10. DUHOUX EFFECT ON INAPERTURATE GYMNOSPERM AND ANGIOSPERM POLLEN GRAINS

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Abstract

The TEM and LM method was used during our investigation. The Duhoux effect was investigated by the TEM on the pollen grains of *Taxus baccata* L. after X-ray irradiation and hydratation. Hydratation was applied for the experimental material (*Larix decidua* MILL., *Metasequoia glyptostroboides* HU et CHENG, *Biota orientalis* ENDL., *Juniperus virginiana* L., *J. chinensis* L., *Taxus baccata* L., *T. baccata* cv. *aurea*, *T. baccata* cv. *compacta*, *Populus alba* L.) at the LM studies. Different kinds of staining and preparation methods were used at the pollen grains of *Taxus baccata*. The new results raised the necessity of further experimental studies in this field to get more LM and TEM data.

Key words: Palynology, recent, Duhoux effect, LM, TEM.

Introduction

DUHOUX (1972, 1975, 1979) established at the inaperturate pollen grains of some *gymnosperm* taxa (*Cupressaceae, Taxaceae, Taxodiaceae*) peculiar in vitro germination. The exine tears after hydratation in consequence of an extreme swelling of the intine. During our previous experimental investigations we observed this phenomenon after X-ray irradiation (KEDVES and UNGVÁRI, 1996) and partial dissolution as well (KEDVES, KÁROSSY and BORBOLA, 1997, KEDVES et al., 1998). Since we have observed the same phenomenon in consequence of different influences we introduced the following term: "Duhoux effect".

In this paper we present some TEM data on the X-ray irradiated and hydrated pollen grains of *Taxus baccata*, and the LM results of the hydrated inaperturate *gymnosperm* and *angiosperm* pollen grains.

The aim of this contribution was to get new ultrastructural data for the Duhoux effect, and further LM data for the hydratation process. Moreover an attempt was made to establish some "post hydratation" alterations in consequence of the different stains, or the TEM fixation and embedding processes.

Materials and Methods

For TEM investigations pollen grains of *Taxus baccata* were chosen. Experiment No: 1/7-1744. Duration of the irradiation: 1 hour with CuKα X-ray (35 KV, 20 mA).

BRON-OM1 apparatus in the Radiological Laboratory of the Department of Mineralogy, Petrology and Geochemistry of the J.A. University. Experiment No: 1/7-1361. Hydratation with 5 ml distilled water at 30 °C during 24 hours. The experimental pollen material was postfixed with OsO_4 (aq.dil.), dehydrated and embedded in Araldite (Durcupan, Fluka). The ultrathin sections were made on a Porter Blum ultramicrotome in the E.M. Laboratory of the Institute of Biophysics of the Biological Research Center of the Hungarian Academy of Sciences. The pictures were taken on a TESLA BS-540 TEM (resolution 6-7 Å).

For LM studies all samples were hydrated as previously. Mounted in glycerine-jelly (g-j), in Araldite (Ar), without coloration (w.c.). The stains are designated as follows: Os for the postfixed material, the name of the other stains are written without abbreviation. The experimental data are as follows: *Larix decidua* MILL., experiment No: 1/7-1450, (Toluidine Blue, g-j); *Metasequoia glyptostroboides* HU et CHENG, experiment No: 1/7-1449 (w.c. and Toluidine Blue, g-j); *Biota orientalis* ENDL., experiment No: 1/7-1446 (w.c. and Toluidine Blue, g-j); *Juniperus virginiana* L., experiment No: 1/7-1444 (w.c. and Toluidine Blue, g-j); *Juniperus chinensis* L., experiment No: 1/7-1445 (w.c. and Toluidine Blue, g-j); *Taxus baccata* L. experiment No: 1/7-1361a (w.c., g-j); 1/7-1361b (Toluidine Blue, g-j); 1/7-1361c (Os, g-j); 1/7-1361d (Os, Ar); 1/7-1428 (w.c., g-j); 1/7-1429 (Bismarck Brown, g-j); 1/7-1430 (Methylene Blue, g-j); 1/7-1431 (Toluidine Blue, g-j); 1/7-1432 (Eosin B, g-j); 1/7-1433 (Methyl Violet, g-j); 1/7-1434 (Hematoxylin, g-j); 1/7-1435 (Safranine T, g-j); 1/7-1436 (Chrysoidine, g-j); 1/7-1437 (Azure A, g-j); 1/7-1438 (Aniline Red, g-j); 1/7-1439 (Azure II, g-j); 1/7-1440 (Astrazal Blue, g-j); 1/7-1441 (Bromthymol Blue, g-j); 1/7-1442 (Amaranth, g-j); *Taxus baccata* L. cv. *aurea*, experiment No:1/7-1452 (Toluidine Blue, g-j); *Taxus baccata* L. cv. *compacta* experiment No:1/7-1453 (Toluidine Blue, g-j); *Populus alba* L. experiment No: 1/7-1443 (Toluidine Blue, g.-j).

Results

1. Transmission electron microscopical results

For the terminology of the ultrastructural elements of the inaperturate *gymnosperm* pollen grains the publication by DUHOUX (1972) was used.

Taxus baccata L.

1.1. Ultrastructure after X-ray irradiation (Plate 10.1., figs. 1,2)

The ultrastructure of the protoplasm is degraded. At the specimen illustrated in Plate 10.1. remnants of the middle layer of the intine (Ie2) were observed. At other pollen grain the Ie2 layer is enclosed within the Ie3 layer (Plate 10.1., fig. 2). Strong degradation was observed at the ectexine and endexine (Plate 10.2., fig. 1), but the originally lamellar ultrastructure of the intine is more or less perceptible.

1.2. Ultrastructure of the hydrated pollen grain (Plate 10.2., figs. 2-6, plate 10.3., figs. 1,2)

The exine ultrastructure after hydratation is similar to those of the irradiated ones (Plate 10.2., fig. 2). A general survey picture illustrated the empty pollen grain (Plate 10.2., fig. 3). The used long hydratation resulted in desintegrated protoplasm (Plate 10.2., figs. 4-6). The preservation of the ultrastructure of the intine is different, but the outest layer (Ie3) and sometimes the exine is well shown, e.g.: Plate 10.3., fig. 1. Ie2 is well illustrated in picture 1, Plate 10.3. Similarly the characteristic lamellar inner layer around the protoplasm (Plate 10.3., figs. 1,2).

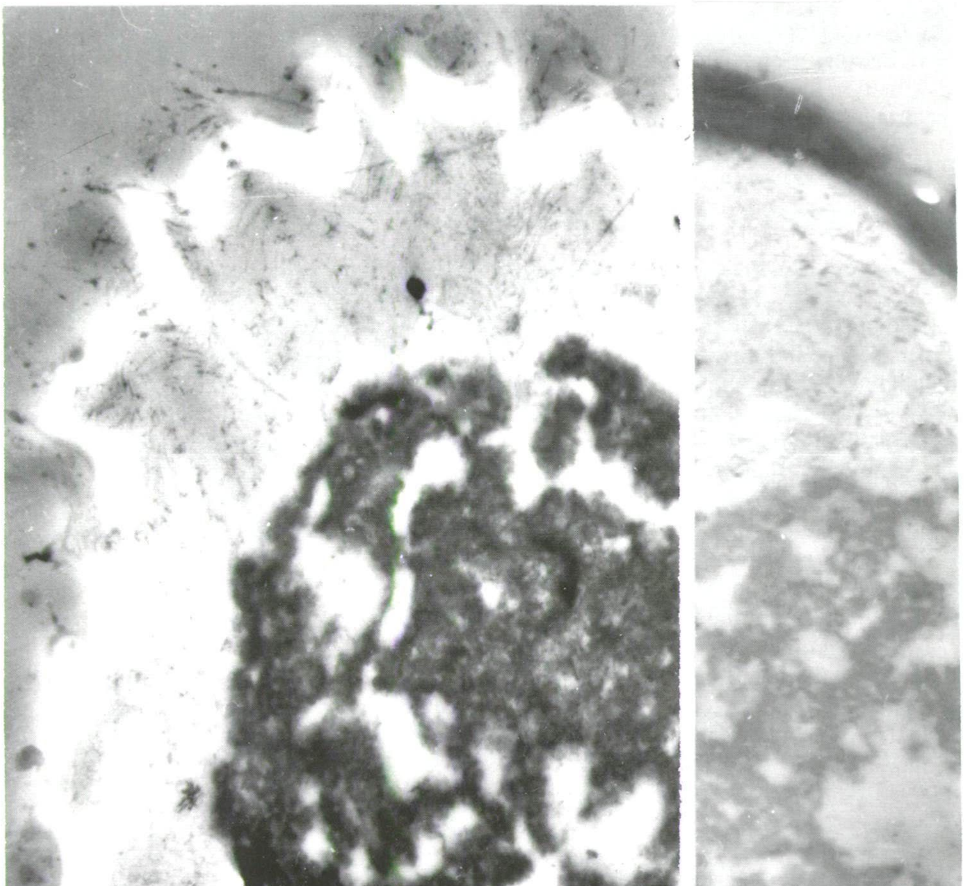

Plate 10.1.

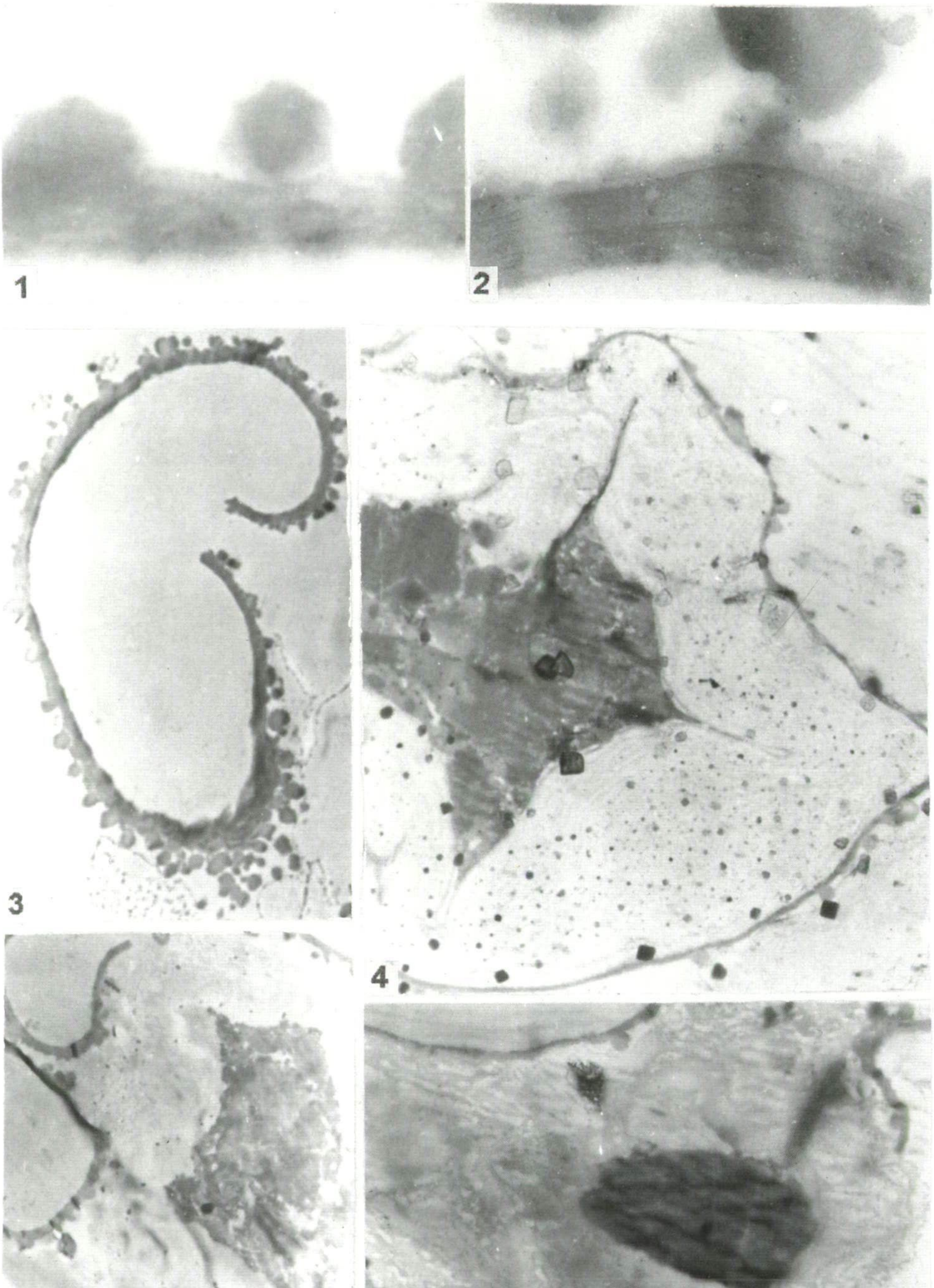

Plate 10.2.

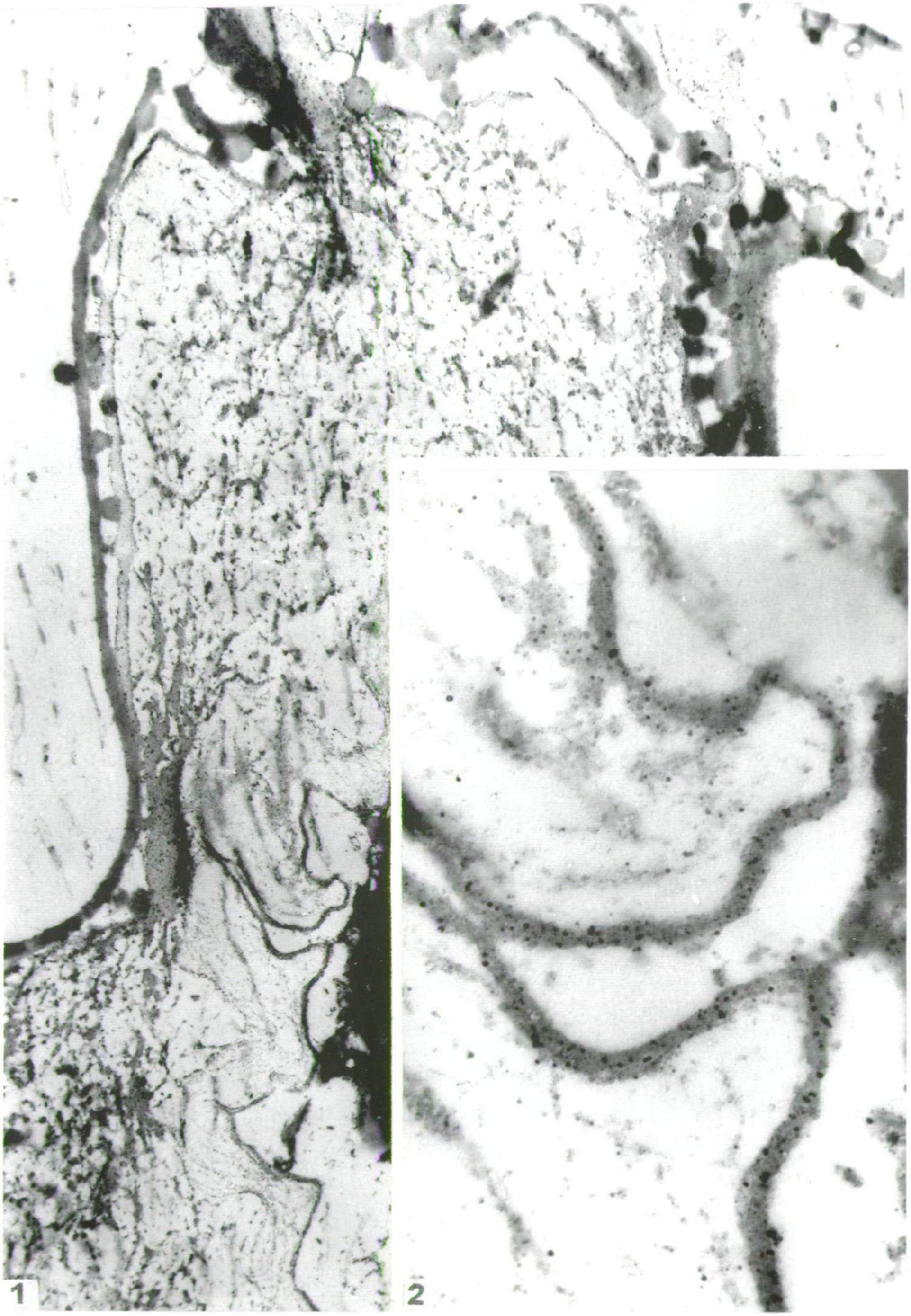

Plate 10.3.

2. Light microscopical results

2.1. *Larix decidua* MILL. (Plate 10.4., figs. 1-4)

No opened pollen grains were observed after hydratation. The thickness of the intine from 5 µm until 22.5 µm, maximum 34% at 10 µm. Average: 11.5 µm. Nucleolus and nucleus were also observed at the hydrated pollen grains (Plate 10.4., figs 1,4).

Remark. - During our previous investigations on partially dissolved pollen grains of *Larix decidua* in several cases the Duhoux effect was observed (KEDVES et al., 1998).

2.2. *Metasequoia glyptostroboides* HU et CHENG (Plate 10.4., figs. 5-11)

The characteristic ligula and the hiatus form was observed in our investigation material (Plate 10.4., figs. 5-7, resp. 8,9). The thickness of the intine was measured at "not opened" pollen grains, so-called dubius form. The thickness of the intine from 2.5 µm until 7.5 µm, maximum 53.0% at 5.0 µm. Average: 5.15 µm.

2.3. *Biota orientalis* ENDL. (Plate 10.4., figs. 12-16)

The hydratation resulted in all kind of secondary forms. In the intine of the pollen grains without exine different kinds of structures (granular, radially oriented fibrillar, network-like) were observed. The thickness of the intine of the "not opened" pollen grains from 5 µm until 10 µm, maximum 42.0% at 7.5 µm. Average: 7.1 µm. The thickness of the intine of the pollen grains without exine from 7.5 µm until 17.5 µm, maximum 26.5% at 12.5 µm. Average: 11.5 µm.

2.4. *Juniperus virginiana* L. (Plate 10.4., figs. 16-21, plate 10.5., figs. 1-3)

Similarly to the previous species all kinds of secondary forms appeared after hydratation. Mostly granular, rarely fibrillar structures are in the intine. The thickness of the intine of the "not opened" forms from 3.75 µm until 7.5 µm, maximum 57.0 % at 5.0 µm. Average: 5,1 µm. The thickness of the intine of the pollen grains without exine from 5.0 µm until 16.25 µm, maximum 24.0% at 10 µm. Average: 10.25 µm.

2.5. *Juniperus chinensis* L. (Plate 10.5., figs. 4,5)

All kinds of secondary froms were observed. Around the protoplasm in the inner half of the intine radially oriented fibrillar or granular differentiations were observed. The thickness of the intine of the "not opened" forms from 3.75 µm until 7.5 µm, maximum 48.5% at 5.0 µm. Average: 5.63 µm. The thickness of the pollen grains without exine from 5.0 µm until 15.0 µm, maxima 25.0% and 25.5 % at 7.5 µm and 10.0 µm. Average: 8.6 µm.

Plate 10.1.

1,2. *Taxus baccata* L. Ultrastructure of the X-ray irradiated pollen grain. Experiment No: 1/7-1744, 1. 10.000x, Negative No: 6240, 2. 25.000x, negative No: 6245.

Plate 10.2.

1-6. *Taxus baccata* L.

1. Ultrastructure of the X-ray irradiated ectexine. Experiment No: 1/7-1744, 50.000x, negative No: 6254.

2-6. Ultrastructure of the hydrated pollen grains. Experiment No: 1/7-361. 2. Detail of the ectexine, 50.000x, negative No: 7267, 3. General survey picture of the empty pollen grain, 5.000x, negative No: 7267, 4-6. Protoplasm, intine and inner part of the endexine, 5.000x, negative numbers: 4. 7261, 5. 7268, 6. 7232.

Plate 10.3.

1,2. *Taxus baccata* L. Ultrastructure of the hydrated pollen grain. Experiment No: 1/7-1761. 1. 10.000x, negative No: 7027, 2. 50.000x, negative No: 7230.

2.6. *Taxus baccata* L. (Plate 10.5., figs. 6-20, plate 10.6., figs. 1-12)

This species was investigated in detail. An attempt was made for revealing the qualitative and quantitative alterations in consequence of the different coloration and/or preparation. Important variations were observed in the morphology of the contracted protoplasm. The different kinds of alterations are illustrated in the above mentioned plates. The qualitative alterations are summarized as follows.

The thickness of the intine of the "not opened" pollen grains

Experiment No	µm from	until	% maximum	µm	µm average
1361/a	2.5	10.0	49.5	7.5	6.7
1361/b	2.5	10.0	47.5	5.0	6.33
1428	3.75	7.5	51.5	5.0	5.28
1429	2.5	8.75	48.5	5.0	5.63
1430	2.5	8.75	44.0	5.0	5.0
1431	3.75	7.5	53.0	5.0	5.44
1432	3.75	7.5	61.5	5.0	5.5
1433	3.75	8.75	48.0	5.0	5.84
1434	3.75	8.75	52.0	5.0	5.65
1435	3.75	8.75	52.0	5.0	5.68
1436	3.75	8.75	49.5	5.0	5.83
1437	5.0	8.75	49.5	5.0	5.84
1438	3.75	7.5	48.0	5.0	5.8
1439	3.75	8.75	55.5	5.0	5.7
1440	2.5	8.75	47.5	5.0	5.75
1441	3.75	8.75	53.0	5.0	5.75
1442	3.75	8.75	57.5	5.0	5.58

The thickness of the intine of the pollen grains without exine

Experiment No	µm from	until	% maximum	µm	µm average
1361/a	7.5	15.0	39.0	10.0	10.8
1361/b	5.0	22.5	32.5	12.5	11.95
1428	7.5	20.0	44.0	12.5	12.73
1429	10.0	18.75	39.5	12.5	13.53
1430	8.75	17.5	42.5	12.5	12.45
1431	7.5	18.75	38.0	12.5	13.28
1432	7.5	18.75	40.0	12.5	14.05
1433	7.5	18.75	39.5	12.5	13.0
1434	10.0	20.0	33.0	12.5	13.95
1435	8.75	18.75	42.0	12.5	12.96
1436	7.5	17.5	44.0	12.5	12.4
1437	10.0	20.0	33.0	12.5	14.3
1438	7.5	18.75	45.5	12.5	13.5
1439	7.5	17.5	40.5	12.5	13.13
1440	7.5	18.75	38.0	12.5	13.25
1441	7.5	18.75	42.0	12.5	12.8
1442	7.5	21.25	28.0	15.0	15.05

The differences in the thickness of the swollen intine are sometimes remarkable.

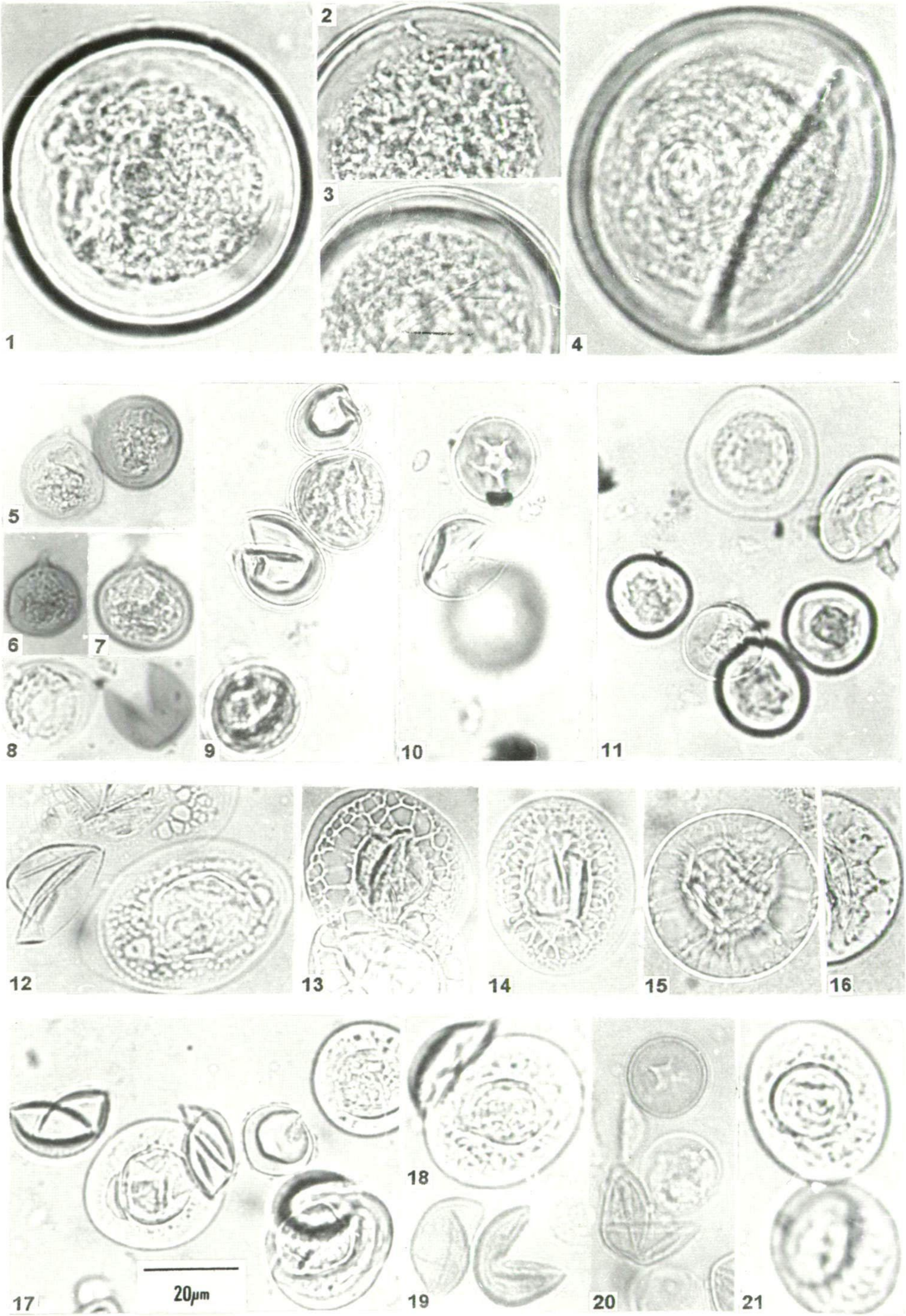

Plate 10.4.

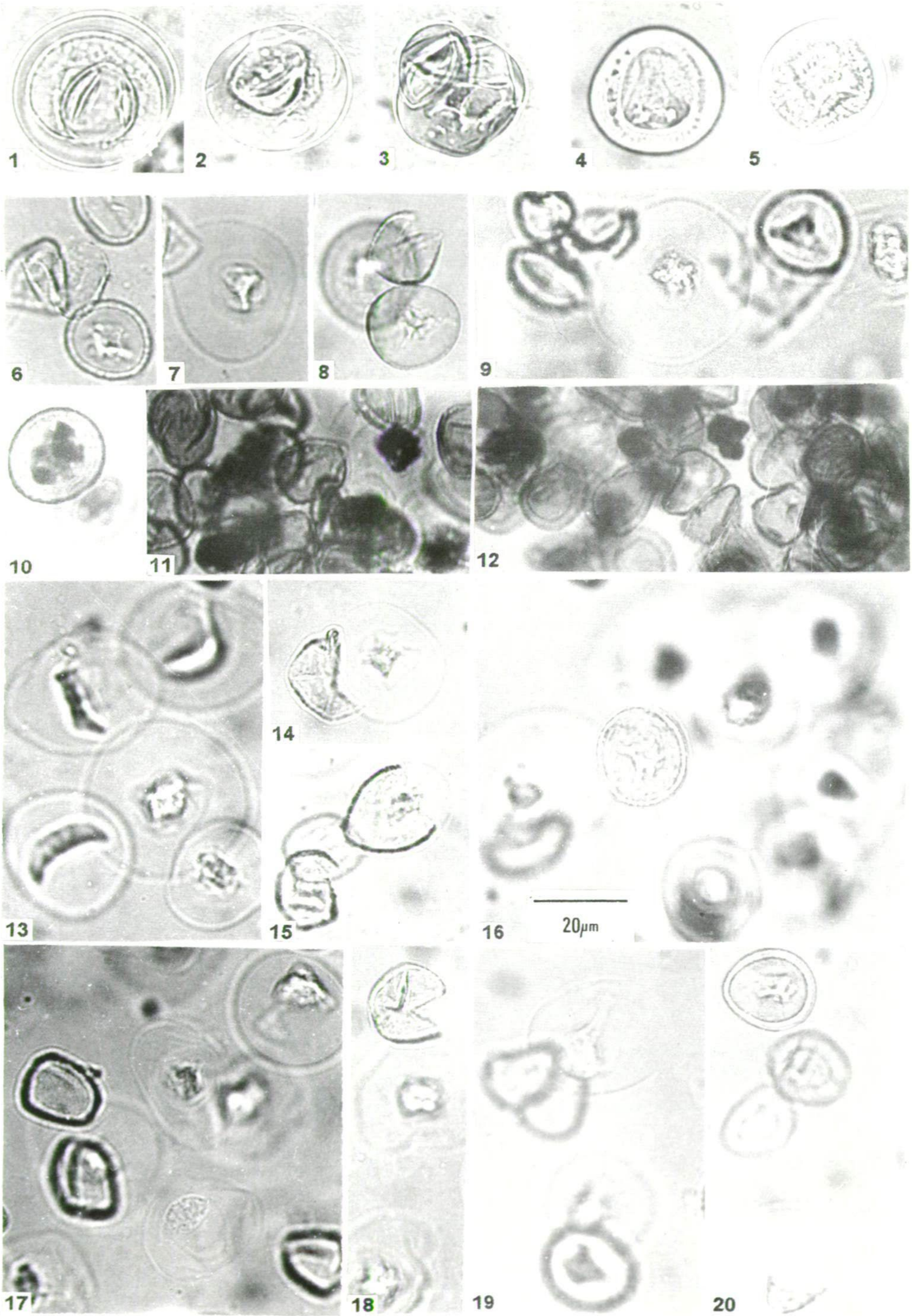

Plate 10.5.

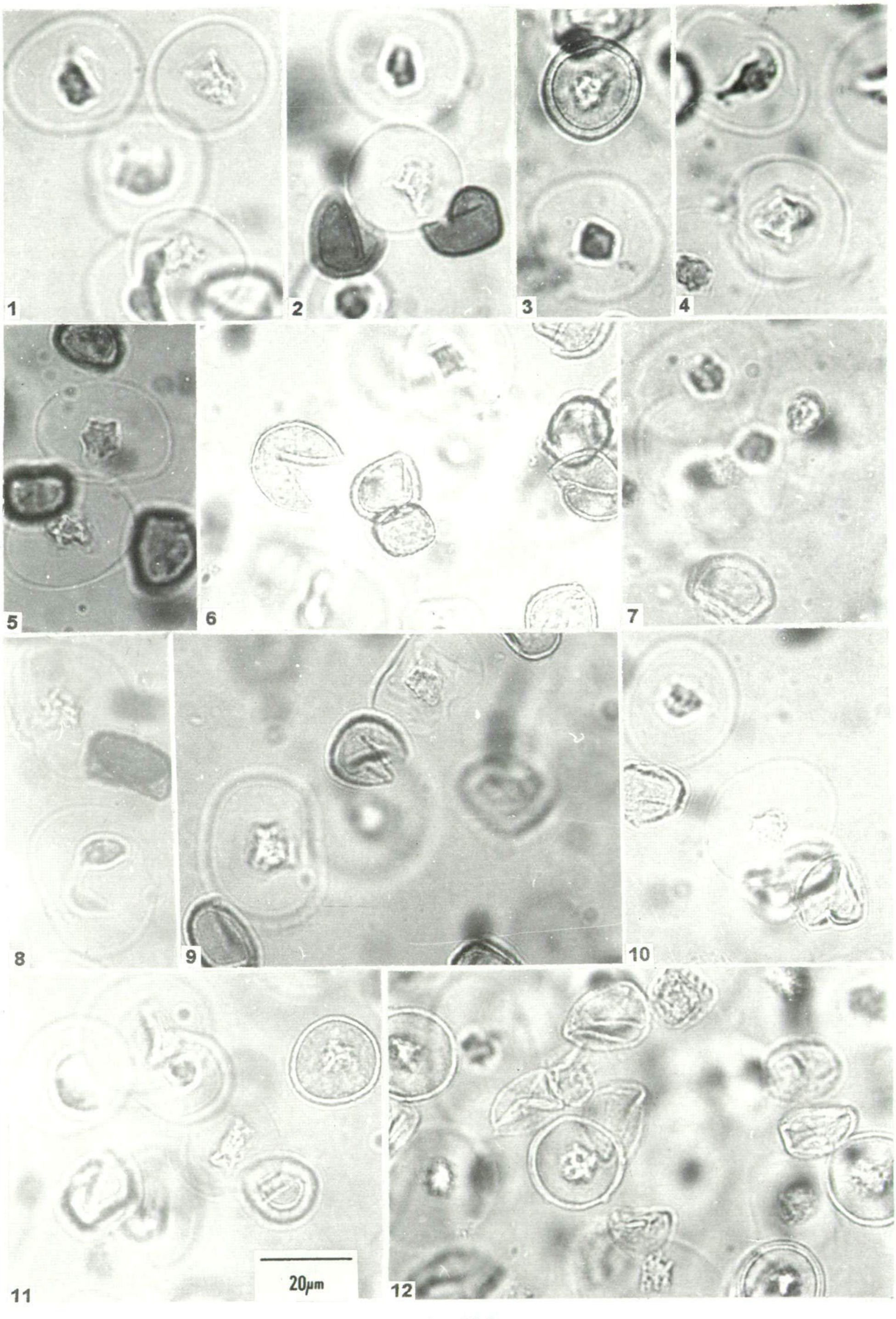

Plate 10.6.

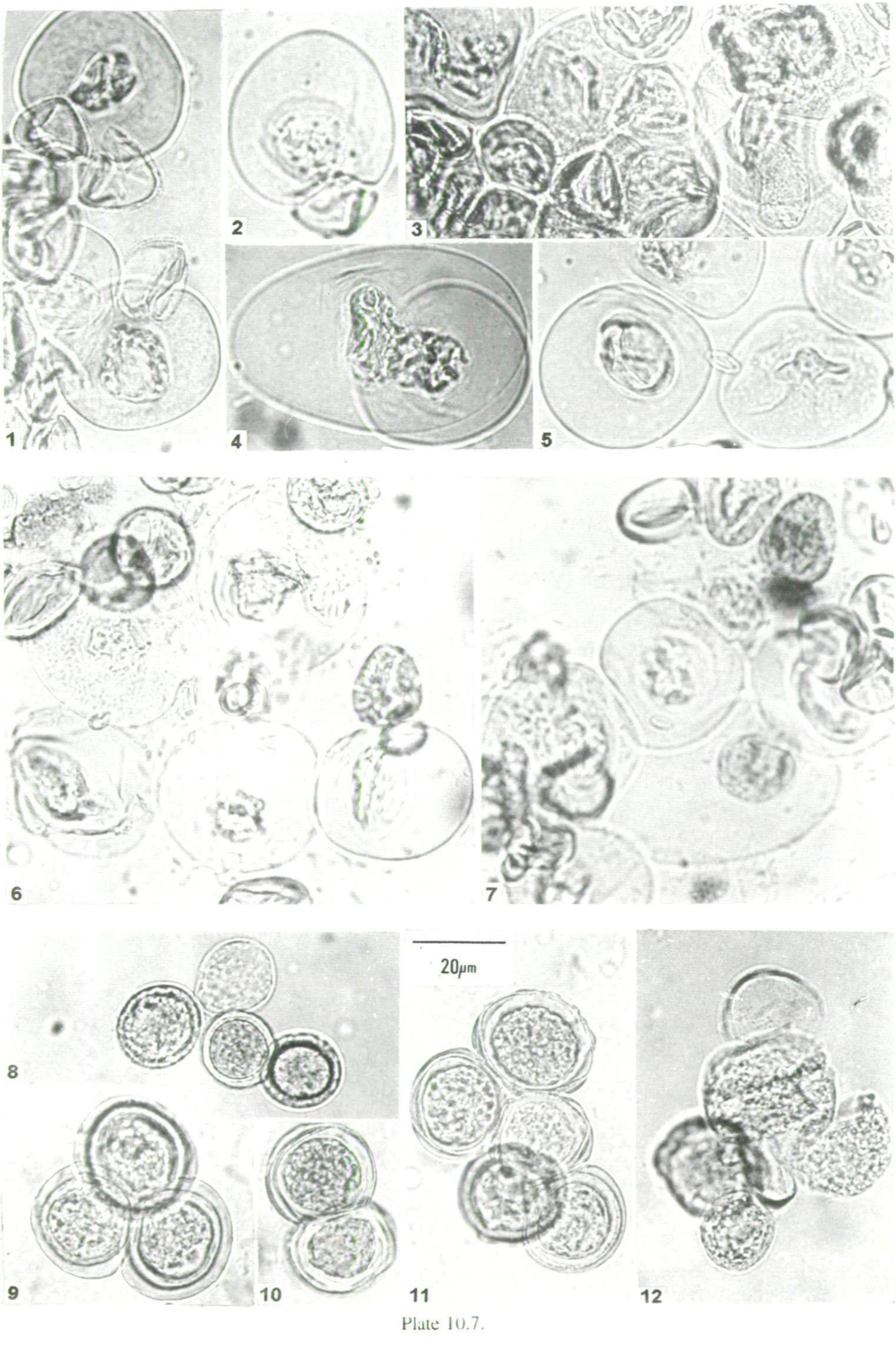

Plate 10.7.

2.7. *Taxus baccata* L. cv. *aurea* (Plate 10.7., figs. 1-5)

All the morphological variations were observed in consequence of the hydratation. The thickness of the intine of the "not opened" forms from 3.75 μm until 10.0 μm, maximum 40% at 5.0 μm. Average: 6.05 μm. The thickness of the pollen grains without exine from 10.0 μm until 26.25 μm, maximum 24% at 15.0 μm. Average: 15.6 μm.

2.8. *Taxus baccata* L. cv. *compacta* (Plate 10.7., figs. 6,7)

The thickness of the intine of the "not opened" forms from 2.5 μm until 10.0 μm, maximum 48.0% at 5.0 μm. Average: 5.65 μm. The thickness of the pollen grains without exine from 10.0 μm until 27.5 μm, maxima 22.5% and 24.0% at 12.5 μm and 15.0 μm. Average:16.5 μm.

2.9. *Populus alba* L. (Plate 10.7., figs. 8-12)

The thickness of the intine of the "not opened" forms from 1.25 μm until 5.0 μm, maximum 56.5% at 2.5 μm. Average: 3.0 μm. The number of the pollen grains without exine was not enough for quantitative evaluations.

Discussion and Conclusions

1. Based on our new results concerning the alterations of the intine ultrastructure we need further experiments, with shorter hydratation at constant temperature and water, after an immediate fixation of the embedding processes. In particular at the hydrated pollen grains of *Taxus baccata* we observed that the desintegration is continuous. A complicated enzymatic and/or microbial effect may also be presumed.

Plate 10.4.

1-4. *Larix decidua* MILL., Experiment No: 1/7-1450.
5-11. *Metasequoia glyprostroboides* HU et CHENG, Experiment No: 1/7-1449.
12-16. *Biota orientalis* ENDL., Experiment No: 1/7-1446.
17-21. *Juniperus virginiana* L., Experiment No: 1/7-1444.

Plate 10.5.

1-3. *Juniperus virginiana* L., Experiment No: 1/7-1444.
4,5. *Juniperus chinensis* L., Experiment No: 1/7-1445.
6-20. *Taxus baccata* L., 6,7. Experiment No: 1/7-1361a; 8,9. Experiment No: 1/7-1361b; 10,11. Experiment No: 1/7-1361c; 12. Experiment No: 1/7-1361d; 13-15. Experiment No: 1/7-1428; 16. Experiment No: 1/7-1429; 17,18. Experiment No: 1/7-1430; 19,20. Experiment No: 1/7-1431.

Plate 10.6.

1-12. *Taxus baccata* L., 1. Experiment No: 1/7-1432; 2. Experiment No: 1/7-1433; 3,4. Experiment No: 1/7-1434; 5. Experiment No: 1/7-1435; 6. Experiment No: 1/7-1436; 7. Experiment No: 1/7-1437; 8. Experiment No: 1/7-1438; 9. Experiment No: 1/7-1439; 10. Experiment No: 1/7-1440; 11. Experiment No: 1/7-1441; 12. Experiment No: 1/7-1442.

Plate 10.7.

1-5. *Taxus baccata* L. cv. *aurea*, Experiment No: 1/7-1452.
6,7. *Taxus baccata* L. cv. *compacta*, Experiment No: 1/7-1453.
8-12. *Populus alba* L., Experiment No: 1/7-1443.

2. To this it is interesting, that the relatively resistant exine altered in the same way after the X-ray and the hydratation effect. But it is also worth of mentioning that during the previous experiments with the *Helix*-enzyme method, the control material was infected by microorganisms, and this microbially degraded exine ultrastructure is similar to those in our present results (KEDVES, 1987, p. 50, Plate I, fig. 1).

3. Our LM results revealed several new data, e.g.: the different form of the contracted protoplasm in consequence of the different colouring at the pollen grains of *Taxus baccata.*

4. In the swolled intine different kinds of structures were observed, which may be pro parte originate from the protoplasm.

5. We hope that our qualitative and quantitative data will be a useful basis for comparison of the further experimental studies.

Acknowledgements

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