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Experimental Investigations on the Pollen Grains of *Quercus robur* L.

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ABSTRACT: Dry, hydrated, and partially degraded pollen grains of *Quercus robur* L. were investigated with the LM method. The TEM method was used for pollen grains partially dissolved with diluted glycerine for 30 days. Based on the new experimental data, the sporopollenin of the ectexine of this pollen grains is less resistant and may be degraded very quickly. The inner body, which consists of the intine and protoplasm is more resistant. The symmetry and other morphological alterations of the pollen grains were investigated statistically.

KEY WORDS: Experimental palynology, Recent, *Quercus robur*. LM, TEM.

INTRODUCTION

The pollen grains of the genus *Quercus* are very important from an evolutionary point of view of the angiosperms, cf. Doyle (1978), Hickey and Doyle (1977), etc. The tricolpate or tricoporoidate pollen type is the second step in the evolutionary lineages (monocolpate - tricolpate - tricolporoidate - tricolporate - early Brevaxones). In the fossil assemblages there are a number of publications concerning pollen grains of quercoide type (cf. Potonié, 1931, Wolff, 1934, Thiergart 1938, Potonié *et al.*, 1950, Thomson and Pflug 1953, Traverse, 1955, Potonié, 1951, 1960, etc.). In Africa and in Asia we have also much fossil data in this respect. For example: Pleistocene of Hoggar Mountains of Africa (Van Campo, 1967), Middle Quaternary of Ougartian, Ougarta Mountains, northwestern Sahara (Beucher, 1967), and late Quaternary of western Iran (Van Zeist, 1967).

There are several publications on recent pollen grains also using LM, TEM and SEM method, e.g.: Monson (1954, 1961), Van Campo and Elhaï (1956), Stanley and Kremp (1959), Planchais (1962), Praglowski (1962), Kupriyanova (1965), Dupont and Dupont (1972), McAndrews *et al.* (1973), Ueno (1975), Nilsson *et al.* (1977), Lieux (1980), Miyoshi (1982), Tarnavschi *et al.* (1987), etc. Sporopollenin structures were investigated by Frederiksen (1978), Rowley and Claugher (1991), and Rowley and Skvarla (1994).

Previously we carried out several experimental investigations on the pollen grains of the genus *Quercus*. The remarkable differences between the fresh and the heated pollen grains of *Quercus robur* L. were established in the polar axis and in the P/E ratio (Kedves *et al.*, 1993). The solubility of the sporopollenin was investigated (Kedves and Gáspár 1994a, b). Later, the

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pollen grains of *Q. robur* L., *Q. libani* Oliver, *Q. pubescens* Milld., and *Q. cerris* L. were reported as partially dissolved with diethylamine and merkaptoethanol (Kedves and Gáspár ,1996). The most surprising one was the unexpected solubility of the exines of the pollen grains of *Quercus robur*. The other new results in this subject were also published (Kedves *et al.* 1998; Kedves and Frey, 2001).

The aim of our new contribution for this paper is the following: 1) To investigate the symmetry polar axis of the dry pollen grains; 2) To investigate the effect of 2-aminoethanol over different lengths of time; 3) To investigate the effect of the hydratation and staining with Methylviolet; 4) To investigate the partially dissolved pollen grains with diluted glycerine, in particular the protoplasm by the TEM method; 5) To obtain new information for the cytological data for the allergenic character of these pollen grains.

MATERIALS AND METHODS

The fresh pollen grains of *Quercus robur* L. were collected by Miss. B. Varga in the Botanical Garden of the University of Szeged on April 12, 2001. The LM investigations were carried out as follows: Pollen grains of dry , fresh unstained and stained with Methylviolet were investigated under LM.

Of 5 mg fresh pollen grains were dissolved in 2 mL 2-aminoethanol at 30 °C for 30 min, 1 hr , 5 hrs 10 hrs, and 24 hrs, respectively. Some of them were unstained and some stained with Methylviolet. Both of them were observed under LM to determine their degree of degradation with 2-aminoethanol.

Of 5 mg pollen grains were dissolved in 5 mL 50% glycerine at 30 °C for 30 days. They were observed under LM and TEM to determine the partially dissolved grains. The other 5 mg hydrated pollen grains were placed in 5 mL distilled water at 30 °C for 24 hrs. They were observed under LM.

The following morphological characteristic features were investigated: position of the pollen grains, P/E ratio in the case when the widest part of the pollen grains was in equatorial position, diameter of the inner body (intine + protoplasm), the alterations in the basic morphology as the consequence of the experimental effects and the fine structure of the protoplasmic organelles and the wall revealed by the TEM method.

RESULTS

Dry pollen grains (Fig. 1)

The pollen grains in dry condition are characteristically of longaxones type. The ornamentation and the furrows are also perceptible. Of 86.5% pollen grains are with the greatest part in equatorial position, and 13.5% in polar position. P/E ratio: 1.23 - 2.42 (maximum: 14.0% at P/E ratio 1.7). Polar axis 35.0 - 47.5 μm (maximum: 36.0% at 42.5 μm).

Fresh pollen gains mounted in glycerine-jelly (Fgs. 2-5, 29)

The pollen grains of fresh unstained (Figs 2 & 3) and stained with methylviolet (Figs 4 & 5) were mounted in glycerine-jelly. They are more or less isodiametric. The furrows are opened (Fig. 2). The greatest part of the pollen grains is in polar position of the unstained pollen grains (Fig. 3). Equatorial axis of the pollen grains is 25.0-35 μm (maximum: 41.5% at30.0 μm). The stain altered the morphology of the pollen grains. Their protrusions were observed in 24.0% of the pollen grains (Figs. 4 & 5). The intine and the protoplasm are

swollen and the furrows are more opened than in the unstained forms. The stained pollen grains had 60% in polar position, and they were 66.5% with protrusions in contrast to the unstained pollen grains. In this case we need to take into consideration a hydration effect during the washing. The equatorial axis of the pollen grains is 20.0 - 32.5 μm (maximum: 44.55% at 30.0 μm). The stain had decreased a little the size of the pollen grains and changed the number of the pollen grains with protrusions.

Pollen grains dissolved with 2-aminoethanol (Figs. 6-15, 29)

All of the observed pollen grains were in polar position. The secondary forms were observed as follows: (1) intact pollen grain more or less in polar position; (2) pollen grains with protrusions; (3) opened ectexine with inner body; and(4) inner body without ectexine.

Treatment with 2-aminoethanol for 30 min: Unstained pollen grains with protrusions were observed at the greatest part (75.0%). The quantity of the opened forms (Fig. 6) is important (9.0%). The ectexine lost was represented with 5.5%. In this way the so-called normal forms were 10.5% only. Equatorial axis is 25.0 - 32.5 µm (maximum: 50.0% at 30.0 μm). In the stained pollen grains (Fig. 7), the number of the different secondary forms are nearly identical with the unstained pollen grains. Equatorial axis is 22.5-35.0 μm (maximum: 47.5% at 27.5 μm). After this experiment pollen grains in polar position without protrusions were not observed.

Treatment with 2-aminoethanol for 1 hr: The secondary alteration of the unstained (Fig. 8) and stained (Fig. 9) pollen grains was the same (Fig. 29). The greatest part of the pollen grains was in polar position with protrusions. Equatorial axis of the unstained pollen grains is 22.5 - 32.5 μm (maximum: 50.5% at 27.5 μm), and that of the stained pollen grains is also 22.5 - 32.5 μm (maximum: 59.5% at 27.5 μm.

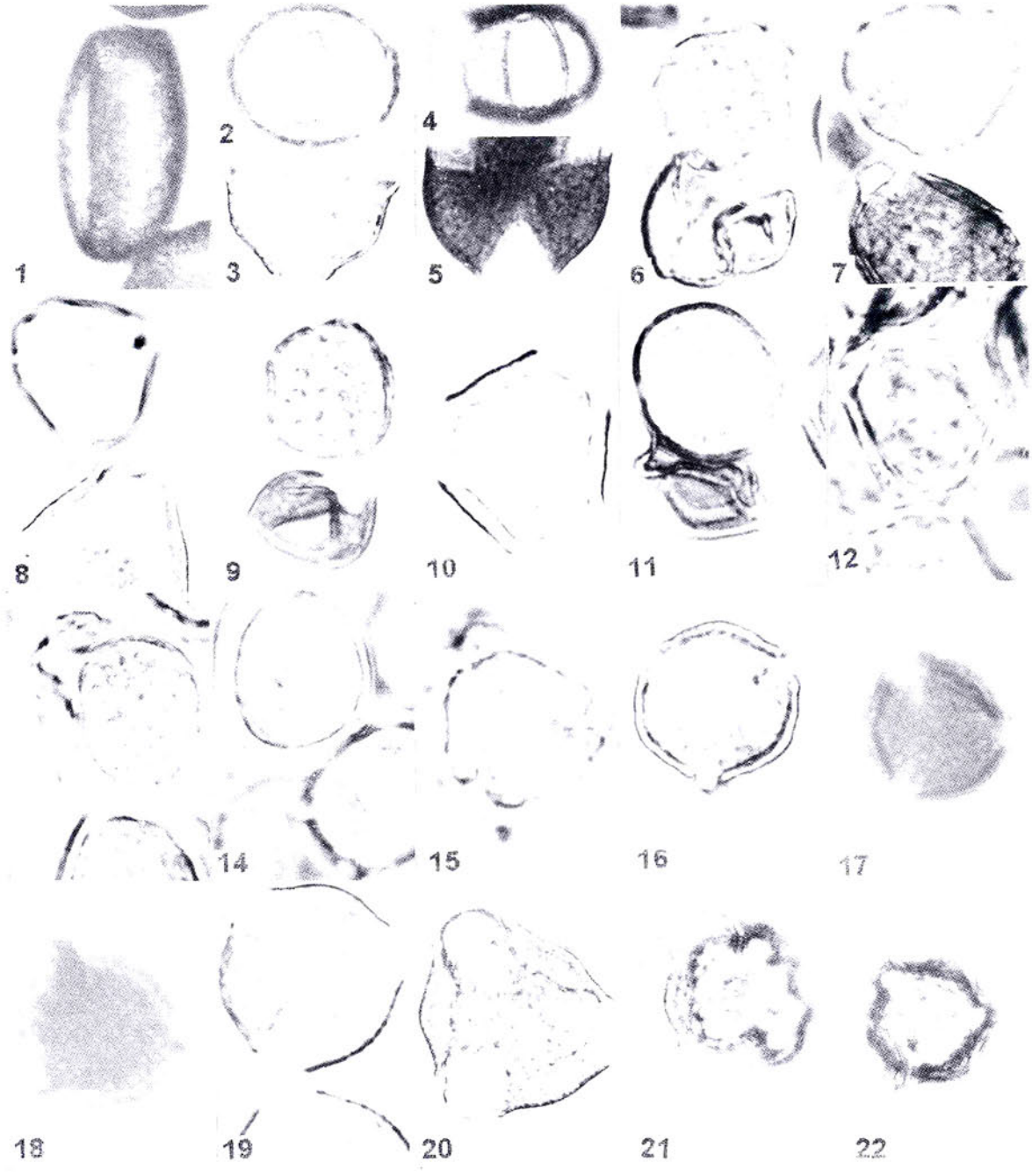
Treatment with 2-aminoethanol for 5 hrs: The quantitative distribution of the secondary altered forms in unstained (Figs. 10 & 29) and stained pollen grains (Figs. 11 & 29) is nearly identical with the previous experiment. Equatorial axis of the unstained pollen grains is 22.5 - 32.5 μm (maximum: 52.0% at 27.5 µm), and that of the stained pollen grains is also 22.5 - 32.5 µm (maximum: 55.0% at 27.5 μm).

Treatment with 2-aminoethanol for 10 hrs: At this experiment the quantity of the opened forms increased in the unstained (12.5%; Figs. 12 & 29) and the stained pollen grains (16.0%; Figs. 13 & 29). Equatorial axis of the unstained pollen grains is 22.5 - 32.5 μm (maximum: 52.5% at 27.5 μm). Equatorial axis of the stained pollen grains is 22.5 - 32.5 μm (maximum: 45.5% at 27.5 μm).

Treatment with 2-aminoethanol for 24 hrs: In general the ectexine disappeared during this experiment. In the unstained pollen grains (Fig. 14) ectexine was observed at 5.5% (Fig. 29). Equatorial axis of the unstained pollen grains is 20.0 - 32.5 μm (maximum: 42.5% at 27.5 μm), and that of the stained pollen grains is 22.5 - 32.5 μm (maximum: 57.5% at 27.5 μm).

LM results of pollen grains partially dissolved with glycerine (50%) over 30 days (Figs. 16-18)

The structure of the ectexine was not altered during this experiment based on the LM investigations. The observed pollen grains were in polar position. The equatorial axis of unstained pollen grains (Fig. 16) is 22.5 - 30.0 μm (maximum: 53.5% at 27.5 μm), and that of the stained pollen grains (Fig. 17) is also 22.5 - 30.0 μm (maximum: 49.5% at 27.5 μm). The equatorial axis of pollen grains embedded in Araldite (Fig. 18) is 22.5 - 30.0 μm (maximum: 46.0% at 25.0 μm).



Figs. 1-22: *Quercus robur* L. fig. 1: Dry pollen grain. figs. 2 & 3: Unstained pollen grains mounted in glycerine-jelly. figs. 4 & 5: Stained pollen grains with methylviolet, mounted in glycerine-jelly. figs. 6-15: Pollen grains partially degraded with 2-amino-ethanol. figs. 6 & 7: For 30 min treatment; fig. 6. Unstained pollen grain. fig. 7: Stained pollen grain. figs. 8 & 9: For 1 hr treatment. fig. 8: Unstained pollen grain. fig. 9: Stained pollen grain. Figs. 10 & 11: For 5 hrs treatment. fig. 10: Unstained pollen grain. fig. 11: Stained pollen grain. Figs. 12 &13: For 10 hrs treatment. fig. 12: Unstained pollen grain. fig. 13: Stained pollen grain. Figs. 14 &15: For 24 hrs treatment. fig. 14: Unstained pollen grain. fig. 15: Stained pollen grain. Figs. 16-18: Pollen grains dissolved with glycerine 50% during 30 days, fig. 16: Unstained pollen grain mounted in glycerine-jelly. fig. 17: Stained pollen grain mounted in glycerine jelly. fig. 18: embedded pollen grain in Atraldite. figs. 19-22: Hydrated pollen grains during 24 hours: figs. 19 & 20 unstained pollen grain. figs. 21 & 22. Stained pollen grains.

Hydrated pollen grains over 24 hrs (Figs. 19-22)

The hydration altered in an important measure of the general morphology of the pollen grains. Peculiar forms are shown in figs. 21 and 22. In general the alterations happened at the apertural area. The equatorial axis of the unstained pollen grains (Figs. 19 & 20) is 25.0 - 32.5 μm (maximum: 54.0% at 30.0 μm), and that of the stained pollen grains (Figs. 21 & 22) is 22.5 - 32.5 μm (maximum: 46.0% at 27.5 μm).

TEM results of pollen grains partially dissolved with glycerine (50%) over 30 days (Figs. 23-28)

The general survey picture illustrates well the interapertural exine, the ectexine and the intine and protoplasm (Fig. 23). The protrusion in the apertural area consisting of the protruded intine and protoplasm is well shown (Fig. 26). An electron dense microbody (starch?) emerges from the protoplasm (Figs. 23 & 24). The ultrastructure of the superficial ornamentation of the tectum in the inter-apertural area is illustrated in Fig. 25. Moreover, the fine details of the two-layered intine and the degraded protoplasm are also well illustrated. In highly magnified pictures (Figs. 27 & 28) with high resolution we can point out that the electron density of the foot layer is more much stronger than that of the other layers of the ectexine. The endexine seems to be degraded, this degradation is not the same in the different specimens investigated. There are some differences in the ultrastructural characteristics features of the intine also. The plasma membrane was not perceptible, probably disappeared during the experiment.

DISCUSSION

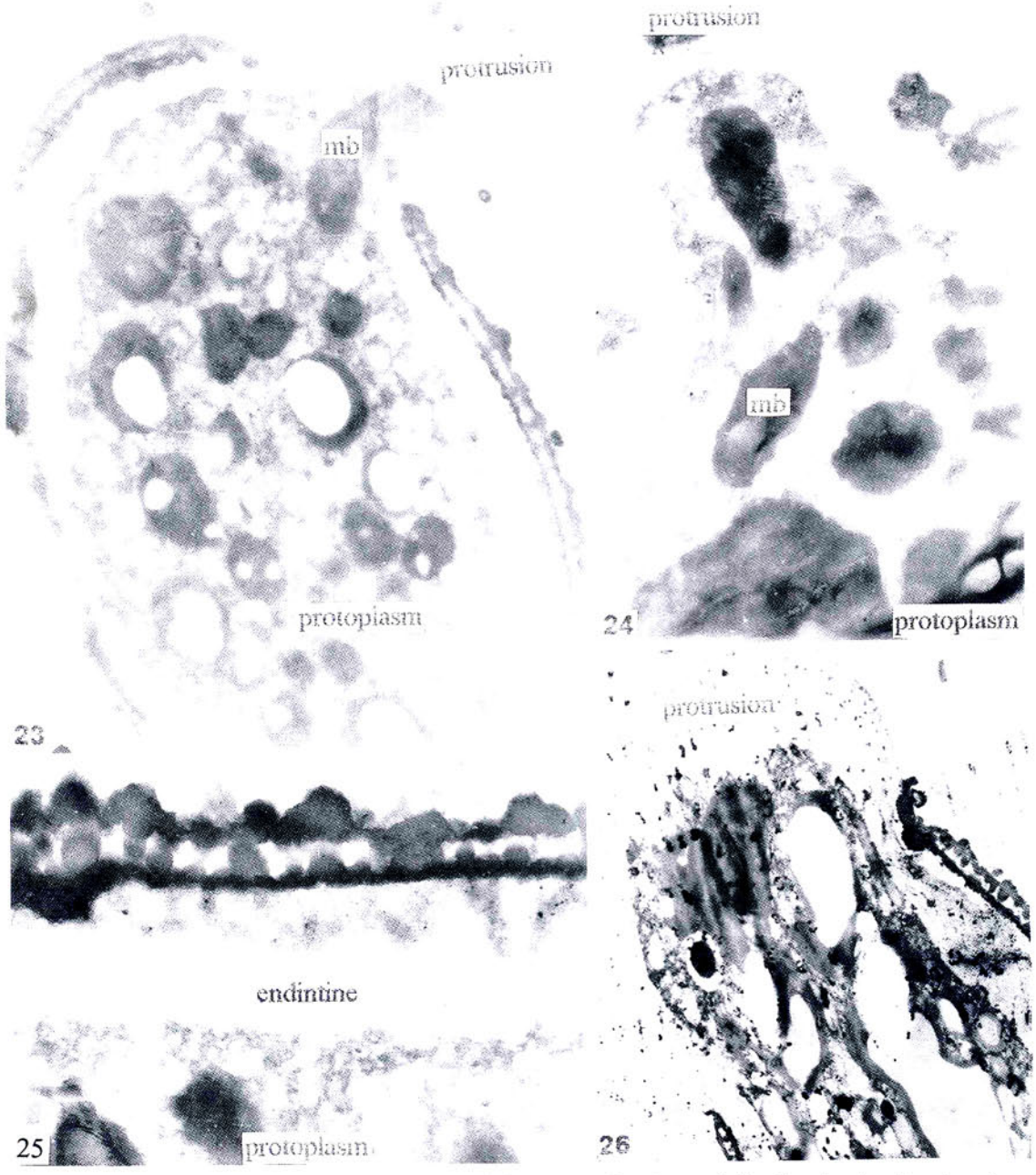
The treatment of *Quercus robur pollen grains* with 2-aminoethanol after 24 hours dissolved the sporopollenin of ectexine layer almost completely in this experimental. Our present experimental result in accordance with the previous experimental studies reconfirm that the sporopollenin of the *Quercus robur* and other *Quercus* pollen grains is less resistant. On the other hand, the sedimentary pollen grains of *Quercus* type from the Lower Cretaceous were more or less abundant than in the Tertiary and Quaternary layers. This is a peculiar contradictory phenomenon mentioned above, which needs further investigations.

The less resistance of the ectexine and the relatively resistant intine is in all probability important in the allergenic effect of this pollen grain, because the place of accumulation of the antigens is firstly in the intine (cf. Knox and Heslop-Harrison, 1970, 1971, Knox *et al.*, 1970).

The relatively short hydration resulted important alterations in the basic morphology of the pollen grains. The alterations happened in the first place in the apertural area. This also may be another constituent of the allergenic effect.

Our TEM results are in accordance with the published data of Nilsson *et al.* (1977). We can emphasize that the secondarily electron-dense foot layer is unusual which might indicate some differences in the molecular composition of the infratectal layer and the foot layer.

It is worth mentioning of the altered endexine, which sometimes is very similar to the intact ones. Further studies are in progress on the pollen grains using the C60 fullerene/benzol solution to get more information on the interesting sporopollenin of the pollen grains of the genus *Quercus*.



Figs. 23-26: Ultrastructure of the pollen grains of *Quercus robur* L. partially dissolved with glycerine, 50%. fig. 23: General survey picture from the apertural area, 15.000x, Negative No. 8962. fig. 24: Detail of the protruding protoplasm, 15.000x, Negative No. 8978. fig. 25: Ultrastructure of the pollen wall and the outer part of the protoplasm, 15.000x, Negative No. 8950. fig. 26. Detail from the protruded intine and protoplasm. 5.000x, Negative No. 8983.

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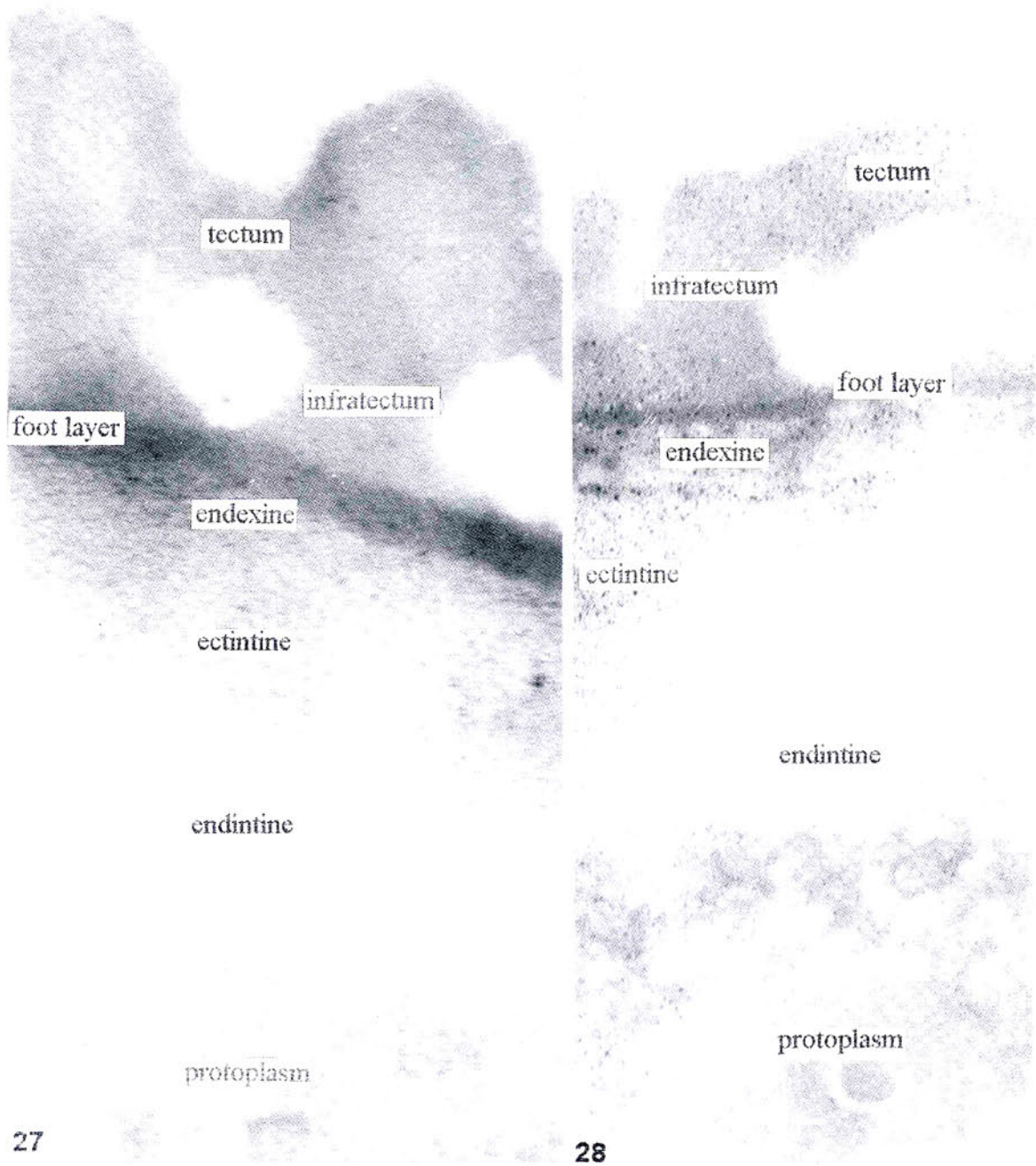

Figs. 27 & 28: Detail from the ultrastructure of the wall and the outermost part of the protoplasm. Fig. 27: 100.000x, Negative No. 8969. fig. 28: 100.000x, Negative No. 8971.

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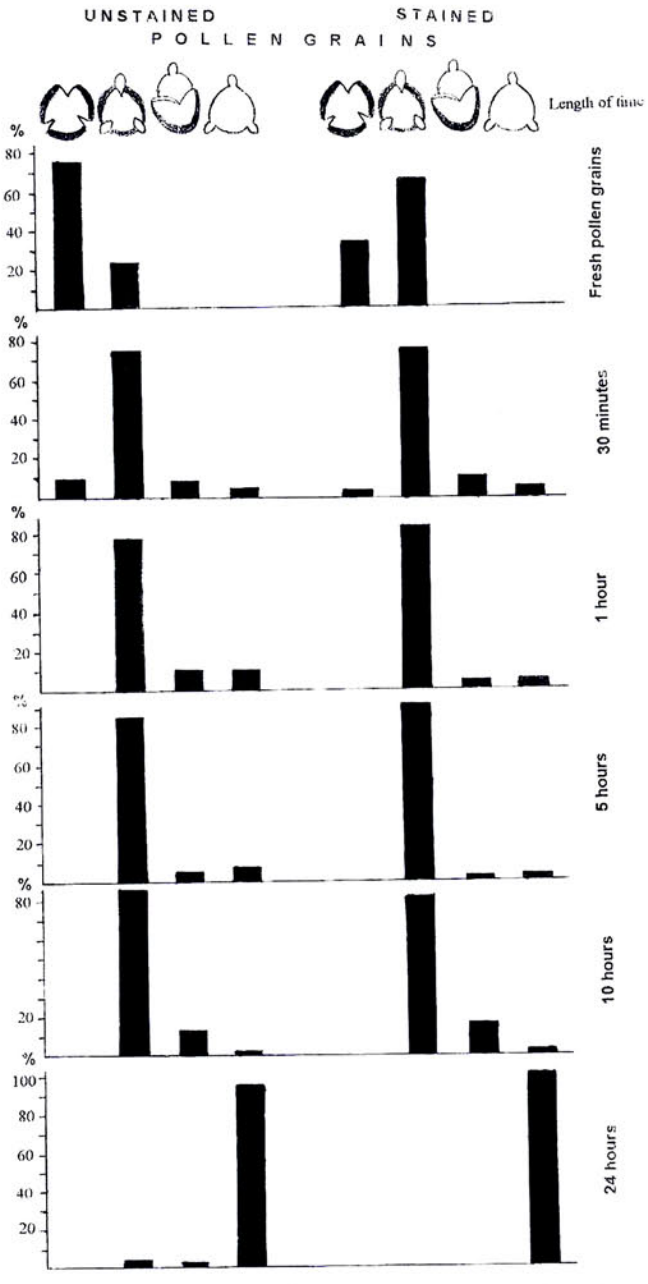

Fig. 29. Quantitative data of the secondary altered forms (Pollen grains partialy degraded with 2-aminoethanol).

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英國櫟 (*Quercus robur* L.) 花粉粒之研究

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摘　　要

乾燥、水解及部分崩解之英國櫟 (*Quercus robur* L.) 花粉粒藉光學與電子顯微鏡加以研究。以稀釋甘油處理 30 天之部分溶解花粉以穿透式顯微鏡研究。根據新實驗資料，英國櫟的孢粉外壁之孢粉質素抗力較差且崩解迅速。孢粉壁內層及原生質抗力較強。統計方法分析花粉粒的對稱性及其他形態的轉換。

關鍵詞：實驗孢粉學、近代、英國櫟、光學顯微鏡、穿透式電子顯微鏡。

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