

### 3. LM INVESTIGATIONS OF PARTIALLY DISSOLVED SPOROMORPHS I.

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#### Abstract

The light-microscopical results of partially dissolved spores and pollen grains of the recent species are summarized in this paper: *Dryopteris filix-mas*, *Juniperus virginiana*, *Betula verrucosa*, *Carya illinoensis*, *Juglans nigra* and *Platycarya strobilacea*. Seven organic solvents – diethylamine, merkptoethanol, methanol, ethanol, n-propanol, n-butanol and i-amyl alcohol – were used. Length of time: 30, 90, 150, 210, 270 and 330 days, temperature 30°C. Taxonomically important alterations were observed at the pollen grains of *Juniperus virginiana*, *Carya illinoensis* and *Betula verrucosa*. In this respect the spores of *Dryopteris filix-mas*, and the pollen grains of *Juglans nigra* and *Platycarya strobilacea* are very resistant. Concerning the results of the pollen grains of *Juniperus virginiana* as a new term, the DUHOUX effect is introduced. This is the extreme swelling of the intine, which breaks the exine and peculiar forms appear. This phenomenon may be the result of different influences first observed by DUHOUX as a result of hydration.

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

#### Introduction

The partial degradation of the sporopollenin is based on differences in the resistance of different components of the biopolymer system, cf. ROWLEY and PRIJANTO (1977), SOUTHWORTH (1985, 1986). The partially degraded sporoderm was investigated with the TEM method, and several kinds of biopolymer units were revealed. To the levels of organization of the biopolymer structure of the sporopollenin KEDVES (1989) published a summarizing schema. Later the importance of the LM method was also recognized in the papers of KEDVES and GÁSPÁR (1994). As a surprising result the dissolution in diethylamine of the exospore of *Equisetum arvense* L. and the exine of the pollen grains of *Quercus robur* L. can be pointed out. The experiment of the pollen grains of the genus *Quercus* was repeated later (KEDVES and GÁSPÁR 1996). Other pollen grains (e. g.: *Juglans*) are resistant. Till this time within our experimental material the teliospores of *Ustilago maydis* (DE CANDOLLE) CORDA proved to be the most resistant.

Last year a new research program started to get more information about the dissolution of the spore and pollen wall of the different taxa. This program may be included to the researches of the biopolymer system of the sporopollenin.

#### Materials and Methods

The following species were the subjects of our investigations: *Dryopteris filix-mas* (L.) RICH.

Locality: Botanical Garden of the J. A. University. Collected: Dr. I. SZÖLLŐSI, on 14.08.1995. Beginning of the experiments: 31.08.1995. Numbers of experiments: 1/7 – 318–360.

*Juniperus virginiana* L.

Locality: Botanical Garden of the J. A. University. Collected: Dr. J. PULICS, on 16.03.1995. Beginning of the experiments: 22.03.1995. Numbers of experiments: 1/7 – 1–43.

*Betula verrucosa* EHR.

Locality: Botanical Garden of the J. A. University. Collected: Dr. J. PULICS on 21.03.1995. Beginning of the experiments: 23.03.1995. Numbers of experiments: 1/7 – 44–88.

*Carya illinoensis* (WANG) K. KOCH

Locality: Botanical Garden of the J. A. University. Collected: I. GÁSPÁR on 27.05.1995. Beginning of the experiments: 08.06.1995. Numbers of experiments: 1/7 – 202–244.

*Juglans nigra* L.

Locality: Botanical Garden of the J. A. University. Collected: I. GÁSPÁR on 20.05.1995. Beginning of the experiments: 25.05.1995. Numbers of experiments: 1/7 – 127–169.

*Platycarya strobilacea* SIEB. and ZUCC.

Locality: Botanical Garden of the J. A. University. Collected: I. GÁSPÁR on 10.06.1995. Beginning of the experiments: 20.06.1995. Numbers of experiments: 1/7 – 247, 272–313.

5 mg dried spore or pollen material was measured into small glasses. 5 ml H<sub>2</sub>O distilled and 0.2 ml diethylamine or merkaptioethanol 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. Length of time and the organic solvents are indicated in Plates 3.1.–3.6.

## Results

*Dryopteris filix-mas* (L.) RICH. (Plate 3.1., figs. 1–42)

Based on light-microscopical investigations, taxonomically important alterations were not observed during these experiments. In some cases the perispore detached from the exospore.

*Juniperus virginiana* L. (Plate 3.2., figs. 1–42)

This series of experiment caused important alterations at this species. The basic alteration is the extreme swelling of the intine. The observed pollen grains are classified into three types: 1. The more or less intact pollen grains, the amb is circular, the ectexine is not splitted; e. g.: Plate 3.2., figs. 1–7. 2. The “hiatus form” together with the protoplasm and the thickened intine, e. g.: Plate 3.2., figs. 24,39. 3. The ectexine lost pollen grains, e. g.: Plate 3.2., figs. 17,18,20,27,41. The protoplasm is surrounded by the secondarily thickened intine, e. g.: Plate 3.2., figs. 17,18,20,27,41. The distribution of the percentages of the different forms are summarized as follows.

It is well shown that important alterations happened in consequence of diethylamine and merkaptioethanol after 210 days of dissolution. Methanol, propanol and butanol resulted also in similar alterations.

TIME/DAYS

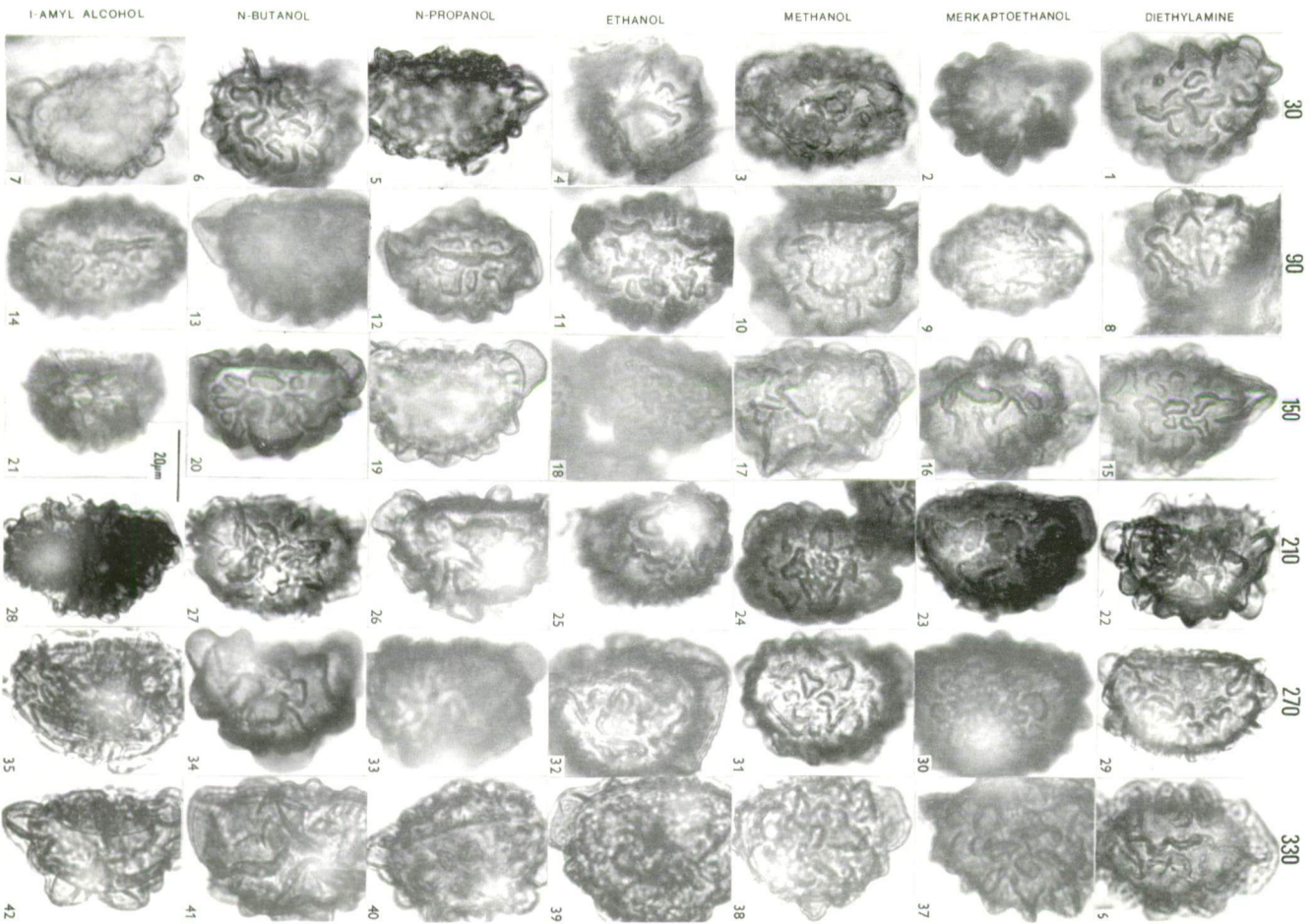


Plate 3.1., 1-42. *Drosophens filix-mus* (L.), RICH.

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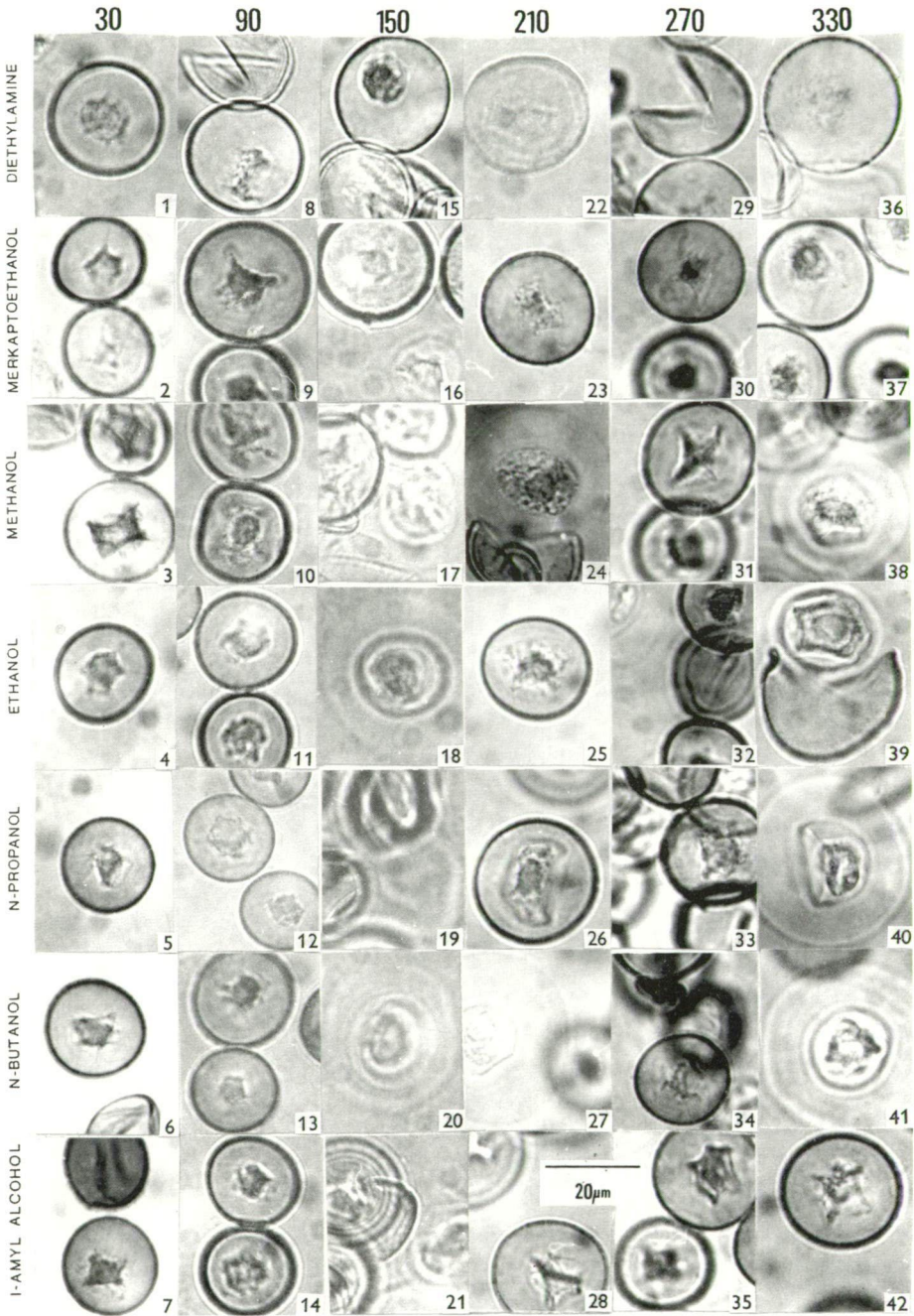


Plate 3.2., 1-42. *Juniperus virginiana* L.

**T I M E / D A Y S**

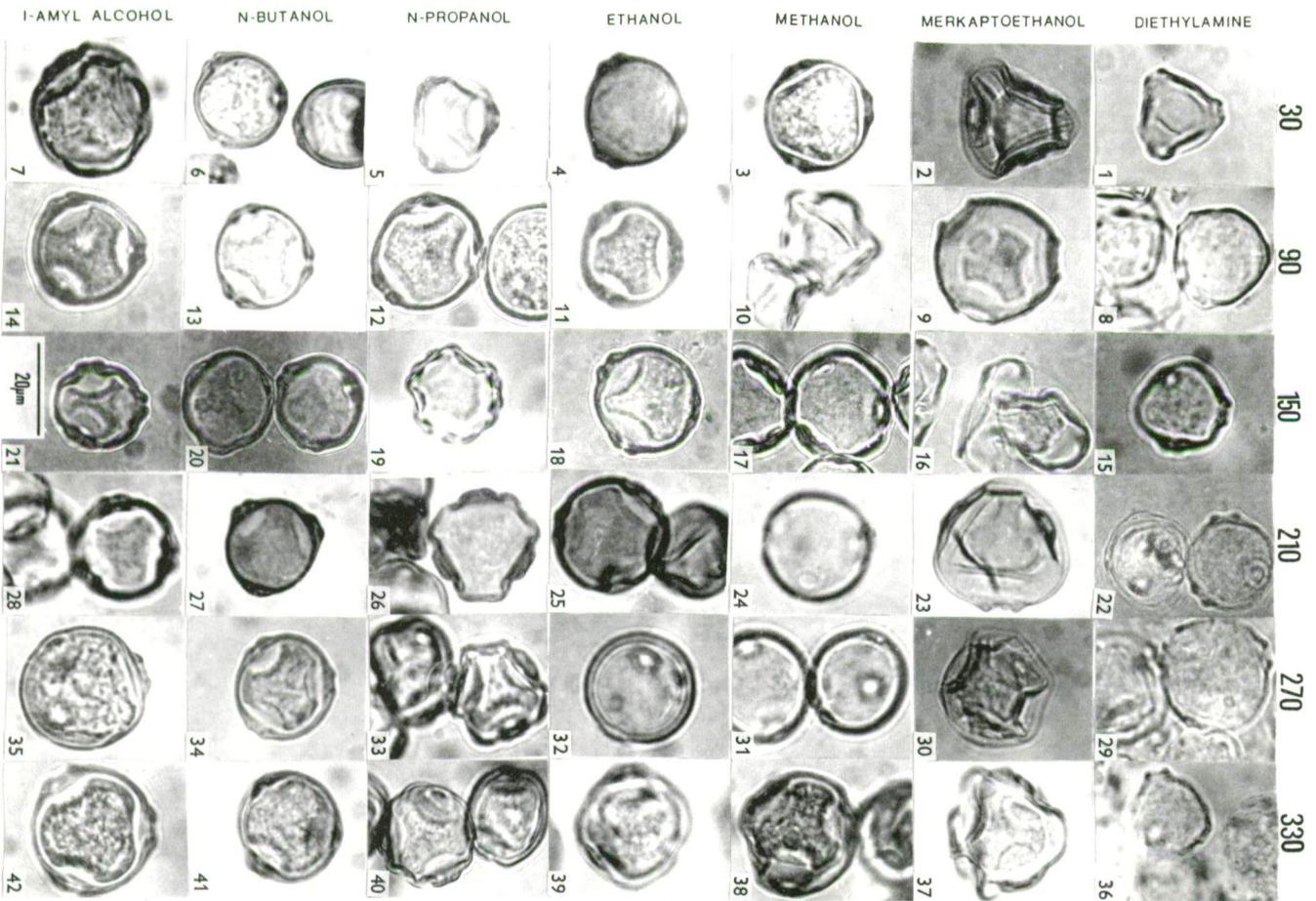


Plate 3.3., 1-42. *Betula verrucosa* EHR.

	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	72.0	26.0	2.0	70.5	28.0	1.5	71.5	27.0	1.5	68.0	8.0	24.0	63.5	10.0	26.5	86.5	0.0	13.5 %
merkptoethanol	68.5	29.0	2.5	86.0	11.0	3.0	64.0	30.0	6.0	73.5	5.0	21.5	50.5	0.5	49.0	89.0	1.0	10.0 %
methanol	72.5	18.0	9.5	89.0	6.0	5.0	75.0	14.0	11.0	66.0	10.0	24.0	96.0	3.0	1.0	76.0	6.5	17.5 %
ethanol	72.0	25.0	3.0	69.0	26.5	4.5	48.5	33.0	18.5	64.5	12.0	23.5	43.0	3.0	54.0	97.0	2.5	0.5 %
n-propanol	75.5	16.5	8.0	71.5	18.0	10.5	51.5	28.5	20.0	84.5	9.0	6.5	54.0	1.0	45.0	55.0	5.0	40.0 %
n-butanol	75.0	23.5	1.5	70.0	17.5	12.5	41.0	20.0	39.0	43.0	5.0	52.0	62.0	0.5	37.5	52.5	5.0	42.5 %
i-amyl alcohol	65.0	21.5	13.5	62.5	32.5	5.0	66.5	21.0	12.5	98.0	0.0	2.0	87.0	1.0	12.0	87.0	11.0	2.0 %

*Juniperus virginiana* L.

Table 3.1.



*Betula verrucosa* EHR. (Plate 3.3., figs. 1-42)

Important qualitative alterations happened during this kind of experimental studies. The pollen grains were grouped into two types; 1. The relatively more or less original, trivestibulate form, e. g.: Plate 3.3., figs. 1,3-5. 2. The altered forms which lost the original morphological characteristic features e. g.: Plate 3.3., figs. 2,19,26,30. The percentages of this two pollen types are summarized in the following.

	30		90		150		210		270		330 days	
	1	2	1	2	1	2	1	2	1	2	1	2
diethylamine	70.0	30.0	99.5	0.5	93.0	7.0	99.0	1.0	97.5	2.5	100.0	0.0%
merkaptioethanol	16.0	84.0	11.5	88.5	9.0	91.0	24.5	75.5	3.5	96.5	13.0	87.0%
methanol	99.5	0.5	100.0	0.0	89.5	10.5	95.5	4.5	100.0	0.0	80.0	20.0%
ethanol	99.0	1.0	100.0	0.0	97.5	2.5	81.0	19.0	100.0	0.0	94.5	5.5%
n-propanol	67.0	33.0	100.0	0.0	4.0	96.0	67.0	33.0	32.0	68.0	92.5	7.5%
n-butanol	96.0	4.0	100.0	0.0	99.5	0.5	75.0	25.0	99.5	0.5	97.5	2.5%
i-amyl alcohol	93.0	7.0	100.0	0.0	97.5	2.5	82.5	17.5	97.0	3.0	96.5	3.5%

*Betula verrucosa* EHR.

Table 3.2.

*Carya illinoensis* (WANG) K. KOCH (Plate 3.4., figs. 1-42)

Important qualitative alterations were observed at this kind of subtriporate *Juglandaceae* pollen type. The pollen grains were classified into two groups: 1. The more or less original but always subtriporate forms, e. g.: Plate 3.4., figs. 1-7, 18-21. 2. Peculiar pseudoplicate forms appearing after several dissolution experiments, sometimes similar to a tetrad mark. In this relation, the position of the subequatorial pores is lateral between two branches of the plicate foldings. The quantitative distribution of these two kinds of pollen forms is summarized in Table 3.3.

	30		90		150		210		270		330 days	
	1	2	1	2	1	2	1	2	1	2	1	2
diethylamine	100.0	0.0	99.5	0.5	100.0	0.0	99.5	0.5	100.0	0.0	100.0	0.0%
merkaptioethanol	61.0	39.0	50.0	50.0	43.5	56.5	25.0	75.0	32.5	67.5	31.5	68.5%
methanol	100.0	0.0	100.0	0.0	100.0	0.0	97.5	2.5	91.5	8.5	100.0	0.0%
ethanol	100.0	0.0	85.0	15.0	99.0	1.0	87.5	12.5	97.0	3.0	100.0	0.0%
n-propanol	100.0	0.0	100.0	0.0	100.0	0.0	89.0	11.0	8.0	92.0	98.0	2.0%
n-butanol	89.5	10.5	100.0	0.0	99.5	0.5	4.5	95.5	6.0	94.0	5.5	94.5%
i-amyl alcohol	100.0	0.0	100.0	0.0	95.5	4.5	99.0	1.0	98.5	1.5	98.5	1.5%

*Carya illinoensis* (WANG) K. KOCH

Table 3.3.

*Juglans nigra* L. (Plate 3.5., figs. 1-42)

No taxonomically important alterations were observed at the pollen grains of this genus.

*Platycarya strobilaceae* SIEB. and ZUCC. (Plate 3.6., figs. 1-42)

This kind of pollen type is also very resistant to the applied dissolution experiments.

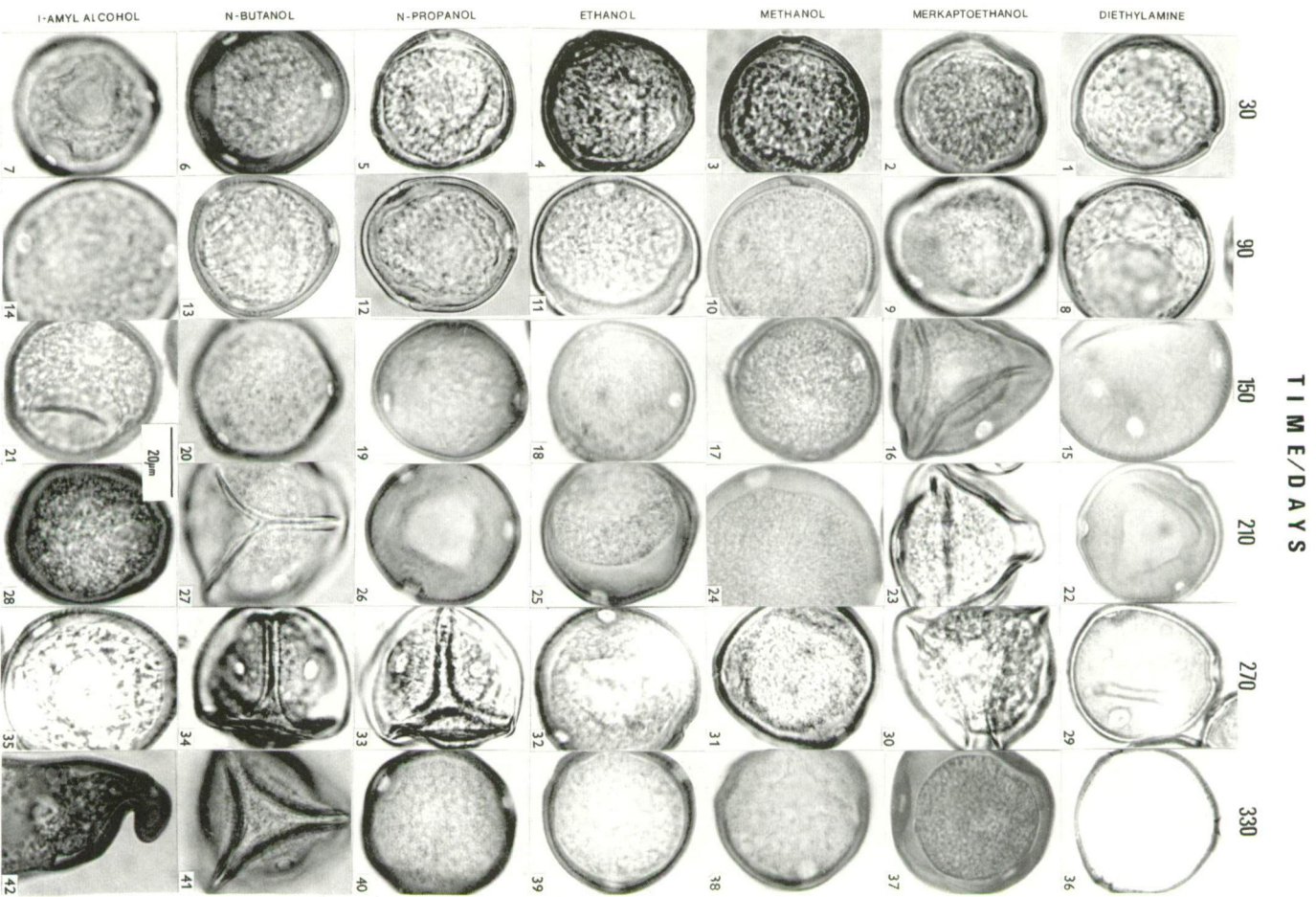


Plate 3.4., 1-42. *Carya illinoensis* (WANG) K. KOCH

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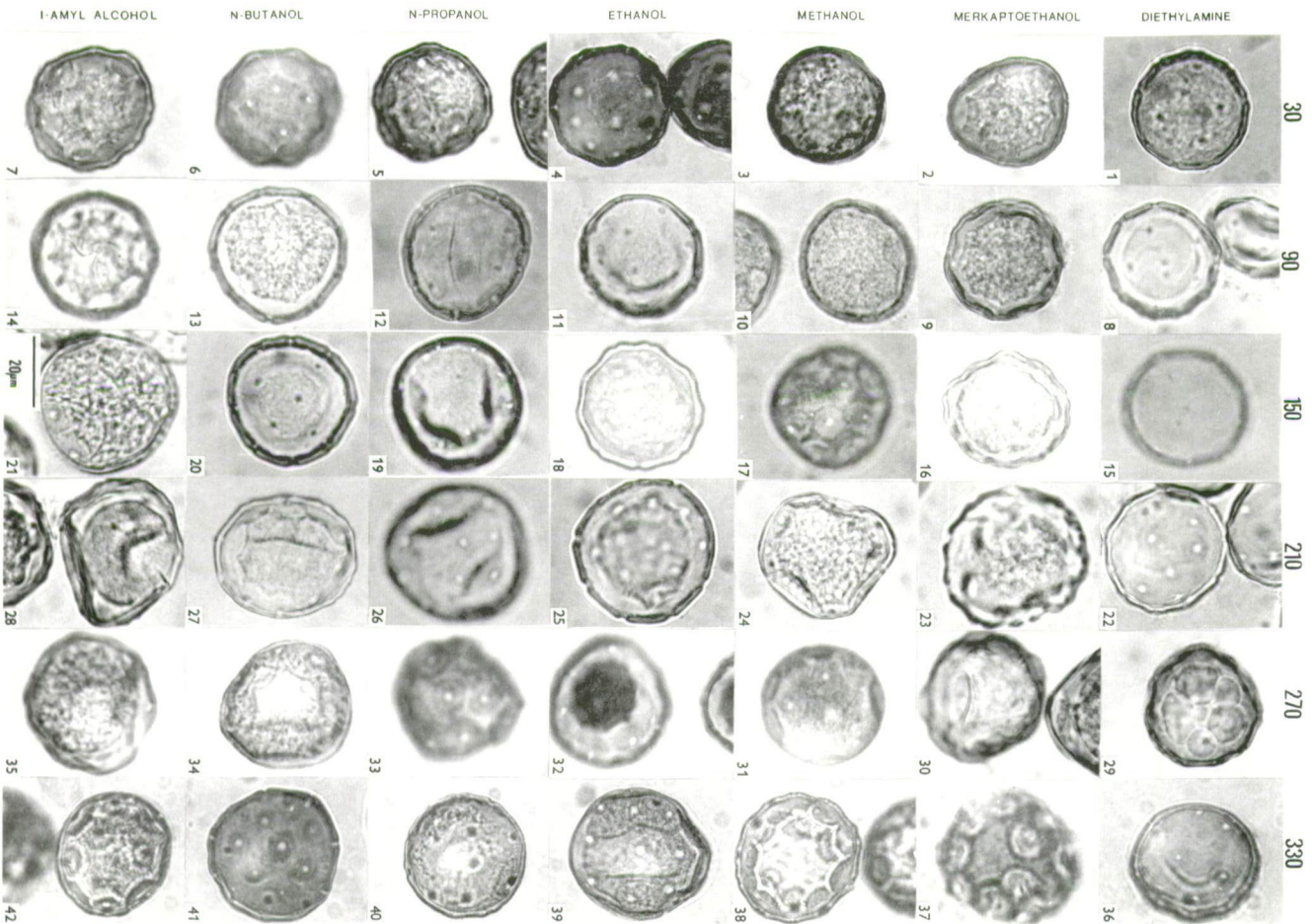


Plate 3.5., 1-42. *Juglans nigra* L.

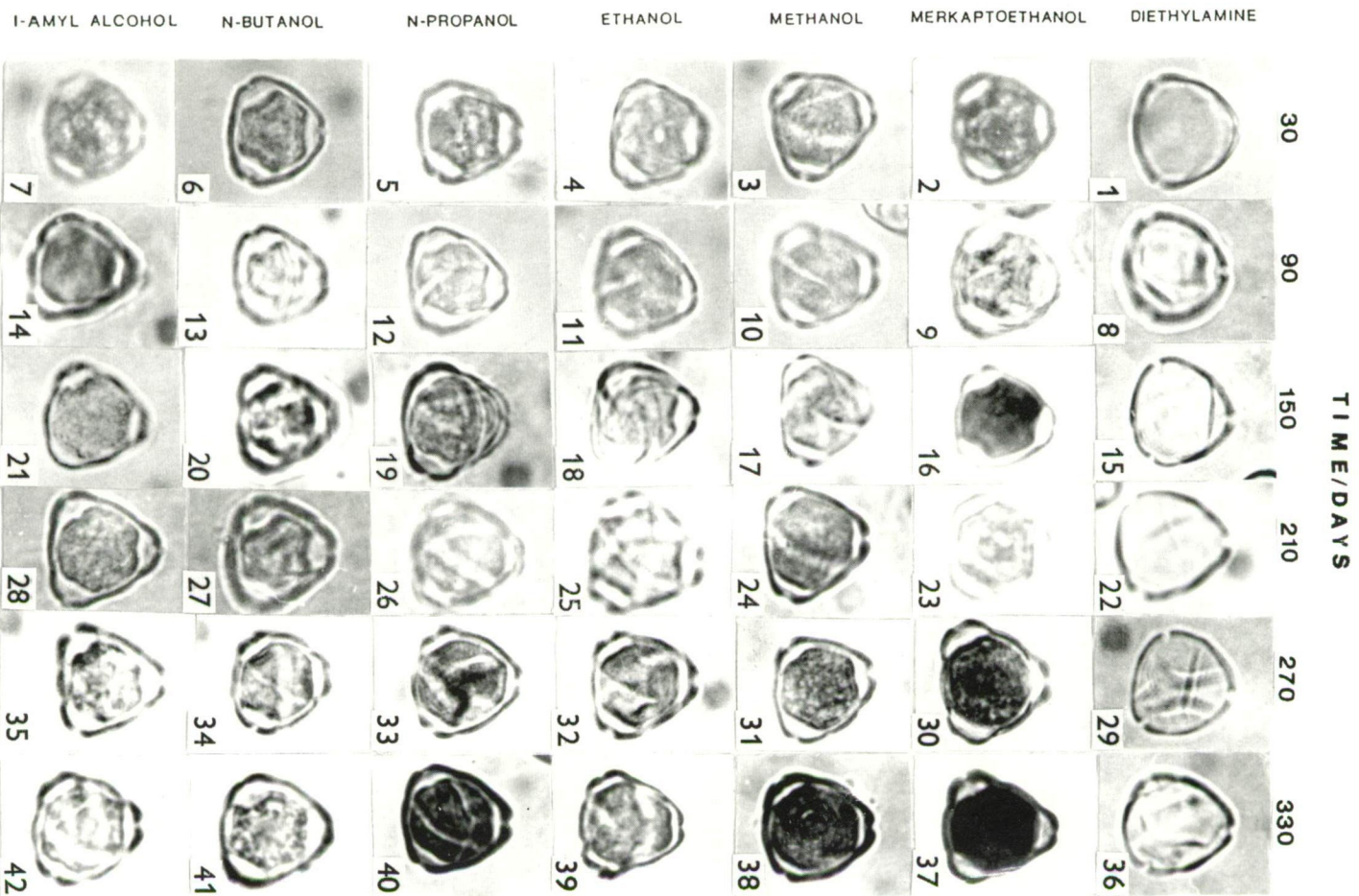


Plate 3.6, 1-42. *Platygygia strobilacea* STEB. and ZUCC., 1000X.

## Discussion and Conclusions

1. The spores of *Dryopteris filix-mas* are resistant as compared to the easily dissolvable exospore of *Equisetum arvense* (cf. KEDVES and GÁSPÁR, 1994). But based on our preliminary TEM investigations the light-microscopically intact spores may be extremely damaged. In all probability its degradation was finished during the embedding processes for the ultrathin sectioning.

2. The results concerning the pollen grains of *Juniperus virginiana* are very interesting. It was DUHOUX (1972, 1975, 1979) who described the hydrated pollen grains which lost the exine in consequence of the extremely swollen intine. This phenomenon was observed by KEDVES and UNGVÁRI (1996) after X-ray irradiation. In this way the same alteration may happen in consequence of different processes. To this we introduce the term named DUHOUX effect as follows:

*The intine of the globular more or less inaperturate or monoporate gymnosperm pollen grains in consequence of exterior influences swelled extremely, disrupt and leave the exine, the protoplasm is surrounded by the extremely thickened intine.*

3. The heterogeneity of the *Juglandaceae* pollen types is interesting in this respect. The resistance of the pollen grains of the genus *Juglans* against different influences was established a long time ago (cf. KEDVES and KINCSEK, 1989). *Carya illinoensis* produced interesting alterations of the dissolution experiments. When during the sedimentation other influences produce similar forms, at least two form-genuses may be established for the pollen grains of the fossil *Carya*. The resistance of the pollen grains of *Platycarya strobilacea* is also interesting. To this it seems to be very important to investigate the pollen grains of the genus *Engelhardtia*, because this is the earliest pollen type within the *Juglandaceae*.

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