5. LM INVESTIGATIONS OF PARTIALLY DISSOLVED SPOROMORPHS II.

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Abstract

This contribution presents LM results of partially dissolved pollen grains of the following species: *Larix decidua* MILL., *Thalictrum minus* L., *Platanus hybrida* BROT., *Tilia platyphyllos* SCOP. and *Castanea sativa* MILL. Diethylamine, merkaptoethanol, methanol, ethanol, n-propanol, n-butanol and i-amyl alcohol were used as solvents at 30 °C, during 30, 90, 150, 210, 270 and 330 days. The Duhoux-effect was observed at the pollen grains of *Larix decidua* MILL. The sporopollenin of the ectexines of *Platanus* hybrida and Tilia platyphyllos were the less resistant to the influence of diethylamine. But not so important qualitative alterations were observed at the investigated *angiosperm* pollen grains. Worth of mentioning is the resistance of the exine of *Castanea sativa* in contrast to the less resistant exine of the genus *Quercus*. The variation of the size was statistically investigated at the pollen grains of *Platanus hybrida*, the peculiar alterations of the pollen grains of *Larix decidua*, finally the pollen tube development at *Tilia platyphyllos*.

Key words: Palynology, recent, pollen grains, partial dissolution, LM method.

Introduction

In our previous paper (KEDVES, KÁROSSY and BORBOLA, 1997) we summarized the problems and our previous results within this research program of the Laboratory. This contribution is part of this research program. The species chosed are in relation with the problems which emerged in connection with to the previous results.

Materials and Methods

Pollen grains of the following species were the subject of our investigations.

Larix decidua MILL.

Locality: Botanical Garden of the J. A. University. Collected: Á. ERDŐDI, on 16.04.1996. Beginning of the experiments: 18.04.1996. Numbers of experiments: 1/7-368-409.

Thalictrum minus L.

Locality: Botanical Garden of the J. A. University. Collected: Á. ERDŐDI, on 12.06.1996. Beginning of the experiments: 13.06.1996. Numbers of experiments: 1/7-521-563.

Platanus hybrida BROT.

Locality: Botanical Garden of the J. A. University, Collected: Á. ERDŐDI, on 30.04.1996. Beginning of the experiments: 02.05.1996. Numbers of experiments 1/7-430-477.

Tilia platyphyllos SCOP.

Locality: Botanical Garden of the J. A. University, Collected: Á. ERDŐDI, on 01.06.1996. Beginning of the experiments: 06.06.1996. Numbers of experiments 1/7-479-520.

Castanea sativa MILL.

Locality: Botanical Garden of the J. A. University, Collected: Á. Erdődi, on 14.06.1996. Beginning of the experiments: 18.06.1996. 1/7-571-612.

The method is completely identical with the earlier one but we repeat it. (P.45)

"5 mg dried spore or pollen material was measured into small glasses. 5 ml H_2O distilled and 0.2 ml diethylamin or merkaptoethanol were added to the dried experimental material. Other alcohols: methanol, ethanol, etc. 5 ml was added to 5 mg spore or pollen material. Temperature: 30 °C."

It's easy to find all data of experiments in Plate 5.1–5.5.

Results

Larix decidua MILL. (Plate 5.1., figs. 1–42, Table 5.1)

Very characteristic Duhoux-effect was observed during the light microscopical investigations. The pollen grains were classified into three groups. (Table 5.1)

1: qualitatively non-altered pollen grains (Plate 5.1., fig. 1),

2: The so-called "hiatus group" (Plate 5.1., fig. 14),

3: Ectexine-lost pollen grains (Plate 5.1., fig. 12).

After thirty days the *"hiatus forms"* occurred in an important quantity and their proportion more or less regularly increased. But the quantities of the ectexine-lost pollen grains changed irregularly in the relation of time and organic solvents.

Thalictrum minus L. (Plate 5.2., figs. 1–42)

No important alterations were observed. Sometimes minor alterations are in the sizes and the thickness of the ectexine.

Platanus hybrida BROT. (Plate 5.3., figs. 1–38, Table 5.2)

The diethylamine dissolved the ectexine relatively quickly. We investigated in detail the alteration of size of the pollen grains. Table 5.2 illustrates well that there is a great variety in the diameter of the pollen grains by the different solvents and the length of time of the dissolution.

Tilia platyphyllos SCOP. (Plate 5.4., figs. 1–37, Table 5.3)

Within the recently investigated species the ectexine of *Tilia platyphyllos* is the less resistant to diethylamine. We have observed a remarkable variation in the diameter of the pollen grains during the qualitative investigations. The pollen tube development indiced by the organic solvents was also investigated. Table 5.3. summarizes these results. In this case also three groups were established.

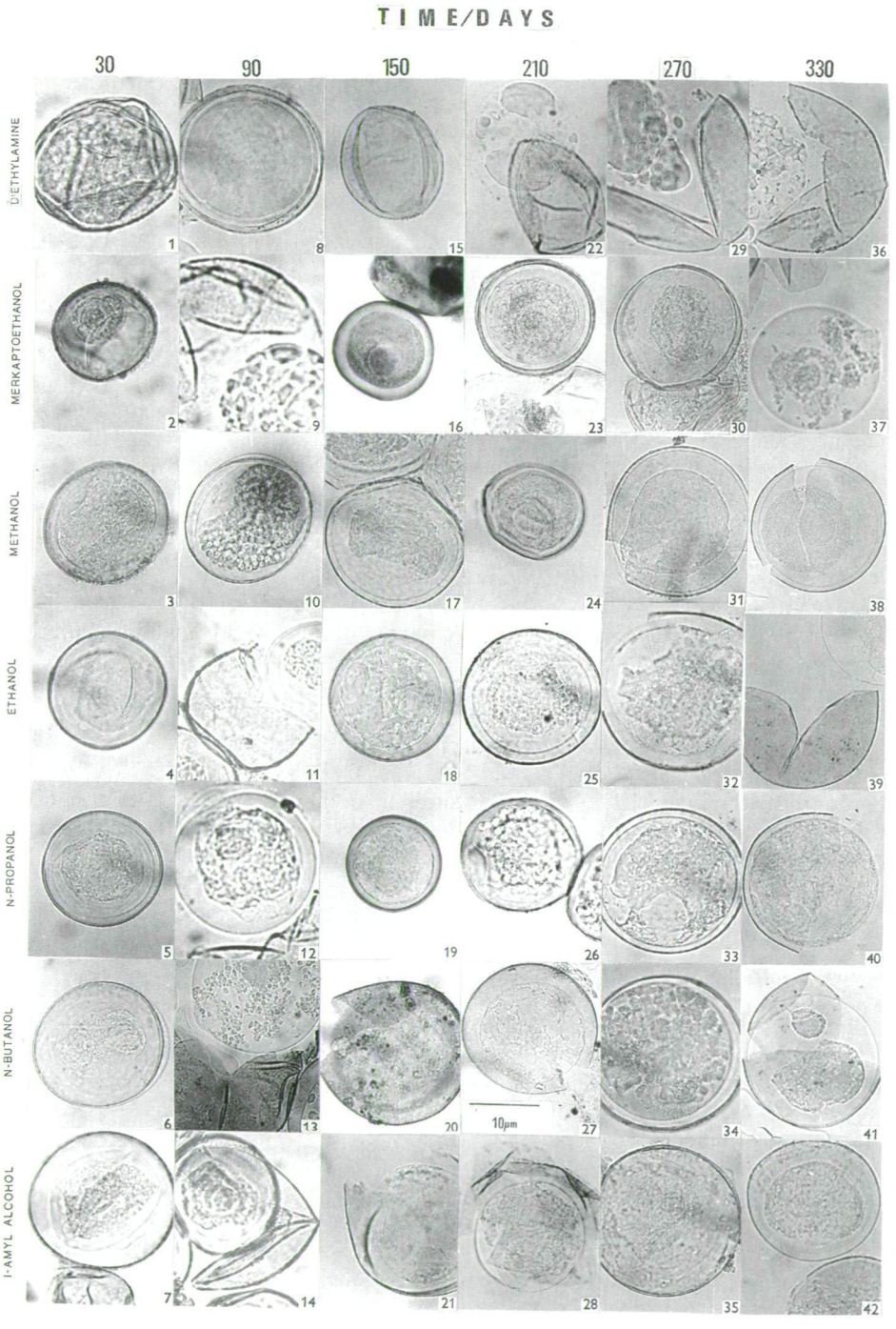

Plate 5.1., 1–42. *Larix decidua* MILL.

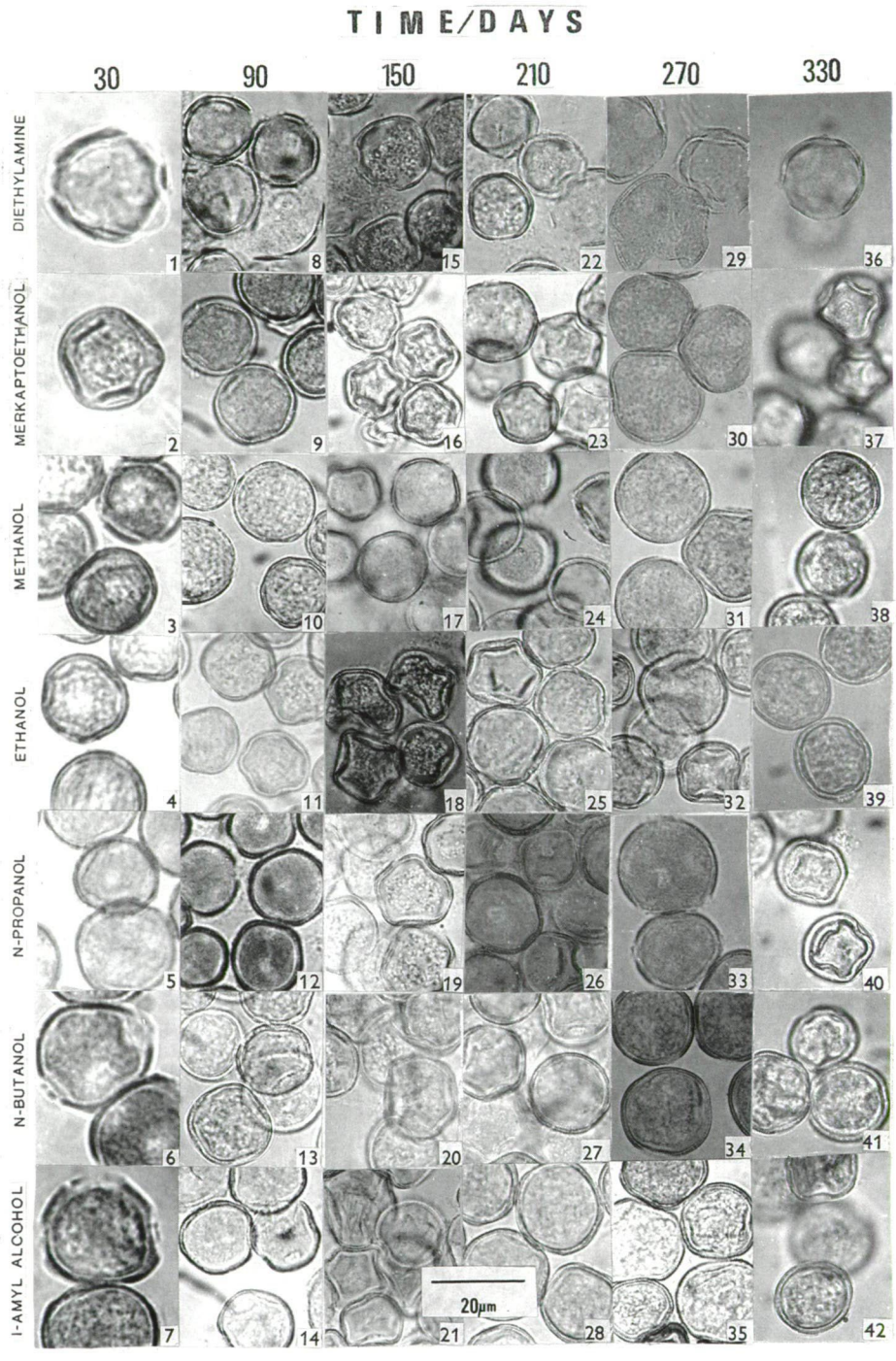

Plate 5.2., 1–42. *Thalictrum minus* L.

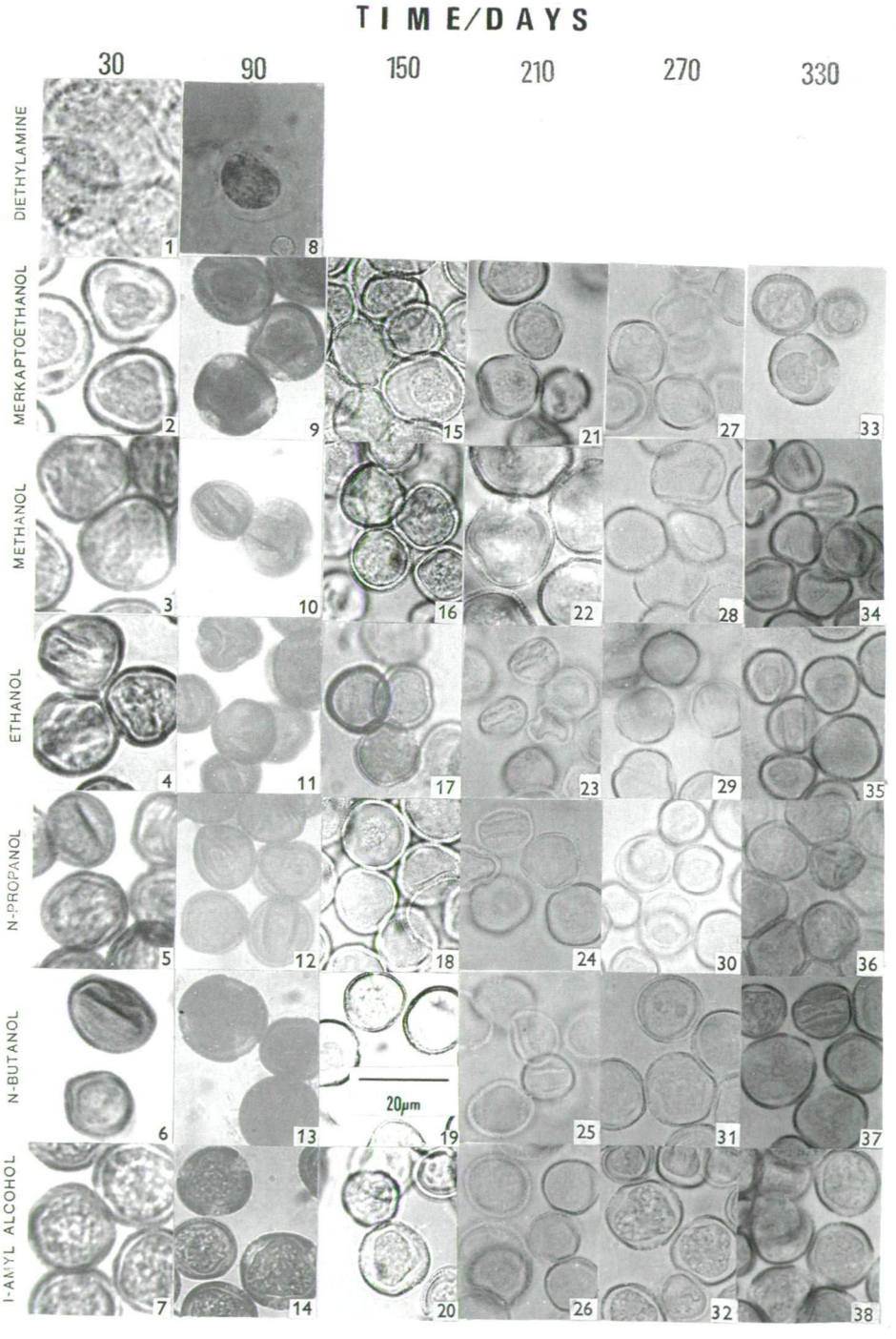

Plate 5.3., 1–38. *Platanus hybrida* BROT.

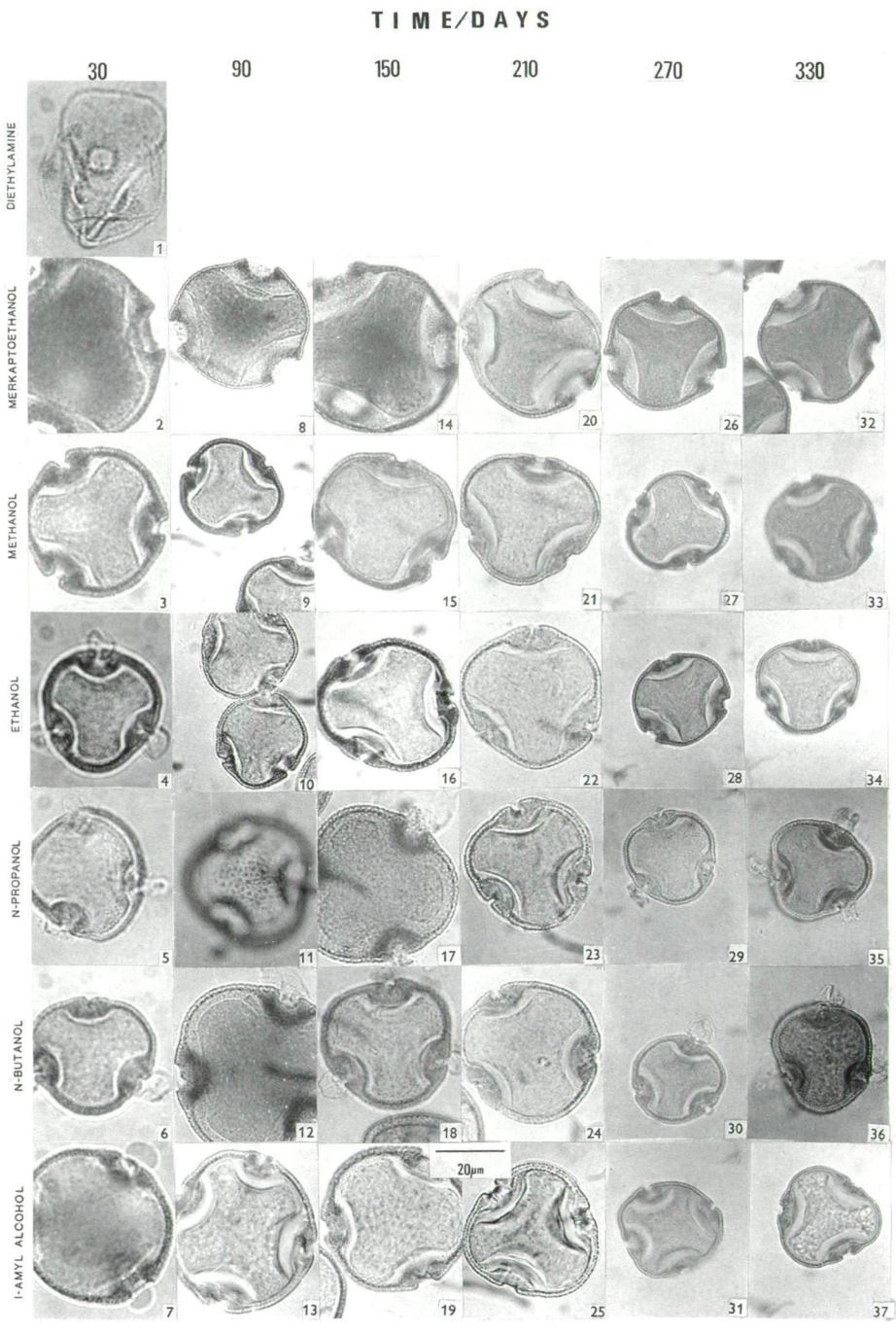

Plate 5.4., 1–37. *Tilia platyphyllos* SCOP.

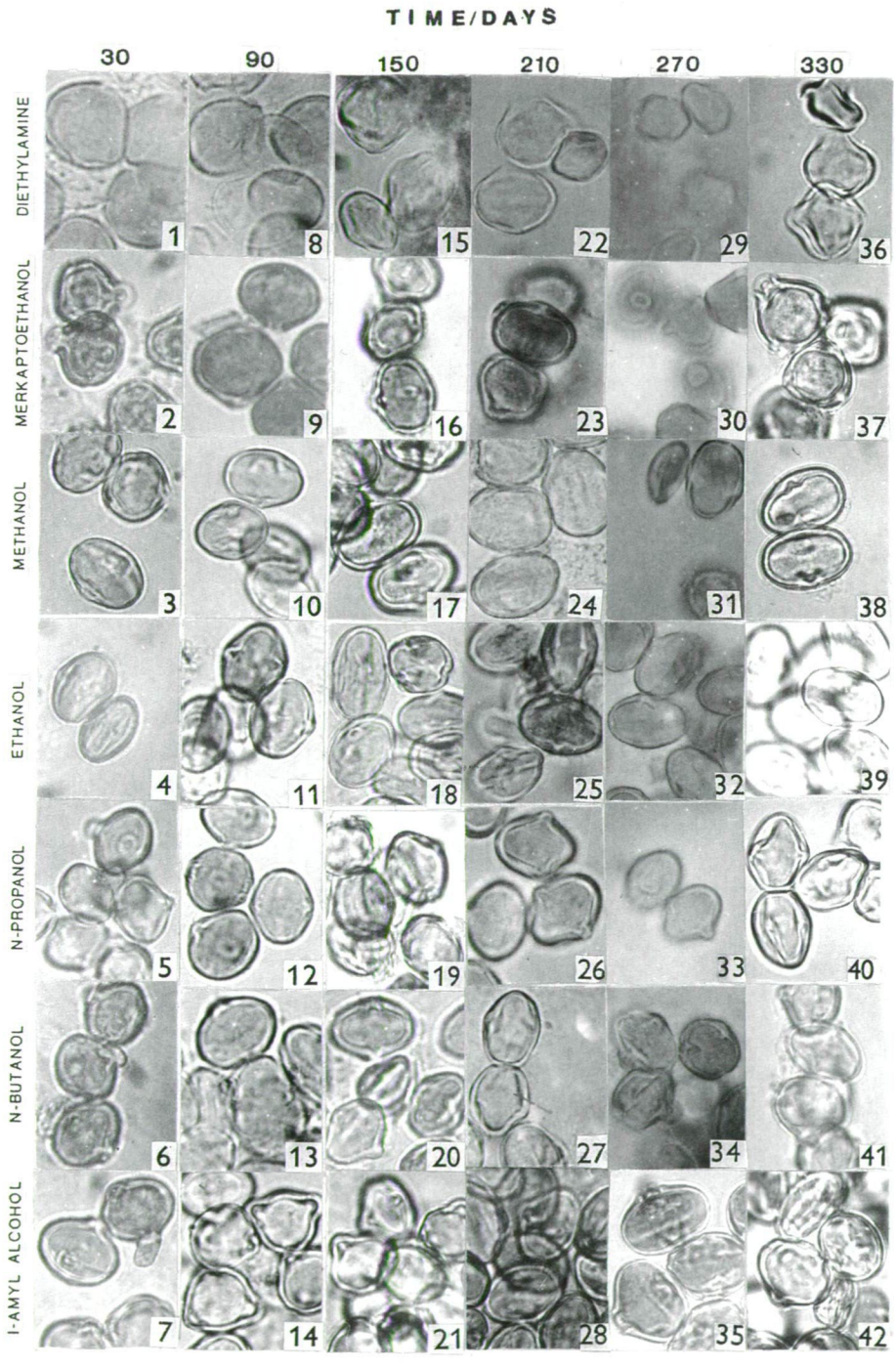

Plate 5.5., 1–42. *Castanea sativa* MILL.

1. pollen grains without pollen tube (Plate 5.4., fig 16)
2. the pollen tube developed at one or two aperture (Plate 5.4., fig. 3)
3. the pollen tube appeared at each apertures (Plate 5.4., fig. 18)

After 30 days the pollen tube developed in a remarkable per cent as the result of the dissolution effect of the solvents as follows. N-propanol, n-butanol and i-amyl alcohol. Merkaptoethanol was effectual after 330 days.

Castanea sativa MILL. (Plate 5.5., figs. 1–42)

Pollen grains of this species are resistant to the used organic solvents. Thinning of the exine (Plate 5.5., fig 1) and protrusions in the apertural area were observed only (Plate 5.5., fig. 5)

Discussion and Conclusions

1. Diethylamine dissolved relatively quickly the ectexine of the pollen grains of *Tilia platyphyllos* and *Platanus hybrida*. In our previous paper (KEDVES and GÁSPÁR 1994, 1995) we pointed out this phenomenon at the exospore of *Equisetum arvense* and at the exine of the genus *Quercus*. In this point of view the resistant exine of the pollen grains of *Castanea sativa* is worth of mentioning. Dissolutions experiments of further *Fagaceae* pollen grains (*Fagus, Pasania, Nothofagus*) seem to be necessary.

2. Alterations of the diameter not included originally within this research program. Previously we observed that there are important changes, we believe, that the presented results of *Platanus hybrida* are worth of mentioning.

3. Pollen tube development was investigated in our Laboratory in the first place after X-ray irradiation. This phenomenon may happen in consequence of other experimental influences.

Acknowledgements

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	30			90			150			210			270			330			days
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
diethylamine	76.5	22.5	1.0	68.5	16.5	15.0	53.0	29.5	17.5	54.0	32.0	14.0	54.0	27.5	18.5	49.0	32.0	19.0	%
merkaptoethanol	89.0	11.0	0.0	70.5	19.0	10.5	70.5	20.0	9.5	68.5	16.0	15.5	71.5	11.5	17.0	52.5	27.0	20.5	%
methanol	91.5	7.0	1.5	83.0	13.5	3.5	68.0	27.5	4.5	79.0	17.5	3.5	84.5	13.0	2.5	73.5	17.5	9.0	%
ethanol	84.5	15.5	0.0	72.0	23.5	4.5	77.0	22.0	1.0	70.0	22.5	7.5	73.0	22.0	5.0	75.0	19.0	6.0	%
n-propanol	89.0	10.0	1.0	84.0	13.0	3.0	76.0	19.5	4.5	84.0	14.0	2.0	71.5	22.0	6.5	88.5	8.0	3.5	%
n-butanol	89.5	10.0	0.5	67.0	24.0	9.0	75.0	21.5	3.5	69.5	17.0	13.5	50.5	36.5	13.0	25.5	53.5	21.0	%
i-amyl alcohol	74.5	12.5	13.0	49.0	28.5	22.5	65.0	24.5	10.5	60.0	31.5	8.5	56.5	25.5	18.0	29.5	41.5	29.0	%

Larix decidua MILL.
Table 5.1.

	30 days		90 days		150 days		210 days		270 days		330 days		standard		average
diethylamine	1.50%	15 μm											8.00%	15 μm	
	22.0%	17.5 μm											65.00%	17.5 μm	
	33.50%	20 μm											25.50%	20 μm	16.67%
	27.50%	22.5 μm											1.15%	25 μm	
	13.0%	25 μm													
	2.50%	27.5 μm													
merkaptoethanol	1.00%	12.5 μm	19.00%	15 μm	4.00%	12.5μm	5.00%	12.5 μm	23.00%	15 μm	1.50%	15 μm	8.00%	15 μm	
	14.5%	15 μm	37.50%	17.5 μm	15.00%	15 μm	26.50%	15 μm	29.50%	17.5μm	14.50%	17.5 μm	65.00%	17.5 μm	
	31.00%	17.5 μm	32.00%	20 μm	27.00%	17.5 μm	39.00%	17.5 μm	32.00%	20 μm	28.50%	20 μm	25.50%	20 μm	18.30%
	44.50%	20 μm	10.00%	22.5 μm	28.50%	20 μm	25.50%	20 μm	13.00%	22.5 μm	26.50%	22.5 μm	1.15%	25 μm	
	8.00%	22.5 μm	1.50%	25 μm	20.50%	22.5 μm	4.00%	22.5 μm	2.50%	25 μm	20.50%	25 μm			
	1.00%	25 μm			5.00%	25 μm					0.50%	27.5 μm			
ethanol	5.00%	12.5 μm	22.00%	15 μm	26.50%	15 μm	29.00%	15 μm	16.50%	15 μm	14.00%	17.5 μm	8.00%	15 μm	
	22.00%	15 μm	45.50%	17.5 μm	48.50%	17.5 μm	41.00%	17.5 μm	42.50%	17.5 μm	48.50%	20 μm	65.00%	17.5 μm	
	55.00%	17.5 μm	25.00%	20 μm	23.50%	20 μm	26.50%	20 μm	32.00%	20 μm	32.50%	22.5 μm	25.50%	20 μm	20.39%
	22.00%	20 μm	7.50%	22.5 μm	1.50%	22.5 μm	3.50%	22.5 μm	8.00%	22.5 μm	5.00%	25 μm	1.15%	25 μm	
	5.00%	22.5 μm							1.00%	25 μm					
methanol	3.50%	15 μm	3.00%	15 μm	20.50%	15 μm	2.00%	15 μm	13.50%	15 μm	10.00%	15 μm	8.00%	15 μm	
	38.50%	17.5 μm	29.00%	17.5 μm	44.00%	17.5 μm	21.00%	17.5 μm	40.50%	17.5 μm	44.00%	17.5 μm	65.00%	17.5 μm	
	53.00%	20 μm	30.00%	20 μm	25.00%	20 μm	58.50%	20 μm	33.50%	20 μm	37.00%	20 μm	25.50%	20 μm	
	5.00%	22.5 μm	24.50%	22.5 μm	10.50%	22.5 μm	17.50%	22.5 μm	3.50%	22.5 μm	6.00%	22.5 μm	1.15%	25 μm	20.66%
			12.00%	25 μm			1.00%	25 μm	3.00%	25 μm	3.00%	25 μm			
			1.50%	27.5 μm											
n-propanol	1.00%	12.5 μm	5.00%	12.5 μm	11.00%	15 μm	13.00%	15 μm	17.00%	15 μm	9.00%	15 μm	8.00%	15 μm	
	8.00%	15 μm	11.00%	15 μm	53.50%	17.5 μm	33.50%	17.5 μm	33.00%	17.5 μm	43.00%	17.5 μm	65.00%	17.5 μm	
	41.00%	17.5 μm	37.00%	17.5 μm	34.50%	20 μm	34.00%	20 μm	34.00%	20 μm	37.00%	20 μm	25.50%	20 μm	
	46.50%	20 μm	41.50%	20 μm	1.00%	22.5 μm	15.00%	22.5 μm	12.00%	22.5 μm	9.00%	22.5 μm	1.15%	25 μm	21.93%
	3.50%	22.5 μm	10.00%	22.5 μm			4.20%	25 μm	3.00%	25 μm	2.00%	25 μm			
									0.50%	27.5 μm					
									0.50%	32.5 μm					

	30 days		90 days		150 days		210 days		270 days		330 days		standard		average
n-butanol	3.50%	15 µm	25.00%	15 µm	5.00%	15 µm	0.30%	15 µm	13.50%	15 µm	0.80%	15 µm	8.00%	15 µm	
	39.50%	17.5 µm	42.00%	17.5 µm	11.50%	17.5 µm	31.50%	17.5 µm	48.00%	17.5 µm	29.00%	17.5 µm	65.00%	17.5 µm	
	53.00%	20 µm	28.50%	20 µm	62.00%	20 µm	37.50%	20 µm	32.50%	20 µm	32.50%	20 µm	25.50%	20 µm	20.69%
	3.50%	22.5 µm	4.50%	22.5 µm	25.50%	22.5 µm	20.50%	22.5 µm	0.60%	22.5 µm	20.50%	22.5 µm	1.15%	25 µm	
	0.50%	27.5 µm			5.00%	25 µm	7.50%	25 µm			0.80%	25 µm			
											0.20%	27.5 µm			
i-pentanol	1.50%	15 µm	15.00%	15 µm	14.50%	15 µm	3.00%	15 µm	0.50%	12.5 µm	0.15%	12.5 µm	8.00%	15 µm	
	27.50%	17.5 µm	24.50%	17.5 µm	31.00%	17.5 µm	44.50%	17.5 µm	14.00%	15 µm	13.00%	15 µm	65.00%	17.5 µm	
	59.00%	20 µm	37.50%	20 µm	48.50%	20 µm	42.00%	20 µm	34.00%	17.5 µm	28.50%	17.5 µm	25.50%	20 µm	19.93%
	12.00%	22.5 µm	19.50%	22.5 µm	6.00%	22.5 µm	9.50%	22.5 µm	41.50%	20 µm	27.50%	20 µm	1.15%	25 µm	
			4.00%	25 µm			0.10%	25 µm	0.90%	22.5 µm	20.50%	22.5 µm			
									0.10%	25 µm	9.00%	25 µm			
50% glycerine	11.50%	15 µm	0.50%	15 µm	3.00%	15 µm	24.50%	15 µm	11.00%	15 µm	20.50%	15 µm	8.00%	15 µm	
	24.00%	17.5 µm	28.50%	17.5 µm	24.50%	17.5 µm	52.00%	17.5 µm	45.00%	17.5 µm	39.50%	17.5 µm	65.00%	17.5 µm	
	34.50%	20 µm	38.00%	20 µm	50.50%	20 µm	22.00%	20 µm	40.50%	20 µm	25.50%	20 µm	25.50%	20 µm	20.53%
	11.50%	22.5 µm	20.00%	22.5 µm	19.50%	22.5 µm	15.00%	22.5 µm	3.50%	22.5 µm	11.50%	22.5 µm	1.15%	25 µm	
	5.00%	25 µm	7.50%	25 µm	2.50%	25 µm					0.30%	25 µm			
			1.00%	27.5 µm											

Platanus hybrida BROT.
Table 5.2.
Compiled by V. KECSKEMÉTI

	30			90			150			210			270			330			days
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
diethylamine	99.0	1.0	0.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	%
merkaptoethanol	87.5	9.0	3.5	88.0	12.0	0.0	85.5	12.5	2.0	97.0	3.0	0.0	92.0	7.5	0.5	54.5	37.5	8	%
methanol	85.5	12.0	2.5	99.5	0.5	0.0	87.5	11.5	1.0	98.0	2.0	0.0	93.5	5.5	1.0	85.0	14.0	1	%
ethanol	87.5	10.0	2.5	90.5	9.5	0.0	90.5	9.5	0.0	79.0	19.5	1.5	90.5	7.5	2.0	79.0	16.5	4.5	%
n-propanol	56.5	34.5	9.0	60.0	35.5	4.5	24.0	56.0	20.0	90.5	9.0	0.5	45.0	37.0	18.0	51.0	32.5	16.5	%
n-butanol	54.5	32.0	13.5	44.5	30.5	25	43.0	46.0	11.0	89.0	9.5	1.5	40.5	41.0	18.5	39	39.5	21.5	%
i-pentanol	70.5	27.5	2.0	88.5	10.5	1.0	90.0	8.5	1.5	97.0	3.0	0.0	86.0	14.0	0.0	89	10.5	0.5	%

Tilia platyphyllos SCOP.
Table 5.3.