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DESCRIPTION OF DIPSACUS FULLONUM L. POLLEN

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ABSTRACT

The pollen morphology of *Dipsacus fullonum* L. is described. Special attention is drawn to the aperture mechanism; provisional comparisons with other genera are made.

Detailed descriptions of the pollen of *Dipsacaceae* are scarce. ERDTMAN (1952) gives a good description of the basic characters of these pollen grains: he divides the *Dipsacaceae* pollen into two groups, the group of *Morina* and the group of the remaining *Dipsacaceae* (*Callistemma*, *Cephalaria*, *Dipsacus*, *Knautia*, *Pterocephalus*, *Scabiosa*, *Succisa*). Since the pollen of *Morina* is very much unlike that of the remaining *Dipsacaceae*, the pollen of *Morina* will be left out of consideration here. Apart from descriptions and palynograms based on intact pollen grains, plates of the following pollen sections are known: *Cephalaria alpina* (L.) Schrad. (in STIX 1960, Taf. XXI Fig. 1), *Scabiosa columbaria* L. (in BRORSON-CHRISTENSEN 1949, Tavl. VIII Fig. 6, Tavl. IX Fig. 7), *Knautia arvensis* (L.) Coulter (in BRORSON-CHRISTENSEN 1949, Fig. 1, Tavl. VIII Fig. 5), and sections of "*Scabiosa palaestina*" (in ERDTMAN 1956, Pl. I Fig. 6–8). Presumably Erdtman's "*Scabiosa palaestina*" was *Scabiosa palaestina* L. (not synonymous with *Callistemma palaestinum* (L.) Hal.). As can be seen from the sections depicted in 1956, the wall construction of *Scabiosa palaestina* differs from that of some other *Scabiosa* species, and resembles more that of *Callistemma* (ERDTMAN, 1952). Good descriptions of sections are even more scarce than plates and nowhere special attention has been paid to the aperture mechanism. It is the opinion of the present authors that the aperture mechanism is very well developed in the genera *Cephalaria* and *Dipsacus*; therefore, a detailed description of the pollen morphology of one of these genera may be of some importance. The pollen of *Dipsacus fullonum* L. was chosen for a thorough investigation. In addition, pollen sections of some other *Dipsacaceae* were studied to obtain a better idea of the diversity in pollen within the dipsacaceous genera; consequently some provisional comparisons between the pollen morphology of *Dipsacus* and that of other genera could be made.

The investigations were carried out with pollen collected in the Botanical Garden of Amsterdam. Pollen preparations were obtained by boiling the material in KOH 10 % before acetolysis; often a portion of the material was subsequently chlorinated to make the wall stratification clearer. The slides of intact pollen were mounted in silicone

oil. Sections of the wall were made according to the method of BRORSON-CHRISTENSEN (1949). To this end the grains were also treated with KOH 10 %, acetolysed and chlorinated; the sections were mounted in Caedax. Both intact pollen and sections were stained with fuchsine or safranine. The observations were made with a Wild microscope (M 11, oc. K 10×, obj. Fluotar 100 × Oel) and a Leitz microscope (Laborlux oc. 8× B, obj. Fl. 95 × Oel).

The terminology proposed by IVERSEN and TROELS-SMITH (1950) will be followed as far as possible. As regards the dimensions reported, it should be noticed that only pollen of a few plants, grown in culture, were studied.

The pollen grains of *Dipsacus fullonum* are rather large. The dimensions of grains prepared after various methods, but all mounted in silicone oil, appeared to differ considerably, cf. Table 1. The smallest size was found when the material was only boiled in KOH 10 % for four hours (as proposed by FAEGRI and DEUSE, 1960); the largest size was measured in material of the same plants (but collected at another date) which was boiled in KOH 10 % for a few minutes, acetolysed and partly chlorinated.

TABLE 1

Method of preparation	Average length	Variation in length	Average breadth	Variation in breadth
KOH 4 hrs	[1]) 57.2	46–72	58.7	46–72
KOH 4 hrs, acetol.	[1]) 63.6	48–76	62.2	48–74
KOH 4 hrs, acetol., chlorin. . . .	[1]) 61.5	52–77	61.9	52–74
KOH 4 hrs, acetol., ½ chlorin. .	71.9	56–89	72.3	56–86

Size data of pollen grains of *Dipsacus fullonum* L. in microns, based on *c.* 100 grains a sample.

[1]) These three methods of preparation were carried out on the same material.

The grains are 3–4-aperturate (porate or colpate, P–Lg/P–Lt 1.1–2.8), subspheroid (Lg/Lt 0.88–1.14); in polar view the grains are more or less rounded triangular, the pores are situated at the corners. The apertures are surrounded by a massive annulus (width about 1.5–2 μ). The pollen wall encircling the annulus differs from the remaining surface of the grain, this part (width about 5–8 μ) is seen as a bright area with a less pronounced structure (Pl. III Fig. 1 and 2). The terminology to be used for this area will be discussed below. The grains are tectate, the ektexine is very thick (4–6 μ) and provided with two types of spines, viz. large clearly visible echinae(protruding about 2 μ beyond the tectum) and very small ones that are difficult to distinguish. In Pl. I Fig. 1–5 sections of the pollen wall are depicted. These sections will be discussed first; thereafter an analytical description of the intact pollen wall will be given as observed in the successive microscopical levels. The aspects of the intact wall will be more easily understood, when the structure of the sections is known. In

Pl. I Fig. 1 and 2 sections of the aperture area are shown, Pl. I Fig. 3 shows a section of the annulus, surrounding the aperture, and Pl. I Fig. 4 and 5 are details of the wall construction. As is seen from Pl. I Fig. 4 and 5 the wall is composed of robust, wide-set, mostly unfurcated columellae (columellae simplices), which end in the tectum. The tectum (2 – 2.5 μ thick) is made up of many very fine radially directed elements that pass into very fine echinae. Just like the radial elements these minute echinae vary in size. The fine radial elements forming the tectum can be compared with similar elements found in the tectum of *Compositae*, though in this family the radial elements do not terminate in minute echinae (STIX, 1960). Therefore it seems justified to speak of tectal bacula after STIX (*tegillare Bacula*, 1960). The bacula are thickened at the base and apex and often also in the middle, or in some cases when the tectum is very well developed (Pl. I Fig. 5) there are some more thickenings in the bacula between base and apex. The thickened apices of the bacula form the clearly visible outer membrane of the tectum with the minute echinae (*Abschluszmembran* after STIX, 1960). Often it could be observed that the remaining thickenings of the tectal bacula are likewise connected, so that an inner tectum membrane is formed and one or more intermediate membranes (*Stützmembran* and *Mittelschicht* respectively, after STIX, 1960). In between the minute echinae, arising from the tectal bacula, large echinae are seen, which are supported by larger columellae. The connection between these large spines and columellae is drawn in Pl. I Fig. 6. From these sections it becomes evident that the shape of the larger echinae varies, the spine being lance-shaped or with a club- or cone-shaped base. A number of fine bacula attach the spine to the hollow apex of the supporting columellae, as can be seen from the drawing (Pl. I Fig. 6). The spines are often more broad than long (length beyond the tectum 1.2 – 2 μ, total length 2 – 2.5 μ, breadth 1.2 – 2.4 μ); the supporting columellae are broader near the apex (about 3.6 μ) than near the base (1.2 μ) and in total 2.4 μ high. The remaining columellae, not provided with spines are somewhat smaller.

When comparing the wall construction of *Dipsacus fullonum* with that of some other species of the family the following has been observed. The construction of the pollen wall of *Cephalaria alpina* is almost the same as that of *Dipsacus fullonum*; the columellae seem to be somewhat longer and less broad, in addition the larger as well as the minute echinae seem to be more acuminate. The wall construction of *Succisa pratensis* Moench and *Scabiosa columbaria* shows more differences. The grains are distinctly bi-echinate, the smaller echinae being very clearly visible in *Scabiosa*. The columellae are furcated and the tectum is built up by the last branches of these widely furcating columellae; these tectal branches end in the smaller echinae. In comparison with the construction of the tectum in *Dipsacus* and *Cephalaria*, it may be thought, that the tectal bacula in *Dipsacus* and *Cephalaria* might likewise be derived from furcated columellae. The wall construction of *Scabiosa* resembles that of *Succisa* to some extent; especially the number

of columellae and furcations is different, the columellae of *Scabiosa* being rather closely packed. The sections of *Knautia arvensis* were not yet made thin enough to get a clear idea of the exact construction of the pollen wall. By the figure given by BRORSON-CHRISTENSEN (1949, Tavl. VIII Fig. 5), one may suppose, that the pollen wall in *Knautia* is constructed in a similar way as in *Scabiosa* and *Succisa*, though at first sight it looks completely different.

The construction of the pollen wall around the aperture is often somewhat different from the remaining wall construction; this is especially true for *Cephalaria* and *Dipsacus*. In *Dipsacus fullonum* the following observations were made. The endexine, which is rather thick (1–2 μ), becomes gradually thinner near the apertures and changes into the tenuous aperture membrane. A large number of robust ektexine elements are found on the aperture membrane (Pl. I Fig. 1; Pl. III Fig. 3). The aperture borders are situated below the exine level, the wall bends inward, while the tectum becomes thicker at the same time. The tectal bacula are longer here and perhaps somewhat thinner; the number of thickenings of the bacula between base and apex increases, the section of the tectum having a slightly retiform aspect in this area. The large spines are less numerous and smaller here; columellae are but rarely present close to the annulus. The annulus itself can be considered to be a massive cylinder-shaped structure belonging to the ektexine, implanted on the endexine membrane. As to its massive internal structure the annulus bears a resemblance to the columellae and to the ektexine elements in the aperture.

Up to now, this cylinder-shaped structure bordering the aperture has been called annulus. No term, however, was used for the area surrounding the annulus, which area differs from the remaining part of the pollen wall by a thicker tectum with a less pronounced structure. The fact that this part of the wall intrudes is irrelevant in this connection, although this renders this area clearly distinguishable. According to the definition given by IVERSEN and TROELS-SMITH (1950) presumably both surrounding structures should be named annulus (viz. annulus 1 and annulus 2). For "annulus" means an area surrounding a pore that is differentiated from the remaining exine of the pollen grain by differences in the ektexine, e.g., a thicker or thinner ektexine. In case the annulus is formed by concentric zones of different structures, these zones can be called annulus 1 and annulus 2 from the inside outwards (IVERSEN and TROELS-SMITH, 1950). However, it does not seem recommendable to use the term annulus for two so completely different structures; the massive structure directly surrounding the aperture and called annulus in this article differing completely from the remaining wall construction, whereas the bright area encircling the annulus can be distinguished only by slight differences in the texture of the tectum. ERDTMAN (1952) uses the term "halo" when describing the aperture border of the *Dipsacaceae*; according to him the term halo can be defined as a rather narrow

area surrounding an aperture and it corresponds with the term annulus of IVERSEN and TROELS-SMITH (1950). Consequently the term "halo" can not be used by the side of the term annulus. CHANDA (1962) encountered the same difficulties, when he described the pollen morphology of some Scandinavian *Caryophyllaceae* (*Stellaria holostea* L.). Chanda retained the term annulus for the innermost clearly distinguishable ring-shaped border of the aperture, which is certainly in agreement with the sense of the definition of IVERSEN and TROELS-SMITH (1950). Chanda introduces the term "extra-annular area" for that special part of the pollen wall surrounding the aperture, which has a less pronounced structure than the remaining part of the pollen wall. In this article the terms extra-annular area and annulus will be used in the sense of Chanda.

In publications no special attention has been paid to the construction of the aperture mechanism, although it is a very essential part of the pollen grain, the harmomegathic movements taking place around the aperture, the exit for the pollen tube. Presumably the construction of a rigid annulus and a flexible extra-annular area, as described for *Dipsacus fullonum*, has to be considered essential to the harmomegathic movements. As appeared from observations by the present authors the same situation is present in *Cephalaria alpina*. In *Succisa pratensis* the annulus and extra-annular area are reduced; the extra-annular area is not present except for a very narrow spineless intruding area along the colp in a few species, whereas the annulus is much shorter than the nearest columellae; consequently the tectum is curved strongly towards the endexine. The aperture of *Scabiosa columbaria* bears a great resemblance to that of *Succisa*, but the annulus is even a little shorter. As regards *Knautia arvensis*, the annulus around the pore is very well developed and has a broad base, but an extra-annular area is absent.

Returning to the pollen grains of *Dipsacus fullonum*, the aspect of the wall of the intact grain still has to be discussed. The successive aspects of the interporium (intercolpium) are drawn in Pl. II Fig. 1 – 5, while the successive aspects of the area around the aperture are given in Pl. II Fig. 6 – 10. These drawings were made with the aid of microphotographs. The highest image of the interporium (Pl. II Fig. 1) shows the tops of the large spines as bright spots; other structures show themselves faintly and are not drawn. The following view (Pl. II Fig. 2) shows the large spines as bright dots with a black lining together with the tops of the minute echinae, drawn as very small white spots. As regards the large spines, the difference in aspect between this level and the previous level is not drawn. In Pl. II Fig. 3 the apices of the columellae supporting the larger echinae are clearly seen as rather bright dots, while in between them the contours of the other smaller columellae begin to be outlined very faintly, covered as they are by the pattern of the tectal bacula; this pattern is represented as small black spots. In Pl. II Fig. 4 not only the larger columellae but also the smaller ones are clearly seen, whereas in Pl. II Fig. 5

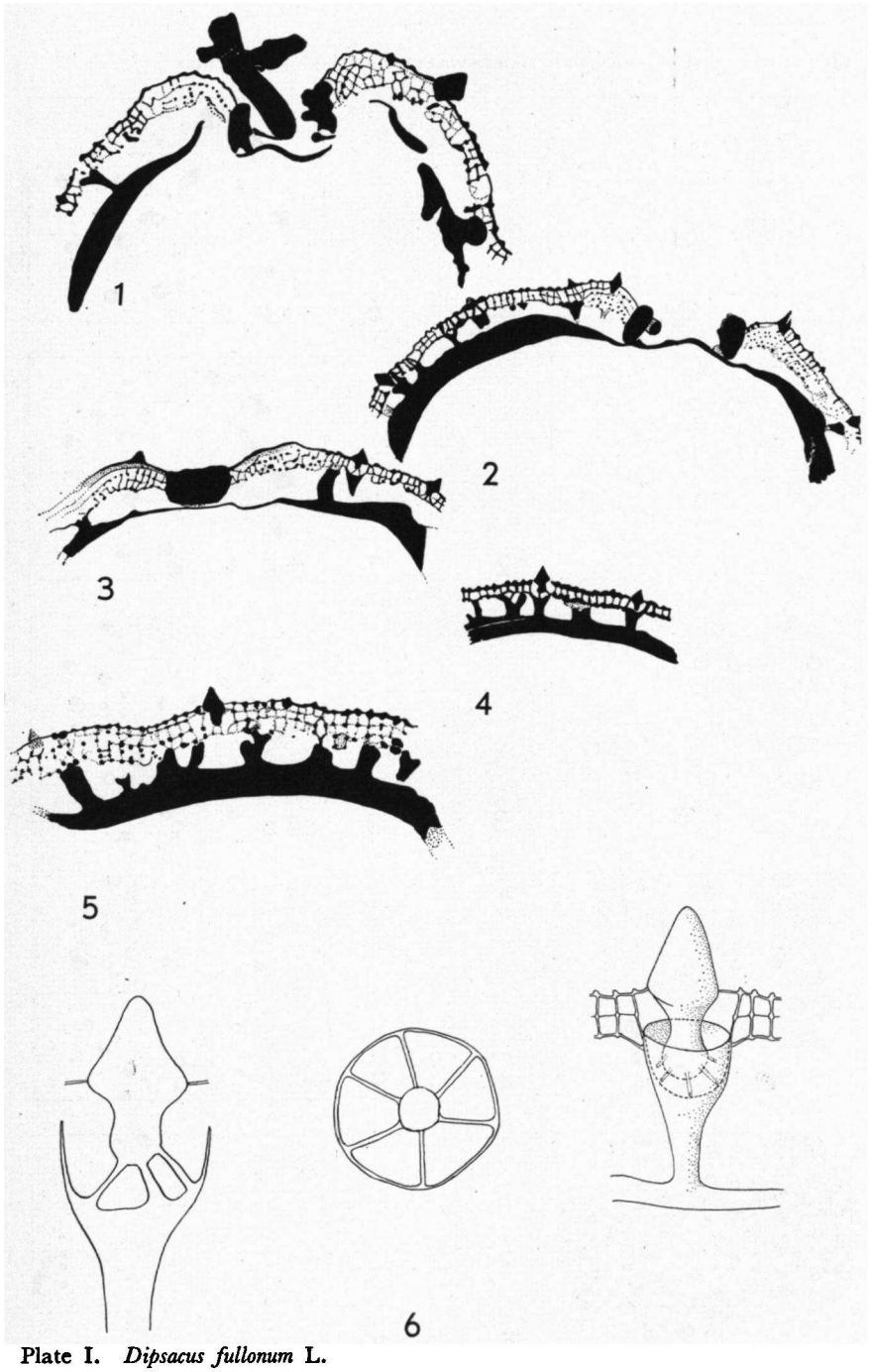

Plate I. *Dipsacus fullonum* L.

Fig. 1–5. Transversal sections through the pollenwall. Fig. 1 and 5, × 1750; Fig. 2–4, × 1450.

Fig. 6. Reconstruction of the connections between a large spine and its supporting columella, Longitudinal section, cross section, total reconstruction resp.

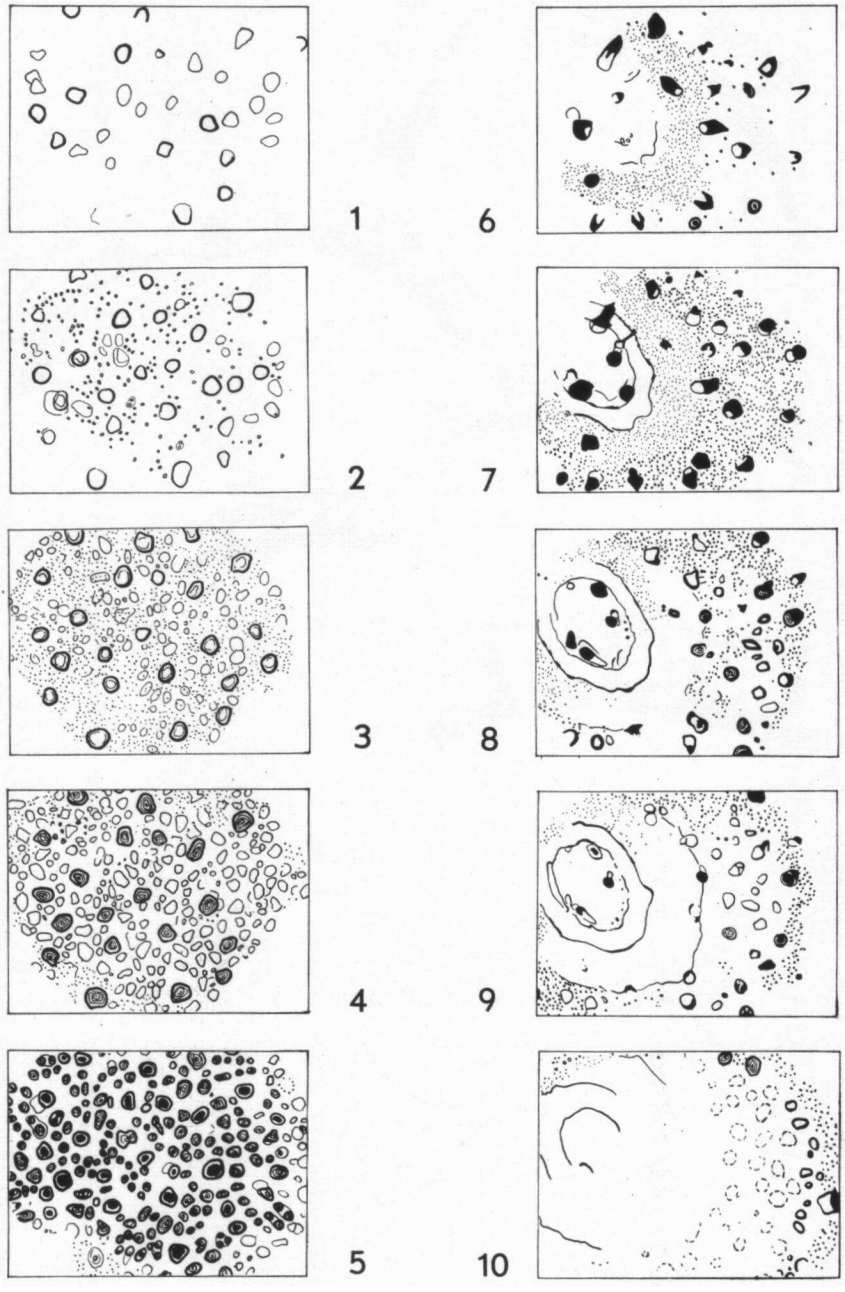

Plate II. *Dipsacus fullonum* L. Exine seen in varying focus (× 1000).
Fig. 1– 5. Exine of the interporium.
Fig. 6–10. Exine of the aperture area.

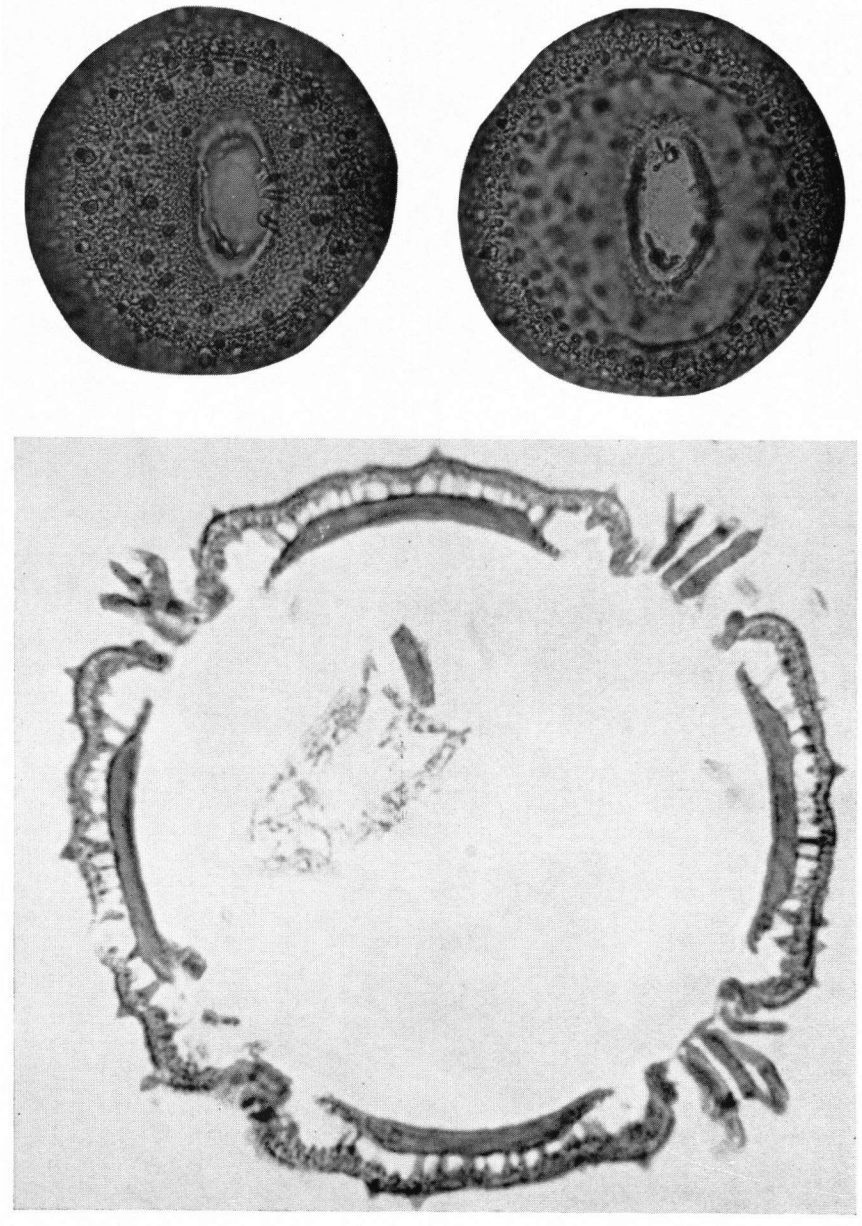

Plate III. *Dipsacus fullonum* L.

Fig. 1. Microphotograph of the aperture area (× 900).
Fig. 2. Microphotograph of the same area at a lower focus.
Fig. 3. Microphotograph of a transversal section through the pollenwa (× 1300).

the bases of the columellae are the only structures left. From Pl. II Fig. 4 and 5 it can be seen that the pattern of the larger columellae remains practically the same, while the pattern of the smaller columellae changes in a few places as a result of the presence of some furcated columellae. The columellae, both the larger and smaller ones, appear to be polygonal in this aspect.

The area round the aperture shows in principle the same successive images. The aperture is situated in the left top corner of the drawings. As a result of the spherical form of the grain the uppermost part of the aperture is not sharp and neither is the wall in the corners of the drawings. In Pl. II Fig. 6 a level of the interporium near the extra-annular area is drawn, comparable to the level depicted in Pl. II Fig. 2. In this area the tops of the smaller echinae are seen as small bright spots; the larger spines are for the greater part folded downwards by the pressure of the coverglass, and consequently the contours of the entire spine can be seen. In the extra-annular area the tectal bacula are present as small dark spots whereas in a small zone next to the annulus these bacula are not yet visible and the annulus can not be seen. Not until the level of Pl. II Fig. 7 the tectal bacula next to the annulus and the annulus itself are distinguishable; likewise the tectal bacula outside the extra-annular area are seen in between the bases of the larger spines. The sections of the aperture (Pl. I Fig. 1, 2 and 3) will give a better idea of the aspects described above. Special attention should be given to the intruding aperture and to the tectum bending strongly inwards quite close to the annulus. Therefore the annulus and tectal bacula immediately surrounding the annulus can only be observed at a lower focus. The spherical form of the grain explains the fact that the tectum of the remaining extra-annular area can be seen sharply before that of the wall outside this area; in addition it should be mentioned that there often is a slight vaulting at the outer border of the extra-annular area. In Pl. II Fig. 6–8, the ektexine elements of the aperture are visible. Pl. II Fig. 8 gives the larger and smaller columellae outside the extra-annular area, which pattern is comparable to that of Pl. II Fig. 3 and 4. Of aperture only the annulus is distinct. Pl. II Fig. 9 gives practically the same image but at a lower level; the outline of the extra-annular area is rather distinct here. Pl. II Fig. 10, finally, gives the lowest level of the wall. The columellae are vague, just like the aperture; there still exists, however, in some places a faint outline of the extra-annular area and of the broad base of the annulus.

Pl. III Fig. 1 and 2 are microphotographs of two different levels of the aperture region. In Pl. III Fig. 1 the extra-annular area with the tectal bacula (black spots) is very clear; this level is comparable to Pl. II Fig. 6 – 7. Pl. III Fig. 2 is a microphotograph of the lowest level of the wall; only the outline of the extra-annular area and the annulus are marked (cf. Pl. II Fig. 9 – 10).

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SUMMARY

The pollen morphology of *Dipsacus fullonum* L. was studied and the following data were obtained.

The pollen grains of *Dipsacus fullonum* are subspheroid (Lg. 46–72 μ, av. Lg. 57.2 μ; Lt. 46–72 μ, av. Lt. 58.7 μ), 3–4-aperturate (porate or colpate), tectate and bi-echinate. The endexine and ektexine are rather thick, 2–2.5 μ and 4–6 μ, respectively.

The ektexine is built up of strong mostly unfurcated columellae (2–3 μ high), a tectum (2–2.5 μ thick) composed of very thin radial elements (tectal bacula), and two types of spines. The tectal bacula pass into the minute echinae; the larger echinae (about 2 μ beyond the tectum) are attached to very strong columellae.

The terminology of the bordering structures of the aperture is discussed. Following CHANDA (1962), the term annulus is used for the innermost massive border, and the term extra-annular area for the outermost border.

The intruding aperture mechanism is built up of the massive annulus and the extra-annular area, the latter being characterized by a thicker tectum and less sculpture than the remaining pollen wall.

The pollen grains of *Dipsacus* and *Cephalaria* resemble each other very much. The wall structure of *Succisa*, *Scabiosa* and *Knautia* is different from that of *Dipsacus*, though the construction may follow an analogous scheme.

The aperture mechanism of *Dipsacus* and *Cephalaria* appeared to be composed of a very well developed annulus and an extra-annular area. In *Knautia* the annulus is also very well developed, whereas the annulus in *Succisa* and *Scabiosa* is rather short. In *Knautia*, *Succisa* and *Scabiosa* the extra-annular area is reduced or lacking.

The construction of the tectum of *Dipsacus* and *Cephalaria* bears a great resemblance to the tectum of some genera of the *Compositae* family (cf. STIX, 1960).

RESUME

Le pollen de *Dipsacus fullonum* L. a été examiné et on a récolté les resultats discutés.

Les grains du pollen de *Dipsacus fullonum* sont subsphériques (Lg. 46–72 μ, Lg. moy. 57.2 μ; Lt. 46–72 μ, Lt. moy. 58.7 μ), 3–4-aperturates (porates ou colpates), tectates et bi-echinates. L'endexine et l'ektexine sont assez épaisses (2–2.5 μ et 4–6 μ respectivement).

L'ektexine se compose d'un nombre de columellae robustes (hautes 2–3 μ), lesquelles ne sont que ramifiées rarement, d'un tectum (épais 2–2.5 μ) formé par un grand nombre des élements radiaux très fins (bacula tectals) et de plus de deux types d'épines. Les bacula tectals se convertissent en épines minues, tandis que les épines larges (hauteur au-dessus du tectum 2 μ environs) sont fixés aux columellae très robustes.

La terminologie pour les structures englobantes l'aperture a été discutée. D'après CHANDA (1962) le terme annulus est employé pour la zone intérieure et le terme "extra-annular area" (région extra-annulaire) pour la zone extérieure.

Le mécanisme de l'aperture, sous le niveau de la paroi, se compose d'un annulus massif et d'une région extra-annulaire; celle-ci est caractérisée par un tectum plus épais et par un sculpture moins prononcé.

Les grains du pollen du genre *Dipsacus* et du genre *Cephalaria* se ressemblent beaucoup. La construction de l'exine des genres *Succisa*, *Scabiosa* et *Knautia* diffère de celle du genre *Dipsacus*, quoique l'analogie du schéme de la construction soit possible.

Le mécanisme aperturale de *Dipsacus* et *Cephalaria* se compose pour tous les deux d'une région extra-annulaire et aussi d'un annulus très bien développés. L'annulus est également bien développé dans le genre *Knautia*, tandis que les genres *Succisa* et *Scabiosa* possèdent un annulus assez court. Une région extra-annulaire ne se trouve pas dans les genres *Knautia*, *Succisa* et *Scabiosa*.

La construction du tectum de *Dipsacus* se rapproche de celle du tectum de quelques genres de la famille des *Compositae* (cf. STIX, 1960).

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