9. LM AND TEM INVESTIGATIONS ON PARTIALLY DEGRADED POLLEN GRAINS OF ALNUS GLUTINOSA (L.) GAERTN.

M. KEDVES[1], Á. PÁRDUTZ[2], M. MADARÁSZ[1], K. PRISKIN[1], A. SZÉCSÉNYI[1], J. SASHALMI[1] and D. TOMBÁCZ[1]

1. Cell Biological and Evolutionary Micropaleontological Laboratory of the University of Szeged, H-6701, P.O. Box 993, Szeged, Hungary, 2. Institute of Biophysics, Biological Research Center of the Hungarian Academy of Sciences H-6701, P.O. Box 521, Szeged, Hungary

Abstract

This paper present the qualitative LM and TEM results of the partially degraded pollen grains of *Alnus glutinosa* (L.) GAERTN. Three series (2-aminoethanol, 2-aminoethanol + $KMnO_4$, 2-aminoethanol + merkaptoethanol) of experiments and glycerine (50%) treatment were carried out. Identities within the series of experiments were established.

Key words: Palynology, recent, *Alnus glutinosa*, experimental ultrastructure.

Introduction

Pollen grains of *Alnus glutinosa* (L.) GAERTN. are allergenic (RICHARD et al. 1986, JÁRAI-KOMLÓDI, 1991, PEHLIVAN, 1995, MOLNÁR,1999, etc.). Pollen grains of this species are included in the book of NILSSON, PRAGLOWSKI and NILSSON (1977) and LM, SEM and TEM pictures were published from the non-experimental pollen grains.

Previously different kinds of experimental studies were carried out in our Laboratory on the pollen grains of the genus *Alnus*. X-ray irradiated pollen grains of *Alnus subcordata* C. A. MEY were investigated by KEDVES and UNGVÁRI (1996). The following was established, p. 80: "The differences in the percentages of the pollen tube development of the genuses *Betula* and *Alnus* are also interesting." TEM studies of X-ray irradiated pollen grains of *Alnus glutinosa* were published by KEDVES and PÁRDUTZ (1992). Pollen grains of *Alnus glutinosa* were investigated with the LM method after partial dissolution with 7 organic solvents (i-amyl alcohol, n-butanol, n-propanol, ethanol, methanol, merkaptoethanol, diethylamine) by KEDVES, HORVÁTH, BORBOLA and TÓTH (1999). The following was concluded, p. 82: "In comparison with *Betula verrucosa* EHRH. there are minor alterations at the pollen grains of *Alnus glutinosa*." The TEM method was used for partially degraded pollen grains by different kinds of methods. The biopolymer organization was investigated by KEDVES and ROJIK (1989) on partially degraded and fragmented exines of *Alnus glutinosa*. Different kinds of organization were observed: filamentous units, basic and highly organized metastable quasi periodic biopolymer structures and helical structures also. The modified Markham rotation method was also used. KEDVES, FARKAS, MÉSZÁROS, TÓTH and VÉR (1991): "Several kinds of the modi-

fied Markham rotation method were used to verify and investigate the symmetry of the basic polygon."

This paper represent a part of our systematic studies on allergenic pollen grains with two kinds of standard methods. The qualitative LM and TEM data are presented in this paper. The aim of this paper is to present data, and comparisons with the previous results in this subject.

Materials and Methods

The investigation material was collected by M. MADARÁSZ on 18. 02. 2000 in the Botanical Garden of the University of Szeged.

The following experiments were carried out:

T-12-10: non-treated pollen grains.

T-12-11: 5 mg dry pollen grains + 1 ml 2-aminoethanol, length of time 24 h, at 30 °C.

T-12-12: 5 mg dry pollen grains + 1 ml 2-aminoethanol, length of time 48 h, at 30 °C.

T-12-13: 5 mg dry pollen grains + 1 ml 2-aminoethanol, length of time 72 h, at 30 °C.

T-12-14: 5 mg dry pollen grains + 1 ml 2-aminoethanol, length of time 24 h and 10 ml $KMnO_4$ during 24 h, at 30 °C.

T-12-15: 5 mg dry pollen grains + 1 ml 2-aminoethanol, length of time 48 h and 10 ml $KMnO_4$ during 24 h, at 30 °C.

T-12-16: 5 mg dry pollen grains + 1 ml 2-aminoethanol, length of time 72 h and 10 ml $KMnO_4$ during 24 h, at 30 °C.

T-12-17: 5 mg dry pollen grains + 1 ml 2-aminoethanol, length of time 24 h and 1 ml merkaptoethanol during 24 h, at 30 °C.

T-12-18: 5 mg dry pollen grains + 1 ml 2-aminoethanol, length of time 48 h and 1 ml merkaptoethanol during 24 h, at 30 °C.

T-12-19: 5 mg dry pollen grains + 1 ml 2-aminoethanol, length of time 72 h and 1 ml merkaptoethanol during 24 h, at 30 °C.

T-12-20: 5 mg dry pollen grains + 5 ml glycerine (50%), length of time 30 days.

LM pictures were taken from unstained and stained pollen grains mounted in glycerine-jelly hydrated and from the pollen grains after embedding in Araldite.

For TEM investigations, the pollen material was washed with distilled water, then 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 Biological Research Center of the Hungarian Academy of Sciences. The microphotographs were made on a Tesla BS-540 (resolution 6-7 Å).

Results

1. Partial degradation with 2-aminoethanol (Plate 9.1., figs. 1-22)

1.1. Experiment No.: T-12-11 (Plate 9.1., figs. 1-8)

LM results. - No taxonomically significant alterations were observed. Thinning of the ectexine and electron dense particles of the protoplasm and intine protrusions are illustrated in pictures 2-4 (Plate 9.1.). In TEM pictures (Plate 9.1., figs. 5-8), there are numerous electron dense microbodies in the protoplasm. Rarely in the intine electron dense globular units are also present (Plate 9.1., figs. 5,7). The protruding protoplasm (Plate 9.1., fig. 8) was also perceptible. Characteristic degradation of the infratectal layer and of the intine is illustrated in pictures 5-7, Plate 9.1.

1.2. Experiment No.: T-12-12 (Plate 9.1., figs. 9-16)

LM results. - Similar to the previous experiment protruding protoplasm was not observed after the treatment (Plate 9.1., figs, 9-12). TEM results (Plate 9.1., figs. 13-16), show the degradation of the infratectal layer (Plate 9.1., figs. 13-15). Rarely electron dense granules are in this layer (Plate 9.1, fig. 14). The endexine is seemingly dissolved. There are numerous electron dense microbodies in the protoplasm (Plate 9.1., figs. 13,15,16).

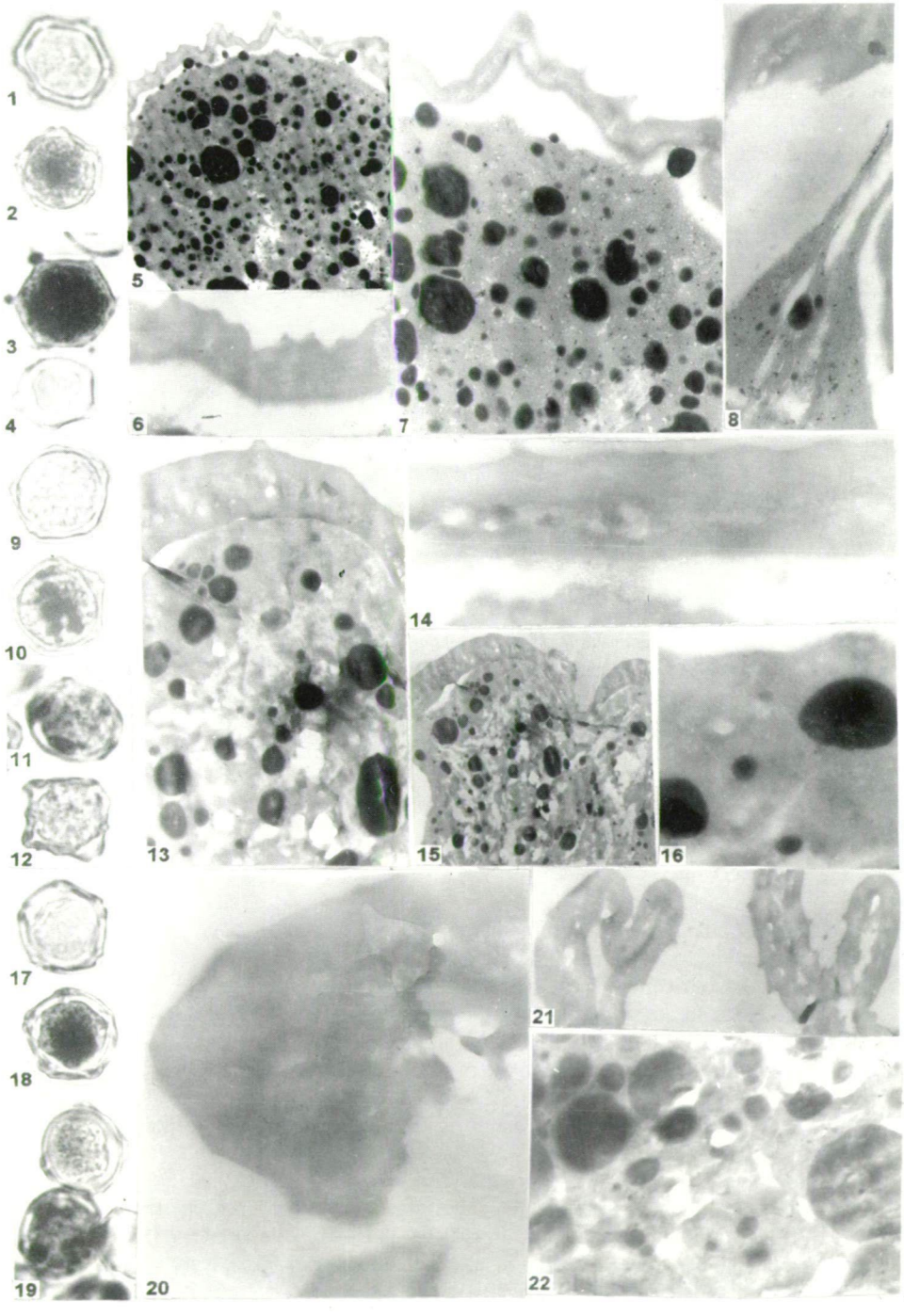

Plate 9.1.

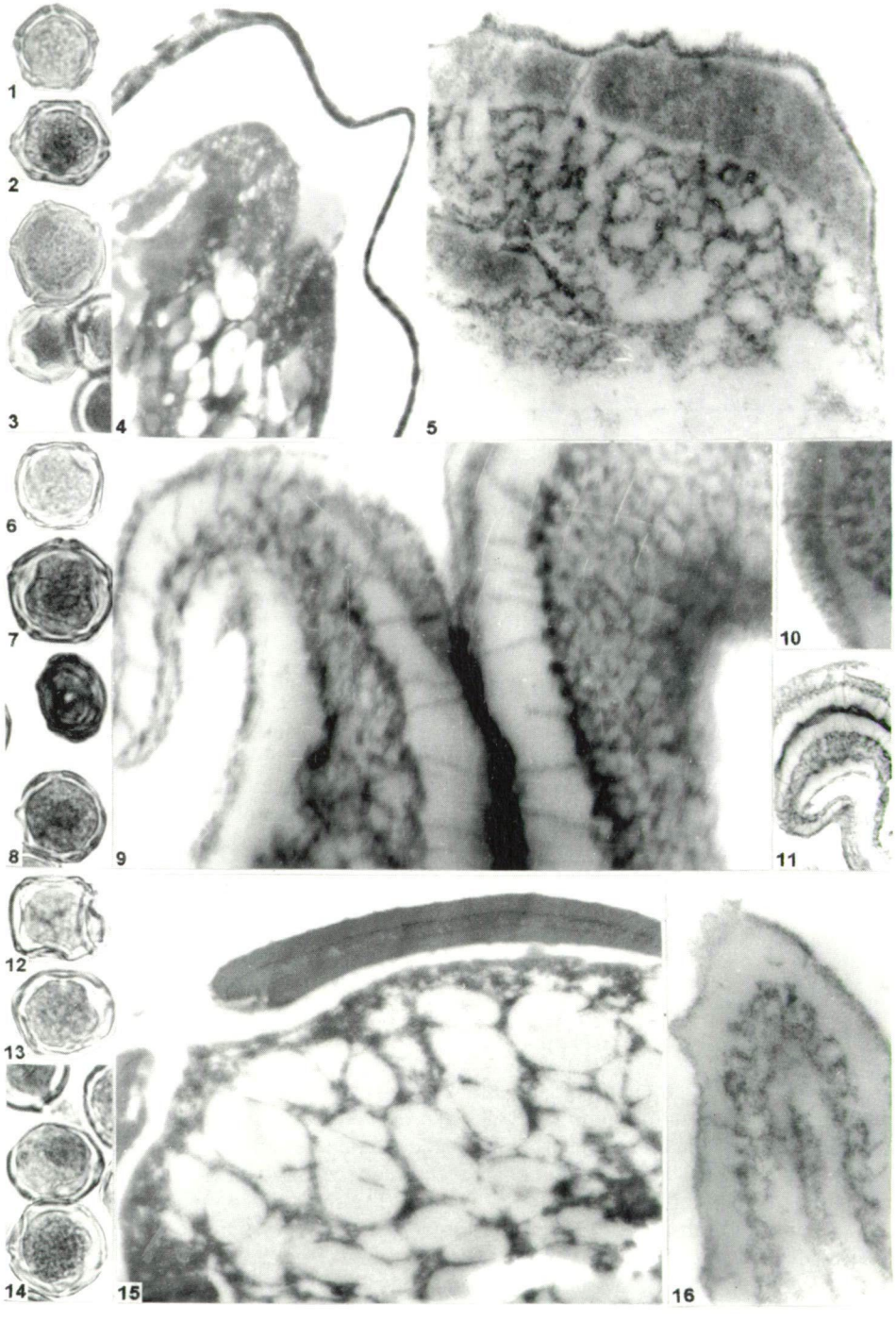

Plate 9.2.

Plate 9.1.

1-22. *Alnus glutinosa* (L.) GAERTN.
1-8. Experiment No.: T-12-11. 1-4. LM pictures, 5-8. TEM pictures, 5. Negative No: 8375, 5.000x., 6. Negative No.: 8379, 15.000x., 7. Negative No.: 8376, 15.000x., 8. Negative No.: 8378, 15.000x., 9-16. Experiment No.: T-12-12. 9-12. LM pictures, 13-16. TEM pictures, 13. Negative No.: 8310, 15.000x., 14. Negative No.: 8309, 5.000x., 15. Negative No.: 8235, 50.000x., 16. Negative No.: 8311, 50.000x., 17-22. Experiment No.: T-12-13. 17-19. LM pictures, 20-22. TEM pictures. 20. Negative No.: 8314, 50.000x., 21. Negative No.: 8213, 15.000x., 22. Negative No.: 8240, 15.000x.

Plate 9.2.

1-16. *Alnus glutinosa* (L.) GAERTN.
1-5. Experiment No.: T-12-14. 1-3. LM pictures, 4,5. TEM pictures, 4. Negative No.: 8292, 5.000x., 5. Negative No.: 8300, 50.000x., 6-11. Experiment No.: T-12-15. 6-8. LM pictures, 9-11. TEM pictures, 9. Negative No.: 8243, 75.000x., 10. Negative No.: 8244, 50.000x., 11. Negative No.: 8242, 15.000x. 12-16 Experiment No.: T-12-16. 12-14. LM pictures, 15,16. TEM pictures, 15. Negative No.: 8318, 10.000x., 16. Negative No.: 8383, 50.000x.

1.3. Experiment No.: T-12-13 (Plate 9.1., figs. 17-22)

LM results. - Identical with the previous one (Plate 9.1., figs. 17-19). The disintegration of the infratectal layer in the apertural area is characteristic (Plate 9.1., fig. 20). Morphological alterations occurred during the embedding processes, which are well shown in picture 21, Plate 9.1. The electron dense microbodies are numerous (Plate 9.1., fig. 22).

1.4. Experiment No.: T-12-14 (Plate 9.2., figs. 1-5)

LM results. - The ectexine investigated by this method seems to be not altered in a remarkable measure. There are light areas (intine) between the foot layer and the plasma membrane (Plate 9.2., figs. 1-3). TEM results. - Sometimes the outest ectexine layers are degraded. The intine, the plasma membrane and the electron dense microbodies in the protoplasm are mostly degraded (Plate 9.2., fig. 4). In the apertural area the surface of the isodiametric and anastomosing elements of the infratectal layer are degraded. Similar superficial alterations were observed on the tectum and on the foot layer. The endoaperture is also altered, remnants of the intine with lamellar ultrastructure are in the inner part of the exoaperture.

1.5. Experiment No.: T-12-15 (Plate 9.2., figs. 6-11)

LM results. - Essentially identical with the previous one (Plate 9.2., figs. 6-8). TEM results. - Alterations in the morphology of the pollen grains were also observed, which may be the consequence of the embedding processes (Plate 9.2., figs. 9,11). Characteristic degradation was observed on all surfaces of the ectexine, well shown at the channels of the tectum also.

1.6. Experiment No.: T-12-16 (Plate 9.2., figs. 12-16)

LM results. - Not so important alterations were observed (Plate 9.2., figs. 12-14), the contraction of the protoplasm is a little advanced in relation of the previous ones. TEM results. - The degradation of the protoplasm and the pollen wall is expressed (Plate 9.2., figs. 15,16). All surfaces, outer and inner of the ectexine are disintegrated these superficial parts are electron dense (Plate 9.2., fig. 16). The intine is completely disappeared. The protoplasm protrudes in the apertural area, the plasma membrane is degraded, the electron dense microbodies are completely disappeared (Plate 9.2., fig. 15).

1.7. Experiment No.: T-12-17 (Plate 9.3., 1-7)

LM results. - The protoplasm was not greatly contracted (Plate 9.3., fig. 2). TEM results. - The degradation of the ectexine is characteristic, particularly in the infratectal

layer (Plate 9.3., figs. 5-7). Electron dense particles of different size represent the infratectal layer. Relatively large electron dense globular units are in the intine. The intine is not completely disintegrated. The electron dense bodies are not degraded in the protoplasm (Plate 9.3., fig. 5).

1.8. Experiment No.: T-12-18 (Plate 9.3., figs. 8-14)

LM results. - The contraction of the protoplasm is well shown and it is heterogeneous in stain acceptance (Plate 9.3., figs. 9,11). TEM results. - Important basic morphological alterations were established in the ultrathin sections, e.g.: Plate 9.3., fig. 12. The ectexine is degraded (Plate 9.3., figs. 12,13). The intine is seemingly completely distroyed, but electron dense globular units are between the ectexine and the protoplasm. The disintegration of the organelles of the protoplasm is heterogeneous in relation to the electron dense microbodies (Plate 9.3., fig. 12). In some part of the protoplasm is filled with electron dense bodies (Plate 9.3., figs. 13,14) in other cases it is full with holes in consequence of the degradation of these particles (Plate 9.3., fig. 12).

1.9. Experiment No.: T-12-19 (Plate 9.4., figs. 15-20)

LM (Plate 9.3. figs. 15-18) and TEM (Plate 9.3., figs. 19-20) results are identical with the previous experiment.

1.10. Experiment No.: T-12-20 (Plate 9.4., figs. 1-7)

LM results. - Sometimes peculiar contraction of the protoplasm was observed (Plate 9.4., figs. 1,2), and the stain acceptance is remarkable (Plate 9.4., figs. 3,4). Dissolution of the ectexine is well illustrated in pictures 5-7, plate 9.4. The intine disappeared, the disintegration of the protoplasm is also well shown (Plate 9.4., fig. 5). There are holes, and a homogenisation of the organelles is characteristic.

Discussion and Conclusions

1. Among the LM results the following are worth of mentioning:

1.1. Protrusion of the protoplasm occurred only at the experiment T-12-11.

1.2. The stain acceptance of the protoplasm is sometimes characteristic.

1.3. Contraction of the protoplasm is important and not uniform. Peculiar contracted protoplasm was observed at the experiment No.: T-12-20 (Plate 9.4., figs. 1,2).

2. There are some identities within the experiment.

2.1. The 2-aminoethanol have not degraded the electron dense microbodies of the protoplasm.

Plate 9.3.

1-20 *Alnus glutinosa* (L.) GAERTN.
1-7. Experiment No.: T-12-17. 1-4. LM pictures, 5-7. TEM pictures, 5. Negative No.: 8267, 50.000x., 6. Negative No.: 8265, 50.000x., 7. Negative No: 8260, 5.000x., 8-14. Experiment No.: T-12-18. 8-11. LM pictures, 12-14. TEM pictures, 12. Negative No.: 8279, 5.000x, 13. Negative No.: 8280, 15.000x., 14. Negative No.: 8274, 5.000x. 15-20. Experiment No.: T-12-19. 15-18. LM pictures, 19,20. TEM pictures, 19. Negative No.: 8282, 15.000x., 20. Negative No.: 8282, 25.000x.

Plate 9.4.

1-7. *Alnus glutinosa* (L.) GAERTN.
Experiment No.: T-12-20. 1-4. LM pictures, 5-7. TEM pictures 5. Negative No.: 8324, 10.000x, 6. Negative No.: 8322, 25.000x, Negative No.: 8286, 50.000x.

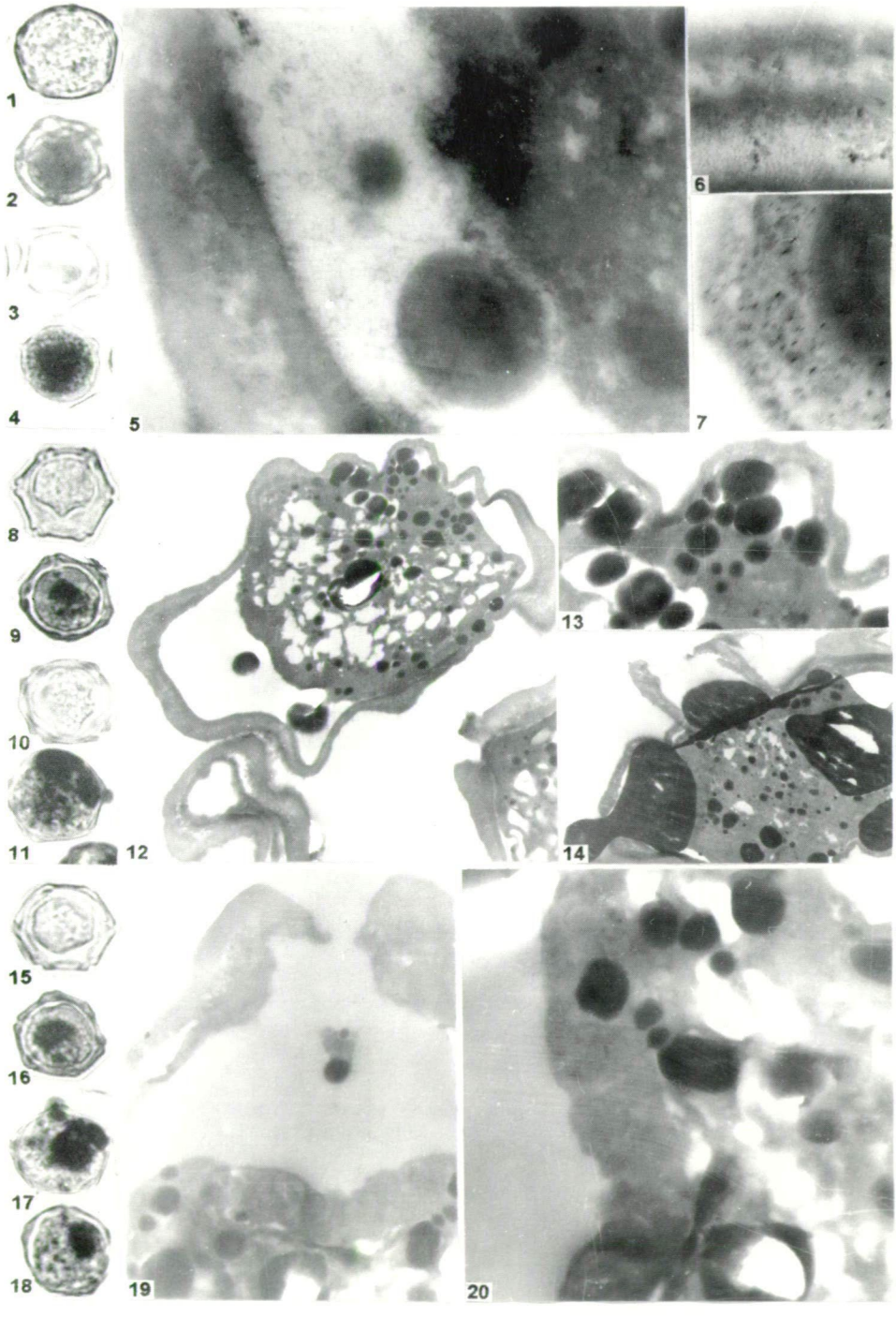

Plate 9.3.

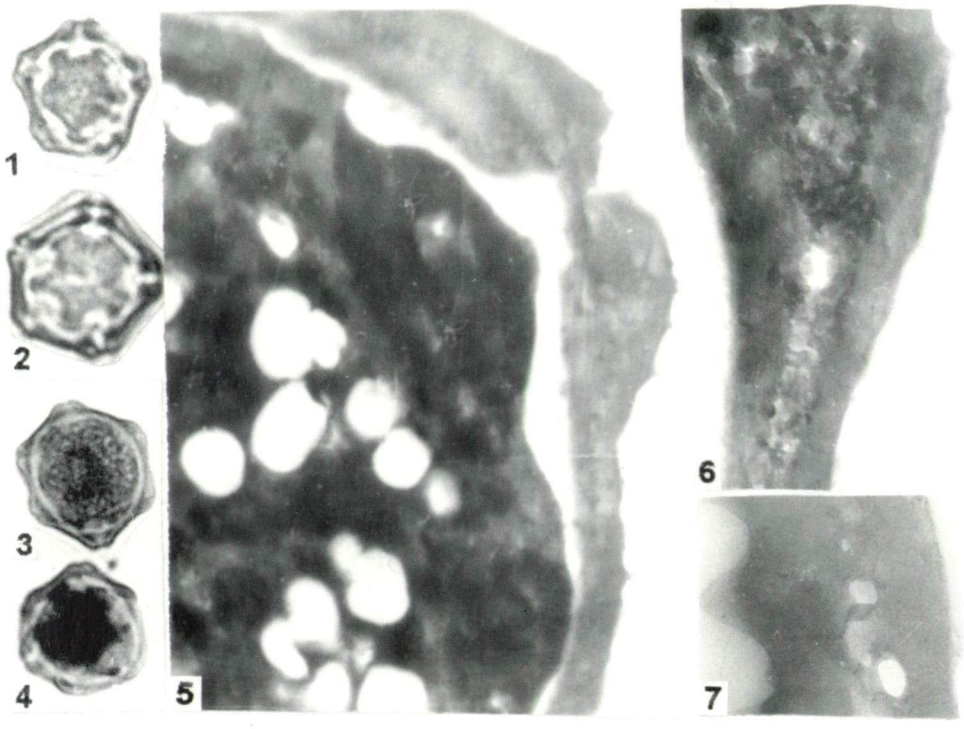

Plate 9.4.

2.2. The $KMnO_4$ after 2-aminoethanol degraded the particles of the protoplasm. The biopolymer system of the ectexine was discovered by these experiments.

2.3. Particular deformations were also observed which are the consequence of the embedding processes, because such deformations were not observed in the LM pictures.

2.4. Concerning the experiments which were combined with merkaptoethanol the electron dense globular units in the intine can be emphasized. These resistant units are in all probability of pollenkitt origin. Similar small electron dense granules were published by NILSSON, PRAGLOWSKI and NILSSON (1977), from non-experimental pollen grains of *Alnus glutinosa* in the onci.

2.5. In contrast to the results on the partially dissolved pollen grains of *Platanus hybrida* protoplasm organelles were not discovered by the partial dissolution with 50% glycerine.

Acknowledgements

This work was supported by Grant OTKA T 031715.

References

JÁRAI-KOMLÓDI, M. (1991): First results of a study on airborne sporomorphs in Budapest, Hungary - Grana *30*, 464-466.

KEDVES, M., FARKAS, E., MÉSZÁROS, K., TÓTH, A. and VÉR, A. (1991): Investigations on the basic biopolymer structure of the ectexine of *Alnus glutinosa* (L.) GAERTN. - Plant Cell Biology and Development (Szeged) *2*, 49-58.

KEDVES, M., HORVÁTH E., BORBOLA, A. and TÓTH, A. (1999): LM investigations of partially dissolved sporomorphs III. - Plant Cell Biology and Development (Szeged) *10*, 75-82.

KEDVES, M. and PÁRDUTZ, Á. (1992): X-ray effect to the exine ultrastructure of *Alnus glutinosa* (L.) GAERTN. - Taiwania *37*, 123-133.

KEDVES, M. and ROJIK, I. (1989): Investigations of the biopolymer organization of partially degraded exines with the fragmentation method. - Acta Biol. Szeged. *25*, 71-80.

KEDVES, M. and UNGVÁRI, E. (1996): X-ray effect to the LM morphology of some gymnosperm and angiosperm pollen grains. - Plant Cell Biology and Development (Szeged) *7*, 75-81.

MOLNÁR, T. (1999): A levegőben található pollenek morfológiai vizsgálata és dinamikája. - Doktori (PhD) Értekezés, DATE. MGT Kar, Növénytani és Növényélettani Tanszék.

NILSSON, S., PRAGLOWSKI, J. and NILSSON, L. (1977): Atlas of Airborne Pollen Grains and Spores in Northern Europe. - Natur och Kultur, Stockholm.

PEHLIVAN, S. (1995): Türkiye'nin alerjen polenleri atlasi. - Ünal Offset, Matbaacilik Sanayi ve Ticaret Ltd. Şirketi, Ankara.

RICHARD, H., BIDAULT, M., KADMIRI, S., SIMON-RIGAUD, M.L., BOTELLA, N., CHAMBAUD, R. et Rougeot, J.C. (1986): Calendrier de la pluie pollinique à Besançon en 1984. - Publ. Lab. de Chrono-Ecol., URA n° *35*, du CNRS, 1-32.