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Ultrastructual Study on the Pollen — Microbial Interactions[1]

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ABSTRACT: The interactions between the pollen grains of *Thalictrum flavum*, and the microscopical fungi of *Gliocladium roseum* were investigated with a TEM instrument of high resolution (2-3 Å). The results are the following: 1. The pollen coat and the outermost part of the fungal cell wall have the same electron density. 2. The endoplasmatic reticulum can be demonstrated in the highly magnified pictures of the fungal protoplasm. 3. On the basis of the new TEM data, it is possible that the bacterial action plays an important role in the investigated microbial-pollen interaction. 4. The pollen coat is easily digested by the enzymes of the microbial organisms. 5. The outermost surface of the tectum is also degradable enzymatically. 6. On high magnification the outermost part of the fungal cell wall, and the tectum is very similar. At this biopolymer level, in some cases it is not easy to distinguish them. 7. In consequence of the microbial-pollen interactions enzymatically exposed microchannels of 2-4 Å in diameter were also discovered. Similar, but more characteristic system was observed on the fungal cell wall. This may play a role in the diffusion of the enzyme.

KEY WORDS: Pollen-microbial interactions, Ultrastructure.

INTRODUCTION

The biological degradation of the extremely resistant spore and pollen wall was the subject of several investigations. Kirchheimer (1933, 1935) pointed out the importance of the microbial action in the alteration of the organic material during the sedimentation. Heinen (1960, 1963) established that the enzymatic destruction began after oxidation. Havinga (1964, 1967, 1971) studied in detail the problem of the degradation processes of the spores and pollen grains. He emphasized that the preservation or the destruction of fossil spores and pollen grains are influenced by many factors such as microbial action, oxidation, mechanical effects and high temperature. Rowley *et al.* (1990) repeated the corrosion experiments of Havinga (1964) with the TEM method. They pointed out that the degradation of exines may be resulted from the direct microbial attack, indirectly from the influence of microorganisms from their residence in a well-aerated soil.

MATERIALS AND METHODS

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On the pollen grains of *Thalictrum flavum* (L.) Gaertn. symptoms of microbial infections were observed (Kedves and Rojik, 1994). In this study we used an TEM with better resolution power to get more information about the relationship of the connected surface between the pollen and fungal cell.

The pollen material with the microscopical fungi was prepared for TEM investigations as follows: Fixation was done in Millonig buffered 1% osmium tetroxide for 1 h. After fixation the material was washed in a 0.2 M Millonig (osmium-free) phosphate buffer overnight. Dehydration was performed in an ascending series of ethanol in 15 min steps, including uranyl acetate staining in 70% ethnol. The samples were embedded in Durcupan (Fluka) araldite epoxy resin in gelatin capsules and polymerized in 56°C thermostat for 3 days.

The ultrathin sections were made in the Electron Microscopical Laboratory of the Institute of Biophysics of the Biological Center of the Hungarian Academy of Sciences on a Porter Blum ultramicrotome. The TEM pictures were taken in an instrument of Zeiss EM-902 by resolution 2-3 Å.

RESULTS

The fungal cells were observed inside and outside of the pollen grains (Plate 1, figs. 1, 2). In some of the holes of the infratectal layer, there are electron dense contents. The peculiar, and rare interbedded layer (Frean, 1973; Kedves and Antunovics, 1975) can also be demonstrated (Plate 1, figs. 3-6; plate 2, fig. 1). On the basis of fine structural observations, we could further characterize the microbial organisms. In a low magnification picture (Plate 1, fig. 6) the microbial cell attached to the surface of the pollen grains may be a fungal cell in a multiply state but in particular based on its wall ultrastructure the possibility of bacterial organism can not be excluded. With the help of highly magnified pictures made with the high resolution EM, we could distinguish two types of the microbial and pollen surface connections. The new results on this subject are summarized as follows:

1. The microbial-pollen interactions with the fungal cell of *Gliocladium roseum* (Link) Bainier sensu stricto (Plate 2, fig. 2; plate 3, figs. 1-5; plate 4, fig. 1).The electron density of the outer part of the fungal cell, and the pollenkitt of the tectum is very similar or the same. In the terminal part of the fungal cell the endoplasmatic reticulum (ER) was observed (Plate 2, fig. 2; plate 3, fig. 1). The enzymatic action has a heterogeneous character. The connection between the fungal cell surface and the pollenkitt of the tectum are very tight (Plate 2, fig. 2; plate 3, figs. 1-5; plate 4, fig. 1). The degradation of the pollen grains starts on a restricted part of the pollenkitt, this is well shown on the fig. 3 of the plate 3. At another kind of destruction is when the enzymatic dissolution starts below the pollenkitt (Plate 4, figs. 2, 4, 5). On biopolymer, or molecular level (plate 5, figs. 1-4) it was established, that there is a great similarity between the fungal cell wall and the tectum of the pollen grain. Both layers are transversed with microchannels of 2-4 Å diameter. In general these microchannels are in a regular arrangement, one central channel is surrounded with about 8 further ones (Plate 5, figs. 3,4, framed part of the pictures). The channeled wall biopolymer system is more much frequent in the wall of the fungal cell. The diffusion of the fungal enzymes may be carried out through this system. It is probable as a consequence of

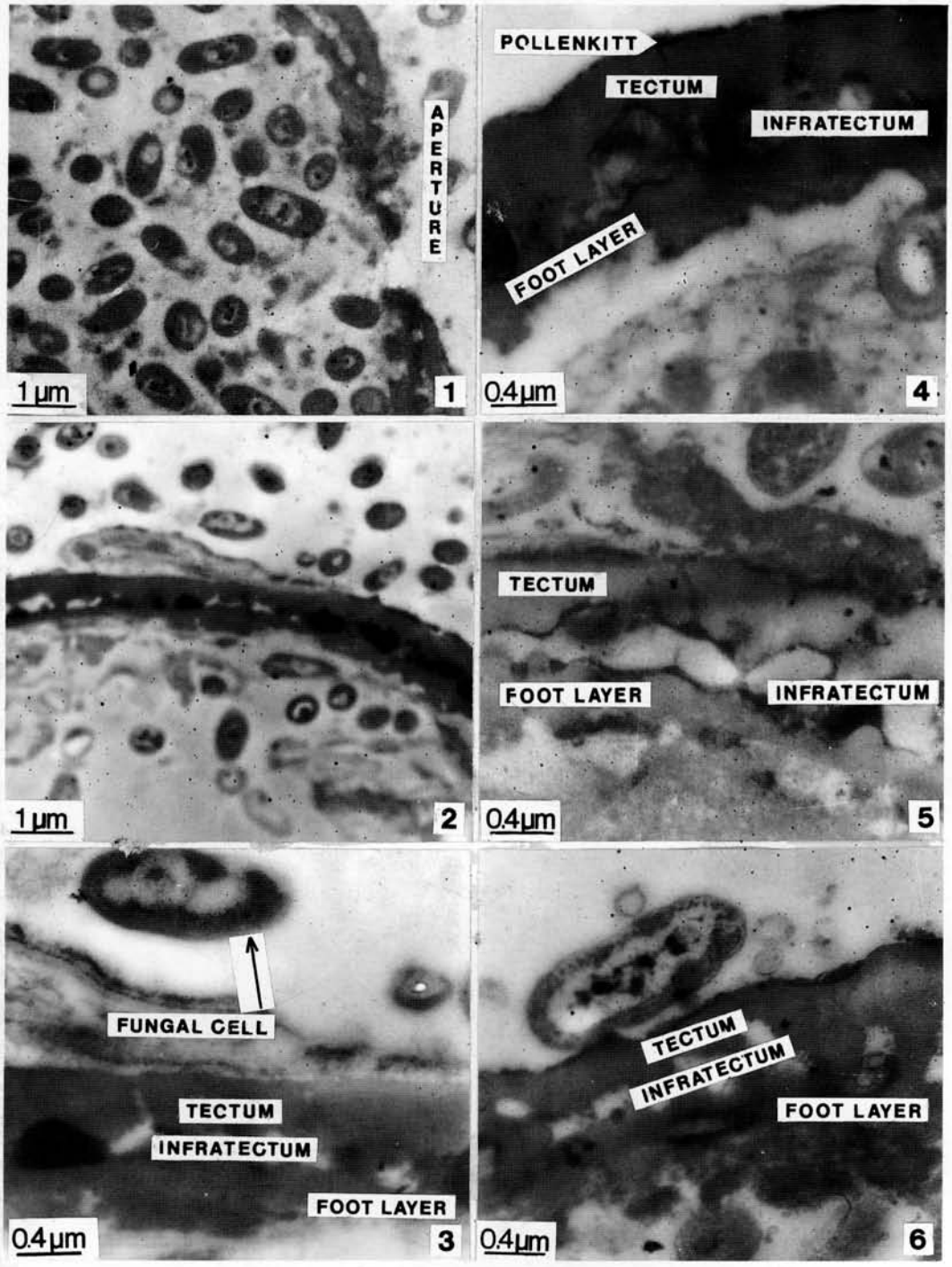

Plate 1:

1. Pollen grain of *Thalictrum flavum* (L.) Gaertn. in the apertural area. The protoplasm is full of fungal cells. Negative no: 3037, x 9.350.
2. Pollen grain in the inter-apertural area. Fungal cells were found outside and inside of the pollen grain. Negative no: 3040, x 9.350.
3. Detail of the inter-apertural exine, with a part of the fungal cell on the surface. Negative no: 3041, x 23.375.
4. Detail of the inter-apertural exine. The fungal cell in the protoplasm, and interbedded zone beneath the foot layer in the intine are illustrated. Negative no: 3031, x 23.375.
5. Fungal cell, closely connected to the surface of the tectum. Negative no: 3096, x 23.375.
6. Peculiar microbial, probably bacterial cell, connected to the pollen surface. Negative no: 3033, x 23.375.

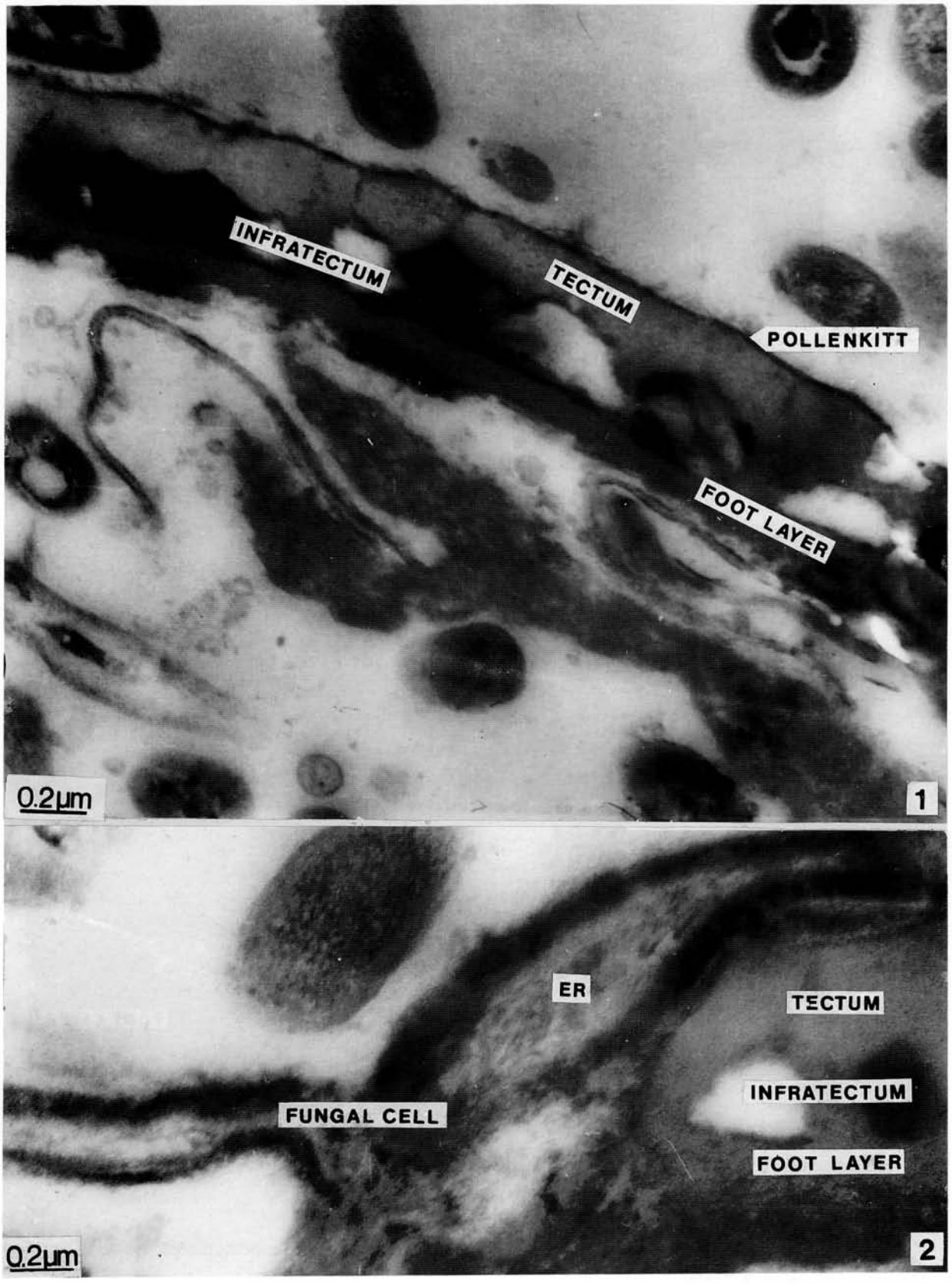

Plate 2:

1. Detail of the inter-apertural exine with fungal cells outside and inside of the pollen grain. Note the presence of the channels in the tectum, the electron dense contents in the holes of the infratectal layer, and the lamellar interbedded zones beneath the foot layer. Negative no: 2311, x 50.000.
2. Detail of the apertural area with closely connected fungal cells. The endoplasmatic reticulum (ER) is shown in the protoplasm of the fungal cell. The introductory phase of the enzymatic destruction of the tectum in clearly demonstrated. Negative no: 2312, x 50.000.

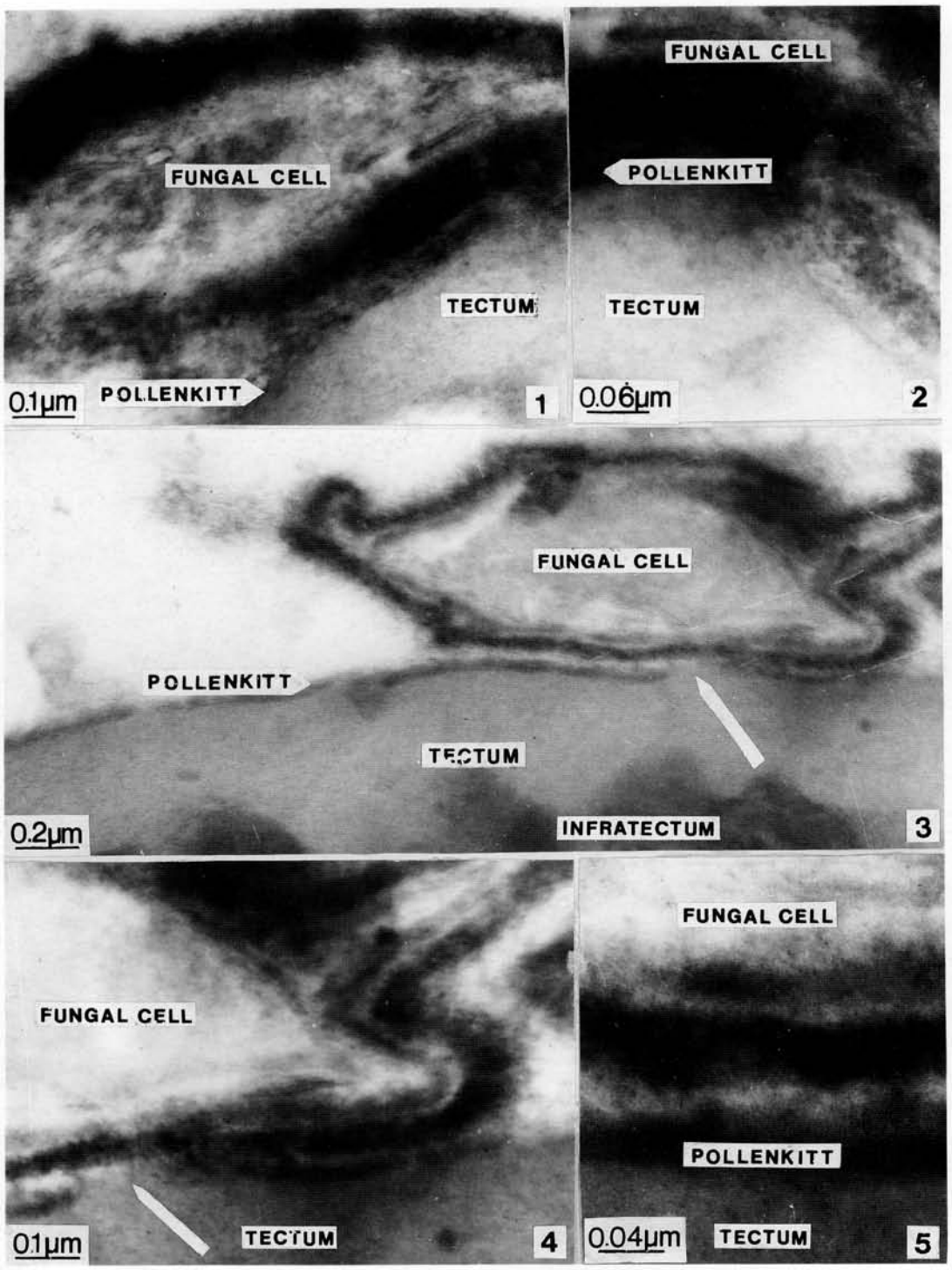

Plate 3:

1. Detail of the endoplasmatic reticulum of the fungal cell, a magnification of the fig. 2 of plate 2, and the connection with the pollen surface. The fine structural characteristic features of the pollenkitt and the fungal cell are well demonstrated. Negative no: 2313, x 86.500.
2. Detail of the fine structure of the fungal-pollen surface connection. Negative no: 2314, x 129.750.
3. General survey picture of the ultrastructure of the ectexine of *Thalictrum flavum* and a fungal cell of *Gliocladium roseum* connected to the pollen grain. The space between the two surfaces and the partial digestion of the pollenkitt are well shown. Negative no: 2317, x 43.250.
4. Detail of the very close connection of the fungal and pollen surfaces. Negative no: 2318, x 86.500.
5. Detail of the not so close fungal-pollen connection. In the highly magnified picture a very interesting connection is well illustrated. "Processes" of molecular dimension of the fungal cell wall are connected with the outer part of the pollenkitt. Negative no: 2319, x 216.250.

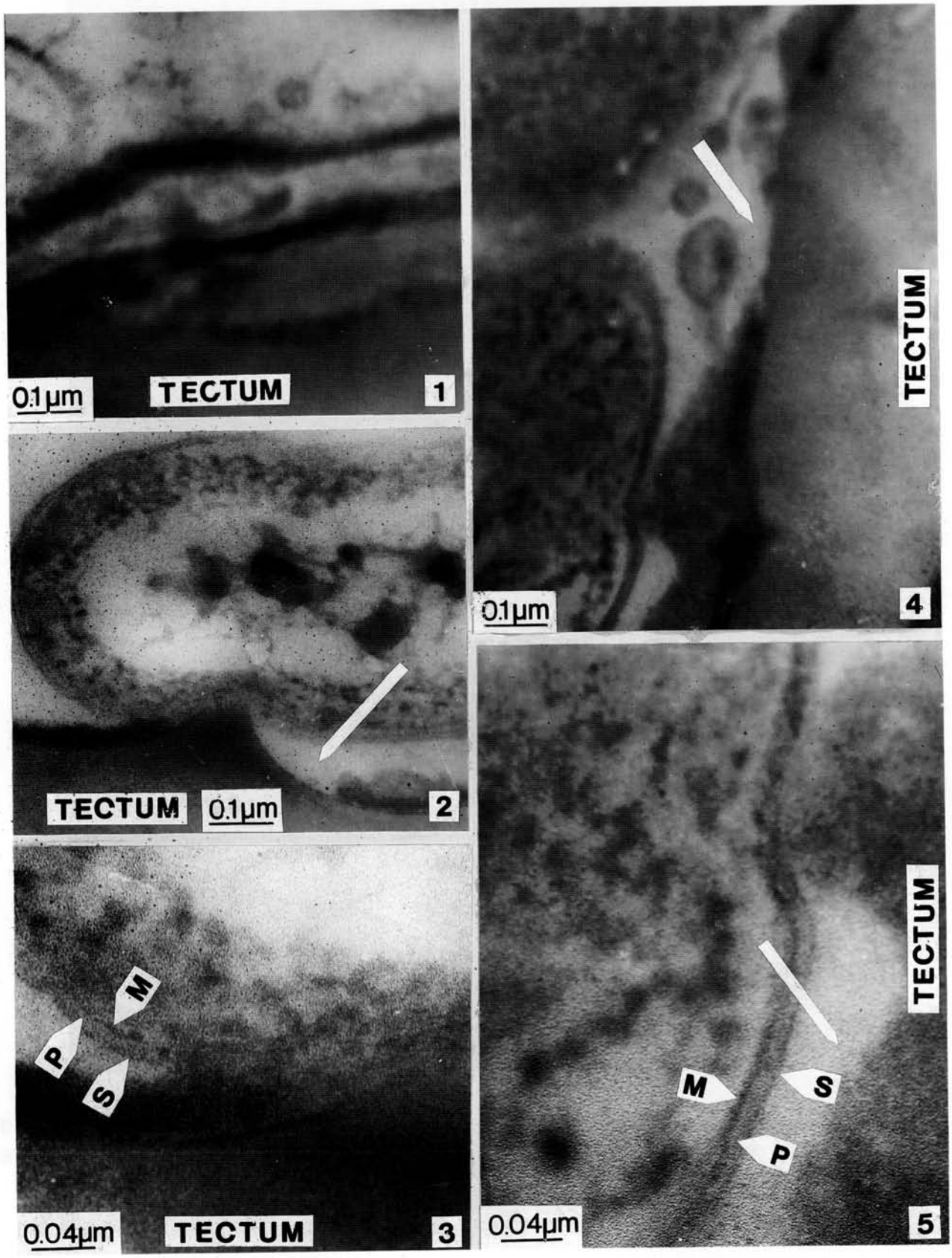

Plate 4:

1. Characteristic destruction of the surface of the tectum. Negative no: 3081, x 100.000.

2-5. In all probability bacterial-pollen interaction. Note the peculiar, and characteristic wall ultrastructure of the microbial cell, and the different kinds of destruction of the pollen surface. (2. Negative no: 3034, x 100.000; 3. Negative no: 3035, x 250.000; 4. Negative no: 3078, x 100.000; *5.Negative no: 3080, x 250.000)*

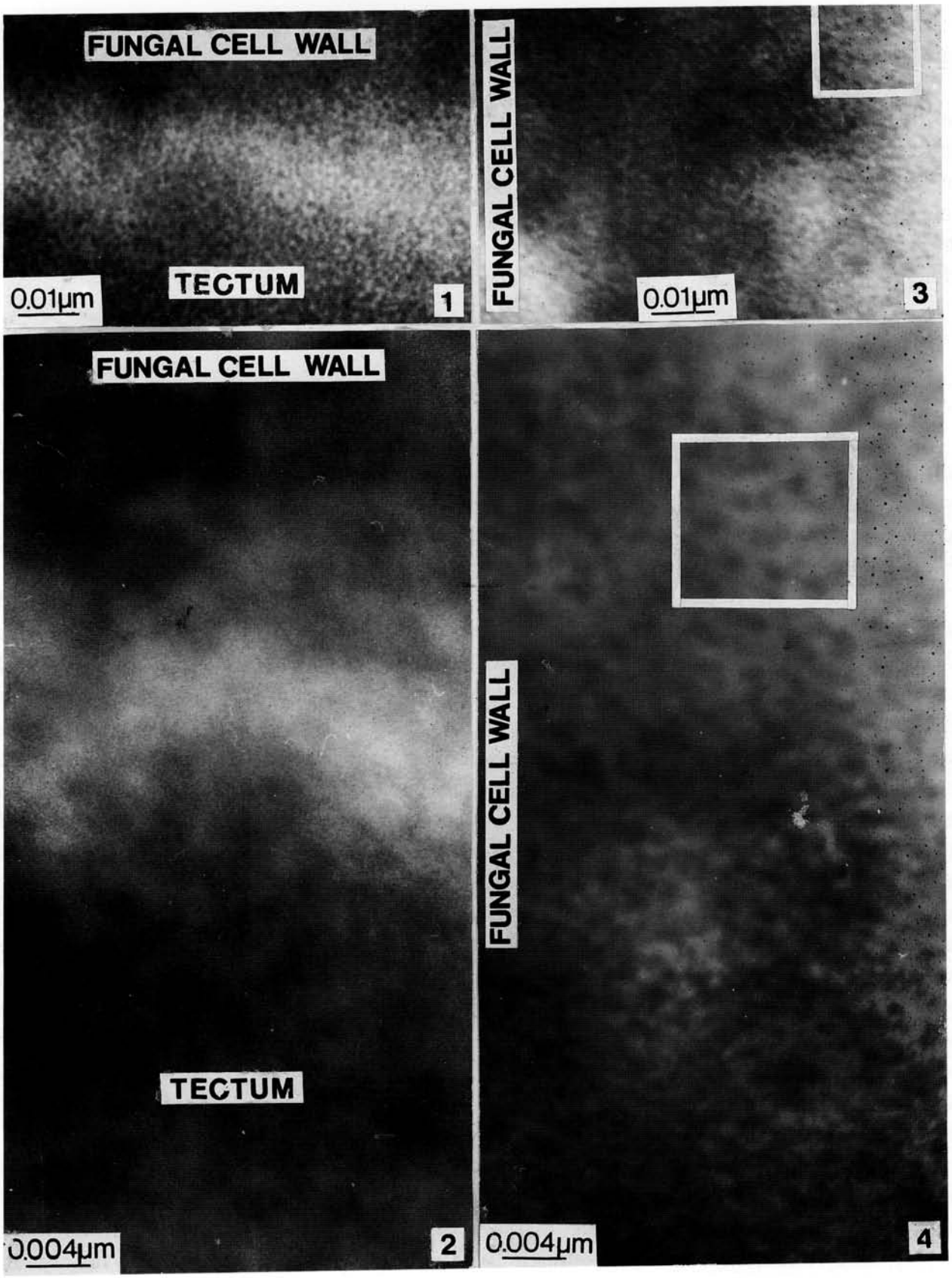

Plate 5:

1,2. Biopolymer structures of the fungal-pollen surfaces. (1. Negative no: 2321, x 1,000.000; 2. Negative no: 2322, x 2,500.000)

3,4. Characteristic molecular microchannel system of the fungal cell wall. (3. Negative no: 2316, x 1,000.000; 4. Negative no: 2316, x 2,500.000)

the enzymatic effect the biopolymer system of the tectum has also this structure as secondary character.

2. Pictures (Plate 1, fig. 6; plate 4, figs. 2-5) represents another kind of microbial action. The illustrated microbial wall ultrastructure is similar to that of some bacterial wall. A very characteristic outer layer of 13-15 Å in thickness, and a large granular inner wall are illustrated (Plate 4, figs 2,5). We could observe diffuse DNA like material in the protoplasm (Plate 4, fig.2). The digestion of the pollenkitt starts at the place of the connections of the microbial and pollen surfaces, the digestion process can be continuously followed on the surface of the tectum (Plate 4, figs. 2,4,5, marked with arrows).

DISCUSSION

The new results, presented in this paper has a methodical aspect too. Namely, the resolution power of the TEM instrument has a peculiar importance in this kind of studies. Several new details have been observed, which could not detect with the transmission electron-microscope used in the first investigations (Kedves and Rojik, 1994). Concerning the new results we pointed out the following:

The different kinds of microbial ultrastructure represent the different ontogenetical phases of the same species, *Gliocladium roseum*. But taking into consideration some new TEM data on the bacterial cells it seems more probable that both microscopical fungi and bacteriophytes might participate in the extramatrical microbial attack. To the bacterial origin of some of the observed microbial cells some selected bibliographical data are as follows. Schussing (1953) following the papers of Guillermond and Hollande published data about the bacterial cell structure. The chromidian and metachromatic granules were described in these classical works from the following species: *Bacillus mycoides, B. radicosus, B. asterosporus, B. alvei.* Similar granular structures are shown in our pictures of the plate 4, figs. 3-5. There are some similarities between the reticulum-like network of *B. cereus* (Frobischer, 1968) and some inner structures illustrated on the fig. 4, plate 4. Concerning the outer part of the wall of the microbial cell (Plate 4, fig. 5) some similarities may be established with the following previous data: two layers, an outer protein array and a peptidoglycan inner layer at *Bacillus polymixa (*Darnell *et al.*, 1986). Among others Beveridge (1988) published TEM pictures of *Escherischia coli*, and described an outer phospholipid-lipopolysaccharide-protein bilayer. This is also similar to our pictures (Plate 4, figs. 2-5). The outer "S", the middle "P" and the inner "N" layers may be recognized on our documents too.

The recently used TEM instrument with a high resolution power demonstrated new details concerning the relations of the *Gliocladium roseum* and *Thalictrum flavum.* Finely lamellar system was observed at the interbedded zone (Plate 2, fig. 1). Characteristic endoplasmatic reticulum was demonstrated in the fungal protoplasm (Plate 3, fig. 1). In every respect it is interesting to note the similarity of the biopolymer structure of the pollenkitt and the outermost part of the wall of the fungal cell wall. This may be the consequence of the similar electron density of these two ultrastructural elements, and previously discussed biopolymer system.

During these new studies we have not observed in the pollen protoplasm such microbial organisms, which may be of bacterial origin. In this way the previously discussed, probably bacterial action is external only. The newest concept of fungal-plant interactions, was reviewed in the book of Isaac (1992). In our point of view the most important establishment is that endophytic fungi may live in the plant tissues without causing symptoms of infection.

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花粉與微生物交互作用之超顯微構造的研究[1]

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摘　　要

以高解像力(2-3 Å)的穿透式電子顯微鏡來研究黃花唐松花粉(*Thalictrum flavum*)與微小真菌(*Gliocladium roseum*)之間的相互作用，所得結果如下：1. 花粉外壁與真菌細胞壁的最外層具有相同的電子緻密度。2. 以較高放大倍率觀察真菌的原生質體，可發現內質網。3. 由穿透式電子顯微鏡觀察結果，可得知細菌可能在花粉與微生物交互作用之間扮演一重要的角色。4. 花粉壁可為真菌分泌的酵素所分解。5. 花粉外壁的最堅硬的外層也可被酵素所分解。6. 在高放大倍率觀察下，花粉外壁與真菌細胞壁的最外部分構造相似，且在某些情況下兩者不易區分。7. 花粉與微生物之間，經酵素分解作用後，可發現直徑 2-4 Å之微細管道，此微細管道系統也可在真菌細胞壁上觀察到，其可能與酵素的擴散有關。

關鍵詞：花粉與微生物交互作用，超顯微構造。

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