7. EFFECT OF THE HIGH TEMPERATURE AND THE C60 FULLERENE/ BENZOL SOLUTION TO THE POLLEN GRAINS OF GINKGO BILOBA L. AND QUERCUS ROBUR L.

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Abstract

Two types of experiments were carried out on pollen grains of *Ginkgo biloba* L. and *Quercus robur* L.: 1. The first experiment noted the affect of a 30 °C temperature on these pollen grains for different length of time: 10 minutes, 1, 5, and 10 hours. The second experiment noted the effect of C60 fullerene/benzol solution on the LM morphology and ultrastructure of the pollen grains.

Key words: Experimental Palynology, recent, LM, TEM.

Introduction

It has been a considerable time that our Laboratory has investigated the effect of high temperature on different kinds of pollen and spores. In the past the pollen spores were subjected to temperatures of 100 °C and usually 200 °C. When subjected to these temperatures important morphological alterations were observed in the studied pollen grains (KEDVES and KINCSEK, 1989, KEDVES, TÓTH and FARKAS, 1991). In view of these results, we decided to investigate the effect of 30 °C temperatures on selected pollen grains inasmuch as these lower temperatures may also have an affect occurring in nature during the dispersion of the pollen before sedimentation.

The study of the biopolymer structure and symmetry is another program currently undertaken in our Laboratory. Different kinds of solvents and oxidizing agents are being used to observe the partial degradation of the sporoderm. Initially, the application of C60 fullerene/benzol solution was used to observe sporoderm degradation (KEDVES and FREY, 2002). Later, the application of the above techniques on *Taxus baccata* pollen also were succesful (KEDVES, PÁRDUTZ, JACSÓ and KOCSICSKA, 2002). The biopolymer symmetry studies of KEDVES, BÉRES, JACSÓ and KOCSICSKA (2002) are presented in this volume.

The initial data on the effect of a 30 °C temperature and the results of the C60 fullerene/benzol solution on the fine structure of the two above mentioned pollen grain species are presented herein. The purpose of these studies, presented herein, is to establish the morphological alterations effects when the pollen of the two above mentioned species are subjected to a temperature of 30 °C and also the changes in the ultrastructure when these two pollen species are subjected to C60 fullerene/benzol solution.

Materials and Methods

Ginkgo biloba L. pollen grains was collected by K. PRISKIN (Szeged, cultivated, date: 15. 04.2001), *Quercus robur* L. by B. VARGA (Botanical Garden of the University of Szeged, date: 12.04.2001).

High temperature experiments, on 30 °C

Ginkgo biloba L. T-12-289, dry, fresh pollen grains, T-12-290, heated for 10 minutes, T-12-291, length of time: 1 hour, T-12-292, 5 hours, T-12-293, 10 hours.

Quercus robur L. T-12-284, dry fresh pollen grains, T-12-285, heated for 10 minutes, T-12-286, length of time: 1 hour, T-12-287, 5 hours, T-12-288, 10 hours.

T-12-306. - *Ginkgo biloba* L. 3 mg pollen grains + 5 ml C60 fullerene/benzol solution + 5 ml benzol, temperature 30 °C, length of time: 1 day T-12-307, 2 days, T-12-308, 3 days, T-12-309, 4 days, T-12-310, 5 days, T-12-311, : 6 days. For LM studies unstained, and Methylviolet stained pollen grains were investigated.

T-12-300. - *Quercus robur* L. 3 mg pollen grains + 5 ml C60 fulerene/benzol solution + 5 ml benzol, temperature 30 C, length of time: 1 day, T-12-3001, 2 days, T-12-302, 3 days, T-12-303, 4 days, T-12-304, 5 days, T-12-305, 6 days.

In this paper we present the results of the LM investigations of these pollen grains, and as a preliminary report the TEM data of the first experiments (T-12-306, and T-12-300).

Results

LM results

Ginkgo biloba L. (Plate 7.1., figs. 1-17)

P/E ratio

Experiment N°	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	
T-12-289		8.0	17.5	23.5	23.5	9.5	9.5	5.5	2.0		0.5		0.5	%
T-12-290		8.5	28.5	20.0	16.0	11.5	9.0	4.0	0.5	1.0	0.5		0.5	
T-12-291		1.0	25.5	16.5	21.5	10.5	8.0	5.5	0.5	1.5	0.5			
T-12-292	4.5	20.0	31.0	13.5	15.0	8.0	4.0	2.5	1.0		0.5			
T-12-293	3.0	14.5	22.5	28.0	14.0	13.0	1.0	1.5	0.5	1.5			0.5	

Polar axis

Experiment N°	20.0	22.5	25.0	27.5	30.0	32.5	35.0	37.5	40.0	42.5	45.0	47.5	50.0	µm
T-12-289	0.5		12.5	44.0	33.5	7.5	1.5	0.5						%
T-12-290		1.0	12.5	38.0	36.0	12.0	0.5							
T-12-291			5.5	46.5	38.0	6.5	3.5							
T-12-292		1.5	11.5	26.0	34.0	20.5	5.0	1.0	0.5					
T-12-293		0.5	7.5	24.0	36.5	22.5	8.0	1.0						
T-12-306A			2.0	8.0	38.0	36.0	12.0	3.0	0.5	0.5				
T-12-306B			2.5	3.5	16.5	27.5	33.5	12.0	2.5			1.0	1.0	
T-12-307A		0.5	2.5	10.5	28.5	36.5	17.0	4.0			0.5			
T-12-307B		0.5	1.5	10.0	24.5	38.0	19.0	4.0	1.0	0.5	1.0			
T-12-308A				2.0	25.5	33.0	30.5	8.0	0.5	0.5				
T-12-308B				8.0	44.0	42.5	4.5	1.0						
T-12-309A			2.0	13.5	39.0	37.5	7.0	1.0						
T-12-309B				3.0	37.5	44.5	12.5	2.5						
T-12-310A				2.5	12.0	42.0	31.5	9.5	2.5					
T-12-310B				0.5	9.0	37.5	43.0	7.5	2.5					
T-12-311A				1.0	14.5	40.5	33.5	9.0	1.5					
T-12-311B				2.0	17.0	35.5	35.5	7.5	2.5					

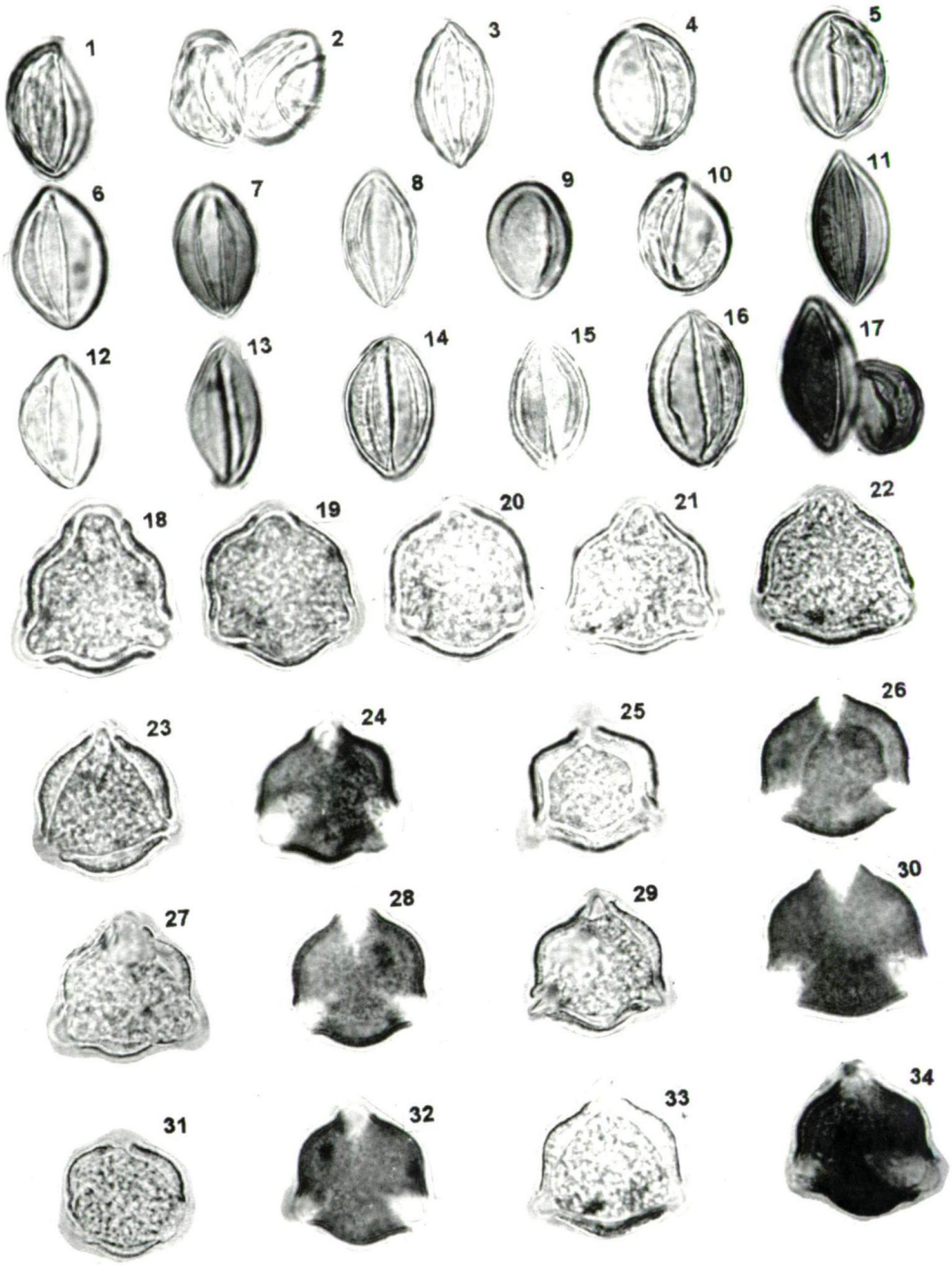
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Plate 7.1.

Plate 7.1.

1-17. *Gingko biloba* L. 1. fresh pollen grain, 2--5..Pollen grains heated on 30 °C. 2. length of time:30 minutes, 3. length of time: 1 hour, 4. length of time: 5 hours, 5. length of time:10 hours. 6-17. Pollen grains partially degraded with C60 fullerene/benzol solution. 6,7. length of time: 1 day, 6. unstained, 7. stained pollen grain, 8,9. length of time: 2 days, 8. unstained, 9. stained pollen grains

18-34. *Quercus robur* L. 18. fresh pollen grain. 19-22. pollen grains heated on 30 °C.19. length of time: 30 minutes, 20. length of time: 1 hour, 21. length of time: 5 hours, 22. length of time:10 hours. 23-34. Pollen grains partially degraded with C60 fullerene/benzol solution. 23,24. length of time: 1 day, 23 unstained, 24. stained pollen grain. 25,26. length of time:2 days. 25. unstained, 26. stained pollen grain. 27,28. length of time: 3 days, 27. unstained, 28. stained pollen grain. 29,30 length of time: 4 days. 29. unstained, 30. stained pollen grain. 31,32. length of time: 5 days.31. unstained, 32. stained pollen grain. 33,34. length of time: 6 days, 33. unstained, 34. stained pollen grain.

The characteristic LM morphology of these pollen grains has not changed after heating (Plate 7.1., figs. 2-5) and after the treatment with C60 fullerene/benzol solution. But there are some alterations in the P/E ratio (Plate 7.1., figs. 6-17). 1.3 and 1.4 was the maximum value at the fresh dry pollen grains, after heating for 10 minutes 1.2, 1.3 was measured. These values were more or less constant at the further experiments.

Quercus robur L. (Plate 7.1., figs. 18- 34)

Pollen gains of this species were observed and investigated in polar position. The diameter was measured.

Diameter

Experiment N°	20.0	22.5	25.0	27.5	30.0	32.5	35.0	37.5	µm
T-12-284			2.0	2.9	53.0	13.0	3.0		%
T-12-285				18.5	65.0	16.5			
T-12-286			1.5	16.5	44.5	33.5	4.0		
T-12-287			1.0	30.0	42.0	14.0	11.5	1.5	
T-12-288			2.0	33.5	56.0	8.5			

The maximal values of the diameter decreased after 5 and 10 hours of heating.

T-12-300A		3.5	45.5	36.0	15.0			
T-12-300B	0.5	6.0	15.0	29.5	38.5	9.0	1.5	
T-12-301A		4.0	14.5	25.0	35.0	17.0	4.5	
T-12-301B			5.5	17.0	41.5	29.0	7.0	
T-12-302A		2.0	11.0	30.5	34.0	17.0	5.5	
T-12-302B		1.0	11.0	15.5	37.0	30.0	5.0	0.5
T-12-303A		3.0	12.0	33.0	43.5	8.0	0.5	
T-12-303B		0.5	8.5	19.0	35.5	29.0	7.5	
T-12-304A			2.0	24.5	45.5	19.0	9.0	
T-12-304B			10.5	22.5	39.0	21.0	6.5	0.5
T-12-305A			7.0	22.5	43.0	22.0	5.5	
T-12-305B			2.0	24.5	43.0	26.0	4.5	

The maximum values of the diameter of the pollen grains after treatment with C60 fullerene/benzol solution increased Sometimes there are differences between the unstained and stained pollen grains at the same experiment. After 5 and 6 days the maximum, values are essentially identical.

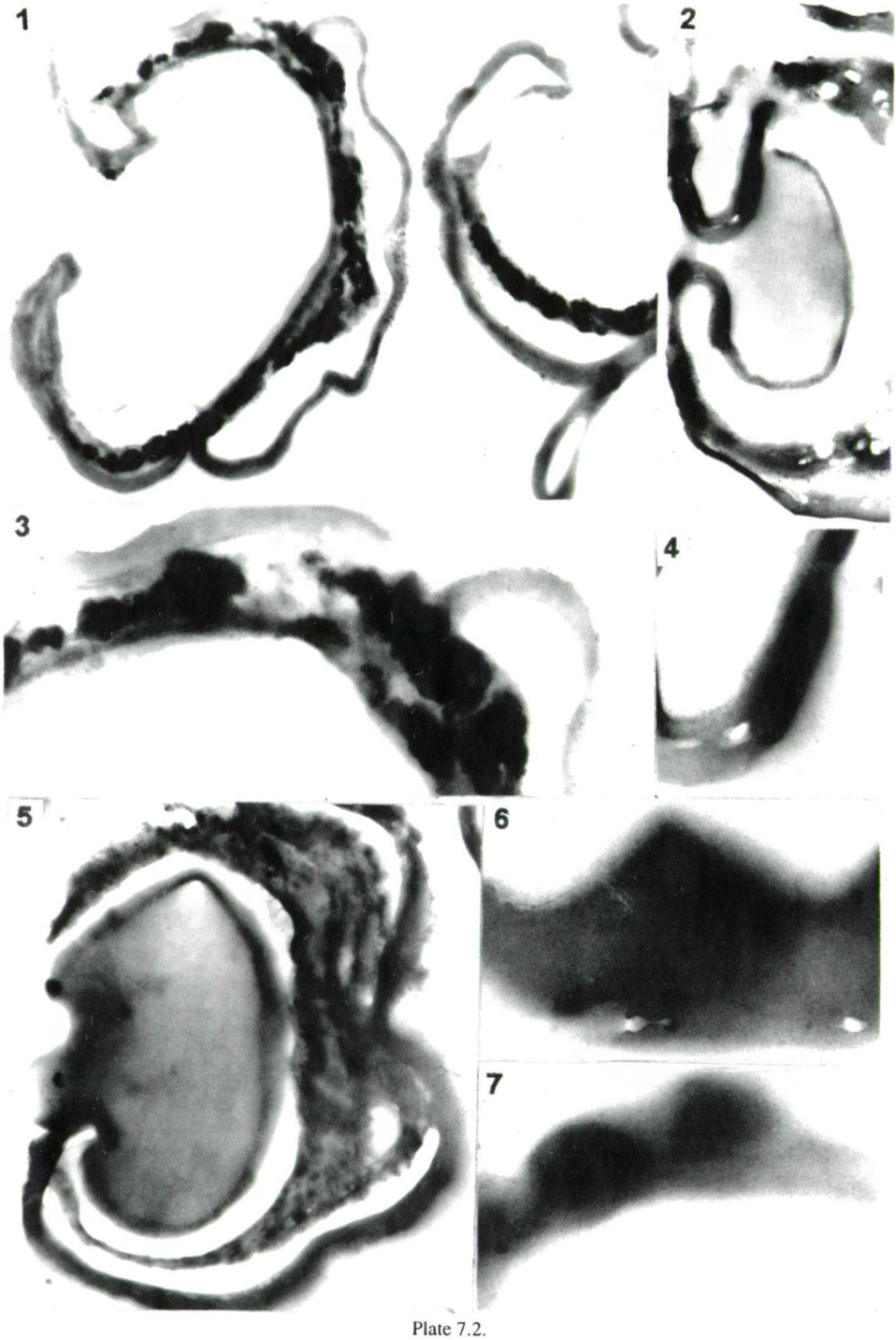

Plate 7.2.

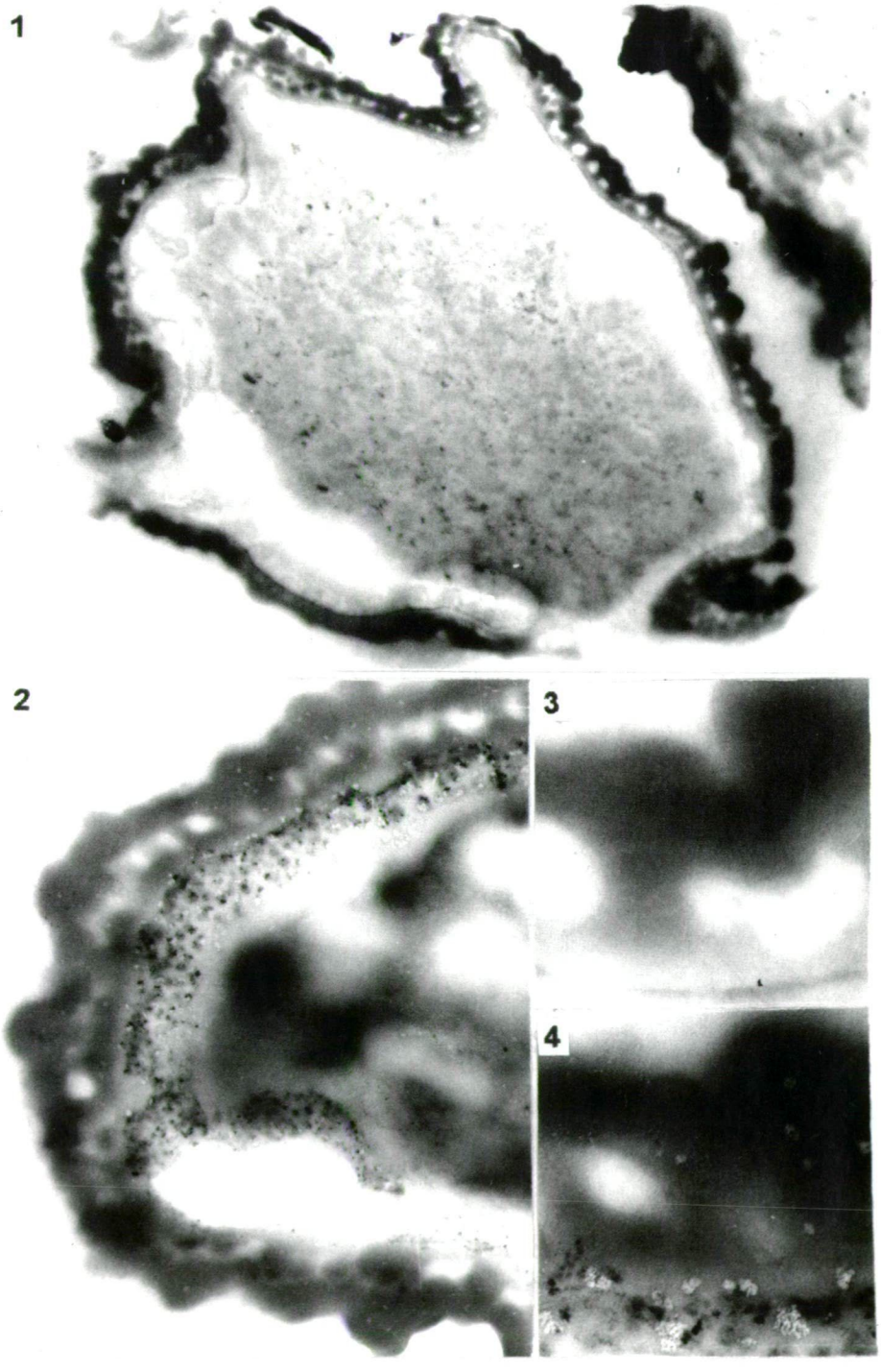

Plate 7.3.

Plate 7.2.

1-7. *Ginkgo biloba* L. Transmission electron microscopy of partially degraded pollen grains with C60 fullerene/benzol solution during 1 day.
1,2,5. General survey picture from the equatorial plane sectioned pollen grains 5.000x., 1. Negative No.: 9621, 2. negative No.: 9592, 5. Negative No.: 9624.
3,4. Details from the exine ultrastructure. 15.000x. 3. negative No.: 9622, 4. Negative No.: 9593.
6,7. Details from the partially degraded ectexine. 50.000x. 6. Negative No.: 9556, 7. Negative No.: 9589.

Plate 7.3.

1-4. *Quercus robur* L. Transmission electron microscopy of partially degraded pollen grains with C60 fullerene/benzol solution during 1 day.
1. General survey picture of the ultrastructure of the pollen grain in equatorial section. 5.000x, Negative No.: 9626.
2. Detail from the exine and protoplasm of the partially degraded pollen grain. 15.000x. Negative No.: 3617.
2,4. Detail from the ultrastructure of the pollen wall. 50.000x. 3. Negative No.: 9669. 4. Negative No.: 9819.

TEM results

Ginkgo biloba L. (Plate 7.2., figs. 1-7)

The ultrastructure of the pollen grains was investigated in equatorial sections. The general survey pictures (Plate 7.2., figs. 1, 2, 5) illustrate well the electron dense ectexine and the protoplasm. The thin apertural ectexine (sulcus wall) is well shown in figs. 2 and 4, (Plate 7.2). The ectexine stratification is more or less well preserved, the tectum, infratectal layer and the foot layer are perceptible. Dissolution or degradation of biopolymer units of the ectexine was not observed (Plate 7.2., figs. 6,7).

Quercus robur L. (Plate 7.3., figs. 1-4)

The general survey picture (Plate 7.3., fig. 1) illustrate well the dark electron dense ectexine, the light intine and the more or less degraded granular protoplasm. The ultrastructure of the ornamental elements of the tectum, the columellar infratectal layer and sometimes the dark ectintine are well shown. Fine structure of the ectexine is illustrated in picture 3 and 4 (Plate 7.3). Well shown are in picture 4, and 2, the light holes at the border of the foot layer and ectintine, and in the intine, indicates the dissolution of globular biopolymer units from this part of the wall. The dark globular units in the intine may be the consequence of the partial dissolution or degradation of the molecular system of this layer.

Discussion and Conclusions

The pollen subjected to a 30 °C temperature did not have a meaningful effect on these grains. There was only a moderate change in the P/E ratio of *Ginkgo biloba* pollen. Therefore, it can be assumed that during wind dispersion the air temperature is not an important factor in changing the morphological characters of these pollen grains.

The TEM data of the partially dissolved pollen grains with the C60 fullerene/benzol solution suggest that the technique is useful and promising for future studies. This technique reveals the ultrastructure elements without the usual fixation of glutar-aldehyde and the postfixation with OsO_4 aq. dil. This technique was succesful in both the relatively resistant ectexinous pollen of *Ginkgo biloba* and the less resistant *Quercus robur.*

As first step in this type of experiment, the results of both the homogeneous consistence of the ectexine and the electron dense protoplasm of *Ginkgo biloba* are also consistent with our early observations on *Taxus baccata* L. pollen.

It is out belief that the partial dissolution of the biopolymer structures at the outermost part of the foot layer and the innermost part of the ectintine indicate the complexity of the biopolymer system of the exinous pollen wall. The tectum, infratectal layer and the outer part of the foot layer are resistant to this treatment. In another experiment (KEDVES, PÁRDUTZ and VARGA, 2002) we observed a separate electron dense part of the foot layer and perhaps also the endexine. The origin of this latter mentioned layer needs further investigation and it may be identified with a less resistant molecular system.

Acknowledgements

The writers are grateful to Professor Edward A. STANLEY (Ardmore, PA, U.S.A.) for critically reading the manuscript and for his valuable comments. This work was supported by Grant OTKA T 31715.

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