ELECTRON MICROSCOPICAL OBSERVATIONS ON POLLEN GRAINS OF SOME GRASS SPECIES

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SUMMARY

The ultrastructure of the pollen surface of fourteen grass species was investigated by studying carbon replicas and ultrathin sections. From the micrographs it was concluded that the investigated grasses can be divided into four groups according to the sculpture types of their pollen surface. One of these types appeared to occur in more than half of the grass species.

Two spatial replicas of pollen grains justified the opinion that the shape of the pollen grains of grasses has value for pollen identification.

It was concluded that for the identification of grass pollen electron microscopy is of less value than light microscopy.

1. INTRODUCTION

The identification of grass pollen has been a problem for a long time. It was generally accepted that grass pollen grains are uniformly shaped, round or oval, whilst they possess only one pore (WODEHOUSE 1935).

VON POST (1929) was the first to express the opinion that palynological investigations of cereal pollen grains would be of interest for the study of the development of agriculture in the course of time. This hypothesis induced FIRBAS (1937) to start an investigation on the morphology of grass pollen grains with the light microscope. Measurement of the diameter and description of some morphological characteristics enabled him to distinguish between two types of grass pollen, *viz.* the wild type and the cultivated type. He treated the pollen grains either with concentrated KNO_3 or with 10% KOH, thus leaving only the resistant exine wall intact, so that comparison with fossil pollen became possible.

FAEGRI & IVERSEN (1950) were able to identify many types of pollen grains from species all over the plant kingdom on the basis of different kinds of exine structure. Regarding grass pollen, however, they proposed one general type of exine structure, *viz.* the psilate (scabrate) type.

In 1956 ERDTMAN suggested the possibility to distinguish grass pollen grains from different species with the phase contrast microscope. GROHNE (1957), applying this method, was able to make a distinction between grass pollen grains of various species, which appeared to diverge as to shape, size and structure of pore and annulus, and with regard to the surface structure of the exine wall. In 1959 ERDTMAN & PRAGLOWSKI, who also employed the phase contrast microscope, divided the pollen grains of cultivated grasses into three types on the basis of differences in structure of the exine wall, *viz.* the *Avena*-type, the *Hordeum*-type and the *Triticum*-type.

During an investigation on the influence of climatic factors on the dispersal of grass pollen, started in 1963 at Schiermonnikoog, one of the Dutch Frisian islands, an identification method of grass pollen grains appeared to be indispensable. For this reason sixteen grass species occurring at Schiermonnikoog were studied. After dehydration of the pollen grains by alcohol and xylene, silicone oil was used as an embedding medium. Pollen grains treated in this way and studied under the light microscope, showed various characteristics concerning (a) their size (b) the structure of the pollen wall and (c) the pore and the annulus. Based on these characteristics an identification key of the sixteen grass species was composed (LIEM *c.s.* 1968).

This investigation made also clear that the finer structure of the pollen grain surface is caused by the presence of tiny punctae, which were observable as light and dark dots. It was not possible, however, to conclude from the observations, whether these punctae are arranged in a distinct pattern. Therefore, it seemed to be of great interest to study the outer exine structure of grass pollen grains more extensively, in order to find an answer to the question, whether it might be of importance for identification. To this end an electron microscopical study was conducted.

The first electron microscopical investigations on grass pollen were carried out by ROWLEY *c.s.* (1959) and by ROWLEY (1960). They established the existence of distinct exine structure types of grass pollen grains. These findings suggest that electron microscopy may be fo value for identification purposes.

2. MATERIAL AND METHOD

Pollen grains of fourteen grass species *(table 1)* were studied anatomically and morphologically by using an electron microscope (Zeiss EM 9). For that purpose ultrathin sections as well as replicas of the pollen grains were prepared.

2.1. Replica method

Pollen grains as such cannot be investigated electron microscopically, because they are too dense for the transmission of electrons. Therefore carbon replicas of the surface of the exine wall were studied.

To obtain these replicas the method as described by MÜHLETHALER (1955) was applied in a slightly modified way. Rectangles of about 2.5 × 1.5 cm, cut from thick mica, were cleaved into two halves which were kept in a Petri dish, lying with their smooth cleavage side aloft. Dry, mature anthers prepared from the flowers, were pulverized and the powder distributed as evenly as possible over the cleavage side of the mica, coarser pieces of the anthers being carefully removed. In case the pollen grains appeared to be still humid and sticked together, they were suspended in alcohol 96%. This suspension was then spread regularly over the mica surface. After the evaporation of the alcohol a monolayer of pollen grains was obtained.

Table 1. Collected grass species[1]).

Species	Locality
Cultivated grasses:	
Avena sativa L.	Rhoon
Hordeum distichon L.	Rhoon
Secale cereale L.	Amsterdam
Zea mays L.	Wolfheze
Wild grasses:	
Agrostis stolonifera L.	Schiermonnikoog
Dactylis glomerata L.	Schiermonnikoog
Elytrigia repens (L.) Nevski	Amsterdam
Festuca rubra L.	Schiermonnikoog
Glyceria fluitans (L.) R. Br.	Amsterdam
Holcus lanatus L.	Schiermonnikoog
Lolium perenne L.	Schiermonnikoog
Molinia caerulea (L.) Moench	Baarn
Phleum pratense L.	Schiermonnikoog
Phragmites communis Trin.	Amsterdam

[1]) Nomenclature according to HEUKELS-VAN OOSTSTROOM (1962).

In a Balzers high vacuum evaporating apparatus a carbon layer of 200 $\pm$ 50 Å thickness was deposited into the mica covered with pollen. With a razor blade the carbon film on the mica was cut into small squares of about 3 $\times$ 3 mm, these small pieces being easier to handle than the carbon film as a whole.

In order to release the carbon film from the surface of the mica, it was caused to float on distilled water. The method by which this was brought about is diagrammatically presented in *fig.* 1. Water reservoir R, connected with funnel F by means of tube Tu, is filled with distilled water. The water supply in F can be regulated by tending tap Ta. The piece of mica was held obliquely in the funnel. By opening tap Ta a little, the water in the funnel F is caused to rise with adjustable speed, so that finally the film is floating on the water surface.

The pollen grains, now being attached to the film, were removed by transferring the carbon squares to a Cr_2O_3-solution (40% in distilled water) with an inoculation needle. In this medium the pollen grains were destructed totally within in two or three hours, leaving behind the carbon-squares containing the replicas

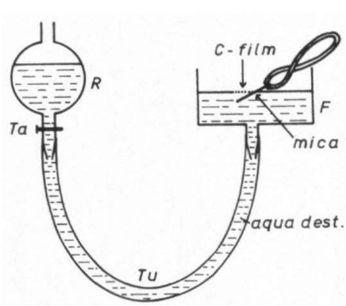

Fig. 1. Release of the carbon film from the mica.

of the pollen grains. The replicas were then washed out several times with distilled water to remove all Cr_2O_3 and other dirt, and finally picked up on uncoated grids. After having dried them in a desiccator, they were ready for observation with the electron microscope.

The most important modification of MÜHLETHALER's method (1955) is the application of mica instead of slides coated with formvar. The present authors never succeeded in causing the carbon film to float on water, when formvar was used. On the other hand, in most cases mica gave positive results: apparently the mica has both the required hardness and smoothness to obtain a successful release of the film. Besides, because of this smoothness no additional structure in the carbon film is brought about; consequently, the pollen replicas can be easily distinguished.

2.2. Ultrathin sections

Anthers with ripe or almost ripe pollen grains were fixed in osmium tetroxide, contrasted in uranyl nitrate, dehydrated, and embedded in Epon-812 (PEASE 1964). Before cutting ultrathin sections on a Reichert ultramicrotome, 2 μ thick sections were studied with a phase contrast microscope to select a suitable cutting area.

3. RESULTS

3.1. Sculpture type of the pollen grain surface

It appeared from the study of ultrathin sections that the wall of grass pollen grains in general shows great uniformity of structure. A diagram of the wall structure is presented in *fig. 2.* The terminology is from BEUG (1961), who classified grass pollen to *Monoporatae*, pollen grains with only one pore.

Generally, the pore (p) is closed by an operculum (op) and surrounded by an annulus (a). The wall of the grain exists of the resistant exine (ex) and the nonresistant intine (int); underneath the pore the latter is thickened to an oncus (on) (HYDE 1955). The exine wall can be subdivided into the ektexine (ektex) and the intexine (intex). The ektexine consists of the tectum (t), called tegillum by ROWLEY (1960), and of the columellae (co). Right across the ektexine and the

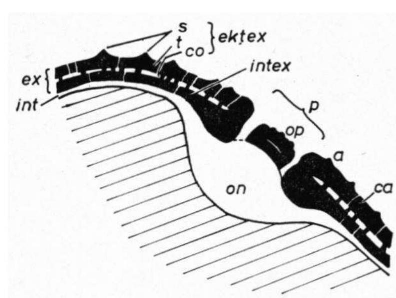

Fig. 2. Wall structure of grass pollen grains.
a = annulus; ca = canal; co = columellae; ex = exine; ektex = ektexine; int = intine; intex = intexine; on = oncus; op = operculum; p = pore; s = scabrae; t = tectum.

intexine, small canals (ca) can be found. On the surface of the tectum scabrae (s) are observable, giving the pollen wall a structural relief.

On the basis of the arrangement of the scabrae, which was studied from the replicas, it appeared to be possible to divide the pollen grains of the investigated species into four groups with different sculpture *(Plate I–VII)*.

3.1.1. Sculpture type I

The pollen grains of *Zea mays (Plate I, 1a, b)* appeared to be representative for this sculpture type. Carbon replicas of this species demonstrate that the scabrae are dispersed regularly over the tectum. Between the scabrae, the tectum surface is rather smooth. These observations are in agreement with those of ROWLEY (1960), who also studied *Zea mays*. He described the smoothness of the tectum in terms of the parallelness of its upper and lower surface. Of the investigated species only *Zea mays* appeared to belong to sculpture type I.

3.1.2. Sculpture type II

This sculpture type is exemplified by the pollen grains of *Elytrigia repens (Plate III, 1a, b)*. From the carbon replicas it can be observed that the dispersion of the scabrae over the tectum is more or less regular. In contrast with sculpture type I, however, the tectum surface between the scabrae is not smooth but

Plate I-VII Micrographs of ultrathin section (a) and carbon replicas (b) of the pollen grains of grasses.

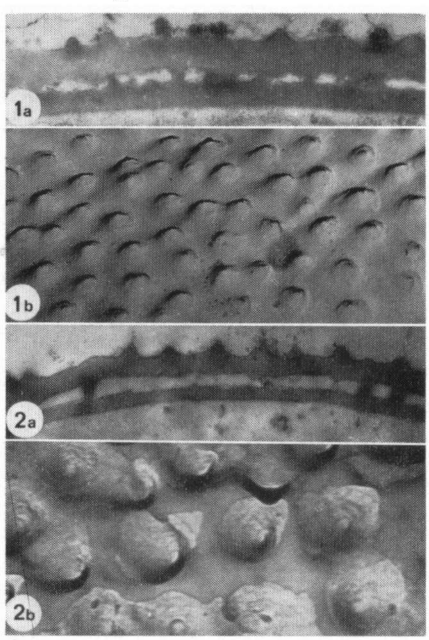

Plate I: 1a, b: *Zea mays* L.;
2a, b: *Secale cereale* L.

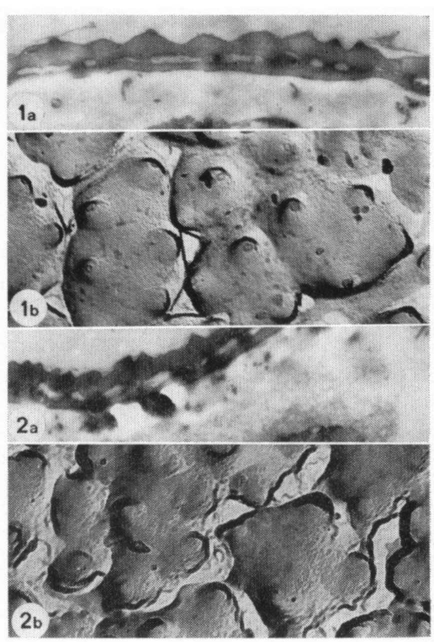

Plate II: 1a, b: *Dactylis glomerata* L.;
2a, b: *Phleum pratense* L.

faintly subsided. These subsidences are found to pass into one another continuously, forming a reticulum, which encloses elevated islets (lacunae, after ROWLEY), each possessing one to several scabrae.

Besides those of *Elytrigia repens*, the pollen grains of another eight of the grass species studied, turned out to belong to this sculpture type, *viz. Festuca rubra (Plate III, 2a, b), Glyceria fluitans (Plate IV, 1a, b), Holcus lanatus (Plate IV, 2a, b), Hordeum distichon (Plate V, 2a, b), Lolium perenne (Plate VI, 1), Molinia caerulea (Plate V, 1a, b), Phragmites communis (Plate VI, 2)* and *Secale cereale (Plate I, 2a, b)*.

The description of this sculpture type is in accordance with that of the grains from *Sorghum vulgare* and *Phragmites communis*, as given by ROWLEY (1960). In the ultrathin sections of all nine grass species, the faint irregularity of the tectum could be corroborated, in particular for *Elytrigia repens*. Here, both upper and lower tectum surfaces run nearly parallel, but less regular than in *Zea mays*.

3.1.3. Sculpture type III

Pollen grains from *Dactylis glomerata (Plate II, 1a, b)* and *Phleum pratense (Plate II, 2a, b)* appeared to represent a third sculpture type. Carbon replicas show an irregular, deeply incised tectum surface. As compared with the preceding type the subsidences are much deeper, forming a continuous reticulum of incisions with islets possessing three to ten scabrae, so that the sculpture shows a rather regular pattern. The honeycomb structure of the pollen grain wall of

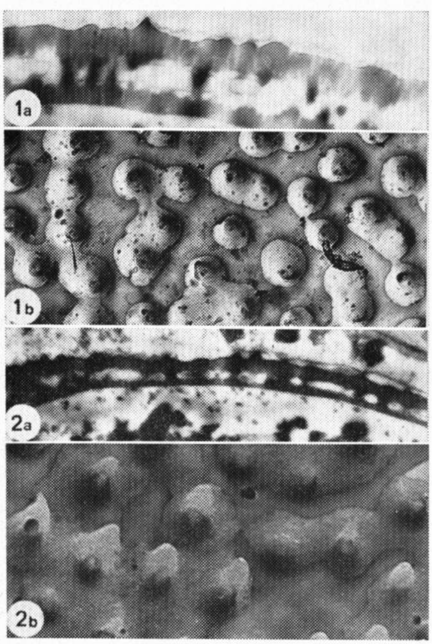

Plate III: 1a, b: *Elytrigia repens* (L.) Nevski; 2a, b: *Festuca rubra* L.

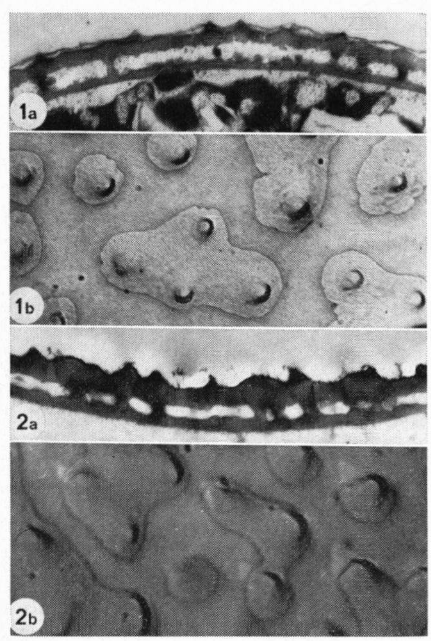

Plate IV: 1a, b: *Glyceria fluitans* (L.) R. Br.; 2a, b: *Holcus lanatus* L.

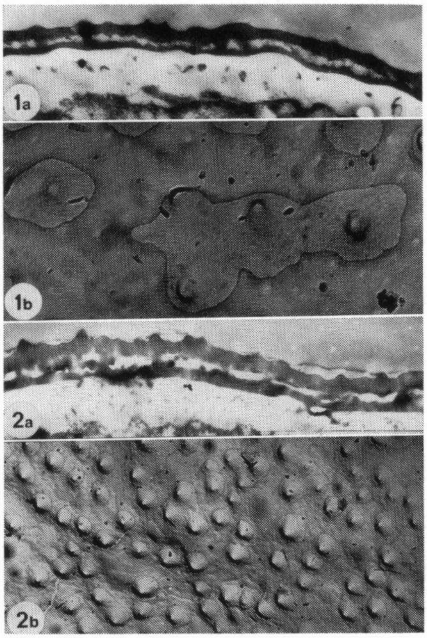

Plate V: 1a, b: *Molinia caerulea* (L.) Moench;
2a, b: *Hordeum distichon* L.

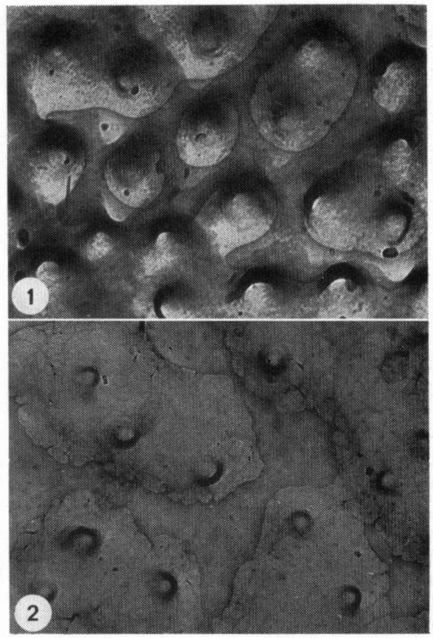

Plate VI: 1: *Lolium perenne* L.
2: *Phragmites communis* Trin. (Only carbon replicas of the grasses were available.)

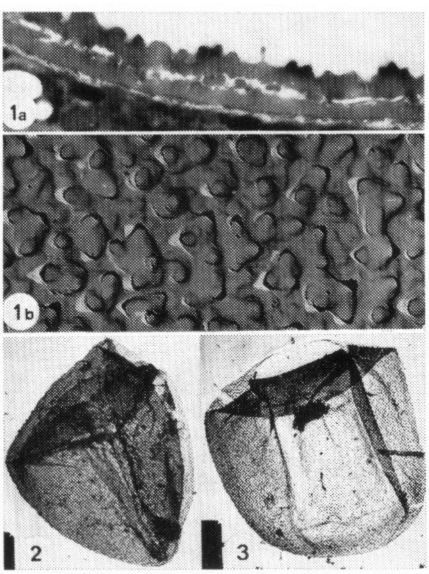

Plate VII: 1a, b: *Avena sativa* L.;
2: *Agrostis stolonifera* L.;
3: *Lolium perenne* L.
(VII 2, 3: carbon replicas of the whole pollen grain.)

Dactylis glomerata observed under the light microscope (LIEM *c.s.* 1968) probably represents the same design.

Micrographs of ultrathin sections show that the lower and upper surfaces of the tectum do not run parallel. *Poa annua*, *Calamagrostis canadensis*, *Cynodon dactylon* and *Phleum pratense*, investigated by ROWLEY (1960) probably belong to this sculpture type.

3.1.4. Sculpture type IV

This type is found in *Avena sativa* pollen grains *(Plate VII, 1a, b)*. From the carbon replicas it could be seen that, contrary to the other sculpture types, the scabrae are irregularly shaped. Their dispersion over the tectum surface, too, was observed to be very irregular; often two to four scabrae stand close together. Furthermore, the incisions of the tectum surface are not continuous. The occurrence of irregular depressions between clusters of scabrae is particularly observable in the ultrathin sections. Apparently, this sculpture type has not been described by ROWLEY (1960).

3.2. Shape of the pollen grain

Usually, it was not possible to get a replica of the entire pollen grain by employing the method described above. Nevertheless, in two cases spatial replicas were obtained, *viz.* from *Agrostis stolonifera (Plate VII, 2)* and *Lolium perenne (Plate VII, 3)*. From the micrographs it can be observed that the two replicas differ greatly as to their shape.

Concerning the shape of grass pollen grains it is worth mentioning that the electron and light microscopical images show close resemblance (LIEM *c.s.* 1968). This reasonably justifies the opinion that the shape of the grass pollen grains has identification value.

4. DISCUSSION

From the preceding sections it can be concluded that the pollen grains of the fourteen investigated grass species belong to four different sculpture types. Sculpture type I has a smooth tectum surface, whilst the tectum of the sculpture types II, III and IV are subsided or incised. Type IV *(Avena sativa)* has incisions of irregular deepness which do not form a continuous reticulum, whereas the incisions of the tectum in types II and III are continuous, forming a reticulum with a regular pattern. Sculpture types II and III are considered as distinct on the basis of differences in depth of the reticulum: the incisions in type III are definitely deeper than in type II.

These observations do not wholly support those of ROWLEY (1960), who distinguished only between two types of exine structure: the smooth and the incised *(table 2)*. Contrary, it appeared to us that the incised type can be subdivided into three distinct types on the basis of relative differences in the pattern and depth of the exine incisions. Furthermore, the suggestion of ROWLEY that cereal and wild grasses might be distinguished on the basis of the surface sculpture of

Table 2. Grass species and their types of pollen exine structure according to ROWLEY (1960) and LIEM & VAN ANDEL.

ROWLEY		LIEM & VAN ANDEL	
Surface	Grass species	Sculpture type	Grass species
Smooth	*Coix lacryma-jobi*	I	*Zea mays*
	Secale cereale		
	Zea mays	II	*Elytrigia repens*
			Festuca rubra
Incised	*Calamagrostis canadensis*		*Glyceria fluitans*
	Cynodon dactylon		*Holcus lanatus*
	Phleum pratense		*Hordeum distichon*
	Phragmites communis		*Lolium perenne*
	Poa annua		*Molinia caerulea*
	Sorghum vulgare		*Phragmites communis*
			Secale cereale
		III	*Dactylis glomerata*
			Phleum pratense
		IV	*Avena sativa*

their pollen (smooth and incised, respectively) is not shared in the present study *(table 2)*.

From the present investigation it can be concluded that sculpture type II is probably the most common, since nine out of the fourteen species belong to this type *(table 2)*.

Replicas of part of the pollen grain wall and also ultrathin sections seem of minor value for identification, many different grass species belonging to the same sculpture type.

In conclusion, the present electron microscopical investigation proved that phase contrast and regular microscope observations are sufficient for identification of grass pollen grains (cf. GROHNE 1957; ERDTMAN & PRAGLOWSKI 1959 and LIEM *c.s.* 1968).

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Magnifications of the micrographs of ultrathin sections (a) and carbon replicas (b) of the pollen grains of grasses (Plate I–VII).

Plate I: 1a, b *Zea mays* L.: × 9000, × 9000.
2a, b *Secale cereale* L.: × 9000, × 28000.
Plate II: 1a, b *Dactylis glomerate* L.: × 9000, × 28000.
2a, b *Phleum pratense* L.: × 6000, × 28000.
Plate III: 1a, b *Elytrigia repens* (L.) Nevski: × 9000, × 16500.
2a, b *Festuca rubra* L.: × 6000, × 28000.
Plate IV: 1a, b: *Glyceria fluitans* (L.) R. Br.: × 9000, × 28000.
2a, b *Holcus lanatus* L.: × 10500, × 28000.
Plate V: 1a, b *Molinia caerulea* (L.) Moench: × 9000, × 28000.
2a, b *Hordeum distichon* L.: × 5000, × 14500.

Plate VI: 1 *Lolium perenne* L.: × 28000
2 *Phragmites communis* Trin.: × 28000.
Plate VII: 1a, b *Avena sativa* L.: × 6000, × 9000.
2 *Agrostis stolonifera* L.: × 3000.
3 *Lolium perenne* L.: × 2700.

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