5. EXPERIMENTAL INVESTIGATIONS ON THE POLLEN GRAINS OF ELAEAGNUS ANGUSTIFOLIA L. AND JUGLANS REGIA L.

M. KEDVES[1], Á. PÁRDUTZ[2], E. EGYÜD[1], L. JACSÓ[1] and D. JACSÓ[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

Pollen grains of *Elaeagnus angustifolia* L. and *Juglans regia* L. were partially degraded with 2-aminoethanol for 30 minutes, 1, 5, 10 and 24 hours. Unstained and stained pollen grains were investigated with the LM method. The weak resistance of the sporopollenin of the ectexine of the pollen grains of *Elaeagnus angustifolia* was established during these experiments. Based on the LM data the pollen grains of *Juglans regia* are relatively resistant. Alterations in the diameter of the pollen grains were established depending on the length of time of the partial degradation. The trend of the alterations was different in the unstained and stained pollen grains. After 24 hours of treatment using the TEM method, moderate disintegrations were observed in the ultrastructure of the ectexine.

Key words: Experimental Palynology, recent, partial degradation

Introduction

Our investigations were carried out on the partially degraded allergenic pollen grains of *Elaeagnus angustifolia* L. and *Juglans regia* L. Based on our previous investigations on the pollen grains of *Elaeagnus angustifolia*, it was established that the sporopollenin of the ectexine of this pollen grain is less resistant to organic solvents (KEDVES and HORVÁTH, 2000). For example, after treatment with diethylamine important alterations were observed in the basic morphology of the pollen grains. No significant alterations were observed after X-ray irradiation of the pollen grains of *Elaeagnus angustifolia* (KEDVES and KÁROSSY, 1998). Later (KEDVES and MADARÁSZ, 2001) investigated the alterations caused by high temperature and 2-aminoethanol in this species.

Based on our previous different experiments, it was established that the ectexine of the pollen grains of the *Juglans* genus is resistant (KEDVES and KINCSEK, 1989, KEDVES and KÁROSSY, 1997, KEDVES, KÁROSSY and BORBOLA, 1997).

Both pollen types are important in the evolution of angiosperm pollen grains, so we hope that the new data presented here will be useful additions to the investigations of allergenic pollen grains and to the investigations of the fossil organic material, primarily with regard to the problems of the preservation and fossilisation of this kind of pollen grains.

The aim of this contribution was to get new, comparative data for the two species, which are different from the point of view of ectexine resistance.

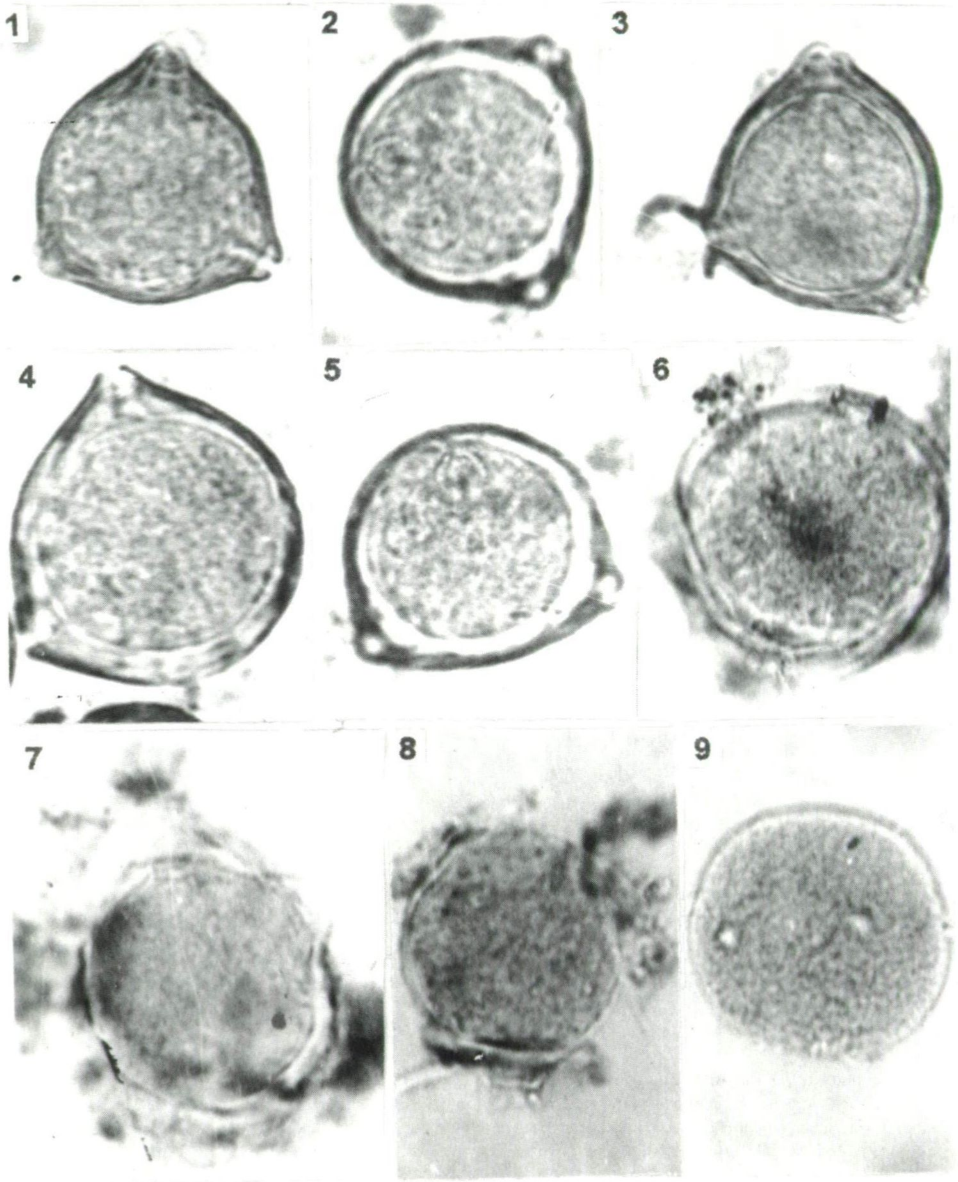

Plate 5.1.

1-9. *Elaeagnus angustifolia* L. LM pictures of the partially degraded pollen grains with 2-aminoethanol for different lengths of time
1-3. Experiment No.: T-12-378, length of time: 30 minutes.
4-6. Experiment No.: T-12-379, length of time: 1 hour.
7-9. Experiment No.: T-12-380, length of time: 5 hours.
Magnification: 1000x.

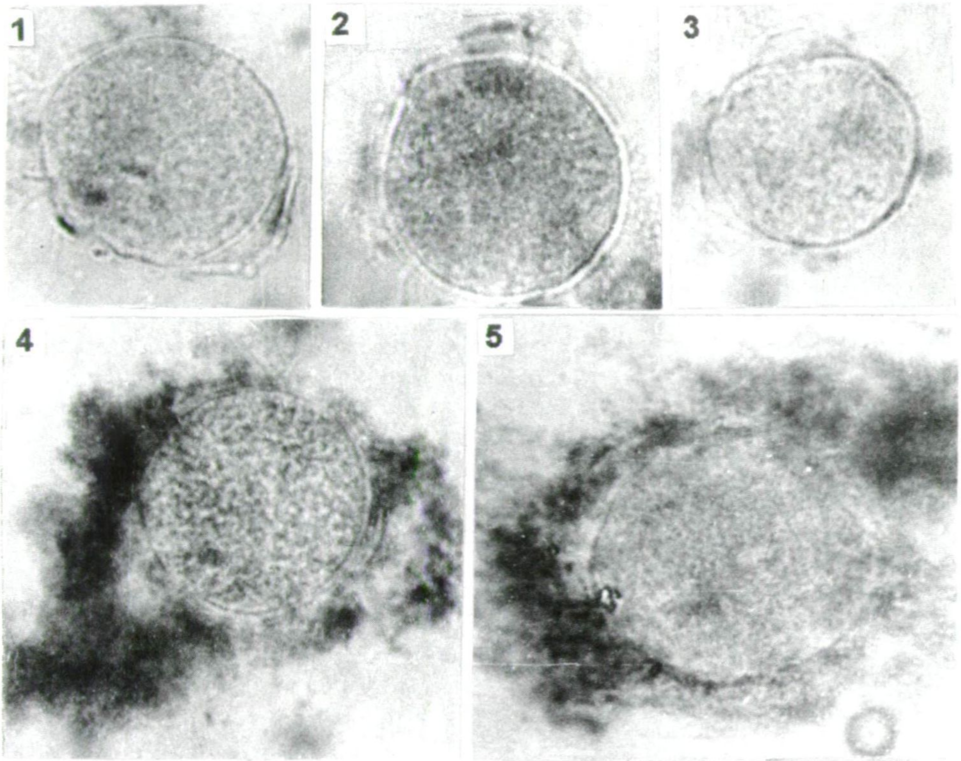

Plate 5.2.

1-5. *Elaeagnus angustifolia* L. LM pictures of the partially degraded pollen grains with 2-aminoethanol for different lengths of time.
1-3. Experiment No.: T-12-381, length of time: 10 hours.
4,5. Experiment No.: T-12-382, length of time: 24 hours.
Magnification: 1000x.

Materials and Methods

Pollen material for our investigations of *Elaeagnus angustifolia* L. and *Juglans regia* L. was collected by ZS. SZÁSZVÁRI. Locality: Szeged, cultivated land. Date of collection: 02.04.03. for *Juglans regia* L. and 02.05.10. for *Elaeagnus angustifolia* L. The experiments were carried out as follows.

Temperature: 30 °C. Quantity of the experimental pure pollen material: 5 mg 2 ml 2-aminoethanol were added for all of the experiments.

Length of time: 30 minutes, 1, 5, 10 and 24 hours. Experiment numbers for *Elaeagnus angustifolia* L.: T-12-378 - 382 and for *Juglans regia* L.: T-12-361 - 365. For LM investigations unstained (A) and stained (B) pollen grains with methylviolet were mounted in glycerine-jelly hydrated at 39.6%. From *Juglans regia* L. some experimental material was prepared for TEM studies and mounted for LM investigations (Ar) also. One experimental material was ultrathin sectioned and investigated with transmission electron microscope. The ultrathin sections were made on a Porter Blum ultramicrotome. The TEM pictures were taken in the EM Laboratory of the Institute of Biophysics of the Biological Recearch Center of the Hungarian Academy of Sciences, Szeged on a Tesla BS 540 (resolution 6-7 Å) and a Zeiss Opton EM-902, resolution 2-3 Å. All pictures are unretouched.

Results

Elaeagnus angustifolia L. (Plate 5.1., figs. 1-9, plate 5.2., figs. 1-5)

Pollen grains treated for 30 minutes (Plate 5.1., figs. 1-3). The greatest part of the pollen grains seems to be non-altered. In polar view triangular amb with convex sides. Sometimes not so characteristic thickening of the intine and in one specimen, the protrusion of the protoplasm was observed. After 1 hour of treatment (Plate 5.1., figs. 4-6), characteristic alterations were observed in some specimens. The sides of the pollen grains are more convex and sometimes circular. The thickening of the intine is characteristic (Plate 5.1., fig. 5). Protrusions of the protoplasm and the degradation of the ectexine was also observed (Plate 5.1., fig. 6). After 5 hours (Plate 5.1., figs. 7-9) the circular amb of the pollen grains is characteristic. At the greatest part of the pollen grains, around the "inner body", remnants of the ectexine are present (Plate 5.1., figs. 7,8). The quantity of the pollen grains without a wall is remarkable (Plate 5.1., fig. 9). The treatment for 10 hours resulted in the same alteration (Plate 5.2., figs. 1-3). Finally, in the last experiment, the greatest part of the pollen grains is represented by the "inner body" and around it, in some specimens, there are remnants of the wall. (Plate 5.2., figs. 4,5).

Juglans regia L. (Plate 5.3., figs. 1-13, plate 5.4., figs. 1-4)

LM results (Plate 5.3., figs. 1-13)

Microphotos on Plate 5.3. illustrate well the resistance of the molecular system of the sporopollenin of the ectexine of this species. In contrast with the previous species, the ectexine was not completely destroyed during the degradation processes. However, in photo 3 and 6 in Plate 5.3. the effect of the fixation and embedding processes is well shown by the LM method. The alteration of the diameter of the pollen grains, dependant on the length of time of the treatment, resulted in the following:

1. The average value of the diameter increased gradually in the unstained pollen grains.
2. The stain effect in this case is unusual, no regularity was observed based on statistical analysis.

TEM results (Plate 5.4., figs. 1-4)

The general survey picture (Plate 5.4., fig. 1) illustrates well the relatively thick tectum with spinae, the characteristic granular or irregular elements of the infratectal layer and the foot layer. The intine seems to be degraded, but before degradation it was swollen. In highly magnified pictures (Plate 5.4., figs. 2-4), degradations in the submicroscopical level were observed. The characteristic channels of the tectum sometimes disappeared. Light parts indicating the place of the channels are marked with an arrow in picture 2. Pictures taken with a high resolution TEM instrument (Plate 5.4., figs. 3,4) illustrate different kinds of alteration in the biomacromolecular system of the sporopollenin. Light and electron dense globular units of 10-15-20 Å were observed (Fig. 4). The 2-aminoethanol revealed some biopolymer units, but a quasi-periodic or quasi-equivalent pattern of the globular biopolymer structures was not observed.

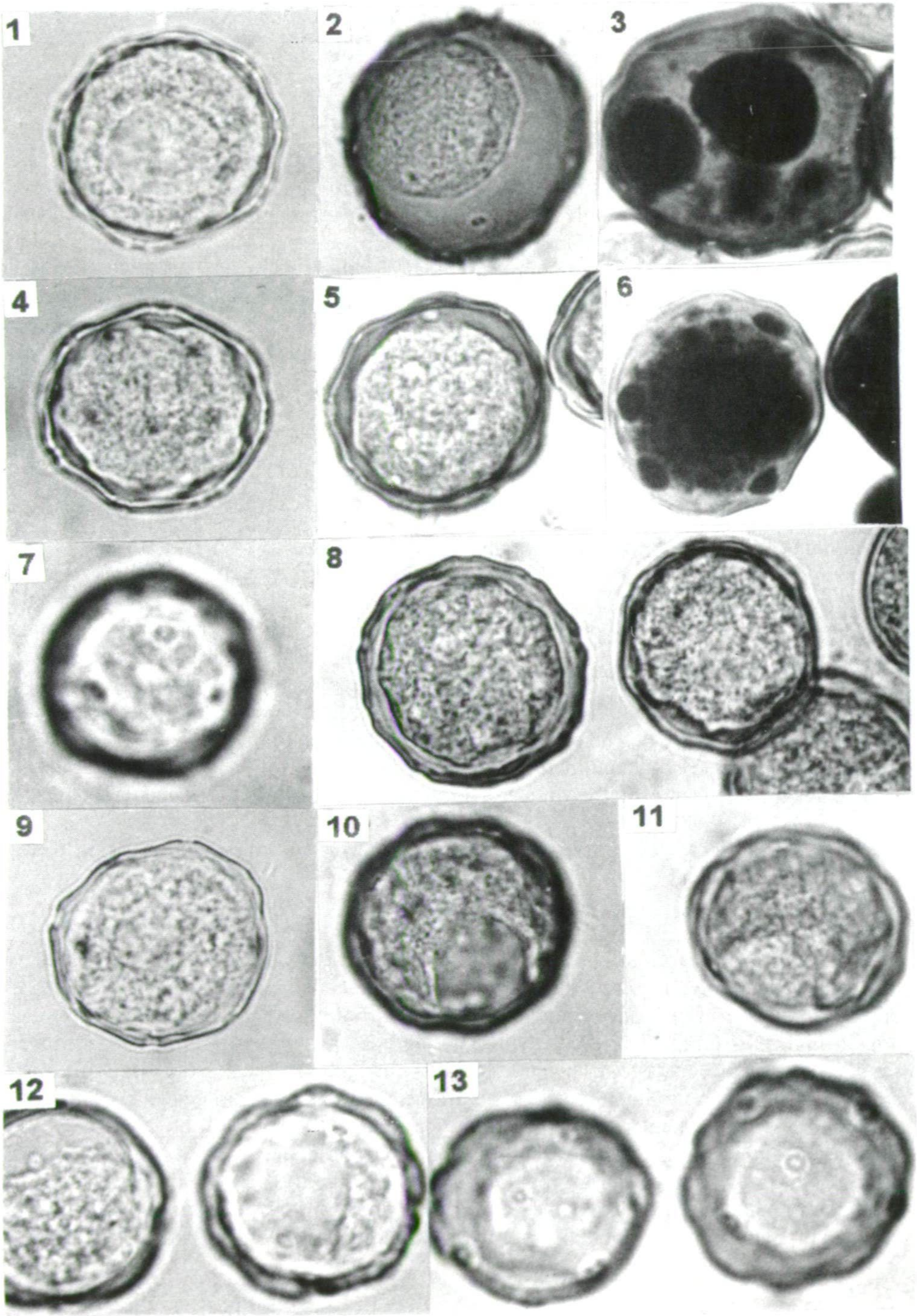

Plate 5.3.

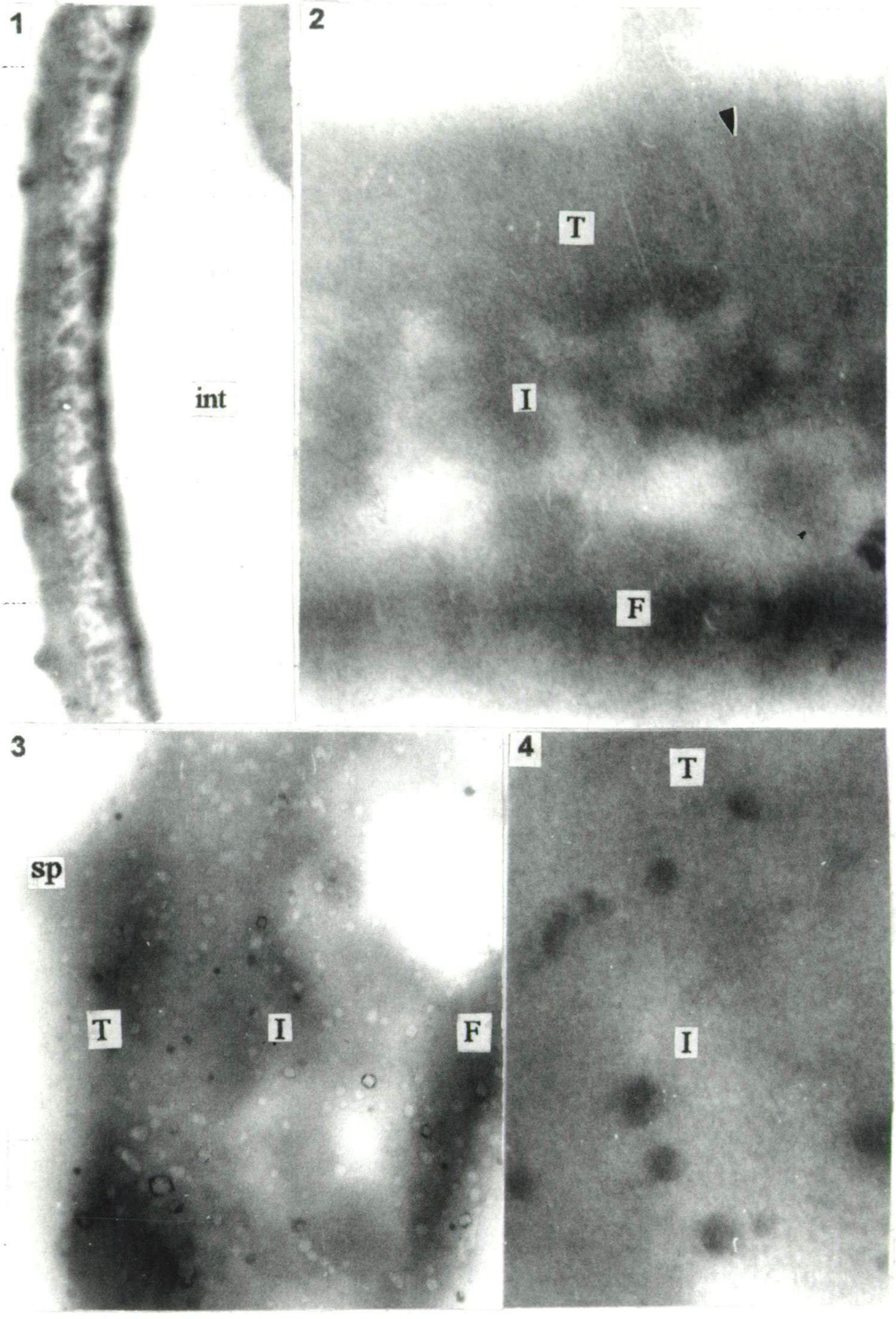

Plate 5.4.

Plate 5.3.

1-13. *Juglans regia* L. LM pictures of partially degraded pollen grains with 2-aminoethanol for different lengths of time.
1-3. Experiment No.: T-12-361, length of time: 30 minutes, 1: A, 2: B, 3: Ar.
4-6. Experiment No:: T-12-362, length of time: 1 hour, 4: A, 5: B, 6: Ar.
7,8. Experiment No.: T-12-363, length of time: 5 hours, 7: A, 8: B.
9-11. Experiment No.: T-12-364, length of time: 10 hours, 9: A, 10,11: B.
12,13. Experiment No.: T-12-365, length of time: 24 hours, 12: A, 13: B.
Magnification: 1000x. Explanation: A = unstained, B = stained pollen grains, mounted in glycerine-jelly. Ar = Pollen grains mounted in Araldite after fixation and embedding processes.

Plate 5.4.

1-4. *Juglans regia* L. TEM pictures of the partially degraded pollen grains with 2-aminoethanol after 24 hours.
1. General survey picture of the ultrastructure of the pollen grain. Magnification: 15.000x. Negative No.: 10190.
2,3. Detail of the ultrastructure of the partially degraded ectexine. Magnification: 100.000x. 2. Negative No.: 10191, 3. Negative No.: 13891.
4. Detail of the partially degraded tectum and infratectal layer. Magnification: 500.000x. Negative No.: 13897.
Explanation: T = tectum, I = infratectum, F = foot layer, int = intine.

Discussion and Conclusions

1. In one of our previous papers (KEDVES and PÁRDUTZ, 1982) we reviewed the most important publications concerning the pollen type of *Elaeagnus angustifolia* both recent (THANIKAIMONI, 1972, etc.) and fossil (KRUTZSCH, 1962, GRAY, 1964, GRUAS-CAVAGNETTO, 1978, *Elaeagnacites* KE et SHI 1978 in SUNG TZE CHEN and TSAO LIU, *Elaeagnuspollenites* HUANG 1980). Based on previous LM, TEM and SEM investigations, it was established that p. 82: "Each of the complex methods, but principally the TEM one suggests that the pollen grains of *Elaeagnus angustifolia* L. may be regarded the morphological analogue of *Complexiopollis* W. KR. 1959 emend. TSCHUDY 1973, without supposing direct botanical relationship between the two." The lamellar foot layer in the apertural area may be one of the early ultrastructural characteristic features of the pollen grain.

2. These new data support again the peculiarities of this kind of pollen type at the molecular level of the ectexine's sporopollenin. The weak resistance aganist the effect of 2-aminoethanol and the relatively good preservation of the protoplasm.

3. Pollen grains of the genus *Juglans* are important stratigraphic elements in the spore-pollen assemblages of Tertiary sediments in the Northern Hemisphere. LM, SEM and TEM morphology of recent *Juglans* pollen grains was investigated by several authors (ERDTMAN, 1952, STONE, REICH and WHITFIELD, 1964, KUPRIYANOVA, 1965, WHITEHEAD, 1965, STONE and BROOME, 1971, VAN CAMPO and LUGARDON, 1973, RYABKOVA, 1987, TARNAVSCHI et al., 1987, BOS and PUNT, 1991). KUTLUK and AYTUG (1999) investigated the fossil fruit from Turkey and, as concerns the dissemination of the pollen grains, emphasized the importance of temperature, relative humidity, precipitation and wind.

4. In general, we have established that the biomacromolecules of the pollen grains of *Juglans* are suitable for the preservation of these pollen grains during sedimentation. The degradations which we have observed in the ultrastructure of the ectexine, namely the disappearance of the tectum channels, may be advantageous for preservation.

5. In summary, the solubility of the biomacromolecules of the ectexine are different in the different taxa and, as we have established previously, (KEDVES and GÁSPÁR, 1996) may also be altered by ecological conditions within one species. This characteristic feature may be taken into consideration in the interpretation of different kinds of palynological investigations.

Acknowledgements

The writers are grateful to Dr. L. F. LAING, Senior Palynologist (Robertson Research International Ltd., Llandudno, U.K.) for critically reviewing the manuscript and for his valuable suggestions. This work was supported by Grant OTKA T 031715.

References

BOS, J.A.A. and PUNT, W. (1991): The Northwest European Pollen Flora 47, Juglandaceae. - Rev. Palaeobot. Palynol. *69*, 79-95.

ERDTMAN, G. (1952): Pollen Morphology and Plant Taxonomy. Angiosperms. - Almqvist and Wiksell, Stockholm.

GRAY, J. (1964): Northwest American Tertiary Palynology: The Emerging Picture. – Ancient Pacific Floras, 21-30.

GRUAS-CAVAGNETTO, C. (1978): Étude palynologique de l'Éocène du Bassin Anglo-Parisien. - Mém. Soc. Géol. France N.S. *56*, 1-64.

KEDVES, M. and GÁSPÁR, I. (1996): New data concerning the solubility of the pollen grains of the genus *Quercus*. L. Short communication. – Plant Cell Biology and Development (Szeged) *7*, 56-61.

KEDVES, M. and HORVÁTH, E. (2000): LM and TEM investigations of partially dissolved and degraded pollen grains of *Elaeagnus angustifolia* L. - Plant Cell Biology and Development (Szeged) *11*, 150-154.

KEDVES, M. and KÁROSSY, Á. (1997): X-Ray effect on the LM morphology of some angiosperm pollen grains I. - Plant Cell Biology and Development (Szeged) *8*, 86-90.

KEDVES, M. and KÁROSSY, Á. (1998): X-Ray effect on the LM morphology of some angiosperm pollen grains II. - Plant Cell Biology and Development (Szeged) *9*, 88-92.

KEDVES, M., KÁROSSY, Á. and BORBOLA, A. (1997): LM investigations of partially dissolved sporomorphs I. - Plant Cell Biology and Development (Szeged) *8*, 44-55.

KEDVES, M. and KINCSEK, I. (1989): Effect of the high temperature on the morphological characteristic features of the sporomorphs I. - Acta Biol. Szeged. *35*, 233-235.

KEDVES, M. and MADARÁSZ, M. (2001): Experimental studies on the pollen grains of *Elaeagnus angustifolia* L. - Plant Cell Biology and Development (Szeged) *13*, 71-75.

KEDVES, M. and PÁRDUTZ, Á. (1982): Complex studies on the pollen grains of *Elaeagnus angustifolia* L. - Acta Biol. Szeged. *28*, 75-83.

KRUTZSCH, W. (1959): Einige neue Formgattungen und Arten von Sporen und Pollen aus der mitteleuropäischen Tertiär. - Palaeontographica *B*, *105*, 125-157.

KRUTZSCH, W. (1962): Stratigraphisch bzw. botanisch wichtige neue Sporen und Pollenformen aus dem deutschen Tertiär. - Geologie *11*, 265-308.

KUPRIYANOVA, L.A. (1965): The Palynology of the Amentiferae. - Nauka, Moscow - Leningrad. In Russian.

KUTLUK, H. and AYTUG, B. (1999): A fossil walnut fruit (*Juglans* L.) from Akçaabar (Trabzon) and ecological-phenological characteristics of walnut. - Geo-Eco-Trop. No. Spécial *(22)*, 83-100.

RYABKOVA, L.S. (1987): Palynography of the flore of Tadzhik SSR. - Nauka, Leningrad. In Russian.

STONE, D.E. and BROOME, C.R. (1971): Pollen ultrastructure: Evidence for relationship of the Juglandaceae and Rhoipteleaceae. - Pollen et Spores *13*, 5-14.

STONE, D.E., REICH, J. and WHITFIELD, S. (1964): Fine structure of the walls of *Juglans* and *Carya* pollen. - Pollen et Spores *6*, 379-392.

SUNG TZE CHEN and TSAO LIU (1978): Early Tertiary spores and pollen grains from the coastal region of Bohai. - Nanjing Inst. Geol. and Paleontol. Acad. Sinica 1-177, Chinese with English summary.

TARNAVSCHI, I.T., SERBÁNESCU-JITARIU, G., N. MITRIU-RADULESCU si RADULESCU, D. (1987): Monografia polennului florei din România. - Acad. Rep. Soc.România, Bucuresti.

THANIKAIMONI, G. (1972): Index bibliographique sur la morphologie des pollens d'Angiospermes. - Inst. fr. Pomdichéry Trav. Sect. Sci. techn. *12*, 1-337.

TSCHUDY, R.H. (1973): *Complexiopollis* Pollen Lineage in Mississippi Embayment Rocks. - Geol. Survey Prof. Paper *743*, C1-C14.

VAN CAMPO, M. et LUGARDON, L. (1973): Structure grenue infratectale de l'exine des pollens de quelques Gymnospermes et Angiospermes. - Pollen et Spores *15*, 171-187.

WHITEHEAD, D.R. (1965): Pollen morphology in the Juglandaceae. II: Survey of the family. - J. Arnold. Arbor. *46*, 369-410.