

7. LM AND SEM INVESTIGATIONS ON PARTIALLY DISSOLVED ALLERGEN POLLEN GRAINS I.

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Abstract

Pollen grains of *Tilia cordata* MILL. were partially dissolved with 2-aminoethanol for 30 minutes, 1 hour, 5, 10 and 24 hours, and investigated with the LM and the SEM method. Alterations in the LM morphology and the fine sculpture of the surface were analyzed and compared with the previous results. After 24 hours of treatment remarkable alterations were established by the LM and the SEM methods.

Key words: Palynology, recent, *Tilia*, partial degradation, LM, SEM.

Introduction

The experimental study by different method on the pollen grains of *Tilia cordata* are included in the research program of our Laboratory. The earlier morphological characteristic features from evolutionary point of view of this kind of pollen grains was pointed out in several papers. On the other hand in case of the pollen grains of *Tilia platyphyllos* SCOP. it was established that the sporopollenin of the ectexine is easily soluble by diethylamine (KEDVES et al., 1998). Pollen grains of *Tilia cordata* were irradiated by KEDVES and KÁROSSY (1998) and a moderate pollen tube development in consequence of the X-ray irradiation was observed. Corroded *Tilia* exines by phase and differential interference contrast were published by ROWLEY, ROWLEY and SKVARLA (1990). The pollen grains of *Tilia* by the SEM data of SKVARLA, ROWLEY and CHISSOE (1996) does not seem to be corroded. Small irregular holes were observed on the tectum (Plate 6, fig. 1). The fractured foot layer of the *Tilia* shows several holes which may be corrosion sites. Corrosion was established at the base of the columellar infratectal layer also. The allergenic character of the pollen grains of the genus *Tilia* was pointed out in several works, e.g.: *Tilia cordata* by NILSSON, PRAGLOWSKI and NILSSON (1977) and RICHARD et al. (1986), *Tilia tomentosa* MOENCH. by PEHLIVAN (1995). LM, SEM and TEM data published by NILSSON, PRAGLOWSKI and NILSSON (1977) are very important for comparison with our data.

Taking into consideration the previous results, in particular the solubility of the ectexine with diethylamine pollen grains of the genus *Tilia* were also included in this research program.

Materials and Methods

Pollen grains of *Tilia cordata* MILL. were collected by Miss M. MADARÁSZ from Szeged (cultivated). The experiments are as follows: T-12-42, fresh pollen grains. During partial dissolution 1 ml 2-aminoethanol was added to 5 mg dry pollen material. Temperature 30 C°, length of times are as follows: 30 minutes (T-12-43), 1 hour (T-12-44), 5 hours (T-12-45), 10 hours (T-12-46), 24 hours (T-12-47). LM investigations were made on unstained pollen grains (a) and stained with methylviolet (b). For SEM investigations the dry pollen grains were covered with gold-palladium and investigated with a Hitachi S-2400 instrument (resolution about 40 Å) in the SEM Laboratory of the Department of Botany of the University of Szeged.

Results

LM results

Qualitative results

T-12-43 - T-12-46. (Plate 7.1. figs. 5,6, 10,11, plate 7.2., figs. 1,2, 6,7). By the LM method there are no important difference in the secondary alterations. The endannulus is characteristic and a plicate form appeared. The endannulus at the coloured pollen grains is characteristic. After 24 hours of dissolution (T-12-47, Plate 7.2., figs. 11,12) important alterations were observed. The endannulus is less characteristic, the exoaperture is ellipsoid pore like and the ornamentation is not well perceptible.

Quantitative results

Experiment number:	Size (μm , %)								Dominant size (μm):	Average: (μm)
	27.5	30	32.5	35	37.5	40	42.5	45		
T-12-42 a		8	39	43.5	9.5				32.5; 35	33.9
T-12-43 a		0.5	30	47.5	22				35	34.8
T-12-44 a	0.5	4.5	28	51	16				35	34.4
T-12-45 a		0.5	14	65	20.5				35	35.1
T-12-46 a		7	22.5	34.5	35	1			35; 37.5	35.02
T-12-47 a					28.5	34.5	37		40; 42.5	40.22
T-12-42 b		20.5	63	16.5					32.5	32.4
T-12-43 b		8	48.5	39	4.5				32.5; 35	33.5
T-12-44 b		11.5	43.5	41	4				32.5; 35	33.43
T-12-45 b		9.5	29	42.5	19				35	34.28
T-12-46 b	1	9.5	22.5	40	26	1			35	34.58
T-12-47 b				8.5	41.5	39	10.5	0.5	37.5; 40	38.83

SEM results (Plate 7.1., figs. 3,4, 7-9, 12,13, plate 7.2., figs. 3-5, 8-10, 13-15)

Plate 7.1.

1-13. *Tilia cordata* MILL.

1-4. Experiment No.: T-12-42. 1,2. LM pictures, 3,4. SEM pictures. 5-9. Experiment No.: T-12-43, 5,6. LM pictures, 7-9. SEM pictures. 10-13. Experiment No.: T-12-44. 10,11. LM pictures, 12,13. SEM pictures.

Plate 7.2.

1-15. *Tilia cordata* MILL.

1-5. Experiment No.: T-12-45. 1,2. LM pictures, 3-5. SEM pictures. 6-10. Experiment No.: T-12-46. 6,7. LM pictures, 8-10. SEM pictures. 11-15. Experiment No.: T-12-47. 11,12. LM pictures, 13-15. SEM pictures.

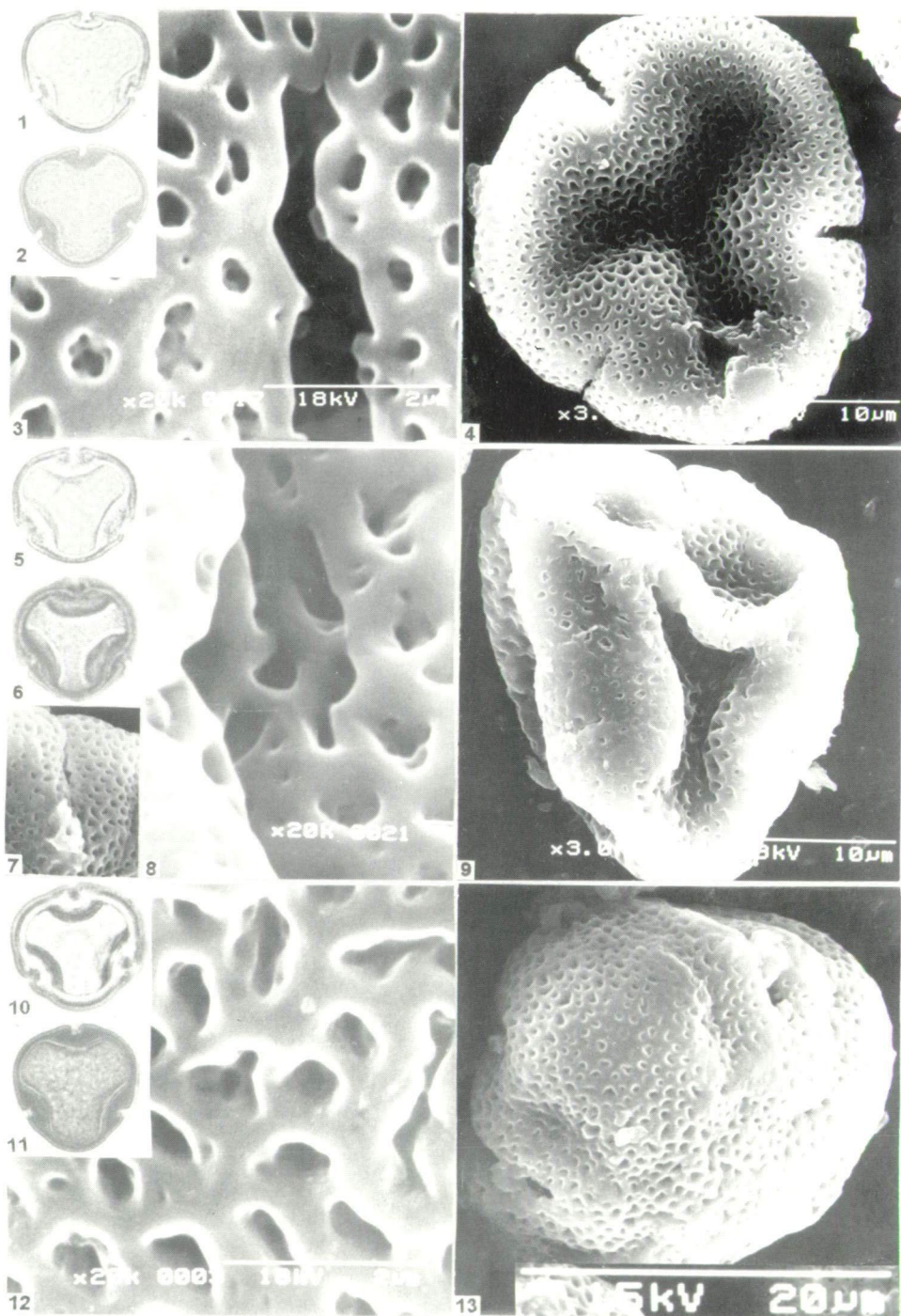


Plate 7.1.

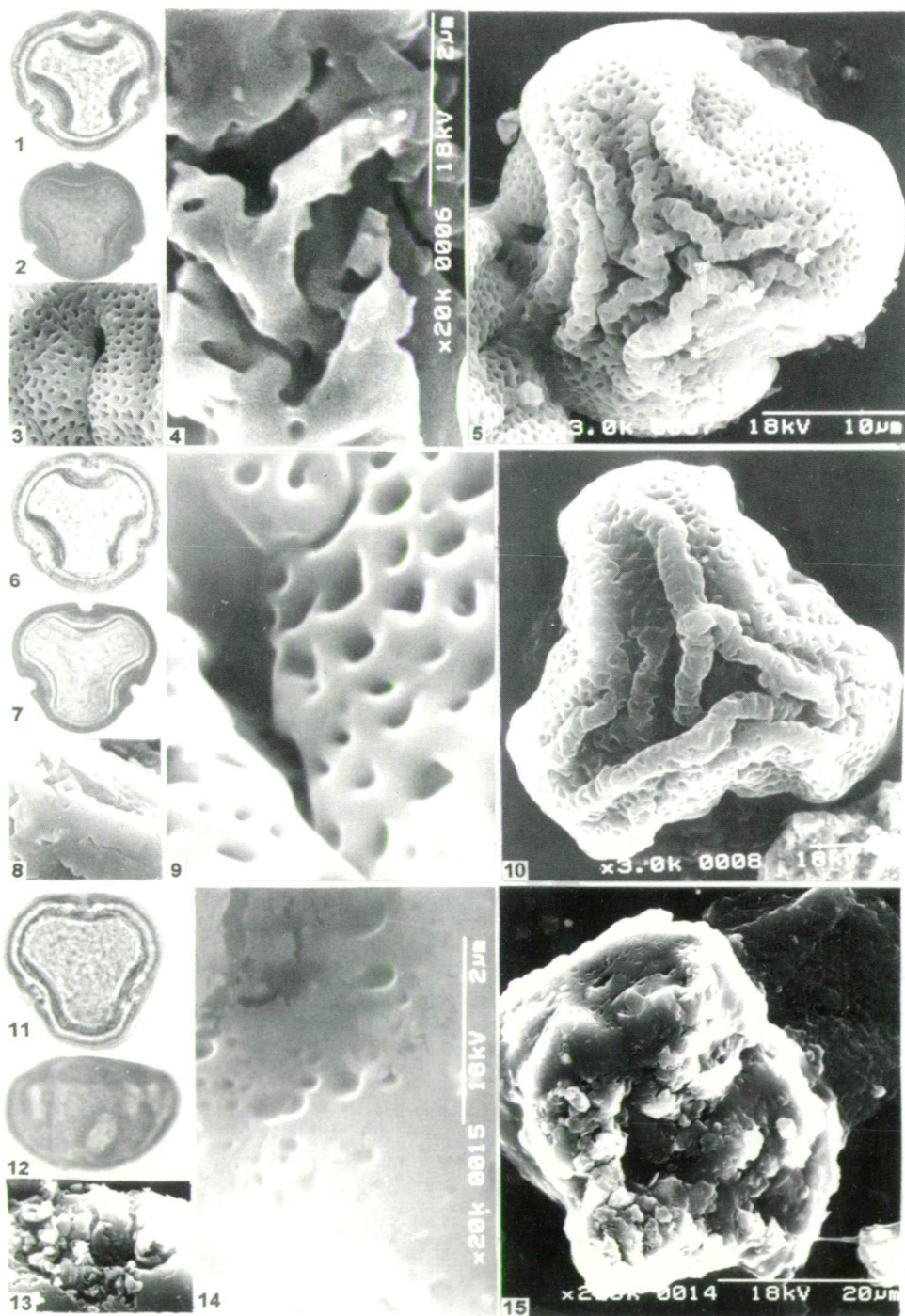


Plate 7.2.

The characteristic exoapertures (short colpi) are well shown in the non-experimental pollen grains. The tectum is not always reticulate sometimes foveolate according to the establishments of NILSSON, PRAGLOWSKI and NILSSON (1977).

Experiments T-12-43-46 resulted the characteristic plicate form which is more complicated depending on the length of time of the experiment (Plate 7.1., figs. 9,13, plate 7.2., figs. 5,10). Some alterations in the exoapertures and in the diameter of the perforations or the mesh of the reticuli are a little larger (Plate 7.1., figs. 7,8,12. plate 7.2., figs. 3,4,8,9).

The dissolution during 24 hours resulted important degradations in the basic morphology of the pollen grains. (Plate 7.2., fig. 15). The apertural area is completely altered (Plate 7.2., fig. 13), the outer layer of the ectexine are degraded. The sculpture is also different from the original one, perforation of different size were observed (Plate 7.2., fig. 14).

Discussion and Conclusions

The alterations in consequence of the 2-aminoethanol are quite different in contrast to the diethylamine. 24 hours of dissolution resulted in really important alterations based on the SEM results. In this way the molecular system of the sporopollenin is more resistant to the influence of 2-aminoethanol.

It is interesting that there are differences in the maximum value of the diameters of the unstained and stained pollen grains. We need to emphasize the very characteristic differences in samples treated for 24 hours.

The SEM method demonstrated also the extremely characteristic alterations after 24 hours of dissolution. But the plicae-like morphology appeared after 30 minutes of dissolution also.

Acknowledgements

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