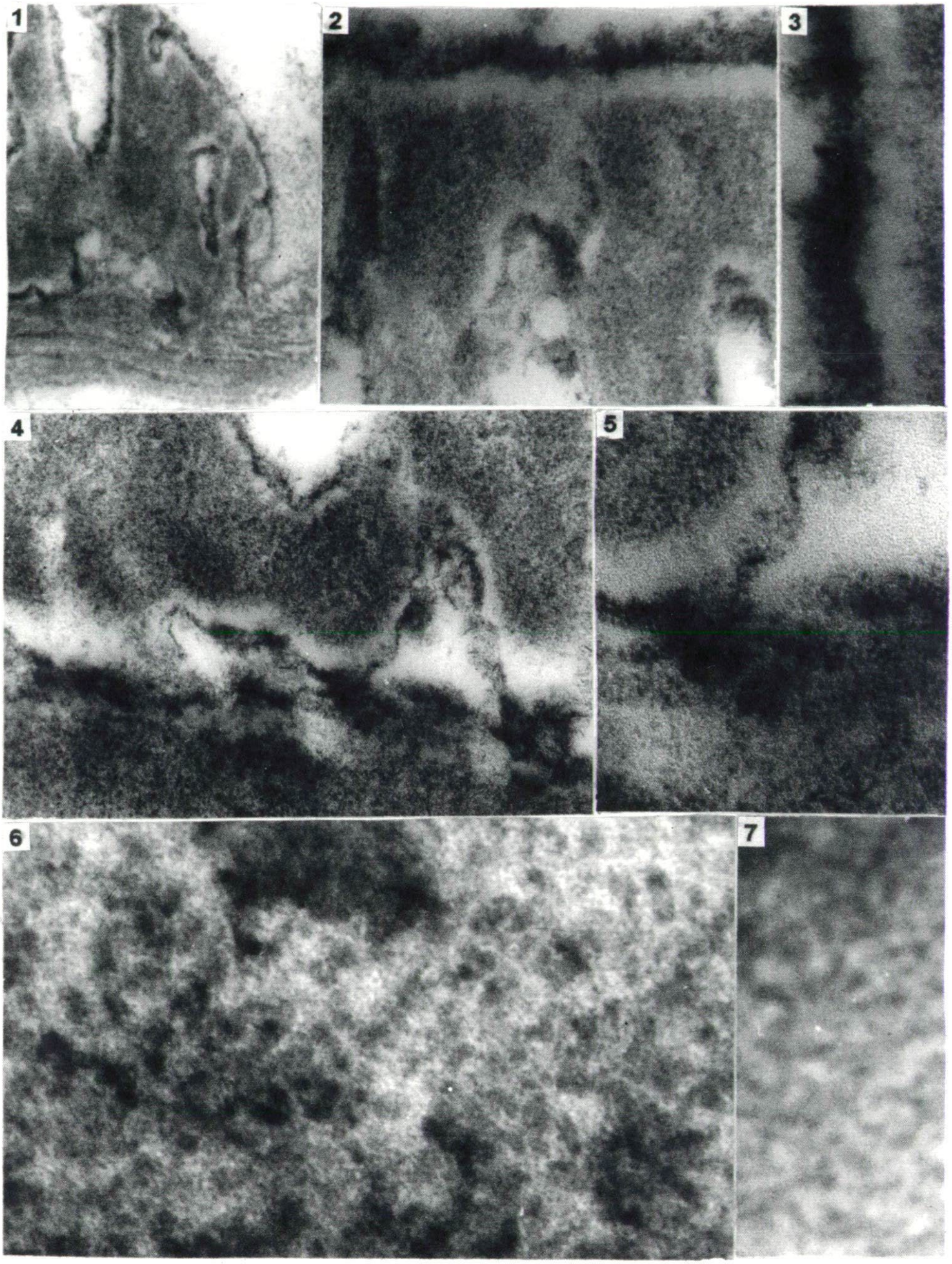

Plate 7.2.

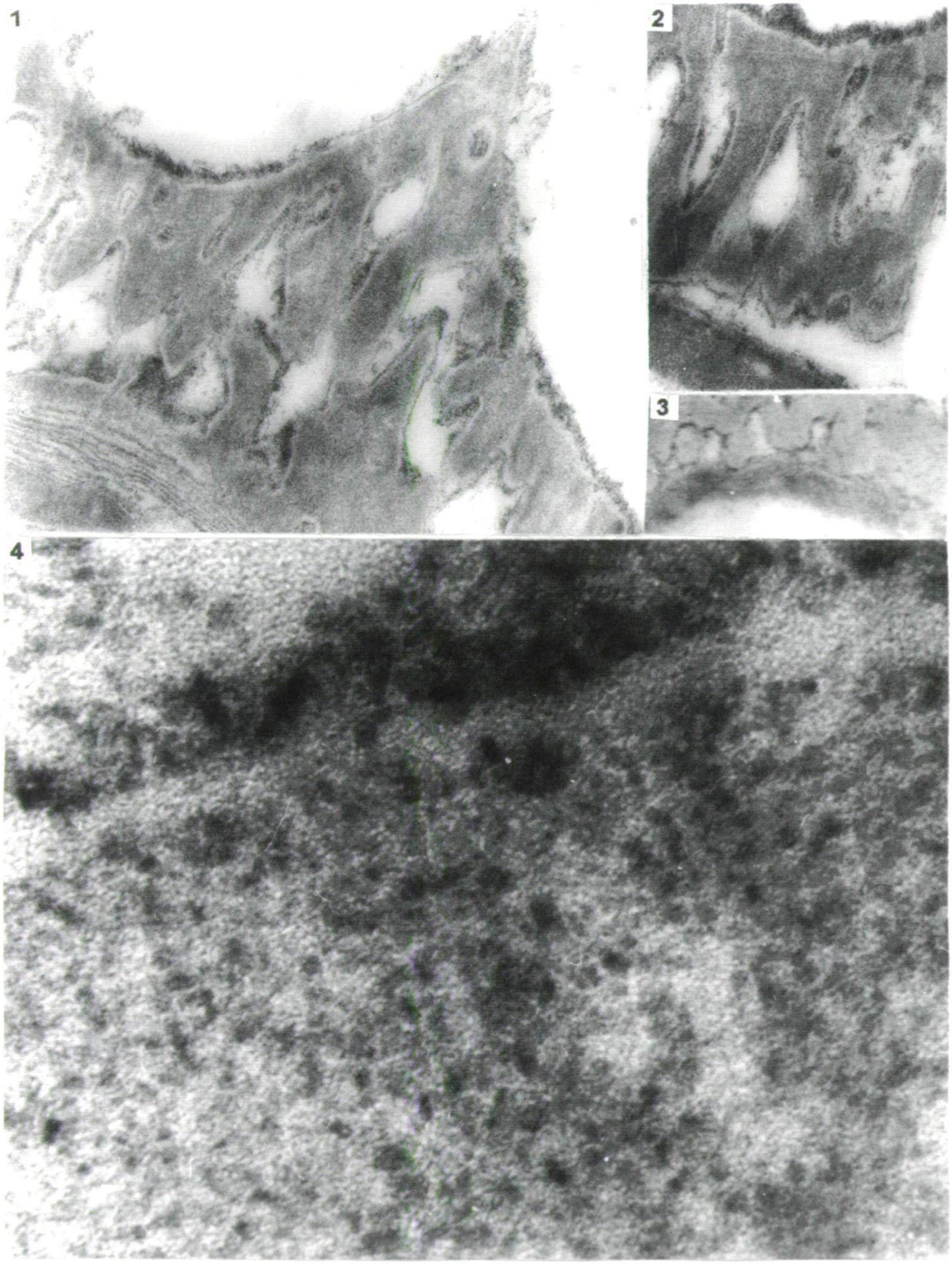

Plate 7.3.

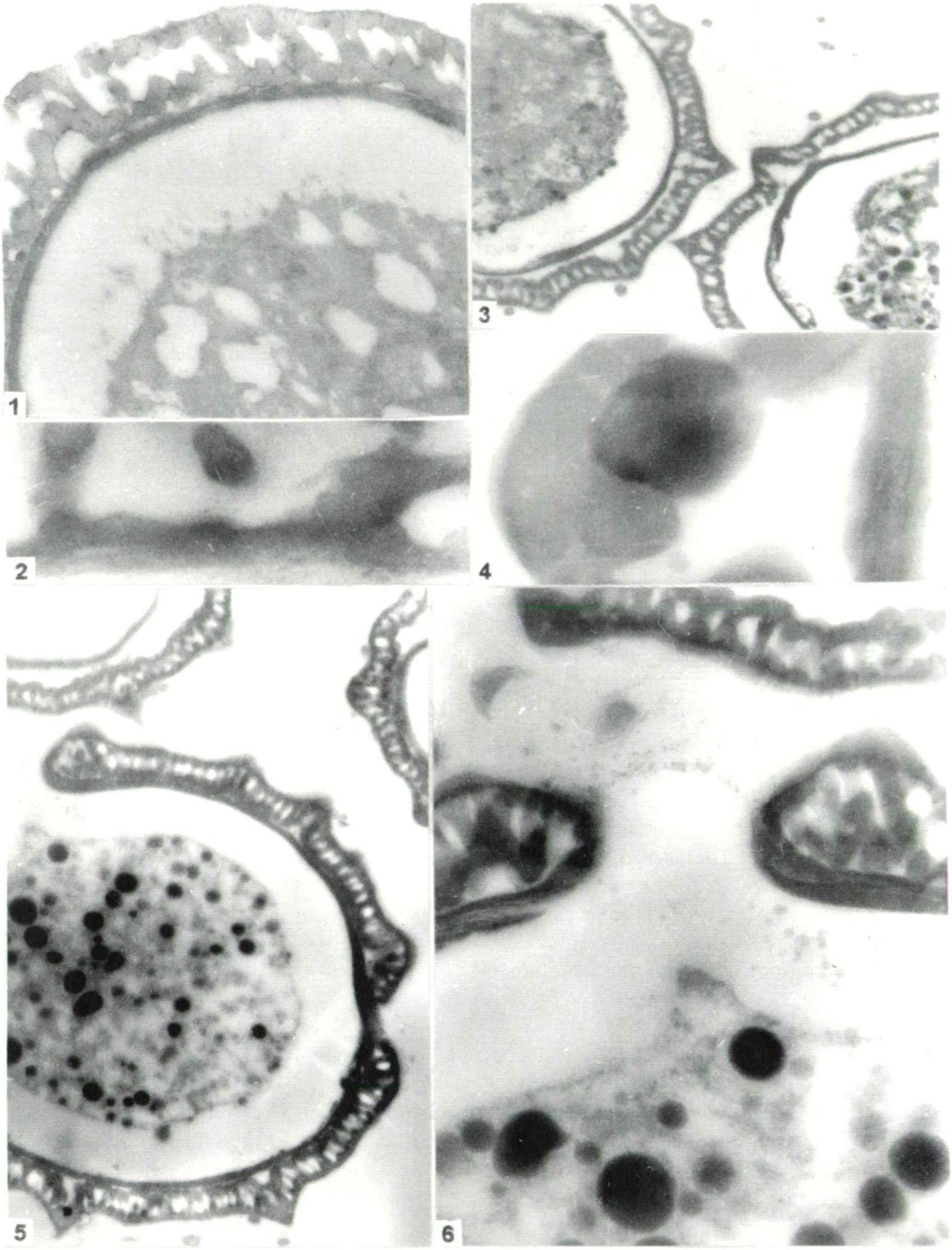

Plate 7.4.

Plate 7.1.

1-6. *Ambrosia artemisiifolia* L. 1. Experiment No.: 1/7-1391, Negative No.: 7385, 12.300x. 2. Experiment No.: 1/7-1391, Negative No.: 7370, 41.000x, 3. Experiment No.: 1/7-1392, Negative No.: 7332, 41.000x. 4. Experiment No.: 1/7-1393, Negative No.: 7338, 41.000x. 5. Experiment No.: 1/7-1393, Negative No.: 7335, 41.000x. 6. Experiment No.: 1/7-1393, Negative No.: 7336, 41.000x.

Plate 7.2.

1-7. *Ambrosia artemisiifolia* L. 1. Experiment No.: 1/7-1394, Negative No.: 7353, 41.000x. 2. Experiment No.: 1/7-1394, Negative No.: 7557, 123.000x. 3. Experiment No.: 1/7-1394, Negative No.: 7558, 205.000x. 4. Experiment No.: 1/7-1394, Negative No.: 7561, 123.000x. 5. Experiment No.: 1/7-1394, Negative No.: 7562, 205.000x. 6. Experiment No.: 1/7-1394, Negative No.: 7563, 820.000x. 7. Experiment No.: 1/7-1394, Negative No.: 7564, 2,050.000x.

Plate 7.3.

1-4. *Ambrosia artemisiifolia* L. 1. Experiment No.: 1/7-1395, Negative No.: 7358, 41.000x. 2. Experiment No.: 1/7-1395, Negative No.: 7357, 41.000x. 3. Experiment No.: 1/7-1395, Negative No.:7401, 41.000x. 4. Experiment No.: 1/7-1395, Negative No.: 7571, 820.000x.

Plate 7.4.

1-6. *Ambrosia artemisiifolia* L. 1. Experiment No.: 1/7-1396, Negative No.: 7380, 12.300x. 2. Experiment No.: 1/7-1397, Negative No.: 7370, 41.000x. 3. Experiment No.: 1/7-1398, Negative No.: 7377, 4.100x. 4. Experiment No.: 1/7-1398, Negative No.: 7373, 41.000x. 5. Experiment No.: 1/7-1399, Negative No.: 7378, 4.100x. 6. Experiment No.: 1/7-1399, Negative No.: 7380, 12.300x.

Partial degradation with 2-aminoethanol and merkaptoethanol

Experiment No.: 1/7-1397 (Plate 7.4., fig. 2). - A remarkable degradation of the intine was observed. The electron dense particles are well presented.

Experiment No.: 1/7-1398 (Plate 7.4., figs. 3-5). - The endexine separates sometimes from the foot layer. Degradation of the intine, sometimes together with the foot layer, was observed. The electron dense particles are present in the holes of the infratectal layer. The intine and the plasma membrane is partially degraded. The protoplasm is full of electron dense particles and light areas, probably with vacuoles.

Experiment No.: 1/7-1399 (Plate 7.4., fig. 6). - The degradation of the plasma membrane is well illustrated together with the different kinds of organelles of the protoplasm. The degradation of the intine is remarkable, but in the apertural area a not so well preserved operculum-like granular structure was observed. The tectum is sometimes dissoluted.

Discussion and Conclusions

Based on our present results, the following may be pointed out:

1. The resistant electron dense particles in the holes of the infratectal layer can be destroyed only by oxidation after the dissolution with 2-aminoethanol.

2. Micro-organisms occur rarely on the perforated tectum, which may be the consequence of the aromatic derivates of the whole plant.

3. The degradation with 2-aminoethanol combined with oxidizing agents discovered several structures of different level of organization of the ectexine.

3.1. On the surface of the tectum and on the inner surfaces also an electron dense layer is present. On the tectum this layer may be composed of radially oriented helical structures. After this layer there is a light zone this is completely new in comparison to the earlier investigated exines.

3.2. The inner part of the tectum and the infratectal layer is of granular structure after partial degradation, namely the biopolymer structures of different organization are well demonstrated.

3.3. The endexine and sometimes the foot layer is finely lamellar after this kind of degradation process.

In comparison with the previous similar degradation experiment it may be emphasized, that there are differences to the previous ones. Namely the infratectal layer was degraded in the first place. The superficial electron dense layer was observed until now in *Phoenix sylvestris* only (cf. KEDVES, BORBOLA, TRIPATHI and KUMAR, 2000).

The ultrastructural data both non-experimental and experimental are useful to understand the allergenic effect of the spores and pollen grains.

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