5. TEM STUDY OF THE "INFECTED" POLLEN GRAINS OF THALICTRUM FLAVUM L. WITH GLIOCLADIUM ROSEUM (LINK) BAINIER

Short communication

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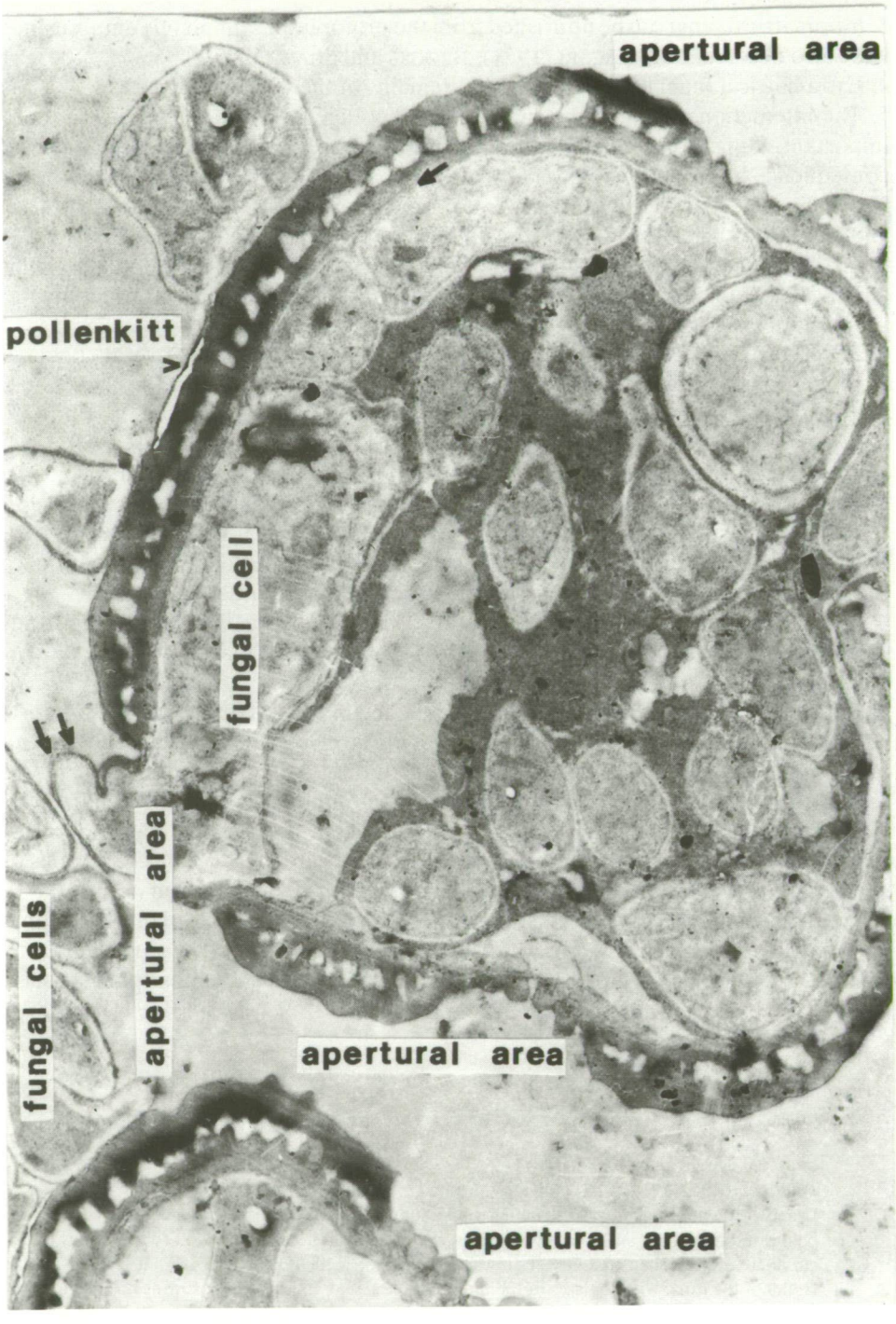
During our experimental investigations of the secondary alterations of the spores and pollen grains (e. g.: KEDVES and KINCSEK, 1989, KEDVES and PÁRDUTZ, 1992a), several taxa were examined. The pollen grains of the interesting, wind-pollinated *Ranalean* genus – *Thalictrum* – were also the subjects of our investigations. On 17. 6. 1988, 20 mg air dried pollen material was mesured six times in small glasses for high temperature effect experiments. Not long after the preparation of the experiment, symptoms of infections were observed. The infected pollen grains were placed into a thermostat at 30 °C for $24^{hrs.}$. The determination of the microscopical fungi was made in the Department of Microbiology of the J. A. University, by Dr. L. MANCZINGER, as *Gliocladium roseum* (LINK) BAINIER. The infected material was fixed (OsO_4 aq. dil. 1%), embedded in Araldite, ultrathin sectioned, and investigated with the TEM method. The electron microscopical pictures were taken with a Tesla BS-500 transmission electron microscope; resolution: 6 Å. Our preliminary results can be summarized as follows.

1. Fungal cells, mostly hyphae were observed intramatrically (inside the pollen grain) and extramatrically (outside the pollen grain); cf. HESSE, KUSEL-FETZMANN and CARNIEL, (1989) (Plate 5.1., plate 5.2., figs. 1,2).
2. Intramatrical fungal cells can grow through the aperture of the pollen grain, and this kind of hypha is similar to the pollen tube; Plate 5.1., marked with two arrows.

Plate 5.1. ▸

Thalictrum flavum L. Recent pollen grain with *Gliocladium roseum* (LINK) BAINIER.

The fungal cells are shown in the protoplasm of the pollen grains and outside the exine. It is worth mentioning that several fungal cells reach the plasma membrane of the pollen grain. Marked with an arrow. One of them is growing out through the pollen aperture pushing out the thin exine layer of the germinal area. Indicated with two arrows. The thin electron dense coat layer on the surface is well shown. Extramatrical fungal cells may be in close contact with the surface of the pollen grain.
Negative no: 8946, 10.000 x.


apertural area
pollenkitt
fungal cell
apertural area
fungal cells
apertural area
apertural area

3. Intramatrical fungi can be nourished from the protoplasm, completely empty exines were also observed. Cf.: FAEGRI (1971), BROOKS and SHAW (1973).
4. Extramatrical fungi can also digest the remains of the tapetal tissues.
5. The interactions between the surface of the pollen grain and the fungal cells are important. Fungal cells can grow directly towards the pollen grain. This can be the consequence of several factors, as follows.

5.1. The peculiar biopolymer system of the pollen surface (cf. KEDVES, PÁRDUTZ, and VÉR, 1991, KEDVES and PÁRDUTZ, 1992b).

5.2. The electrostatic charge of the pollen surface (ROWLEY, 1971), namely the anionic character, which results in the accumulations of the cations, particularly the thorium on the surface.

5.3. The peculiar characteristics of the pollen coat (tryphine). Several concepts were published concerning this question; EHRLICH (1958), FREYTAG, (1967), SKVARLA and ROWLEY (1970), DICKINSON and LEWIS (1973), HESLOP-HARRISON (1975), MASCARENHAS (1975), GILLISSEN and BRANTJES (1978), HESSE (1978a, b, c, 1979, 1980, 1984, 1991), AUDRAN and BATCHO (1981), ZAVADA (1983), BLACKMORE and BARNES (1984), LOEWUS et al. (1985), SOUTHWORTH (1985), FERNÁNDEZ and RODRIGUEZ-GARCIA (1988), HARLEY (1988, 1991), DICKINSON and SHELDON (1990), VAN DER HAM (1990), ROMAN, BLANCA and ROMERO (1992). The chemical interaction between the surfaces of the fungal cell and the pollen grain is also a factor in this phenomenon.

5.4. The molecular sieving character of the exine (ROWLEY, 1973) is also important and so is the basic concept of ROWLEY (1987–88) which says that the "pollen wall" is an integrated part of the plasma membrane.

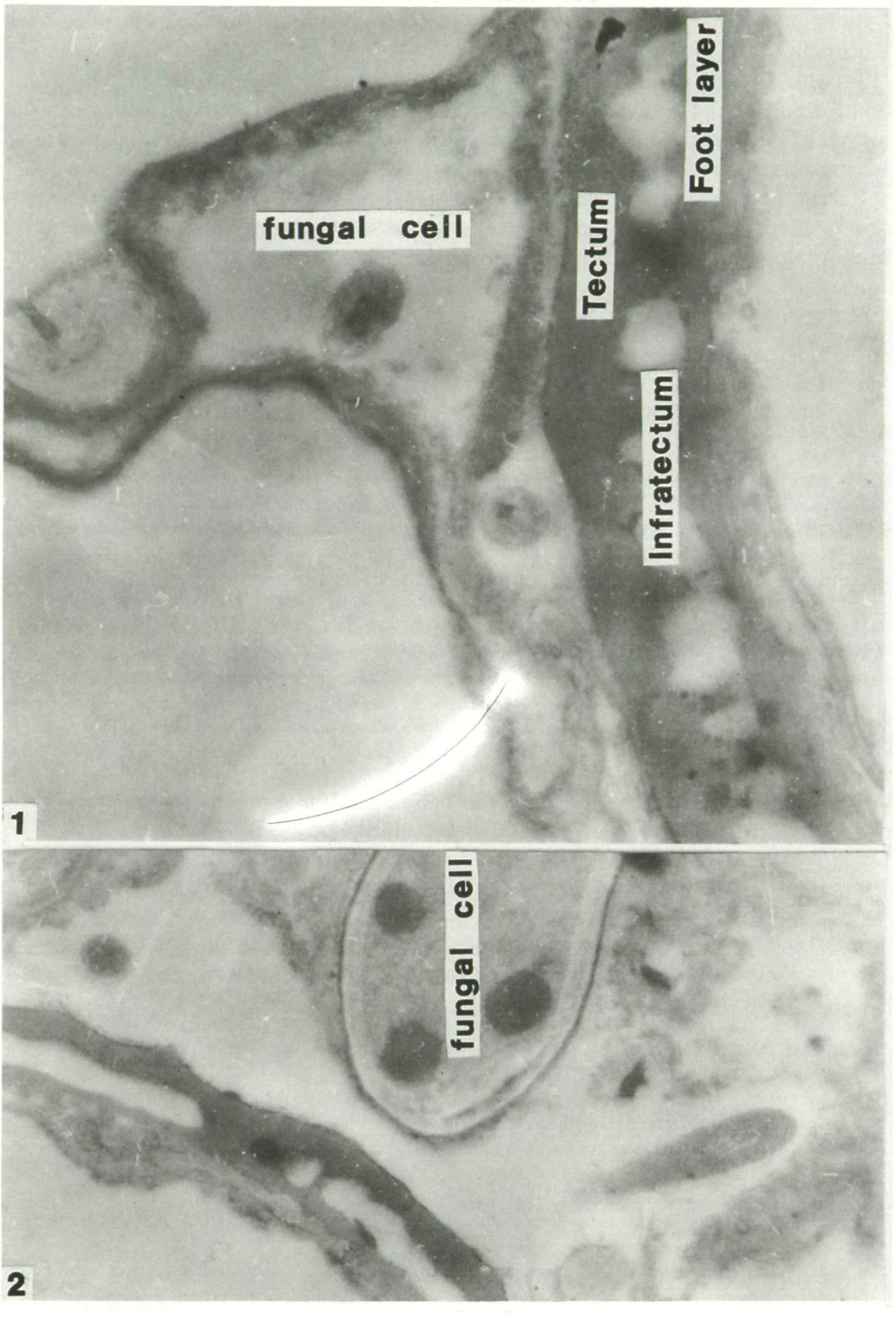
5.5. The plasma membrane and the tapetal membrane glycocalyx are also important in this interaction. The fungal cell, which is in connection with the plasma membrane (glycocalyx), can "imitate" the pollen tube.

6. According to the newest concept of fungal-plant interactions, the following can be cited from the book of ISAAC (1992), p. 316: "There is presently a great deal of discussion concerning the extent to which endophytes are in fact latent pathogens of exampes of fungi co-evolving with plants from parasitism to mutualism (CLAY, 1988). However in many cases mutualistic relationships between host plants and fungi endophytes are suspected and in many instances such associations have been identified." P. 320. "Many endophytes live almost entirely within the host plant tissues, often without causing any visible signs of infection."

Plate 5.2. ▸

Thalictrum flavum L. Recent pollen grain with *Gliocladium roseum* (LINK) BAINIER.

1. Detail of the pollen exine ultrastructure with a fungal cell on the surface. The contact between the pollen and fungal cell surfaces is very close in some parts. The disintegration (digestion by fungal enzymes) of the pollen coat is well shown. negative no: 8614, 40.000x.
2. Detail of the exine with remains of the tapetal tissues. One fungal cell is growing directly towards the pollen exine. On the tectum, the thin electron dense layer, the pollen coat is well illustrated. Negative no: 8589, 20.000x.


fungal cell
Tectum
Foot layer
Infratectum
1
fungal cell
2

In resumé, the observed fungal-pollen interaction is a complicated, multifactorial biochemical and physiological relationship.

Finally it is necessary to emphasize that to get more information about the relationship of the connected surfaces of the pollen and the fungal cell, we need further TEM data with an instrument of better resolution power. These investigations are in progress, and the new results will be published in the near future.

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References

AUDRAN, J.-C. and BATCHO, M. (1981): Cytochemical and infrastructural aspects of pollen and tapetum ontogeny in *Silene dioica (Caryophyllaceae)*. – Grana *20,* 65–80.

BLACKMORE, St. and BARNES, S. H. (1984):Transmission electron microscope preparations from unacetolysed herbarium pollen: A comparison of methods. – Pollen et Spores *26,* 137–146.

BROOKS, J. and SHAW, G. (1973): The role of sporopollenin in Palynology. In: Problems of Palynology, Nauka, Moscow, 80–91.

DICKINSON, H. G. and LEWIS, D. (1973): The formation of the tryphine coating the pollen grain of *Raphanus* and its properties relating to the self-incompatibility systems. – Proc. R. Soc. (London) *183,* 149–165.

DICKINSON, H. G. and SHELDON, J. M. (1990): The cell biological basis of exine formations in *Lilium* sp. – Polen esporas y sus aplicaciones, VII Simposio de Palinologia, 17–29.

EHRLICH, H. G. (1958): Electron microscope studies of *Saintpaulia ionatha* WENDL. pollen walls. – Experimental Cell Research *15,* 463–474.

FAEGRI, K. (1971): The preservation of sporopollenin membranes under natural conditions. In: Sporopollenin eds.: J. BROOKS, P. R. GRANT, M. MUIR, P. VAN GIJZEL and G. SHAW. – Academic Press, Lonodn, New York, 256–272.

FERNÁNDEZ, M. C. and RODRIGUEZ-GARCIA, M. I. (1988): Pollen wall development in *Olea europaea* L. – New Phytol. *108,* 91–99.

FREYTAG, K. (1967): Über doppelbrechende Lipoide in der Exine und im Pollenöl. – Grana Palynologica *7,* 3–9.

GILLISSEN, L. J. W. and BRANTJES, N. B. M. (1978): Function of the pollen coat in different stages of the fertilization process. – Acta Bot. Neerl. *27,* 205–212.

HARLEY, M. M. (1988): Observations on the colours of fresh pollen with the light microscope and macroscope. – Polen, esporas y sus aplicaciones VII Simposio de Palinologia A. P. L. E. Granada 26/30 Septbre 1988 Programa y Resumenes, 31.

HARLEY, M. M. (1991):The pollen morphology of the *Sapotaceae.* – Kew Bulletin *46,* 379–491.

HESLOP-HARRISON, J. (1975): The Cronian Lecture, 1974 The physiology of the pollen grain surface. – Proc. R. Soc. London B, *190,* 275–299.

HESSE, M. (1978a): Der Feinbau der Pollenkleibstoffe: Preparative Probleme bei der Strukturerhaltung, Grundfragen zur Nomenklatur und zur Begriffsabgrenzung. – Linzer Biol. Beitr. *9,* 181–201.

HESSE, M. (1978b): Entwicklungsgeschichte und Ultrastruktur des Pollenkitts bei *Tilia (Tiliaceae).* – Pl. Syst. Evol. *120,* 13–30.

HESSE, M. (1978c): Entwicklungsgeschichte und Ultrastruktur von Pollenkitt und Exine bei nahe verwandten entomophilen und anemophilen Angiospermensippen: *Ranunculaceae, Hamamelidaceae, Platanaceae* und *Fagaceae.* – Pl. Syst. Evol. *130,* 13–42.

HESSE, M. (1979): Entwicklungsgeschichte und Ultrastruktur von Pollenkitt und Exine bei nahe verwandten entomophilen und anemophilen Sippen der *Oleaceae, Scrophulariaceae, Plantaginaceae* und *Asteraceae.* – Pl. Syst. Evol. *132,* 107–139.

HESSE, M. (1980): Ultrastruktur und Entwicklungsgeschihte des Pollenkitts von *Euphorbia cyparissias, E. palustris* und *Mercurialis perennis*. – Pl. Syst. Evol. *135,* 253–263.

HESSE, M. (1984): Pollenkitt is lacking in *Gnetatae: Ephedra* and *Welwitschia:* Further proof for its restriction to the *Angiosperms*. – Pl. Syst. Evol. *144,* 9–16.

HESSE, M. (1991): Cytology and Morphogenesis of Pollen and Spores. – Progress in Botany *52,* 19–34.

HESSE, M., KUSEL-FETZMANN, E. and CARNIEL, J. (1989): Life cycle and ultrastructure of *Ducellieria chodati (Oomycetes).* – Pl. Syst. Evol. *165,* 1–15.

ISAAC, S. (1992): Fungal-Plant Interactions. – Chapman and Hall, London – New York – Tokyo – Melbourne – Madras.

KEDVES, M. and KINCSEK, I. (1989): Effect of the hight temperature on the morphological characteristic features of the sporomorphs I. – Acta Biol. Szeged. *35,* 233–235.

KEDVES, M. and PÁRDUTZ, Á. (1992a): X-ray efect to the exine ultrastructure of *Alnus glutinosa* (L.) GAERTN. – Taiwania *37,* 123–133.

KEDVES, M. and PÁRDUTZ, Á. (1992b): Transmission electron microscopy of partially dissolved exines of different bisaccate *gymnosperm* pollen grains. In: Plant Cell Biology and Development *3,* ed.: M. KEDVES, 38–66, Szeged.

KEDVES, M., PÁRDUTZ, Á. and VÉR, A. (1991): Biopolymer organization of partially degraded exines of saccate *gymnosperm* In: Plant cell Biology and Development *1,* ed.: M. KEDVES, 32–34, Szeged.

LOEWUS, F. A., BALDI, B. G., FRANCESCHI, V. R., MEINERT, L. D. and MCCOLLUM, J. J. (1985): Pollen Sporoplasts: Dissolution of Pollen Walls. – Plant Physiol. *78,* 652–654.

MASCARENHAS, J. P. (1975): The biochemistry of *Angiosperm* pollen development. – Bot. Rev. *41,* 259–344.

ROMAN, J. M., BLANCA, G. and ROMERO, A. T. (1992): Consideraciones sobre la presencia de trifina en el polen de *Cruciferas.* – 9 Simposio de Palinologia, A. P. L. E., Resumenes, 86.

ROWLEY, J. R. (1971): Implications on the nature of sporopollenin based upon pollen development. In: Sporopollenin eds.: J. BROOKS, P. R. GRANT, M. MUIR, P. VAN GIJZEL and G. SHAW. – Academic Press. London, New York, 174–219.

ROWLEY, J. R. (1973): Translocation through the pollen wall. – J. Ultrastructure Research *44,* 449–450.

ROWLEY, J. R. (1987–1988): Substructure within the endexine, an interpretation. – J. of Palynology *23–24,* 29–42.

SKVARLA, J. J. and ROWLEY, J. R. (1970): The pollen wall of Canna and its similarity to the germinal apertures of other pollen. – Amer. J. Bot. *57,* 519–529.

SOUTHWORTH, D. (1985): Pollen exine substructure. I. *Lilium longiflorum.* – Amer. J. Bot. *72,* 1274–1283.

VAN DER HAM, R. W. J. M. (1990): *Nephelieae* pollen *(Sapindaceae):* Form, Functions and Evolution. – Proefschrift, Rijksherbarium/ Hortus Botanicus, Leiden.

ZAVADA, M. S. (1983): Pollen wall development of *Zamia floridiana.* – Pollen et Spores *25,* 287–304.