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The Easiest Proof for the Presence of Pollenkitt

By

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With 46 Figures

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Summary

TEPPNER H. 2009. The easiest proof for the presence of pollenkitt. – Phyton (Horn, Austria) 48(2): 169–198, with 46 figures.

Pollenkitt, as a viscous fluid, behaves like an optical gel and forms in air bright contact plaques between pollen grain and cover slip, which can be seen easily in the LM. In preparations without pressure on the pollen grains, the size of the contact plaques can be used as a relative means for the amount of pollenkitt. Medium amounts were found in streukegel-blossoms (*Solanum lycopersicum, Onosma*), small amounts (*Poaceae*) or no traces (*Luzula, Alnus*) of pollenkitt in anemophilous plants. The results were compared with available TEM data (in angiosperms, traces of pollenkitt even when not visible in the LM) and the appearance of pollenkitt in water and other fluids. It is concluded, that the presented method is not only the easiest, but also the only exact way to prove pollenkitt and its relative amounts in compara-

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tive studies. Within the thecae of *Antirrhinum majus* long, fine threads contribute to hold the pollen mass in the open anther.

Zusammenfassung

TEPPNER H. 2009. The easiest proof for the presence of pollenkitt. [Der einfachste Nachweis für das Vorhandensein von Pollenkitt]. – Phyton (Horn, Austria) 48(2): 169–198, mit 46 Abbildungen.

Pollenkitt, als eine viskose Flüssigkeit, verhält sich ähnlich einem optischen Gel und bildet in Luft helle Kontakthöfe zwischen Pollenkorn und Deckglas, welche leicht im LM zu sehen sind. Werden die Pollenkörner ohne Druck auf das Korn präpariert, kann die Größe der Kontakthöfe als relatives Maß für die Pollenkitt-Menge genommen werden. Mittlere Kittmengen wurden in Streukegelblumen (*Solanum lycopersicum, Onosma*) gefunden, geringe Mengen (*Poaceae*) oder keine Spuren (*Luzula, Alnus*) wurden in Windblütlern beobachtet. Die Ergebnisse wurden mit vorhandenen TEM Daten (in Angiospermen Spuren von Pollenkitt auch dann, wenn im Lichtmikroskop nicht sichtbar) und dem Erscheinungsbild des Pollenkittes in Wasser und anderen Flüssigkeiten verglichen. Es wird geschlossen, daß die vorgestellte Methode nicht nur die einfachste, sondern auch die einzige exakte ist, die in vergleichenden Studien Angaben über relative Pollenkittmengen erlaubt. In den Theken von *Antirrhinum majus* sind lange, feine Fäden vorhanden, die zum Zusammenhalten der Pollenmasse beitragen.

1. Introduction

In the case of large amounts of pollenkitt there is usually no problem to prove its presence, especially when in water or water-based solutions droplets are formed. Even KNOLL 1930: 616, who created the term pollenkitt (HESSE 1978a: 182–193, TEPPNER 2007a:233), has noted that small amounts of pollenkitt can form a thin coat: „Diese flüssige Haut kann so dünn und dabei so innig mit der festen Unterlage verbunden sein, daß sich die als Klebstoff wirkende Flüssigkeit auch dann nicht in einzelne scharf umgrenzte Tropfen zerlegen und dadurch sichtbar machen läßt, wenn man die frischen Pollenkörner in Wasser oder in geeigneten wässerigen Lösungen für einige Zeit untertaucht und allenfalls das Ganze erwärmt.“ [This liquid skin can be so thin and so closely connected with the firm undersurface that the glue-like liquid does not form sharply defined drops, which would allow their visibility, even when the fresh pollen grains are submerged in water or in a suitable aqueous solution for a certain time, or the mixture is warmed.]

The first important records of oily substances on the surface of pollen grains are probably those of NEEDHAM 1743, 1750, KOELREUTER 1761:§ 2–8 and BRONGNIART 1827. The first modern description (modern in the sense that it contains many correct elements) seems to be that of MEYEN 1839: 174 ff. In other respects, the history of the knowledge of pollen and pollenkitt is treated in MOHL 1834: 1–9 and TROLL 1928: mainly p. 328–331, so that a repetition is not necessary.

TROLL 1928 dusted pollen with the help of a paintbrush on a slide and used the pollen dispersion figures as a criterion for the relative amount of pollenkitt in the pollen of a given species.

POHL 1929 spread pollen grains on a cleaned slide, removed the grains, and checked the eventually remaining patches as a measure for presence and amount of pollenkitt [„Abklatschpräparat“ (cast preparation), KNOLL 1930: 611]. On the other hand he observed the pollen grains in Sudan III (alcoholic solution), and used the dimension of the droplets on the surface of the grains as a standard for comparisons of the amount of pollenkitt in different species. For e. g., he proved small amounts of pollenkitt in *Poaceae* and the absence of it in *Alnus*.

The main aim of KNOLL 1930 was a standardized measurement of the adherence of pollen grains together. He used the dispersal of grains fallen from a height of 1.59 m („Fallbild“, fall figure) in his legendary and sophisticated „Fallvorrichtung“ (fall apparatus). KNOLL 1930 concluded the presence of pollenkitt indirectly from the clumps of the grains and their adherence on glass, but in some cases (wind pollination) the amount of the pollenkitt present must have been so small, that a direct proof by available methods was not possible (e. g., for *Alnus* p. 621, for *Taxus* p. 650).

HESSE 1978b: 17 elaborated the experiment of adherence on glass to a three-steps scale as a relative measurement for the ability of pollen to adhere.

DOBSON 1988 has found that neutral lipids dominate in pollenkitt and detected a high diversity in their composition between species. This diversity was not investigated in relation to the morphological appearance of pollenkitt. She used Sudan IV (scarlet red) for staining pollenkitt (p. 171). HENNING & TEUBER 1992 checked the amount of pollenkitt in different *Medicago sativa* lines by evaluating presence and size of pollenkitt globules in water with Sudan IV.

PACINI & HESSE 2005: 405–406 mention the droplet formation and extrusion from exine cavities in water, dissolution in oils (visible in coloured pollen); observation in air and without a cover slip shows pollenkitt in exine cavities or as a thin coat of the whole grain. Staining with Sudan dyes is problematic, because exine stains too, but later than the pollenkitt. The method to dust pollen on a slide and check the droplets of pollenkitt after removing the grains is also mentioned.

To a small extent, possibilities for the proof of the presence of pollenkitt are mentioned in books for methods in flower ecology (such as KEARNS & INOVYE 1993 or DAFNI, KEVAN & HUSBAND 2005). In every case no method for the proof of the presence of pollenkitt in the LM in cases with thin coats which do not form droplets in water or diluted acetic acid, and for very small amounts seem to be mentioned anywhere. If strong solvents like alcohol (e. g., in Sudan III) are used, there is always the uncertainty, that the pollen grain contents extrude. If grains are observed in air without cover slip, it is not possible to distinguish between pollenkitt droplets or other adhering material. Thus, because of problems with answering the question of the presence of pollenkitt in *Mimosaceae* the idea originated, if optical characteristics of a viscous fluid such as pollenkitt can be used for its identification in the LM (Teppner 2007b: 4). On very few selected examples the principle will be demonstrated here.

2. Material and Methods

The pollen of the following species were used in this paper (sequence as in chapter 3):

Antirrhinum majus L. (*Antirrhinaceae*, former *Scrophulariaceae*). Styria, Graz-Gries, self-sowing garden-population.

Acacia cyclops A. Cunn. ex G. Don (*Mimosaceae-Acacieae*). Origin: Western Australia, 'Collected in South-west Botanical Province'. – Kings Park & Botanic Garden, Perth, Western Australia 1995: 2225. – Grown in the cool greenhouse of the Bot. Garden at the Institute of Plant Sciences, University of Graz.

Leucaena leucocephala (Lam.) de Wit (*Mimosaceae-Mimoseae*). Origin: Jardin de Aclimitación de la Orotava, Puerto de la Cruz, Tenerife, Hispania, 1991. – Grown in the cool greenhouse (during summer in the open) of the Bot. Garden at the Institute of Plant Sciences, University of Graz (grown as *Albizia polyphylla*).

Anthoxanthum odoratum L. (*Poaceae-Poeae*, former *Aveneae*) spontaneously growing in meadows in the Botanic Garden at the Institute of Plant Sciences of the University Graz.

Lolium perenne L. (*Poaceae-Poeae*) spontaneously growing in meadows in the Botanic Garden at the Institute of Plant Sciences of the University Graz.

Luzula campestris (L.) DC. (*Juncaceae*) spontaneously growing in meadows in the Botanic Garden at the Institute of Plant Sciences of the University Graz.

Alnus alnobetula (Ehrh.) K. Koch [*A. viridis* (Chaix) DC.]. (*Betulaceae*). Origin: W. Styria, Koralpe, Reinischkogelregion, Sommereben above Stainz, S. Wassermann, c. 890 m; 17.4.2003, leg. H. Teppner. – Grown in the Botanic Garden at the Institute of Plant Sciences of the University of Graz.

Solanum lycopersicum L. 'Mirabell' (*Solanaceae*). Origin: Seed trade, firma Julius Wagner, 69120 Heidelberg, Germany. – Grown in a private garden in Graz-Gries.

Onosma heterophylla Griseb. (*Boraginaceae-Lithospermeae*), tetraploid Peloponnes-populations in the sense of Teppner 1991: 39(3). Origin: Greece, Peloponnisos, Langada Gorge; 27.6.2005; leg. R. Karl. – Grown in the Bot. Garden at the Institute of Plant Sciences, University of Graz, cult. no BOR 1152/5, sown 19.12.2005.

Onosma frutescens Lam. (*Boraginaceae-Lithospermeae*). Origin: Turkey, Becin near Milas; 25.5.2005; leg. F. Wolkinger. – Grown in the Bot. Garden at the Institute of Plant Sciences, University of Graz, cult. no BOR 1168/1, sown 19.12.2005.

Vouchers in the herbarium GZU.

Inflorescences or flowers were pushed with a needle so that the pollen fell out on a slide (cleaned with ethanol). The cover slip should be held at a distance from above the slide a little larger than the pollen grains. In the case of polyads or larger pollen grains addition of fragments of a cover slip is the simplest way. In the case of small grains thin hairs are suited (danger of contamination with fat should be kept in mind). Later, the front-lens of the objective should not touch the cover slip when grains in the second layer, lying on the slide, are observed. A cover slip is put thereon and surrounded with wax, vaseline or something else. Then the slide is inverted and knocked against a table edge. Thus, pollen grains will fall and adhere to the lower side of the cover slip and the contact between grain and cover slip can be observed in the LM. In this way pressure on the pollen grains, which would flatten the grains and enlarge the contact zones, is avoided. On the other hand, in critical cases, it is possible to observe in the same preparation also the upper side of the pollen grains which lie on the slide only and to compare the upper side of grains with and without contact with the cover slip.

For comparison, preparations of fresh pollen in water (eventually addition of Sudan III) and in 45 % acetic acid were also used. All preparations were investigated immediately after production. After days or weeks the image can change, for e. g., in air the contact plaques can increase!

TROLL 1928: 328 used methylene-green-acetic-acid (1g methylene-green in 50 ml dest. water, then a drop of glacial acetic acid added) in *Hippeastrum* to visualize pollenkitt. I tested the method in four of the ten species; the pollen was nicely coloured but in respect of the identification of pollenkitt the results seemed in only one case (*Onosma*) to be significantly different to that in water and acetic acid.

For the LM investigations a ZEISS Photomikroskop III was used, provided 1978 by the Fonds zur Förderung der wissenschaftlichen Forschung (Austria). The negatives were scanned with Epson Perfection 2400 Photo and the figures were edited with Adobe Photoshop CS3.

3. Results

3.1. *Antirrhinum majus* L.

As an example for copious pollenkitt the pollen of *A. majus* is choosen. It does not fall out from the anther but must be scratched from the thecae. During this preparation long, fine threads can be seen, which originate from the dissolved septum and the margin of the stomium, and contribute to maintain the pollen as a compact mass in the open thecae. At least in our material, these threads are so clear and easy to see, that it is surprising that *Antirrhinum* is not mentioned in the survey of HESSE & al. 2000. The pollen forms large clumps and sticks well on the slide so that, with the described method, only few grains adhere to the lower side of the cover slip. They show large, apparent contact plaques roundishly circumscribed or the border is somewhat modified by the exine sculpture (Fig. 1–3). Pollenkitt is located all around the grains (plaques in all positions).

The plaques appear homogenous when focused (because then the exine structure of the grain lies below the optical plane) and are surrounded by a dark line with a narrow bright halo. They are so thick, that the optical plane does not touch the exine. Thus, from the reticulate structure of the exine only a blurred figure of the negative image (on LO-analysis see, e. g., ERDTMANN 1969: 46–47) is seen, when the plaque is focused (Fig. 2).

Fig. 1. *Antirrhinum majus*, a clump of pollen grains hanging on the lower side of a cover slip, with contact plaques. – Scale bar equals 25 µm.

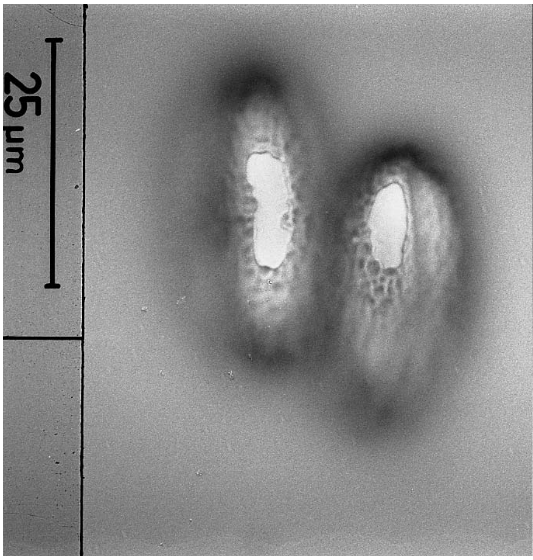

Fig. 2.

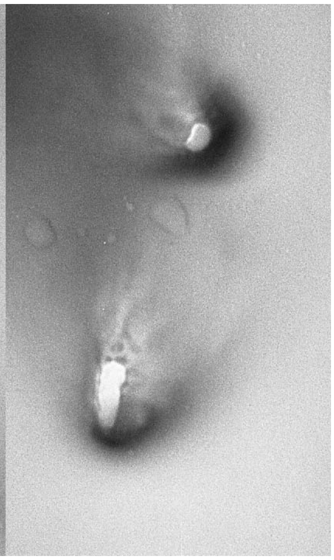

Fig. 3.

Fig. 2–3. *Antirrhinum majus*, pollen grains on the lower side of a cover slip. – Fig. 2. Two pollen grains with one large contact plaque on the intercolpium each. Unsharp negative image of the exine structure. – Fig. 3. Two pollen grains with contact plaques at and near a pole respectively. Some patches of pollenkitt on the cover slip.

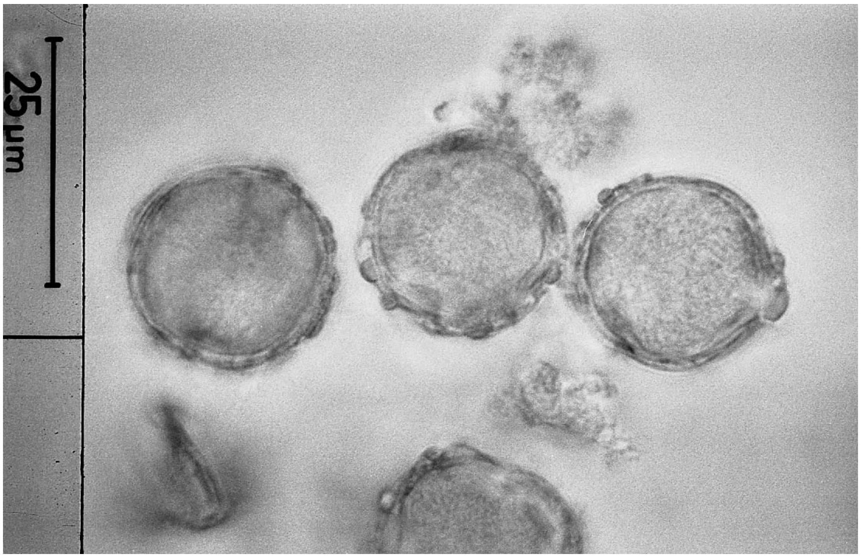

Fig. 4. *Antirrhinum majus*, pollen grains in water, optical section, oily drops on the surface.

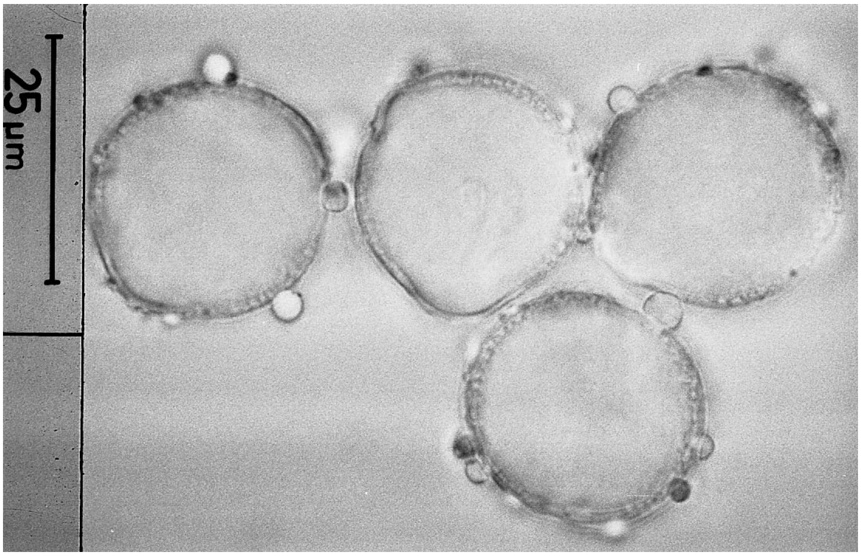

Fig. 5. *Antirrhinum majus*, pollen grains in acetic acid, optical section, oily drops on the surface.

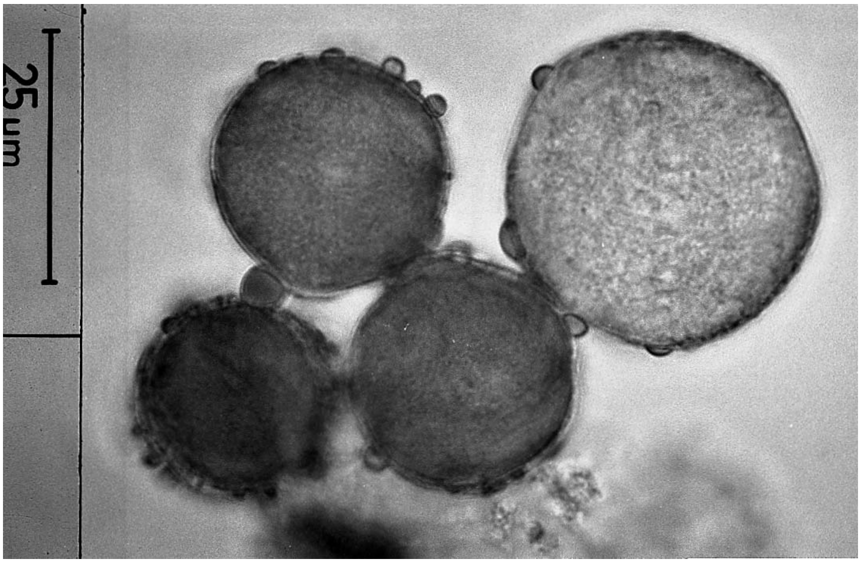

Fig. 6. *Antirrhinum majus*, pollen grains in methylene-green-acetic-acid, optical section, oily drops on the surface.

In water the pollen grains show many, more or less hemispherical light refracting droplets (Fig. 4). In diluted acetic acid many, apparently light refracting, predominantly roundish droplets cover the grains (Fig. 5); many free themselves from the grains, ascend and assemble in a layer under the cover slip. In methylene-green-acetic-acid also many hemispheric to round droplets are visible (Fig. 6). A high amount of pollenkitt was observed directly in air, all pollen grains show droplets in water etc., so it is clear that all or at least the largest part of the droplets must be pollenkitt.

3.2. *Acacia cyclops* A. CUNN. ex G. DON

The polyads of *A. cyclops* do not show droplets of pollenkitt, neither in water nor in diluted acetic acid. Because of the strong diffraction effects in the LM at the periphery of the polyads no clear layer of pollenkitt is discernible. In polyads ahering to the under side of a cover slip always small to very small, bright contact plaques between the polyad and the glass are formed (Fig. 7, 8). The plaques arise in all positions of the polyad, on the flat side as well as on the narrow margin and appear on the central arch of the single grains, as well as on the peripheral ridges. Thus the whole polyad is surrounded by a homogenous layer of pollenkitt; the layer is very thin, as can be seen from the always small contact plaques. From the acacias we investigated, *A. cyclops* is the species with the smallest amount of pollenkitt (smallest contact plaques). So the adherence of the polyads on

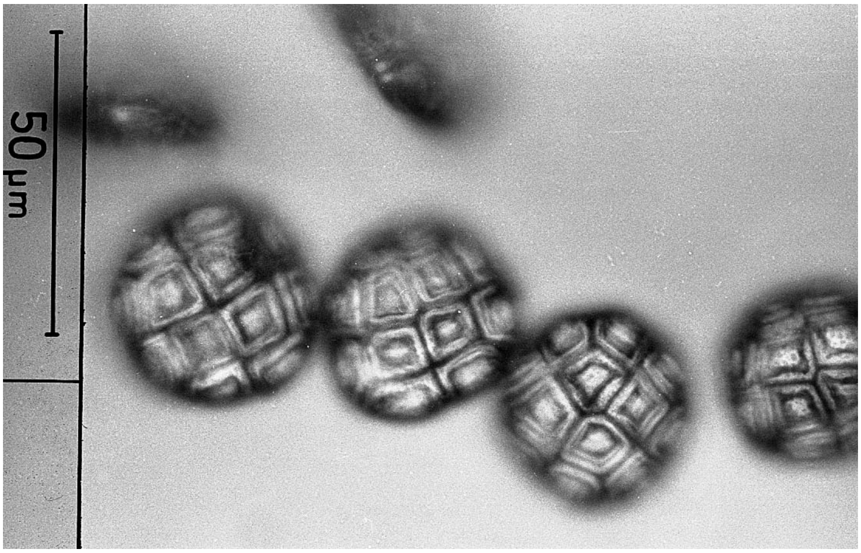

Fig. 7. *Acacia cyclops,* four polyads in face view, two in side view, all with one to seven more or less small contact plaques on the arch or on the marginal bulges of the single grains.

Fig. 8. *Acacia cyclops,* one polyad in face view with two larger and seven very small contact plaques. Polyads in side view with two or three contact plaques, respectively.

the inner side of the valves in the open theca is sufficiently explained, even if other *Acacia* species often show larger amounts of pollenkitt (larger contact plaques, e. g. *A. celastrifolia*, TEPPNER 2007b: 37,38.

3.3. *Leucaena leucocephala* (LAM.) DE WIT

L. leucocephala has sticky pollen grains which form large clumps in the anther or when pushed out on the slide. The pollen adheres well on the under side of a cover slip. The grains show distinct, often large contact plaques of pollenkitt. The contours of the plaques are more or less roundish at the smooth poles or strongly modified by perforations, furrows and roughness of the tectum (Fig. 9–11) on the intercolpi. At the places with most distinct structure in the centre of the intercolpi, contact plaques appear disintegrated (Fig. 12–13) and therefore in this case are often difficult to discern. Nevertheless a layer of pollenkitt seems to cover the whole grain but with the traditional methods I was not able to prove pollenkitt: In water and 45 % acetic acid a fine line surrounds the whole grain (pores included; Fig. 14, 17) which I interpret as diffraction ring. Then a thin hyaline layer lies close to the tectum (Fig. 14, 17) which is not shown in acetolyzed material (GUINET 1966: 39, SORSA 1969); thus I suppose this to be pollenkitt but unfortunately this layer is not stained by Sudan III (Fig. 17). Furthermore in water and much more in acetic acid, strongly refracting droplets are formed (stained by Sudan III) whereas the thin hyaline layer appears intact, even after weeks; the droplets are most abundant and largest in and on apparently degenerated grains (Fig. 16). They occur also in the intact grains and can be observed here above the grain, in the optical section of the grain and within the grains, especially under the pores (Fig. 14, 15, 17). In spite of careful cleaning of slide and cover slip with ethanol, many such droplets appear in the upper layer of the preparation, especially in acetic acid. What is the origin and nature of these droplets ? I don't think they can be pollenkitt, at least not all of them, when simultaneously the outer most, thin, hyaline layer seems to be intact. In methylene-green-acetic-acid (Fig. 18, 19) only the thin hyaline layer seems to be disorganized with the time and only rarely traces of it can be seen after some weeks, whereas many droplets cover the grain.

3.4. *Anthoxanthum odoratum* L.

If prepared in the given manner, in this grass the bulk of the grains lies on the slide and a minority hangs on the lower side of the cover slip. From these a part of the grains show small but distinct contact plaques (Fig. 20, 21, 23) whereas the other part adheres by adhesion (Fig. 22; or is the amount of pollenkitt so small that it is not visible ?). The contact plaques are ± roundish and sharply limited; irregular margins occur in old preparations where pollenkitt probably has desiccated. The tips of arches and

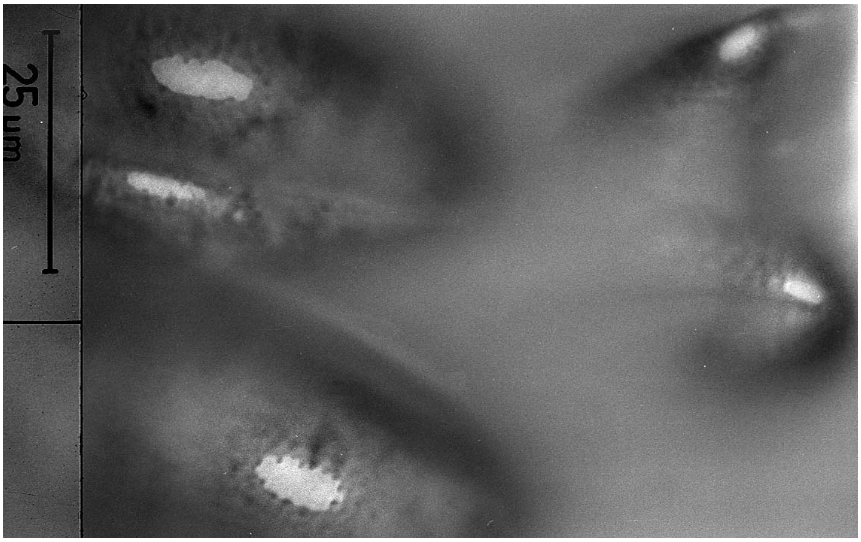

Fig. 9. *Leucaena leucocephala,* clump of pollen grains in air, hanging on the lower side of a cover slip. In the left, contact plaques on the side of grains (intercolpia), above two, below one contact plaque with indentions at the tectum pores. Right two grains with contact near the poles, contact plaques smaller and more roundish borders.

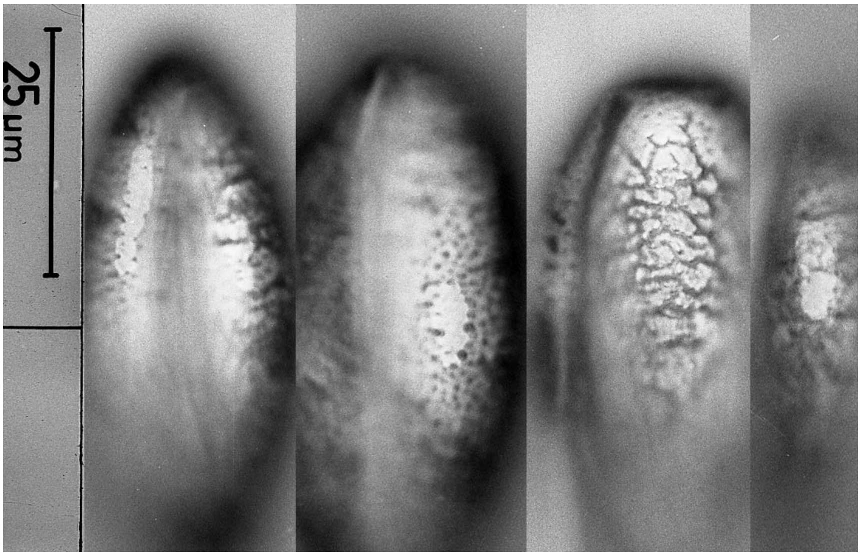

Fig. 10. Fig. 11. Fig. 12. Fig. 13

Fig. 10–13. *Leucaena leucocephala,* pollen grains in air, hanging on the lower side of a cover slip. – Fig. 10. Two lateral contact plaques on the intercolpia with indentions at the tectum pores. – Fig. 11. As in Fig. 10, with one contact plaque at the intercolpium. – Fig. 12. Disintegrated contact plaque in the centre of the intercolpium. – Fig. 13. Partly disintegrated contact plaque in the centre of the intercolpium.

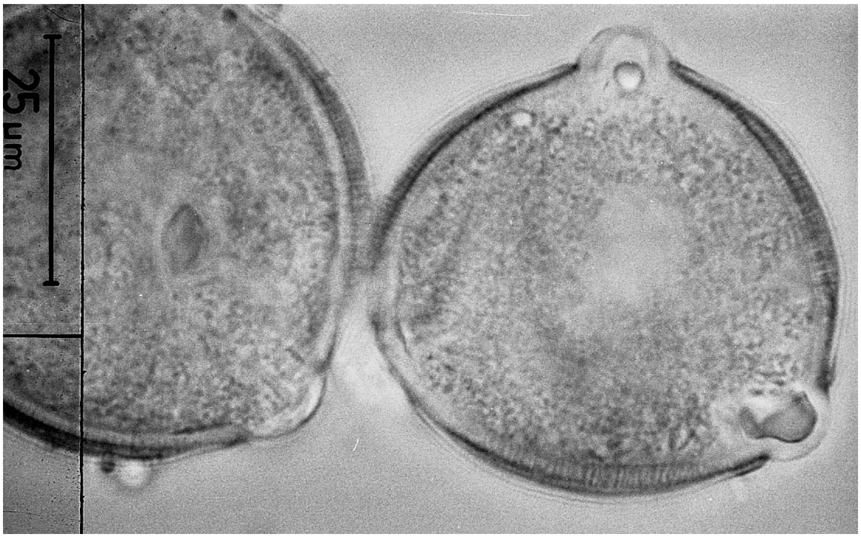

Fig. 14. *Leucaena leucocephala*, two pollen grains in acetic acid. Oily droplets within the grains, especially under the pores and outside (below left). – The outermost hyaline layer ending near the pores is interpreted as pollenkitt.

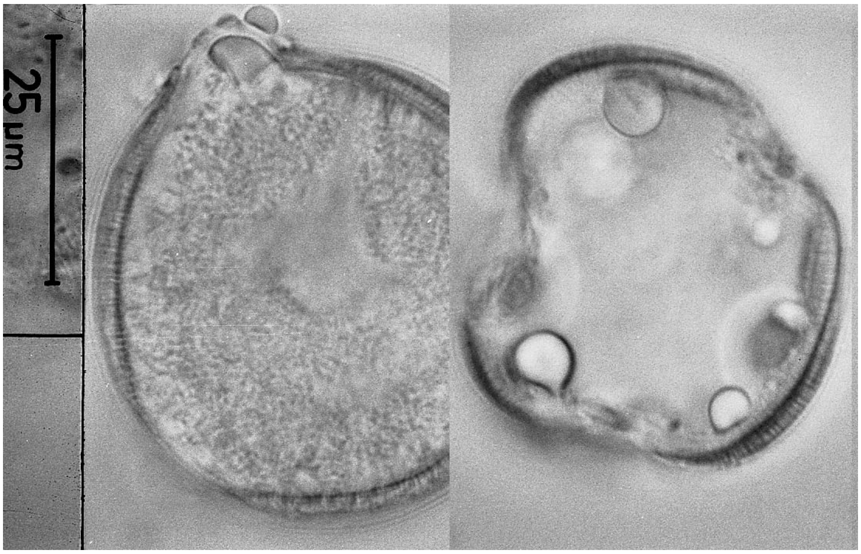

Fig. 15. Fig. 16.

Fig. 15–16. *Leucaena leucocephala*, pollen grains in acetic acid. – Fig. 15. Oily drops inside and outside of a pore. – Fig. 16. A degenerated pollen grain with oily drops within the grain. – The outermost hyaline layer ending near the colpi or pores is interpreted as pollenkitt.

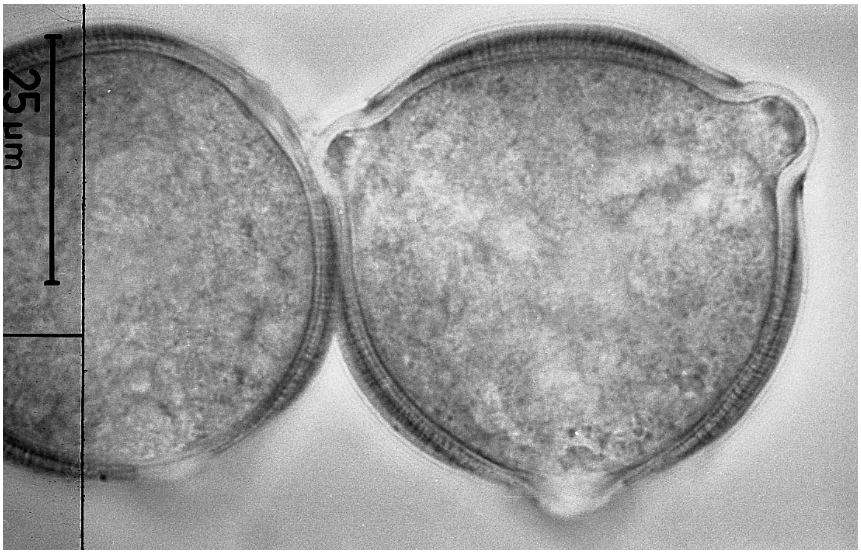

Fig. 17. *Leucaena leucocephala*. Two pollen grains in water with Sudan III, oily drops especially under the pores. The outermost hyaline layer ending near the pores is interpreted as pollenkitt.

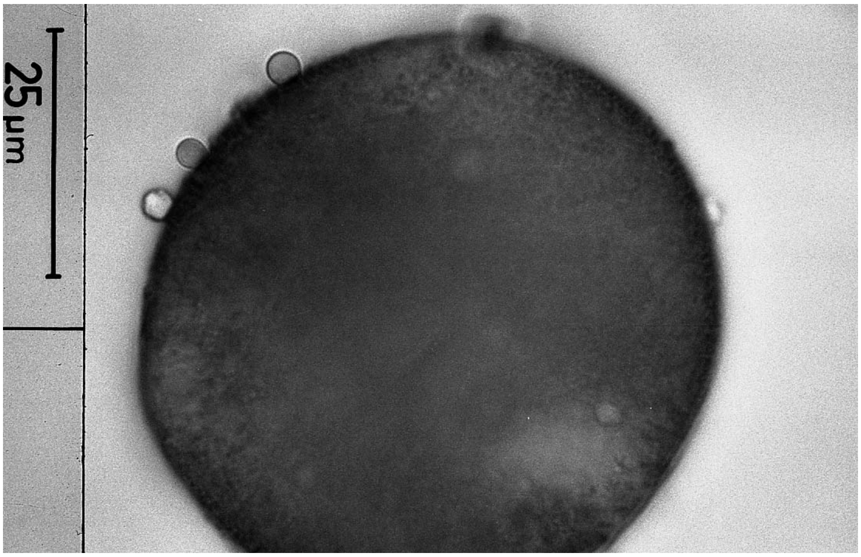

Fig. 18. *Leucaena leucocephala*. Pollen grain in methylene-green-acetic-acid, optical section. Oily drops on and within the grain. At the right side traces of the hyaline layer seen in Fig. 14–17.

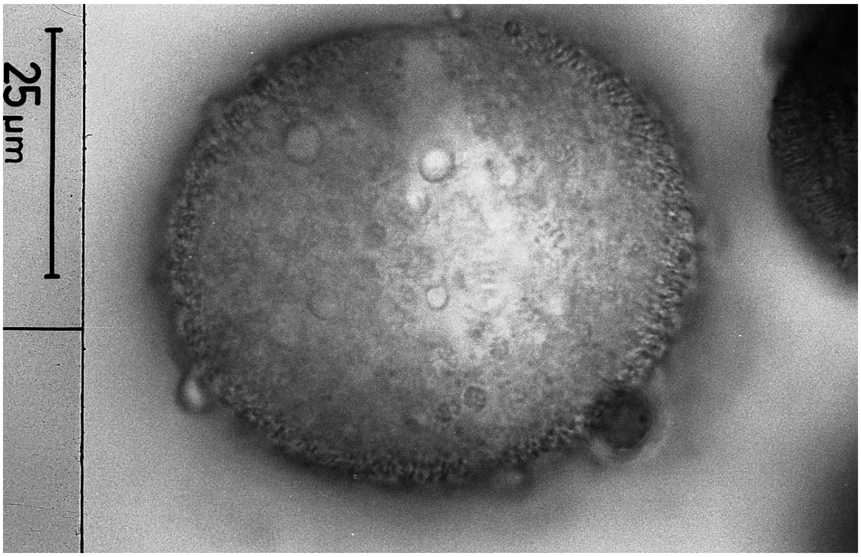

Fig. 19. *Leucaena leucocephala*. Pollen grain in methylene-green-acetic-acid, many oily droplets within (centre) and on the grain (periphery).

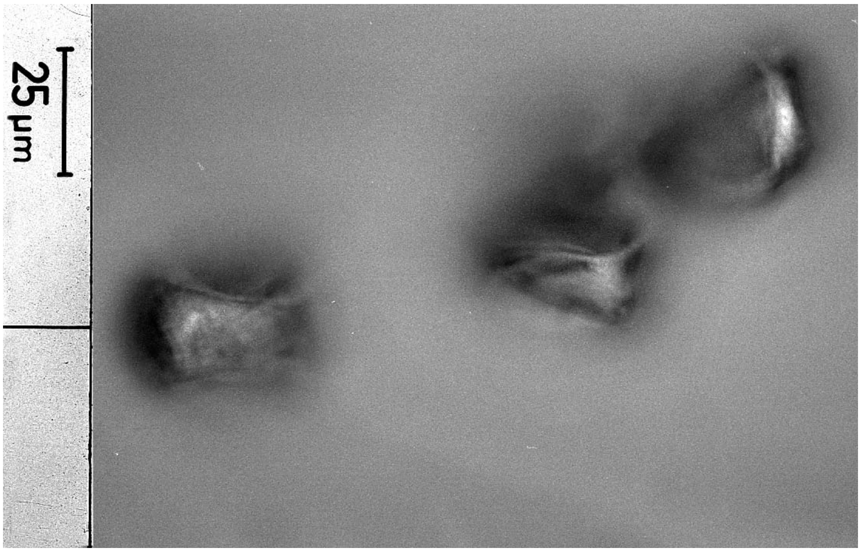

Fig. 20. *Anthoxanthum odoratum,* three grains hanging on the lower side of a cover slip, left and right grain with small contact plaques. On the grain in the middle the plaque is so small that it was seen directly in the microscope, only.

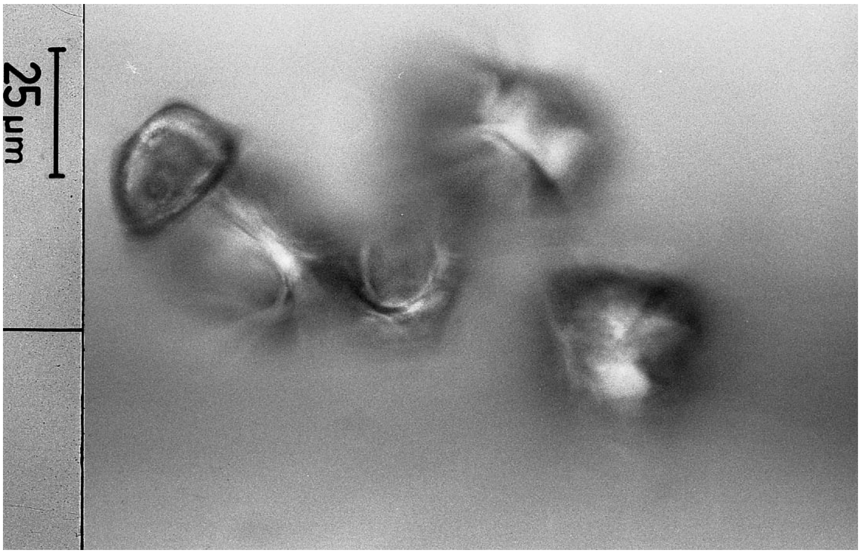

Fig. 21. *Anthoxanthum odoratum*, small, dinstict contact plaques on the lower three grains.

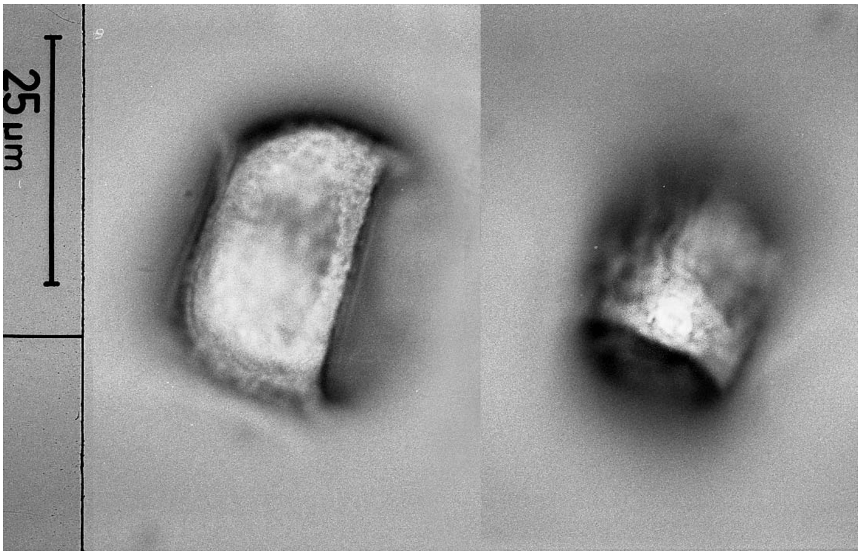

Fig. 22. Fig. 23.

Fig. 22–23. *Anthoxanthum odoratum*, pollen grains in air hanging on a cover slip. – Fig. 22. Pollen grain without contact plaque. – Fig. 23. Pollen grain with contact plaque.

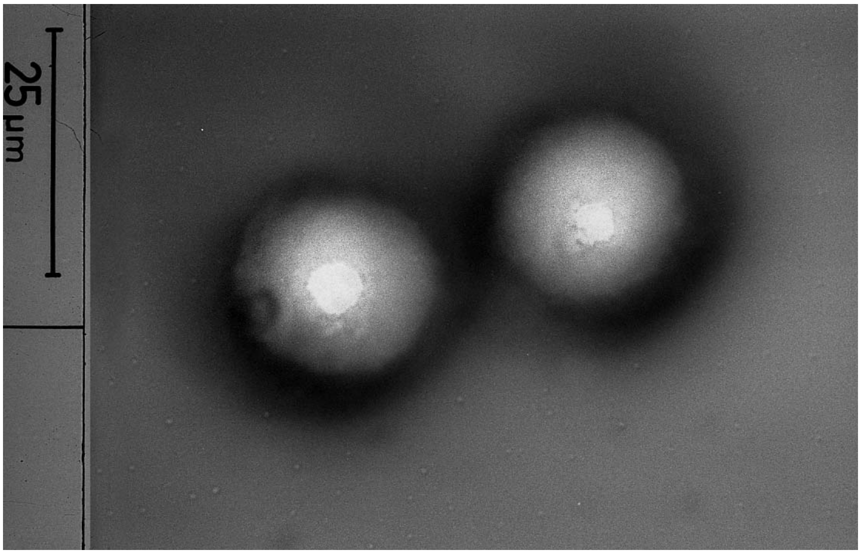

Fig. 24. *Lolium perenne*, two pollen grains with one round contact plaque at the cover slip, each. Left, a porus out of the optical plane.

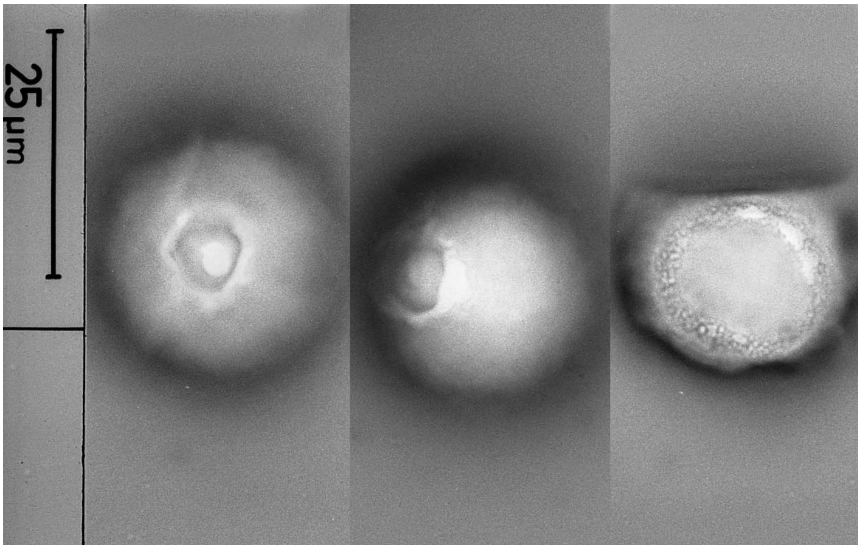

Fig. 25. Fig. 26. Fig. 27.

Fig. 25–27. *Lolium perenne*, pollen grains in air hanging on the lower side of a cover slip. – Fig. 25. One large contact plaque at the operculum, smaller ones at the annulus. – Fig. 26. One contact plaque at the annulus. – Fig. 27. Grain with a depression and three contact plaques on its right margin. Otherwise the exine structure in the optical plane.

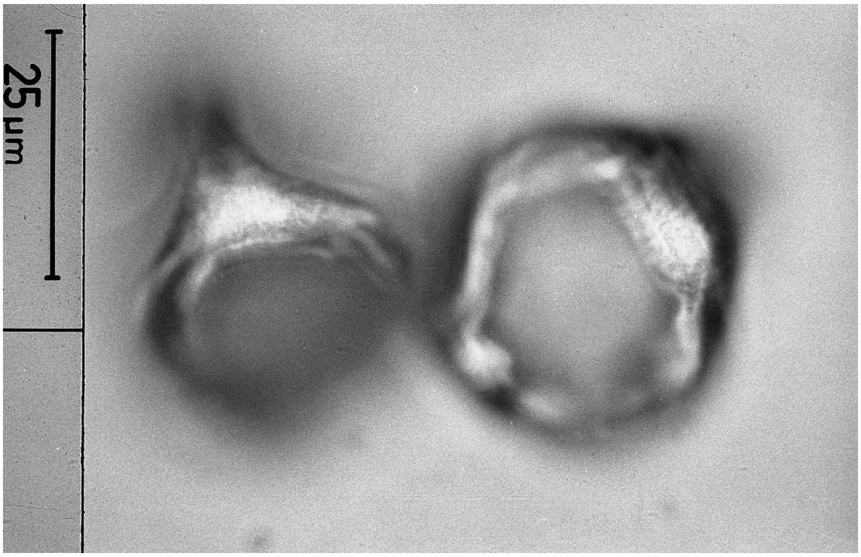

Fig. 28. *Luzula campestris*, tetrads in contact with the lower side of a cover slip. View of the edge between three grains (left) and on the distal side of a grain (right), without contact plaques, the light patches are results of lens effects.

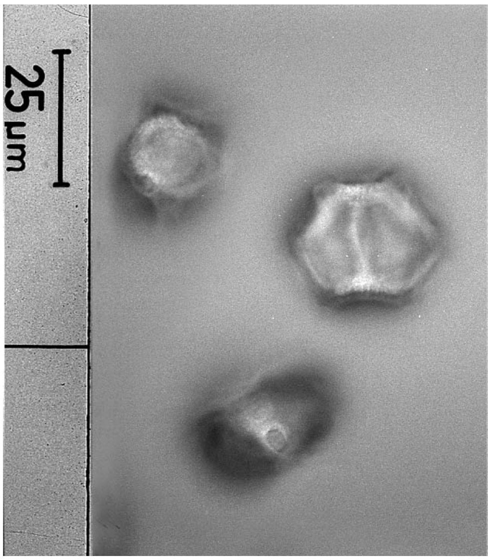

Fig. 29.

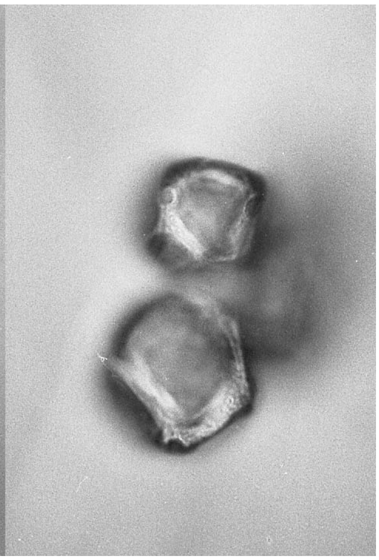

Fig. 30.

Fig. 29–30. *Alnus alnobetula*. Pollen grains hanging on the lower side of a cover slip without contact plaques. Lens effects and exine structure visible.

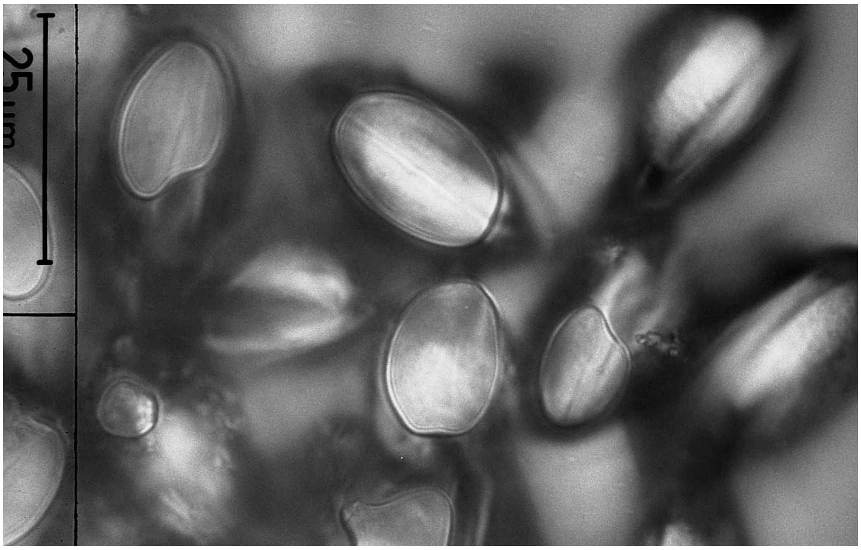

Fig. 31. *Solanum lycopersicum*, a clump of pollen grains in air, in contact with the lower side of a cover slip, showing large contact plaques. Some grains without contact.

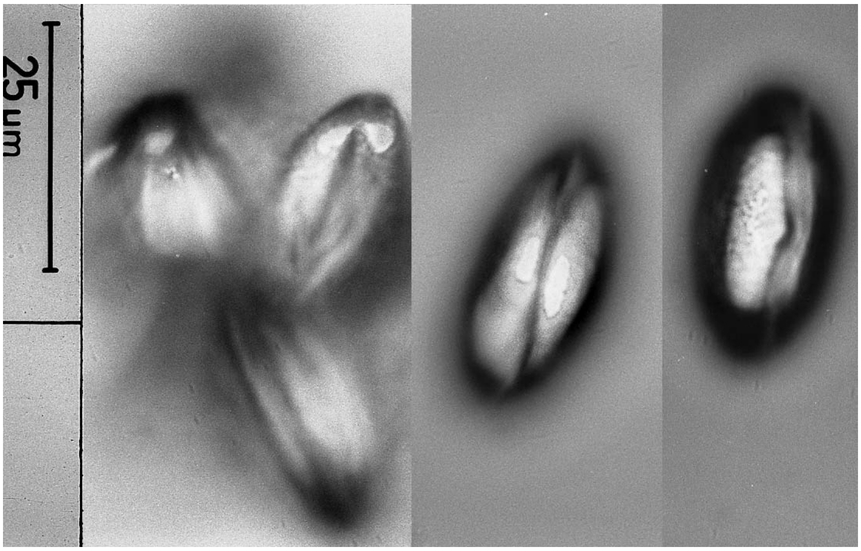

Fig. 32. Fig. 33. Fig. 34.

Fig. 32–34. *Solanum lycopersicum* pollen grains in air. – Fig. 32. Clump of pollen grains, the upper two with one and two contact plaques, respectively, near a polar end. – Fig. 33. Grain with compact contact plaques on both sides of the colpus. – Fig. 34. Grain with a somewhat irregular contact plaque and distinct LO-pattern on a intercolpium.

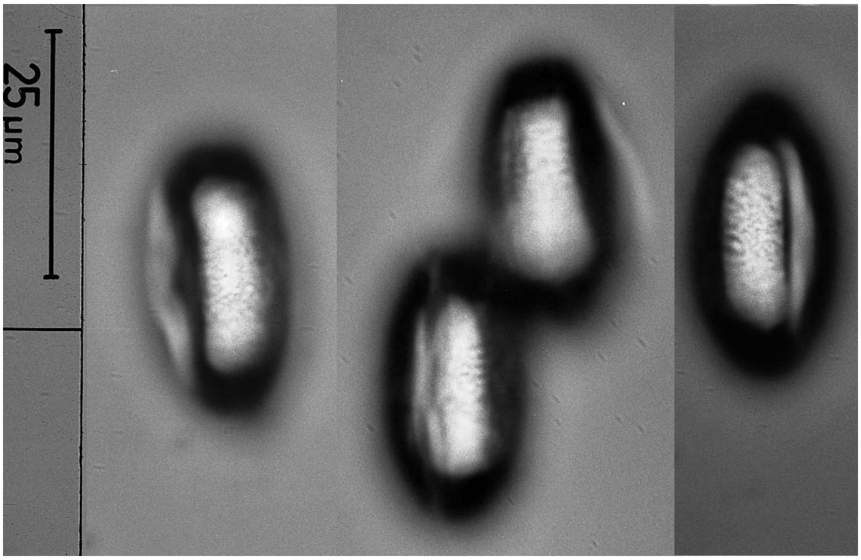

Fig. 35. Fig. 36. Fig. 37.

Fig. 35–37. *Solanum lycopersicum* pollen grains in air, with largely partite contact plaques, in Fig. 37 not to be distinguished from the LO-pattern.

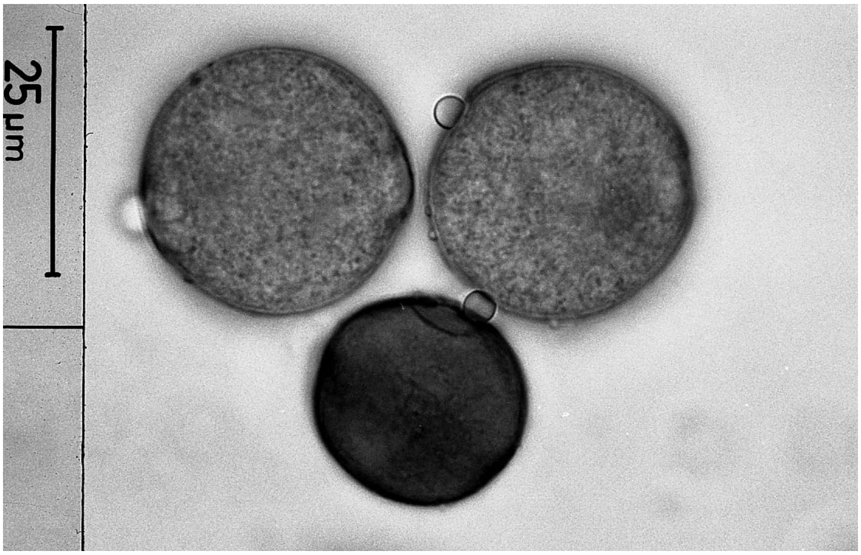

Fig. 38. *Solanum lycopersicum* pollen grains in methylene-green-acetic-acid, exceptionally with distinct droplets.

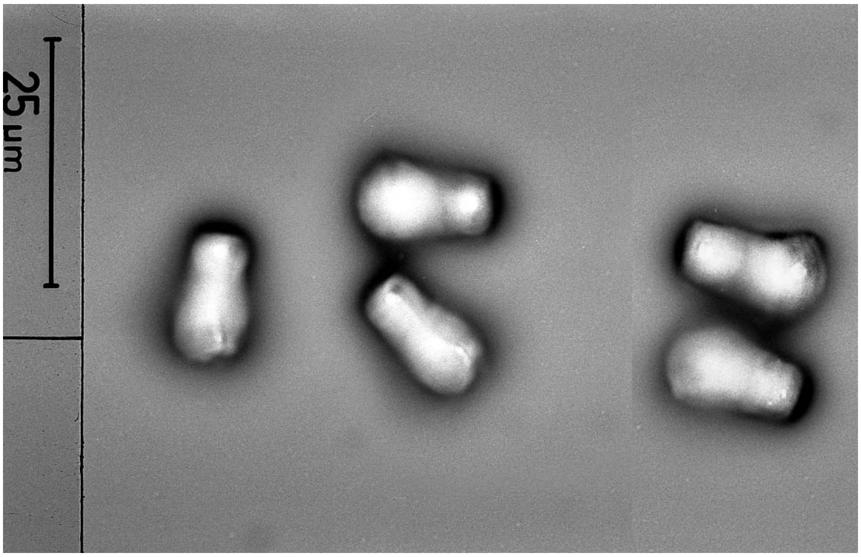

Fig. 39. Fig. 40.

Fig. 39–40. *Onosma heterophylla*, pollen grains in air with contact plaques on the lower side of a cover slip. – Fig. 39. Three grains with one contact plaque each at the sides of the narrow (proximal) end. – Fig. 40. The upper grain with two contact plaques.

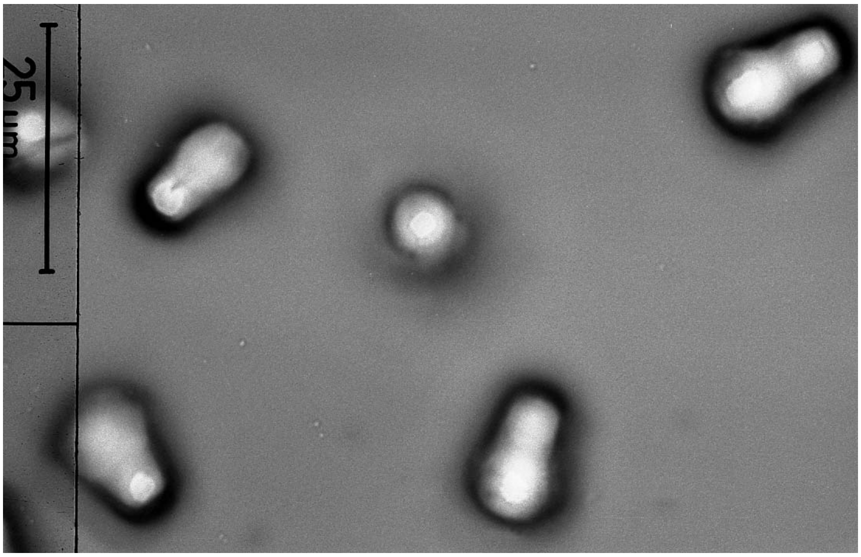

Fig. 41. *Onosma heterophylla*, pollen grains with contact plaques on the lower side of a cover slip. Four grains in side view, one with two, three with one contact plaque each; two grains in polar view with one contact plaque at the proximal end.

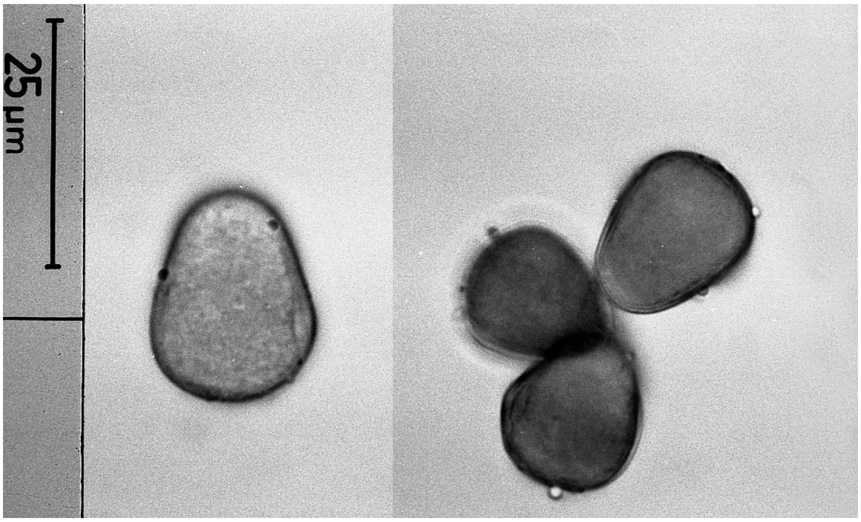

Fig. 42. Fig. 43

Fig. 42–43. *Onosma heterophylla*, pollen grains in methylene-green-acetic-acid. – Fig. 42. Minute droplets immediately after preparation. – Fig. 43. Three grains after one hour with distinct droplets.

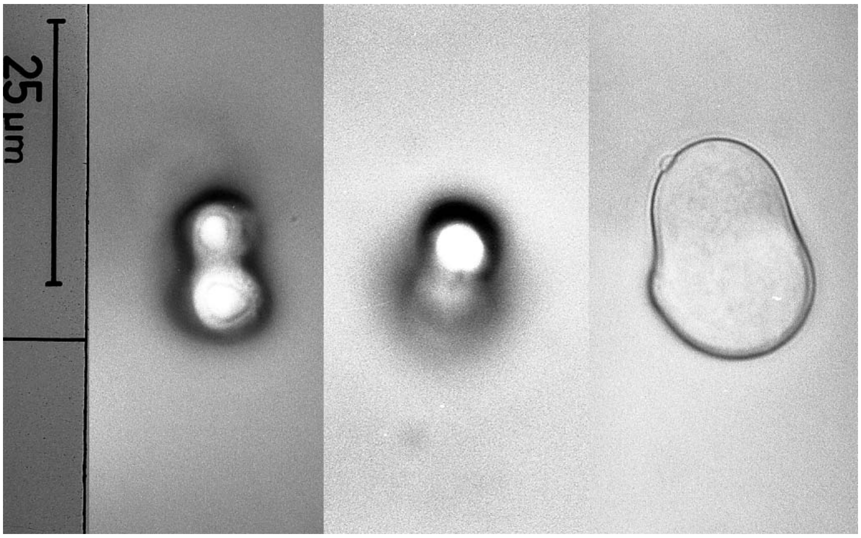

Fig. 44. Fig. 45. Fig. 46.

Fig. 44–46. *Onosma frutescens*. – Fig. 44. Pollen grain in air in side view with two contact plaques. – Fig. 45. Oblique polar view with one contact plaque at the proximal end. – Fig. 46. Pollen grain in water, side view, with one droplet near the proximal pole.

bulges of the grain produce lens effects and appear also bright; they have gradual transitions marginally and the first to be seen at the glass when focusing from above is the negative image of the LO-pattern. So presence or lack of contact plaques usually is easy to distinguish. Because of the small amount of pollenkitt in the case of the thinnest plaques the optical plane is very near the exine when focused on the plaques. Thus, beside the plaque the exine structure is visible at the same time especially in form of their negative image. From the grains without discernible contact plaque and the low number of grains attached to the lower side of the cower slip it can be concluded that only patches of pollenkitt are present on the surface of the grains and not a complete layer or that a part of the grains remain free of it.

3.5. *Lolium perenne* L.

In this grass, as in *Anthoxanthum*, only a small part of the pollen grains hangs on the lower side of the cover slip. From these, only in few grains no contact plaques are present. In average, the plaques are a little larger (Fig. 24–27) than in *Anthoxanthum*. It can be concluded also, that otherwise the same is true as said for the latter. *Lolium* has been already investigated by POHL 1929: 296.

3.6. *Luzula campestris* (L.)DC.

In different preparations few to surprisingly many tetrads adhered to the cover slip. In not a single tetrad a trace of a contact plaque was found, at least when slide and cover slip were cleaned with ethanol. All bright spots are caused by lens effects and the first sharply visible structure when focusing from above is always exine structure (Fig. 28). Thus, visible amounts of pollenkitt are apparently completely lacking in *Luzula campestris*, as was already stated by POHL 1929: 297.

3.7. *Alnus alnobetula* (EHRH.) K. KOCH

A small percentage of grains adhered to the cover slip. Archs and bulges of the ± folded grains cause bright spots by lens effects; the transition of the spots to the surrounding area is gradual. When focusing from above the bright spot, exine structure is always the first to be seen (Fig. 29, 30). Never any trace of a contact plaque was to be seen, therefore we can conclude that pollenkitt is not present, at least not in visible amounts.

3.8. *Solanum lycopersicum* L.

The pollen released from the anther tube on the one hand is dusty, on the other hand clumps fall out. Approximately the same quantity of pollen was lying on the slide as was hanging on the cover slip. In clumps the grains show large contact plaques of sticky material (Fig. 31, 32); it is not sure, if this are contaminations from the stigma or the hairs in the anther tube or – much more probably – the pollenkitt is not evenly distributed within the thecae.

In the single grains of the dusty part of the pollen only small contact plaques (Fig. 33) can be seen in all positions around the grains (grains exactly perpendicular to the slide excepted because of adverse optical effects). Such ± compact plaques occur in the minority of the grains hanging on the cover slip. Usually the contact area is interrupted (Fig. 34, 35). The layer of pollenkitt usually is so thin, that the negative of the LO-pattern of the exine is visible beside the contacts (Fig. 34). Often the contact is so finely partite that it appears similar to the LO-pattern of the exine (Fig. 35–37). May be the pollenkitt extrudes from the tectum pores and the quantity is too small for flowing together of the single elements to homogenous plaques. In these cases contact and LO-pattern are no longer to be distinguished without doubt and pollenkitt can no longer be proven directly (Fig. 37). The situation is similar to that in *Leucaena leucocephala* (Chapter 3.3.) but is more difficult to demonstrate because of the smaller grains and finer exine structure. Often the area around the contact is so bright due to lens effects, that it can also be difficult to discern the contacts (Fig. 35, 36).

In water small light refracting structures (droplets) are very rare. In diluted acetic acid also no sure traces of pollenkitt were to be ascertained. Rarely, on single grains, 1-3 very small light refracting droplets can be seen, but the number of such structures is so small, that they can in no case be the pollenkitt, which is apparently present on most of the grains. In methylene-green-acetic-acid the situation was similar: droplets are very rare, only degenerated grains show verkittung ('cementation'; term used, for e. g., by HESSE 1981). At only one place in the preparation, more droplets were seen (Fig. 38, corresponding to a clump of pollen ?).

3.9. *Onosma heterophylla* GRISEB.

The majority of the pollen grains adheres to the under side of the cover slip. These grains show minute to medium contact plaques which, in part, can be so small that it becomes difficult to distinguish them from the LO-pattern of the exine (Fig. 40, lower grain). Contact plaques are possible in all positions of the grain, near the proximal (narrow) end (Fig. 39, 41) as well as near the wide (distal) end of the grain (Fig. 41, below, right) sometimes near both ends (Fig. 40, 41). Thus pollenkitt must be present all around the grain.

In water sporadically the one or the other light refracting drop appears in few grains. In diluted acetic acid in the minority of grains many small droplets are distributed over the surface of the grains. How is this compatible with the fact, that pollenkitt was seen in air in nearly all grains ?

In methylene-green-acetic-acid immediately after preparation the situation is similar, only a small part of the grains show one to few small droplets (Fig. 42). But c. one hour later most of the grains show a number of droplets (Fig. 43). Are all these droplets pollenkitt or are they extruded from the grains ?

3.10. *Onosma frutescens* LAMARCK

Only the smaller part of the grains adhered to the under side of the cover slip, in spite of the fact that, in average, the contact plaques (Fig. 44, 45) were a little larger than in *O. heterophylla*.

In water most of the grains appear with one to few light refracting droplets (Fig. 46). In acetic acid there are abundantly more small dots which can be seen in surface view but are usually not apparent in the optical section. How does this complement with the relatively rich presence of pollenkitt in nearly all grains ?

4. Discussion

Spiny and otherwise strongly sculptured pollen is related with large amounts of pollenkitt, thus its proof is usually no problem. Thus the more or less smooth grains in which all transitions from large amounts of pollenkitt to lack of pollenkitt may occur, are the subject of this paper. The aim is, to check the possibilities with the LM. The proof of the presence of pollenkitt with the TEM is another issue, with good results (e. g., HESSE 1980, for review PACINI & HESSE 2005), but not suited as routine method because of the high efforts.

Pollenkitt as a viscous fluid shows the respective characteristics. If a grain approximates to the cover slip, the pollenkitt leaps to the glass and forms a contact plaque, surrounded by a hollow. Due to the properties of an optical gel, similar as in the case of immersion oil, the plaque appears bright and surrounded by a dark line and a bright halo. The plaques appear ± homogenous, because the optical plane lies above the exine. In the cases of very few pollenkitt and therefore thin plaques, the optical plane approaches to the exine or lies in the tectum, so that, besides the plaque, the negative image of the exine structure is visible. Due to the lack of exine structure (and sharp margin) plaques are to be distinguished from brightness caused by lens-effects.

For, e. g., in *Acacia* some authors write about the absence of pollenkitt (e. g., BERNHARDT 1989, PACINI & FRANCHI 1993:6). If one observes anther opening and pollen presentation, than it can be seen that the polyads adhere to the valves of the thecae. Why ? Really, in *Acacia* it is extremely difficult to prove the presence of the pollenkitt and I was not able to do so with the usual methods. Using the characteristics of an optical gel, it is easy to show respectable amounts of pollenkitt all around the polyads and the presentation of *Acacia* polyads on the valves becomes understandable.

In the cases of ± round grains or polyads the possibility for size and form of the contact plaques is the same all around the grain. In flattened polyads or elongated grains the possibilities are very different. At the flat sides of polyads or in the equatorial region of elongated grains large plaques are possible. At the narrow margins of polyads or tips of elongated grains only small plaques will be possible. In acacias with pseudocolpi the central arch of the grains shows large, whereas the marginal bulges only small contact plaques.

In plants with the wind pollination syndrome species with small amounts of pollenkitt occur as well as such without visible amounts of pollenkitt. This was already proven by POHL 1929, especially pollenkitt in *Poaceae* and its lack in *Alnus* and *Luzula* was ascertained by him. With the help of the TEM HESSE 1980: 252–255 has shown traces of pollenkitt in and on the exine of *Alopecurus* and *Sesleria*. It is often said (e. g., PACINI 1997: 1453, PACINI 2000: 33, 37) that in strictly anemophilous species a tapetal membrane with orbicules is present, whereas pollenkitt should be lacking. *Poaceae*, well investigated examples for orbicules (e. g., BANERJEE 1967, EL-GHAZALY & JENSEN 1986) clearly possess pollenkitt too (h. l. and POHL 1929). Probably in grasses, in calm, the pollen would fall immediately from the opened anthers to the soil and get lost if not a minimum of pollenkitt would be present. Such a danger is not given in *Luzula* with more or less erect flowers or in *Betulaceae*, where the scales of the catkin gather the pollen. KNOLL 1930 supposed, that in all cases of pollen grains, which form threads or clumps, pollenkitt must be present, even if nothing can be seen, and thus contradicted POHL in the case of, e. g., *Alnus* and *Taxus*. *Alnus* is probably not investigated in the TEM, but in the anemophilous *Quercus robur* (*Fagaceae*) HESSE 1978b: 32–34 has shown pollenkitt in the exine and in minute amounts on the tectum.

If grains with and without contact plaques are to be observed, then it is either, that the small amounts of pollenkitt are unevenly distributed and do not cover the whole grain or pollenkitt is completely lacking in a part of the grains. If no contact plaques can be seen in a given angiosperm species, the next step would be the investigation in the TEM for small amounts far from the visibility in the LM. So HESSE 1980 has shown pollenkitt traces on the intectate exine in *Luzula sylvatica* whereas I have not found any trace in *L. campestris* in the LM. If such traces have any effect in relation of floral ecology is another question, but is doubtful to improbable in my opinion. Adhesion and electrostatic forces (for review: VAKNIN & al. 2000) may be important in such cases.

It lies in the nature of the subject, that in cases with few pollenkitt not in all grains contact plaques must be distinct or present and a larger number of grains has to be checked for a correct impression. The smaller the amount of pollenkitt (the contact plaques), the higher is the probability for only small dots in the dimension of the LO-pattern of the exine. So it will become more or less impossible to decide about the presence of pollenkitt.

In the streukegel blossoms (scatter cone blossoms) with its powdery pollen one would expect few or no pollenkitt – as often said in the literature. For *Galanthus nivalis* SCHOENICHEN 1922: 40, 56, TROLL 1928: 341 and especially KNOLL 1930: 660–663 have proven the presence of a distinct amount. KNOLL describes the pollen grains in air as smooth, whereas in water after some minutes many oil-droplets appear; he had no doubt, that the droplets correspond to pollenkitt. Surprisingly HESSE 1981: 146–147 in the TEM has found pollenkitt only in the exine cavities of *Galanthus* pollen and not on the tectum and in the tectum pores. The other descriptions of streukegel blossoms in SCHOENICHEN 1922: 40–42 do not include indications of pollenkitt. *Solanum lycopersicum* and *Onosma* possess small to medium amounts of pollenkitt, distinctly more than wind pollinated plants with pollenkitt (*Poaceae*). KNOLL 1930: 663 postulates that the streukegel blossoms of *Galanthus* and *Leucojum* in respect to amount and viscosity of pollenkitt are intermediate between wind-pollen and typical insect-pollen. In *Solanum lycopersicum* apparently groups of pollen grains with more pollenkitt than in the remaining anther occur (as shown in *Secale cereale* by POHL 1929: 296). Presence of pollenkitt in *S. lycopersicum* was already mentioned by KING & FERGUSON 1994: 481 (accepted by DOBSON & BERGSTRÖM 2000: 68). They have also shown, that from the *Actinidia* anthers by buzzing clumps of pollen grains are ejected and discuss the importance of some stickiness for the pollen collecting bees. Such a need is to be evaluated, because bees are also able to collect wind-pollen. CORBET & al. 1988: 154 who advocate the powderiness, probably have not checked the elements of the 'cloud of pollen'. From the few but very different examples it seems (in accordance with KNOLL), that, in general, pollenkitt is present in streukegel blossoms and other flowers which require vibratory pollen collection. According to PACINI & HESSE 2005: 403 in *Solanum peruvianum* pollenkitt should be lacking, but this should be reinvestigated in view of the results in *S. lycopersicum*.

In pollen grains in air, pollenkitt is easily to visualize. The pollenkitt can be seen directly in form of the contact plaques between the grains and the cover slip. If preparation is made in such a manner, that no pressure acts on the grains, than the size of the plaques in connection with the size of the grains eventually can be a relative measure for the amount of pollenkitt present. Otherwise, in our few experiments, the adherence of the grains on the cover slip was not always proportional to the size of the contact plaques. The intensity of adherence on the slide and variable electrostatic power of the glass influence the adherence on the cover slip.

Apparently not all pollenkitt forms droplets (*Acacia, Leucaena, Onosma* ?) and not all light refracting droplets are pollenkitt (*Leucaena, Solanum* ? *Onosma* ?).

In wet preparations sometimes pollenkitt is not to be discerned (if no droplets arise). In other cases artefacts are produced: Abundantly, pollenkitt appears as droplets in water, diluted acetic acid, methylene-green-acetic-acid etc. The mentioned examples show discrepancies in the amount of plaques and droplets (e. g. in *Solanum lycopersicum* and *Onosma*), the droplets loose from the grains or droplets extrude from the grains. It is difficult to correlate directly observed plaques with localization and appearance of droplets in water etc. In every case no sure, exact statements about amount and nature of the droplets can be made.

Thus the analysis of the contact plaques of pollen grains in air is not only the easiest method but also the only method which enables a clear and doubtless evaluation of the sticky material which is effective in floral ecology. (The proof with the TEM will only rarely be applicable). If pollen grains are sampled directly from the anthers, this will be always pollenkitt.

Pollen adhesives which would also give contact plaques are produced outside the tapetum (and outside the anthers usually) (TEPPNER 2007a, VOGEL 1984, 2002) and thus, are not to be considered in pollen taken directly from the anthers (only exception: *Calliandra*, in which pollen adhesive is attached to the polyad within the anther, TEPPNER 2007b, TEPPNER & STABENTHEINER 2007).

Thus I hope to stimulate more exact information about occurrence and amount of pollenkitt. The most interesting progress would be, if all species for which TEM data for pollenkitt are available, will be investigated with the method presented here. This would bring a good standardization of this method and thus a welcome basis for all further comparative studies.

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