5. LM AND SEM INVESTIGATIONS ON PARTIALLY DISSOLVED ALLERGEN POLLEN GRAINS II.

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Abstract

Pollen grains of *Taxus baccata* L. were investigated with LM and SEM methods after dissolution with 2-aminoethanol for 30 minutes, 1 hour, 5, 10 and 24 hours. Alterations in the LM morphological characteristic features and the degradation processes of the submicroscopic superficial ornamentation and the ectexine layers are presented. Special attention was made to the alterations, which happened as a consequence of the swelling of the intine.

Key words: Experimental, Palynology, recent, *Taxus baccata*, LM, SEM

Introduction

Pollen grains of *Taxus baccata* have been recognized as allergenic pollen grains, e.g.; PEHLIVAN (1995), LEJOLY-GABRIEL (1986), JÁRAI-KOMLÓDI and MEDZIHRADSZKY (1993). There are several publications concerning this pollen grains in different ways. Alterations as a consequence of hydration were recognized by several authors. SOUTHWORTH (1986) pointed out that in certain Coniferophytina (Pinaceae, Cupressaceae, Taxaceae and Taxodiaceae) the exine separates from the intine and protoplasm. In this regard, she cited several papers: WODEHOUSE, (1935), MÜLLER-STOLL, (1948), VASIL and SAHNI, (1964), DUHOUX (1972, 1982), VILLAR, KNOX and DUMAS, (1984). In this paper she emphasized that purified exines can be isolated for chemical analysis by hydration. During our experimental investigations on different kinds of inaperturate pollen grains, we observed the same phenomenon as a consequence of the effect of X-rays (KEDVES and UNGVÁRI, 1996) and after partial dissolution by different organic solvents (KEDVES, KÁROSSY and BORBOLA, 1997). In this paper the term of the Duhoux effect was introduced. Later this effect was investigated on inaperturate gymnosperm and angiosperm pollen grains by KEDVES et al. (2000). In this paper the ultrastructure of X-ray irradiated and hydrated pollen grains of *Taxus baccata* were also published. In this regard, the papers of DUHOUX (1972, 1975, 1979) were used. The high temperature effect on some inaperturate gymnosperm pollen grains (*Juniperus virginiana* L., *Taxus baccata* L.) was also used. The most important alterations of the pollen grains of *Taxus baccata* are the plicate "Normapolles-like" types. At the terminal parts of the "plicae" pores or pseudopores may also occur. Two secondary types may be distinguished, the so-called "*Plicapollis* form" and the "*Interpollis* type".

Another problem, that is the distinction of the Cupressaceae and Taxaceae pollen grains, was pointed out by BELMONTE et al. (1999) as follows; p. 39: "The pollen grains from Cupressaceae cannot be differentiated under light microscopes, even at the genus level, nor can the pollen grains of Taxaceae (*Taxus baccata*, native to the Iberian peninsula) or Taxodiaceae (*Cryptomeria japonica*, frequently planted as an ornamental). In the aerobiological studies, all these taxa appear under the name Cupressaceae."

The results of UENO (1974) concerning the inaperturate gymnosperm pollen grains are important. Ectexine lost pollen grains of *Cryptomeria japonica* germinated under in vitro conditions in consequence of different carbohydrates.

There are several EM data. TAKEOKA (1966) investigated the surface of the pollen grains of *Taxus cuspidata* by using the methylmethacrilate carbon two-staged replica method. A SEM picture from *Taxus cuspidata* SIEB. et ZUCC. var *nana* REHD. was published by MIYOSHI (1980). Further SEM data were published by XI YI-ZHEN (1986) from *Taxus chinensis* (PILGER) REHD., *T. cuspidata* SIEB. et ZUCC. and *T. yunnanensis* CHENG et L.K. FU. TEM data from *Taxus yunnanensis* were published in the paper of XI YI-ZHEN (1986).

Experimental TEM data from the pollen grains of *Taxus baccata* were published by KEDVES (1987a,b). Partially degraded and fragmented exines, using a magnetic stirrer, of *Juniperus virginiana* L. and *Taxus baccata* L. were investigated with the transmission electron microscope. Several kinds of levels of the biopolymer structure of the sporopollenin were observed, including the basic regular pentagon and also the so-called Penrose units.

Materials and Methods

The material for investigation was collected by Miss M. MADARÁSZ on the 07.03.2000. Locality: Szeged, Honvéd Square cultivated. Non-experimental fresh (T-12-21) and partially degraded pollen grains were investigated, 1 ml 2-aminoethanol was added to 5 mg pollen grains. Temperature: 30 °C, length of time: 30 minutes (T-12-22), 1 hour (T-12-23), 5 hours (T-12-24), 10 hours (T-12-25) and 24 hours (T-12-26). Non coloured pollen grains (a) and stained with Methylviolet (b) were mounted in glycerine-jelly, hydrated to 39.6%. The morphological alterations, the diameter of the pollen grains and the ratio of the diameter/intine thickness were investigated with the LM method. For scanning electron microscopical investigations the dry pollen grains were covered with gold-palladium. The pictures were taken in the SEM Laboratory of the Department of Botany of the University of Szeged on a Hitachi S-2400 instrument, resolution about 40 Å. All pictures are unretouched.

Results

LM results (Plate 5.1., figs. 1,2,5,6,9,10, plate 5.2., figs. 1,2,5,6,10,11)

The climate was extremely unusual during the autumn of 1999, and the winter of 2000. Relatively mild periods started the growth of staminate flowers and cooler days stopped this development. This kind of change in the temperature repeated several times during the final development of the flowers to the stage of mature pollen grains. Without doubt, this was the reason that in the slides several aberrant forms of pollen grains were observed. This question may be the subject of another investigation, in this place the relative abundance of the "normally" aberrant dyads may be emphasized. One pollen grain was relative big, the other one was small and probably not fertile.

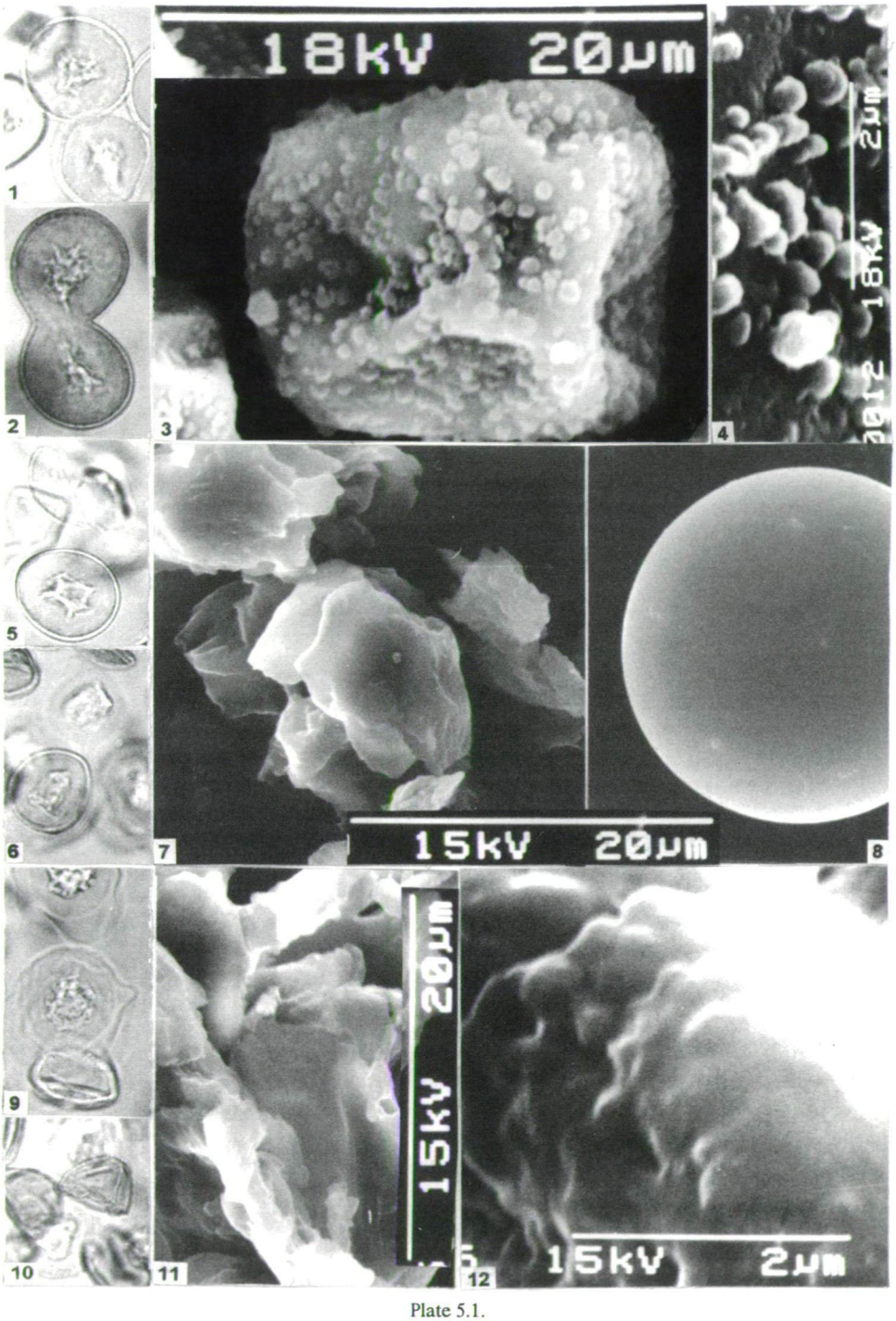
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Plate 5.1.

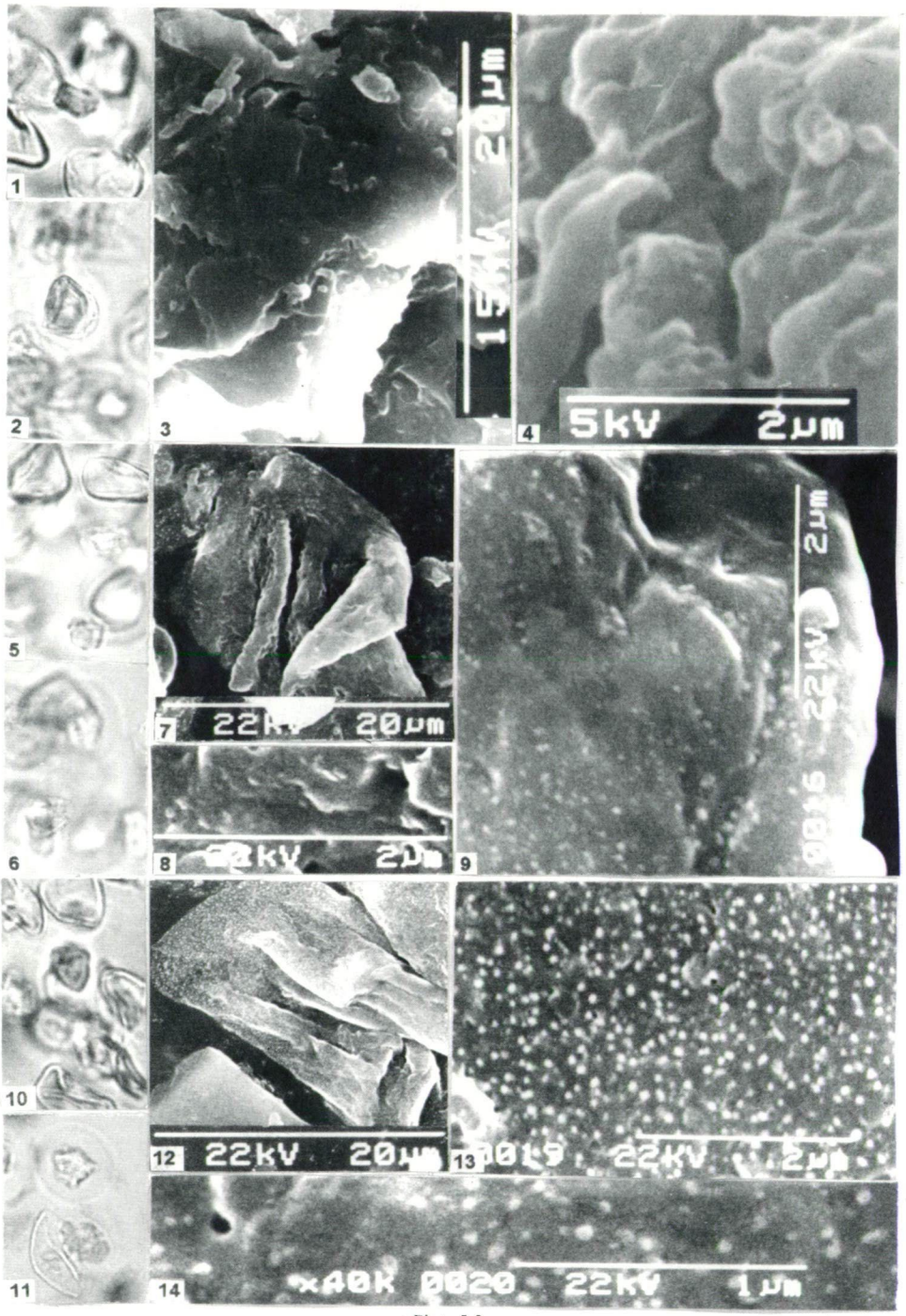
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Plate 5.2.

Plate 5.1.

Figs. 1- 12. *Taxus baccata* L.
Figs. 1-4. Fresh (non-experimental) pollen grains.
Figs. 1,2. LM pictures, fig. 1. unstained (T-12-21, a), fig. 2. stained pollen grain with Methylviolet (T-12-21, b), 750.x.
Figs. 3,4. SEM pictures, fig. 3, illustrate a general survey of the surface of the pollen grains with orbiculi, fig. 4.a highly magnified picture of the orbiculi and the finely granular surface of the ectexine.
Figs. 5-8. Partially degraded pollen grains with 2-aminoethanol during 30 minutes (T- 12-22).
Figs. 5,6. LM pictures, fig. 5. unstained (a). fig. 6. stained pollen grains with Methylviolet.(b), 750x.
Figs. 7,8. SEM pictures, fig. 7. illustrate the disappearance of the orbiculi, and the fragility of the exine. The greatest part of the pollen grains was broken under the SEM method. Fig. 8 was taken from the inner body (ectexine lost) pollen grain.
Figs. 9-12. Partially degraded pollen grains during 1 hour (T-12-23).
Figs. 9,10. LM pictures, fig. 9. unstained, fig. 10. stained pollen grains with Methylviolet, 750x.
Figs. 11,12. SEM pictures, fig. 11. illustrate the damaged and broken ectexine. Fig. 12, in this highly magnified picture the degradation of the outest part of the ectexine is represented.

Plate 5.2.

Figs. 1-14. *Taxus baccata* L.
Figs. 1-4. Partially degraded pollen grains with 2-aminoethanol during 5 hours.(T-12-24).
Figs. 1,2. LM pictures, fig. 1 unstained (a), fig. 2. stained pollen grains with Methylviolet (b), 750x.
Figs. 3,4. SEM pictures, fig. 3 illustrate the degraded and brokened ectexine, in the highly magnified picture (fig. 4) the details of the degraded outer surface of the ectexine are well shown.
Figs. 5-9. Partially degraded pollen grains with 2-aminoethanol during 10 hours (T-12-25).
Figs. 5,6. LM pictures, fig. 5 unstained (a), fig. 6. stained pollen grains with Methylviolet (b), 750x.
Figs. 7-9. SEM pictures, fig. 7 illustrate a "hiatus form" In the highly magnified picture (fig. 9) small granular ornamental elements appeared.
Figs. 10-14. Partially degraded pollen grains with 2-aminoethanol during 24 hours (T-12-26).
Figs. 10,11. LM pictures, fig. 10 unstained (a), fig. 11. stained pollen grains with Methylviolet (b), 750x.
Figs.12-14. SEM pictures, fig. 12 illustrate a "hiatus form", with tiny granules on the surface. In highly magnified pictures (figs. 13, 14) the small granules are better illustrated.

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1. Morphological alterations of the pollen grains during the experiments

Explanation: a - fresh, b - coloured pollen grains,1. - pollen grains with ectexine, 2. - opened ectexine with intine and protoplasm, 3. - ectexine lost pollen grains, 4. - the empty, ectexine, hiatus form, 5. - different kinds of aberrant forms.

1		2		3		4		5		Experiment No
a	b	a	b	a	b	a	b	a	b	
90.0	37.0	0.0	0.0	0.0	35.0	0.0	21.0	10.0	7.0	T-12-21
6.5	6.0	0.0	1.0	43.0	60.0	50.5	33.0	0.0	0.0	T-12-22
4.0	3.5	0.0	0.0	47.5	50.0	48.5	46.0	0.0	0.5	T-12-23
3.5	1.5	0.0	0.5	54.0	51.0	42.5	47.0	0.0	0.0	T-12-24
3.0	1.5	0.0	0.0	47.5	56.5	49.5	42.0	0.0	0.0	T-12-25
3.5	1.5	0.0	0.0	39.0	63.0	57.5	35.0	0.0	0.5	T-12-26

It is well shown that the stain changed the morphology of the pollen grains. In contrast to the 90% of the unstained pollen grains with ectexine, this form is represented with only 37.0% at the stained ones. The effect of the 2-aminoethanol resulted in large numbers of the ectexine lost pollen grains (inner bodies) and the so-called hiatus forms. The quantity of the two forms must be theoretically equal. Important differences were observed in experiment No.: T-12-26 with the coloured pollen grains. It is worth mentioning that the aberrant forms seem to be less resistant against staining and dissolution with 2-aminoethanol. 10% was observed in the slides of the fresh, unstained pollen grains, 7.0% in the stained and the maximum in the dissoluted material was 0.5%.

2. Diameter of the pollen grains

Fresh (non-coloured) pollen grains (a)

17.5	20.0	22.5	25.0	27.5	30.0	32.5	35.0	37.5	µm	Experiment No
		2.0	15.5	33.0	31.0	14.5	3.0	1.0	%	T-12-21
	12.0	26.5	32.0	22.0	6.5	1.0				T-12-22
2.5	17.0	32.0	32.0	13.0	3.5					T-12-23
0.5	26.5	30.5	30.0	9.5	2.5	0.5				T-12-24
1.5	18.5	35.5	27.5	13.0	2.0	1.5				T-12-25
0.5	12.5	31.5	27.5	16.0	11.0	1.0				T-12-26

The diameter of the pollen grains diminishes during the partial degradation.

Stained pollen grains (b)

17.5	20.0	22.5	25.0	27.5	30.0	32.5	35.0 µm		
	3.0	20.0	42.5	27.5	4.5	2.5		%	T-12-21
1.0	14.5	27.5	30.5	17.0	9.0	0.5			T-12-22
	8.5	22.0	34.5	24.5	8.5	1.5	0.5		T-12-23
2.0	21.5	31.5	24.0	15.0	4.5	1.5			T-12-24
2.5	17.5	23.5	32.0	15.5	7.0	1.0			T-12-25
	21.0	33.0	25.0	12.0	7.0	2.0			T-12-26

In the stained pollen grains, nearly the same trend was observed as with the unstained ones.

3. Ratios of diameter/intine thickness

With the non-stained and stained pollen grains, this ratio increased more or less regularly. It is worth mentioning that with the non-experimental pollen grains there are differences in the percentages of the stained and unstained pollen grains.

SEM results (Plate 5.1., figs. 3,4,7,8,11,12, plate 5.2., figs. 3,4,7,9,12-14)

The characteristic orbicules were observed exclusively on the non-experimental material (Plate 5.1., figs. 3,4). After 30 minutes of dissolution important alterations happened in the basic morphology (Plate 5.1., fig. 7). An ectexine lost so-called "inner body" of the pollen grain was also observed. The surface seems to be finely punctate/granulate. Results after experiments during 1 and 5 hours (Plate 5.1., figs. 11,12) and (Plate 5.2., figs. 3,4) respectively are essentially identical. Secondarily rugulate-verrucate sculpture appeared on the surface of the strongly damaged ectexine. (Plate 5.1., fig.12, plate 5.2., fig. 4). Relatively numerous "hiatus forms" were observed after dissolution during 10 and 24 hours (Plate 5.2., figs. 7, 12). In the highly magnified pictures small globular units appeared, which may be larger biopolymer units or the elements of the middle part of the ectexine.

%	2	2.16	2.2	2.25	2.4	2.5	2.6	2.75	2.8	3.0	3.25	3.3	3.5	3.6	4.0	4.3	4.5	5.0	5.5	6.0	7.0	8.0
T–12–21 a		0.5	1.0		2.5	4.5	3.0	19.0	2.5	21.5	10.0	11.5	0.5	13.0	7.5	1.5	1.0	0.5				
T–12–22 a			0.5			4.5	1.5	3.0		13.0	0.5	20.0		16.0	14.0	0.5	15.0	7.5	3.5	0.5		
T–12–23 a						2.0	3.0	2.5		16.0		22.5	2.5	9.0	15.5		18.0	8.0	1.0			
T–12–24 a	0.5			3.5	1.0	5.5	5.5	7.0		10.0	0.5	16.5	0.5	2.0	20.5		18.0	7.5	1.0			0.5
T–12–25 a			0.5			0.5	1.0	3.5		8.5	1.0	11.5	0.5	7.5	17.5	0.5	28.5	15.5	2.0		1.0	0.5
T–12–26 a			0.5			0.5	0.5	4.5		12.5	0.5	11.5	0.5	8.0	16.0		24.5	15.5	3.0	0.5		1.5
T–12–21 b	1.5		1.0	4.5		21.0	2.5	18.5		16.0	1.0	18.0		8.0	4.0		1.5	2.5				
T–12–22 b			0.5		0.5	0.5	2.0	5.0		17.0	0.5	18.0	1.0	5.0	18.0		15.0	14.0	2.0	1.0		
T–12–23 b						0.5		2.5		17.5	1.0	22.0	0.5	19.0	13.5		17.5	12.5	3.0	0.5		
T–12–24 b					1.0		1.0	5.0		10.5	1.0	10.0	2.0	7.0	22.5		22.5	14.5	3.0			
T–12–25 b	0.5				2.0	3.5	3.0	6.0		10.0		12.5	1.0	8.5	15.0		16.5	15.5	1.0	0.5	2.0	2.5
T–12–26 b								1.0		10.0	2.0	12.0		10.0	22.0		25.0	13.0	2.0	2.0		1.0

Discussion and Conclusions

1. Based on our present results we observed the effect of climatic factors on the ontogenesis of the pollen grains of *Taxus baccata*. In consequence of the unusual changes in temperature during the autumn of 1999 and the winter of 2000, several kinds of aberrant forms were observed in the fresh pollen material. It is worth mentioning that after dissolution with 2-aminoethanol, the aberrant forms disappeared, so we can presume, that the molecular structure of the sporopollenin of these forms is less resistant. This may be a disturbed ontogenetical stage, which disturbed the biosynthesis or the precursors or the polymerization processes.

2. The stain used and partial degradation altered the LM morphological characters of the pollen grains of *Taxus baccata*.

3.From the SEM data, we can point in the first place to the disappearance of the orbiculi after 30 minutes of degradation. In the degradation process of the ectexine, two major stages may be distinguished.

3.1. Pollen grains treated during 1-5 hours may be characterized by the uneven surface of the ectexine without orbiculi.

3.2. After 10 and 24 hours of treatment, the tiny granules on the more or less smooth surfaces of the pollen grains are characteristic. These tiny granules may be larger globular molecular units or elements of the inner part of the ectexine.

Finally we can conclude that the ecological conditions may be taken in consideration, to this we can cite one of our papers concerning the solution of the sporopollenin of the ectexine of the pollen grains of *Quercus*.(KEDVES and GÁSPÁR, 1996).

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