

17. TRANSMISSION ELECTRON MICROSCOPY OF PARTIALLY DISSOLVED EXINES OF PSEUDOTSUGA MENZIESII (MIRB.) FRANCO

M. KEDVES and A. BORBOLA

Cell Biological and Evolutionary Micropaleontological Laboratory of the Department of Botany of the J.A. University, H-6701, P.O.Box 993, Szeged, Hungary

Abstract

Pollen grains of *Pseudotsuga menziesii* (MIRB.) FRANCO were partially dissolved with organic solvents and investigated with transmission electron microscope. In this paper the most important TEM results of the partially dissolved exines are presented. The resistance of the exine may be pointed out in particular against diethylamine. The very early exine ultrastructure of the recent *Pseudotsuga* genus was compared with the ultrastructure data of fossil pollen grains.

Key words: Palynology, recent, *Pseudotsuga menziesii*, partial dissolution, TEM.

Introduction

Pollen grains of the genus *Pseudotsuga* were first investigated with transmission electron microscope by GULLVÄG (1966). In consequence of the importance of the *Pseudotsuga-Larix* pollen types in the fossil spore-pollen assemblages isolated from sediments of different ages these pollen grains were investigated in our Laboratory by different methods. High temperature effect of *Pseudotsuga menziesii* was studied by TÓTH (1997), *Larix decidua* MILL. by BORBOLA (1997). The organic solvent effect was investigated later (KEDVES et al. 1998) and the Duhoux effect was also observed at some experiments, e.g.: diethylamine, merkptoethanol, ethanol, i-amyl alcohol.

We carried out several dissolution experiments with different kinds of organic solvents on the pollen grains of *Larix decidua* and *Pseudotsuga menziesii*, for transmission electronmicroscopic investigations.

In this paper the most important results obtained on the pollen grains of *Pseudotsuga menziesii* (MIRB.) FRANCO are presented.

Materials and Methods

The pollen material for these investigations was collected by Miss Á. ERDÓDI in the Botanical Garden of the J.A. University, Szeged, Hungary, on 01.04.1997. 20 mg pollen material was used for each dissolution experiment, during 30 days at 30 °C.

The experiments started on 11.05.1997, and ended on 11.05.1997.

No: 1/7 - 803. - 20 mg pollen grains + 0.2 ml diethylamine + 5 ml H₂O

No: 1/7 - 804. - 20 mg pollen grains + 0.2 ml merkptoethanol + 5 ml H₂O

- No: 1/7 - 805. - 20 mg pollen grains + 5 ml methanol
No: 1/7 - 806. - 20 mg pollen grains + 5 ml ethanol
No: 1/7 - 807. - 20 mg pollen grains + 5 ml n-propanol
No: 1/7 - 808. - 20 mg pollen grains + 5 ml n-butanol
No: 1/7 - 809. - 20 mg pollen grains + 5 ml i-amyl alcohol

Partially dissolved pollen grains were postfixed with 1% OsO₄ aqueous dilution and embedded in Araldite (Durcupan, Fluka). The ultrathin sections were made on a Porter Blum ultramicrotome in the EM Laboratory of the Hungarian Academy of Sciences Biological Research Center. The TEM pictures were taken on a Tesla BS-540, resolution 6-7 Å.

General problems

GULLVÁG (1966) described the ultrastructure of the pollen grains of *Pseudotsuga taxifolia* as follows, p. 439: "The outer layer consists of a three-dimensional network of rounded bars. The inner laminated layer is 0.3-0.4 μ. In the laminated layer triplets of the 50-60 Å unit lamella are rather common, but the individual lamella has also been observed...". In the same year: 1966, PETTITT published some information of the ultrastructure of *Archaeopteris* cf. *jacksonii* (*Archaeopteridales*, *Progymnospermophyta*). The described structure is similar to that of recent *Pseudotsuga taxifolia* published by GULLVÁG (1966). In this way the earliest ultrastructural characteristic features of the ectexine, namely the irregular spongy network of the ectexine and the lamellated endexine which appeared in the Devonian - Carboniferous was conserved in recent taxa of the genus *Pseudotsuga*. During the exine ultrastructure investigations on Mesozoic *Gymnospermatophyta* pollen grains similar exine ultrastructure was described from *Araucariacites hungaricus* DEÁK 1964 (KEDVES, 1985, 1994), and *Balmeiopsis limbatus* (BALME 1957) ARCHANGELSKY 1977 (KEDVES and PÁRDUTZ, 1974, KEDVES 1994). Further information in this subject is in the papers of ARCHANGELSKY (1994), and BATTEN and DUTTA (1997).

Results

As general establishment we can point out, that the sporopollenin of this pollen grains is very resistant against the used organic solvents, in this way we will not describe in detail all results, because most of them are essentially identical.

Experiment No: 1/7 - 803 (Plate 17.1., plate 17.2., figs. 1-3). – The ectexine is thick, and composed of irregular, globular or elongated elements. Based on our TEM data there are some differences in the details:

Plate 17.1., the elements are more or less globular and anastomose, in this way different patterns may be observed, such as linear, spongy and/or irregular. The superficial ectexinous elements are about two times larger than the inner ones. The ectexine is not completely closed, but the outer part composed of larger units may be interpreted as a peculiar tectum. The foot layer may be established by its electron density to the other parts of the ectexine. The foot layer is connected to the outer dark lamella to the ectexine by granular or irregular units. Six dark endexine lamellae may be well seen in our TEM picture. The light lamellae of the endexine are more or less degraded; there are darker substances within the light lamellae. On the inner surface in some parts there are

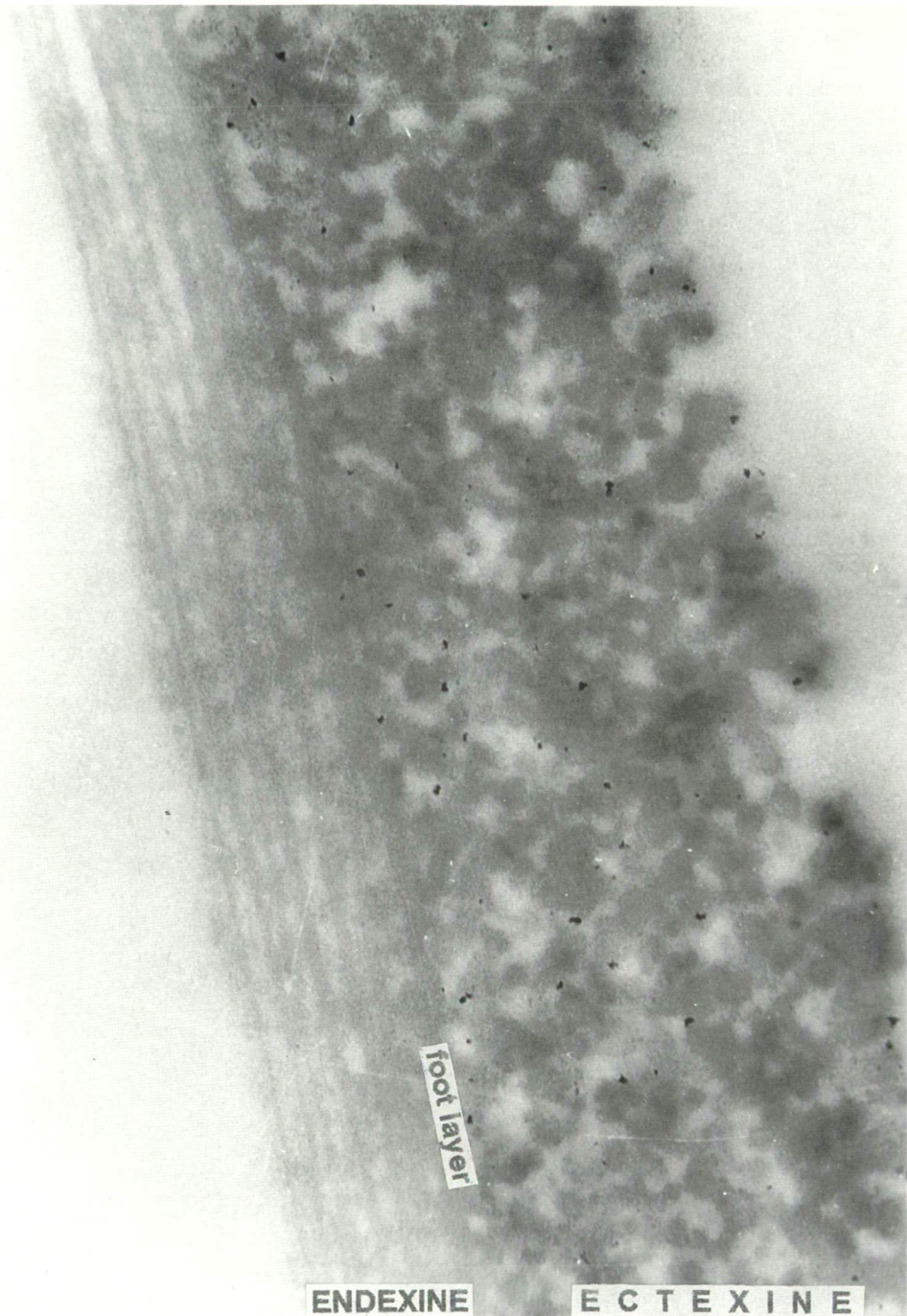


Plate 17.1.

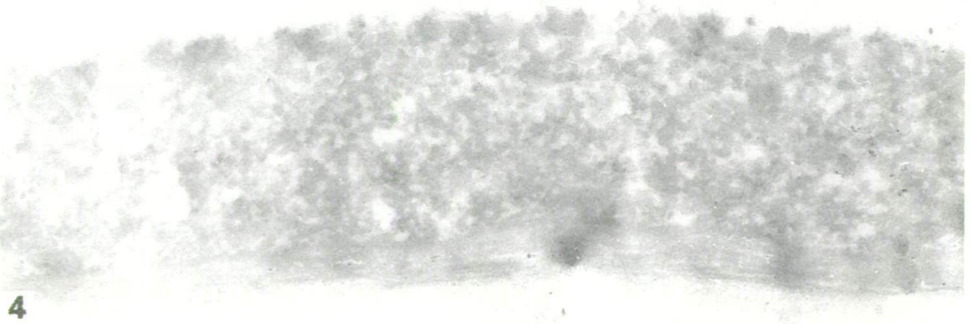
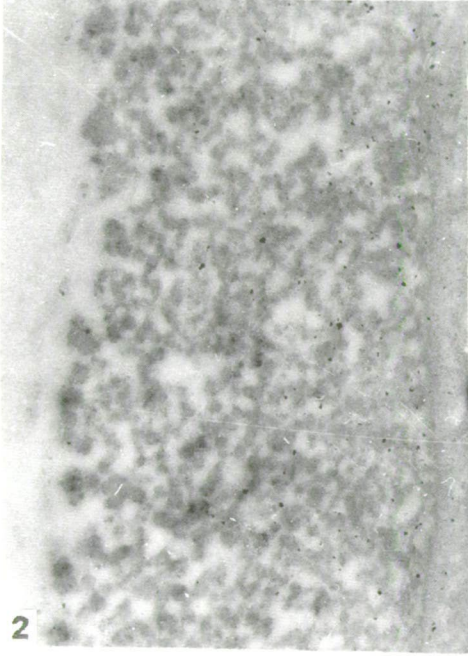
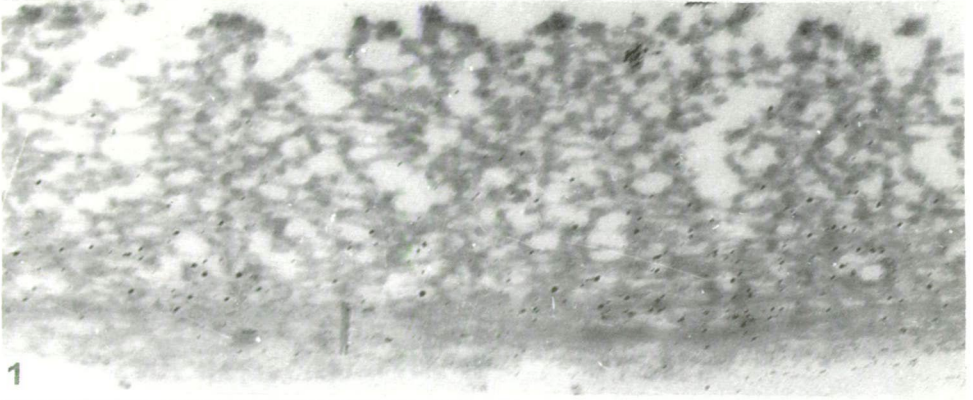


Plate 17.2.

degradations. Plate 17.2., fig. 1. - The electron density of the elements of the outer part of the ectexine is stronger than previously. Moreover, there are globular elements in the inner part of the ectexine with different electron density. The substance is rather network than spongy composed of irregular or globular elements. Degradation of the foot layer and the endexine is well shown. Plate 17.2., fig. 2. - illustrates superficial lamellae on the outer part of the ectexine. The electron density of the outer part of the ectexine - "discontinuous tectum" - is stronger than the inner part. The inner part of the ectexine is composed more or less anastomosing globular elements. The foot layer is quite perceptible, the endexine lamellae are degraded. The above mentioned ultrastructure characteristic features of the outer part of the ectexine are illustrated in a highly magnified picture in the Plate 17.2., fig. 3.

Experiment No: 1/7 - 807 (Plate 17.2., fig. 4). - In general, dissolution of the elements of the exine may be established, but the basic ultrastructural characteristic features are perceptible. So the stronger electron density of the "discontinuous tectum", and the foot layer are well shown. The lamellae of the endexine are also well seen in our TEM picture.

Discussion and Conclusions

1. The sporopollenin of the exine of *Pseudotsuga menziesii* is resistant against organic solvents in comparison to other investigated pollen grains particularly of the *angiosperms* (*Quercus*, KEDVES and GÁSPÁR, 1996, *Platanus*, *Tilia*, KEDVES et al., (1998), etc.).

2. The ectexine is not completely closed, probably the resistant molecular system may be explained with this characteristic feature. But on the other hand similar resistance was established at the tectate *Cycadaceae* pollen grains cf. KEDVES, PÁRDUTZ, TERBE and HORVÁTH (1999). This problem needs further investigations.

3. The basic ultrastructure characteristic features of the ectexine and the lamellar endexine is of the earliest type. To this we have several ultrastructural data from the pollen grains Paleozoic and the Mesozoic sediments. The similarity between the earliest fossil ultrastructure data to the recent species of the genus *Pseudotsuga*, may be emphasized which is an evolved *gymnosperm* pollen grain.

Plate 17.1.

Pseudotsuga menziesii (MIRB.) FRANCO, experiment No: 1/7-803. Negative no: 6980, 75.000x.

Plate 17.2.

1-4. *Pseudotsuga menziesii* (MIRB.) FRANCO, Recent.

1. Experiment No: 1/7-803. Negative no: 6983, 25.000x.
2. Experiment No: 1/7-803. Negative no: 6981, 25.000x.
3. Experiment No: 1/7-803. Negative no: 6984, 75.000x.
4. Experiment No: 1/7-807. Negative no: 6991, 25.000x.

References

- ARCHANGELSKY, S. (1994): Comparative ultrastructure of three Early Cretaceous *gymnosperm* pollen grains: *Araucariacites*, *Balmeiopsis* and *Callialasporites*. - Rev. Palaeobot. Palynol. 83, 185-198.
- BATTEN, D.J. and DUTTA, R.J. (1997): Ultrastructure of exine of *gymnospermous* pollen grains from Jurassic and Basal Cretaceous deposits in Northwest Europe and implications for botanical relationships. - Rev. Palaeobot. Palynol. 99, 25-54.
- BORBOLA, A. (1997): High temperature effect on the pollen grains of *Larix decidua* MILL. - Plant Cell Biology and Development (Szeged) 8, 69-75.
- DEÁK, H.M. (1964): A Dunántúli Középhegység apti üledékeinek palynológiai vizsgálata. (Recherches palynologiques des dépôts aptiens de la Montagne Centrale de Transdanubie). Geol. Hung. Ser. Palaeontologica 29-32, 9-105.
- GULLVÁG, B. (1966): The fine structure of some *gymnosperm* pollen walls. - Grana Palynologica 6, 435-475.
- KEDVES, M. (1985): Structural modifications of degraded fossil sporomorphs. - Micropaleontology 31, 173-178.
- KEDVES, M. (1994): Transmission electron microscopy of the fossil *gymnosperm* exines. - Szeged.
- KEDVES, M. et GÁSPÁR, I. (1996): New data concerning the solubility of the pollen grains of the genus *Quercus* L. - Plant Cell Biology and Development (Szeged) 7, 56-61.
- KEDVES, M., HORVÁTH, E., MÉSZÁROS, E., MÉSZÁROS, R., RONTÓ, G., SZLÁVIK, N., GAUDÉNYI, SZ. and KALMÁR, Á. (1998): LM investigations of partially dissolved sporomorphs II. - Plant Cell Biology and Development (Szeged) 9, 76-87.
- KEDVES, M. and PÁRDUTZ, Á. (1974): Ultrastructural studies on Mesozoic inaperturate *Gymnospermatophyta* pollen grains. - Acta Biol. Szeged. 20, 81-88.
- KEDVES, M., PÁRDUTZ, Á., TERBE, ZS. et HORVÁTH, ESZTER (1999): Microscopie électronique à transmission de l'exine dégradée de grains de pollen de quelques *Cycadales*. - Actes du IV^{ème} Symposium Internationale de Palynologie Africaine - 15-30 Avril 1999 Sousse/Tunisie, 35.
- TÓTH, A. (1997): High temperature effect on the pollen grains of *Pseudotsuga menziesii* (MIRB.) FRANCO. - Plant Cell Biology and Development (Szeged) 8, 76-80.