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Ultrastructure of the ascus top and the ascospore wall in Fimaria and Pseudombrophila (Pezizales, Ascomycotina)

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Morphological and developmental studies on the ascus top and the ascospore wall of *Fimaria theioleuca, F. cervaria, Pseudombrophila obliquerimosa*, and *P. deerata* were carried out with light and electron microscopy. Ascus tops are found with roughly delimited ascostomes and opercula, without indentations, preformed weakened zones, or subapical rings. Dehiscence of the ascus takes place in an eroded, slightly thinner zone of the outer layer and next to a corresponding wrinkled region in the inner layer. This corresponds with the structure and dehiscence mechanism found in genera of Pyronemataceae studied thus far. During ascospore development in all species at first a smooth electron-dense secondary wall is formed. In *F. theioleuca* and *F. cervaria* this layer is permanent, but in *P. obliquerimosa* and *P. deerata* it usually breaks up to form an ornamentation. The presence of smooth and rough ascospores in the same species is explained by assuming a common process of development followed by a further final ripening in the rough-spored ones. The presence of two types of septa is recorded from excipular cells and paraphyses of *F. theioleuca*.

In view of the preparation of a taxonomic revision of the genera *Fimaria* Vel. and *Pseudombrophila* Boud. but the almost complete absence of knowledge about the fine structure of the species of these genera, a study on the ultrastructure of asci, ascospore walls, and septa has been undertaken.

The comparative structural study of the dehiscence mechanism of operculate Ascomycetes started with the aid of light microscopy. Especially investigations by Boedijn (1933), Chadefaud (1942, 1944, 1946), and Le Gal (1946a, 1946b) of living and revived material yielded the first beginnings of understanding of the structure of this mechanism. As subject of these first investigations especially representatives of the family Sarcoscyphaceae were chosen. Here relatively thick walls were found in the top of the asci characterized by a very strong, but unequal, swelling of the different parts in water and other media.

Especially many of the post-mortem observations on revived exsiccata and on material conserved in liquid have led to wrong interpretations of the structure and the mechanism of the ascus dehiscence. Boedijn's (l.c.) observations on living asci of *Cookeina sulcipes* (Berk.) O.K. are contrasting examples of lasting value. The strongly asymmetrical structure of the ascus top with an eccentrically oriented operculum in this species which was also studied by Le Gal (1946a), make it difficult to homologize the structural details described with those observed in symmetrical asci.

The ultrastructure of the ascus top has now been investigated in more than 40 genera of Pezizales (e.g. Schrantz, 1970; Wells, 1972; van Brummelen, 1974, 1975, 1978;

Samuelson, 1975, 1978a–d; Hung, 1977; Bellemère, 1977; Samuelson & Kimbrough, 1978; Kimbrough & Benny, 1978; Samuelson & al., 1980).

Based on the fine structure of the ascus top in the Pezizales van Brummelen (1978) distinguished eight principal types of asci, while Samuelson (1978d) even concluded that 'no two genera share an identical apical apparatus.' Although the latter conclusion would seem to be somewhat extreme, comparative study of the structure of the ascus top can certainly help in determining taxonomic affinities at familial and sometimes even at generic level.

The structure of the ascospore wall and of its ornamentation in Pezizales have been the subject of extensive studies by Le Gal (1947) with light microscopy and by Merkus (1973, 1974, 1975, 1976) with electron microscopy. They, however, did not study species of the genera under consideration here.

In *Fimaria* and *Pseudombrophila* there occur taxa with smooth as well as with ornamented ascospores. In some taxa this character seems to be inconstant. Here smooth and rough ascospores can be found in the same, apparently homogeneous, collection. The ultrastructure of the ascospore wall may give an explanation for this phenomenon.

MATERIAL AND METHODS

For the present study fresh material was collected in the Netherlands, France, and Italy. The following list gives more details about the specimens and their origins. *Fimaria theioleuca* (Roll.) Brumm. — *van Brummelen* s.n., on sheep dung, Elspeet, Gelderland, the Netherlands, 7 XII 1972 (L); *Fimaria cervaria* (Phill. apud J. Stevenson) Brumm. — *van Brummelen* s.n., on hare dung, Vogelenzang, North Holland, the Netherlands, 4 VII 1974 (L); *Pseudombrophila deerata* (P. Karst.) Seaver — *Donadini*, on sheep dung, Aubagne, Bouche du Rhône, France, 25 IV 1981 (L); *Pseudombrophila obliquerimosa* Harmaja — *Lucchini & van Brummelen 6263*, on rotten vegetable debris mixed with cow dung, near Selva di Trissino, Veneto, Italy, 2 V 1981 (L).

Living isolated asci or bundles of gently spread out asci were observed in water or in a weakly hypotonic solution of glucose in distilled water. The slides were studied with phase contrast and Normarksi's interference contrast optics.

For light microscopy asci and ascospores were stained with e.g. Congo red, trypan blue, and methyl blue. For critical observations monochromatic light was used of a wavelength equal to that of the maximum absorption value of the stain used. Also, sections 0.2–0.5 μm thick of material embedded in epoxy resin and stained with toluidine blue proved to be of value.

For electron microscopy, small squares of the hymenium of apothecia at different stages of ripening were cut and fixed.

One part of the material was fixed for 3 hours in 1% glutaraldehyde buffered at pH 7.2 with 0.2M cacodylate at 4°C. Another part was fixed for 1 hour in 1–1.5 % $KMnO_4$ in distilled water. All material was post-fixed for 1 hour in 1% buffered OsO_4 at 4°C.

Fixed material was dehydrated in an ethanol graded series and embedded in Epon. During dehydration the material was stained for 5 minutes in a solution of 1% uranyl

acetate. Longitudinal median sections of asci were cut with a diamond-knife on an LKB Ultratome III. The grids were normally contrasted with Reynold's lead citrate and uranyl acetate, and occasionally also with barium permanganate. The ultrathin sections were viewed with an Philips EM 300 electron microscope.

OBSERVATIONS

The ascus top

In the species under observation, structural differentiation in the top of the ascus can only be observed in mature asci shortly before the moment of spore discharge. At this stage minor changes in the osmotic pressure of the medium may easily cause the discharge.

In mature undehisced asci the upper ripe ascospore is located in the top just under or against the apical wall. Since the endospore of mature ascospores in Pezizales becomes resistant to fixation, embedding, and thin sectioning, it is often difficult to study ascospores and apices of asci properly at the ripest stage. Consequently the study of the ascus top is especially based on ripening asci, on mature asci where the spores have accidentally been retracted from the top, and on dehisced asci.

Since ample material of *Fimaria theioleuca* from cultures was available, the structure of the ascus of this species is described and illustrated in the first place.

The shape of the asci is cylindrical with a rounded tip, 150–200 × 13–15 μm.

In the young ascus and during early ascospore formation, the ascal wall appears to be still undifferentiated, thicker throughout the lateral face of the ascus and thinner at the tip (Fig. 1A).

At the inner face of the lateral wall no protuberances are found in the apical or subapical region of the ascus.

In the apical epiplasm, also called acroplasm (Chadefaud, 1942), an apical funnel continuing as a tract downwards to the first ascospore can be found (Fig. 3A). Sometimes the tract can be followed further downwards along the lower ascospores. These structures can best be detected with phase contrast or interference contrast optics.

In 0.2–0.5 μm thick sections the apical and subapical regions of the ascus wall stain strongly with toluidine blue, especially after dehiscence.

Also electron microscope observations of asci at different stages of development did not reveal a subapical ring or protuberances other than lomasomes at the inner face of the ascal wall.

In young asci of permanganate-OsO_4-fixed material the wall at the immediate region of the tip is 150–170 nm thick, subapically the ascal wall reaches a thickness of 200–500 nm. At the outside of the ascus a thin electron-dense periascus is present from the beginning.

During the ripening of the ascospores an inner layer becomes discernible over the full length of the ascus. This layer is not contrasting much in electron-density with the outer layer, but it is marked by a contrasting boundary line. The formation of the inner

layer is completed at the moment of spore maturity. In the apical region, or the future operculum, the inner and outer layers are then of about the same thickness, both 100–120 nm, as well in glutaraldehyde-OsO_4 as in permanganate-OsO_4-fixed material. In the subapical region there is a considerable but gradual change in the thickness of the ascal wall and in both of its layers. The ascal wall thickens from 240–270 nm closely behind the tip to 460–520 nm more downwards. This is due to changes in the thickness of the outer layer which increases from 170–200 nm in the upper part to 450–500 nm in the lower part of the subapical region. Over the same distance the inner layer reduces in thickness from 70–100 nm near the tip to only 20–25 nm lower down.

In the outer layer of the lateral wall, at some distance behind the apex, two strata can be distinguished: an outer stratum 310–344 nm thick and an inner stratum 120–155 nm thick (Fig. 2B).

Even at full maturity no trace of an indentation or a preformed weakened zone demarcates the place of the future operculum. The only indication of the formation of an operculum at the top of the ascus is the presence, at a short distance behind the tip, of a zone with a slightly thinner ascal wall and with some irregular erosion at the surface of the outer layer, corresponding with a region of wrinkling of the inner layer (Fig. 1B).

The operculum opens forcibly by a fracture in this zone of the outer layer and by a fracture in the inner layer next to the wrinkled region. As a result of this fracture, the margins of the ascostome (Seaver, 1928) and the operculum look rather irregular and rough, while in the operculum the outer layer usually overlaps the inner one (Fig. 1D, 2A, C, D).

Abbreviations used in figures. — AS, ascostome; AW, ascus wall; CM, condensed material; E, epiplasm; EN, endospore; EP, epispore; ER, endoplasmatic reticulum; F, fracturing line; FU, funnel; IL, inner layer; IM, investing membrane; M, mitochondrion; N, nucleus; O, operculum; OL, outer layer; P, periascus; PM, plasma membrane or plasmalemma; PW, primary spore wall; S, ascospore; SP, sporoplasm; SW, secondary wall; T, tract or funiculus; WZ, weakened zone.

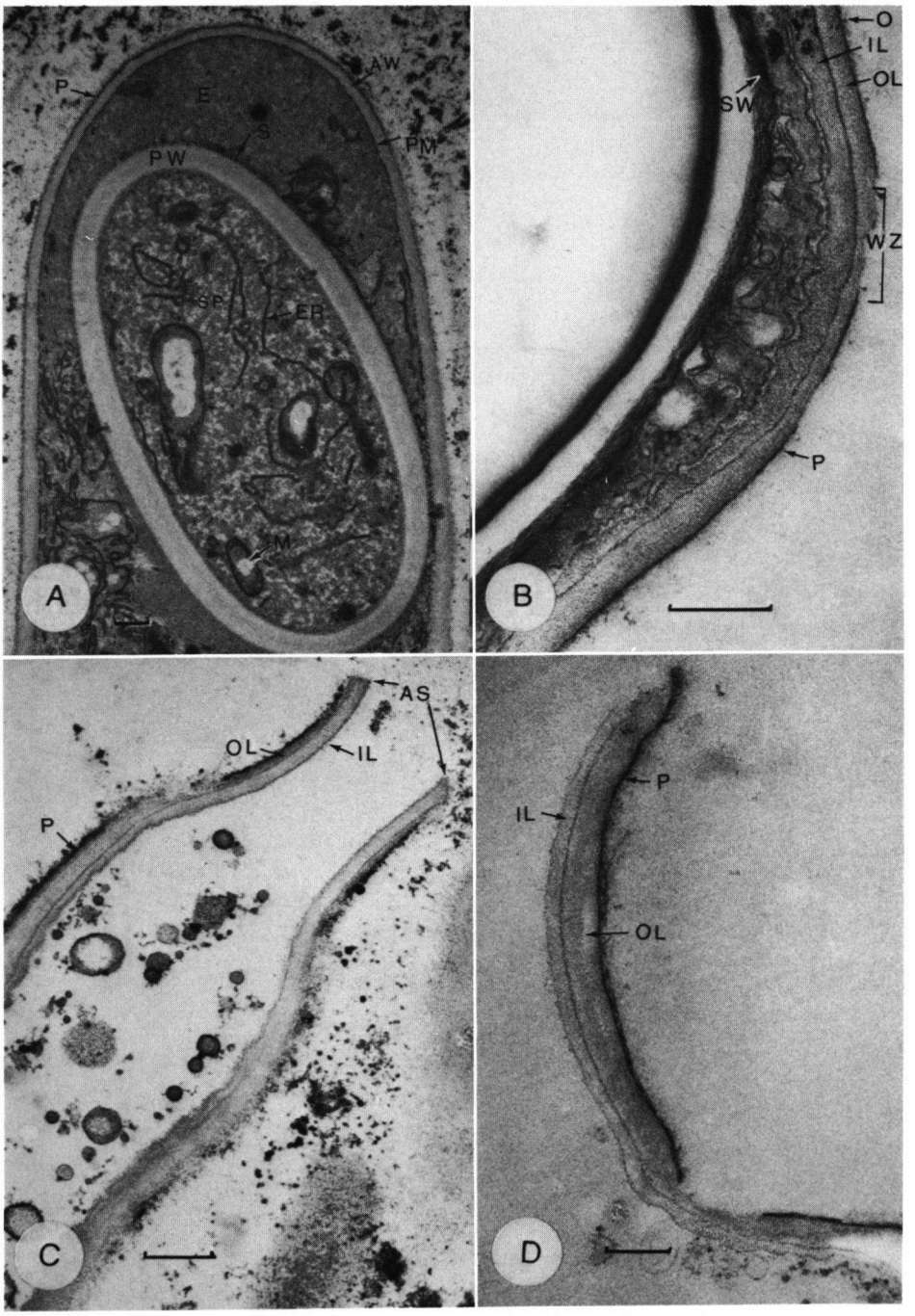
The scale markers in all figures equal approximately 0.5 μm.

Fig. 1. *Fimaria theioleuca*, electron micrographs of ripening and emptied asci. — A. Median section of the distal portion of ripening ascus, fixed in 1% $KMnO_4$ and 1% OsO_4. — B. Detail of apex of almost mature ascus just before dehiscence, with empty space of fallen out uppermost ascospore, fixed in 1% glutaraldehyde and 1% OsO_4. — C. Apex of emptied ascus without operculum, fixed in 1% $KMnO_4$ and 1% OsO_4. — D. Operculum of emptied ascus, fixed in 1% OsO_4.

Fig. 2. *Fimaria theioleuca*, electron micrographs of emptied asci. — A. Median section of apex of emptied ascus with operculum, fixed in 1% glutaraldehyde and 1% OsO_4. — B. Transverse section of lateral wall, fixed in 1% glutaraldehyde and 1% OsO_4. — C. As A but fixed in 1% $KMnO_4$ and 1% OsO_4.— D. Detail of operculum, fixed in 1% glutaraldehyde and 1% OsO_4.

Fig. 3A, B. *Pseudombrophila obliquerimosa*, electron micrographs of ripening asci, fixed in 1% glutaraldehyde and 1% OsO_4. — A. Detail of apical epiplasm, showing tubular structure of tract. — B. Operculum region.

Figs. 3C–E. *Fimaria theioleuca*, electron micrographs of ascospore development, fixed in 1% $KMnO_4$ and 1% OsO_4. — C, D. Development of the secondary wall. — E. Id., also showing development of the endospore and the epispore.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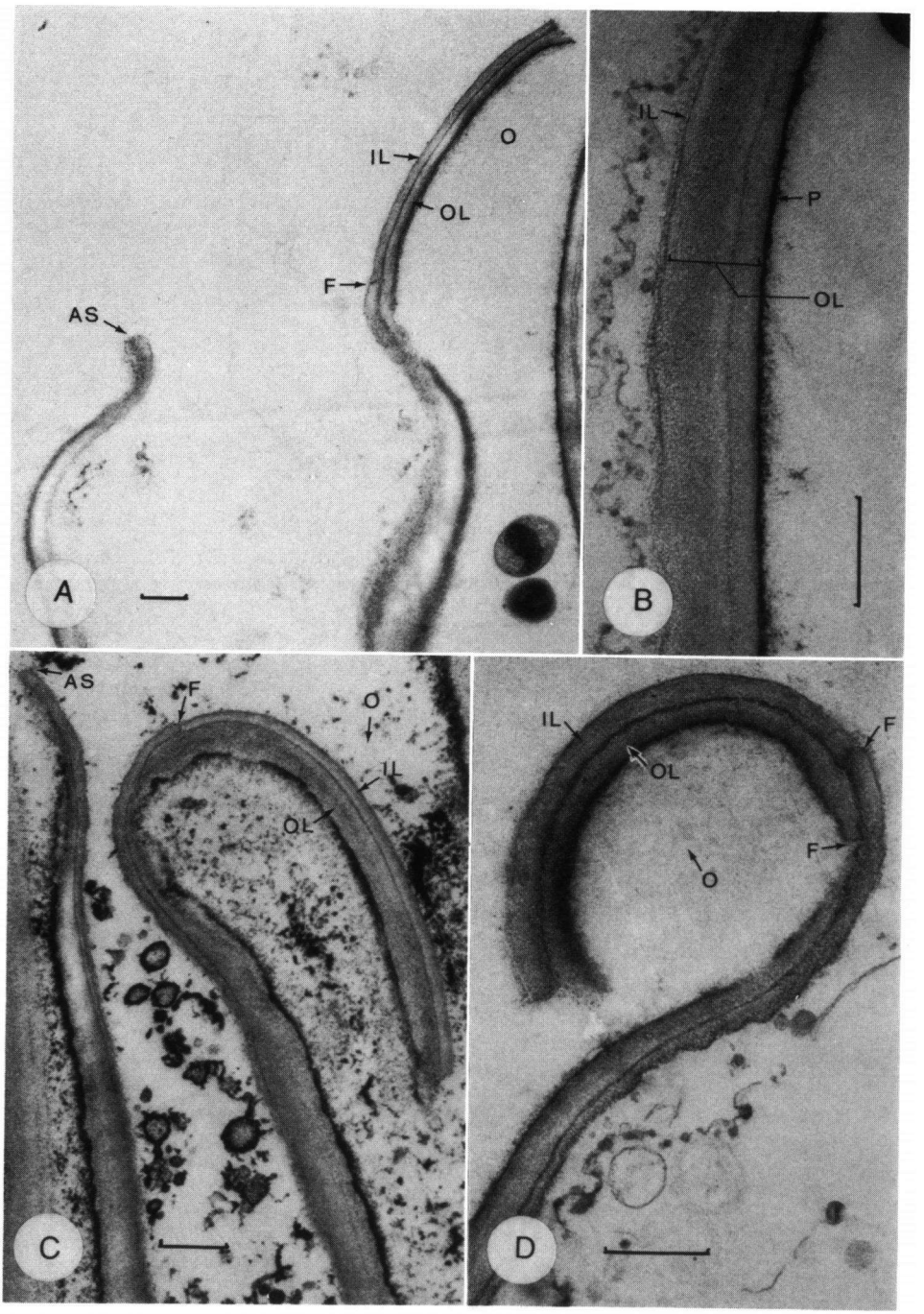
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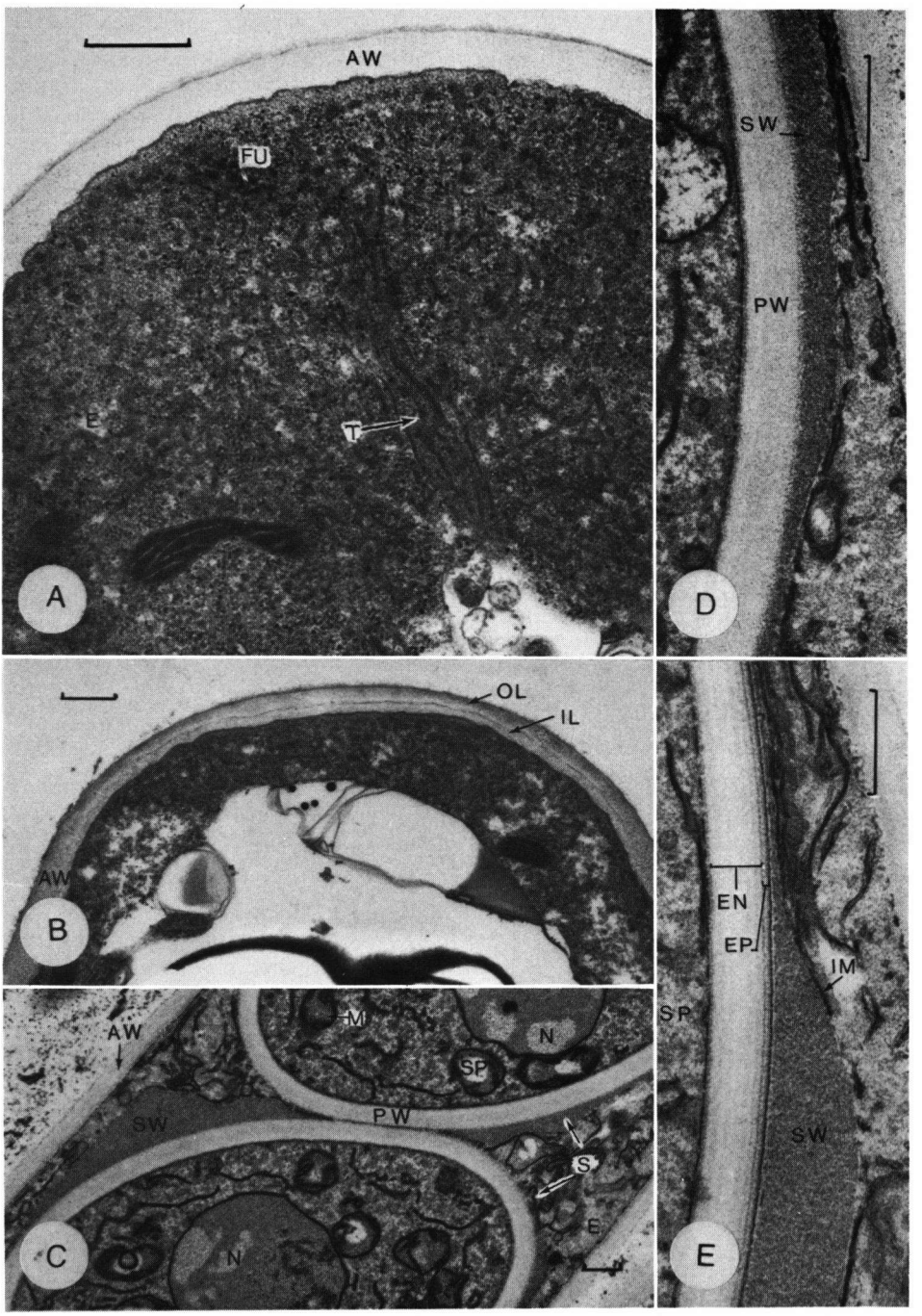
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After the violent discharge of the ascospores the operculum remains attached to the rest of the ascus by a narrow hinge. In the species studied, the place of the hinge seems to be fully arbitrary. Even in the hinge the lines of fissuring can often be recognized in one or both layers (Fig. 2A, C, D). While the layer of the operculum remains more or less constant in thickness, 85–140 nm, the outer layer may swell up to double its original size, from 120 to 280 nm.

In the subapical region, somewhat behind the ascostome, often a zone of irregular swelling and very low electron-density can be distinguished in the wall (Figs. 2A, C). In this zone it is difficult to trace the boundary lines between layers and strata. It corresponds exactly with the sites of optimal staining with e.g. toluidine blue in light microscopy.

Also with electron microscopy a funnel and a tract can be distinguished in the apical part of the epiplasm; both consist of subparallel anastomosing electron-dense tubules 14–17 nm wide (Fig. 3A). In the top of the ascus the tract reaches a diameter of 270–350 nm.

The asci of *Fimaria cervaria*, *Pseudombrophila deerata*, and *P. obliquerimosa* have also been studied in detail, showing that, apart from some minor differences in the dimensions of the asci, there are no significant differences between the four species in the structure of the ascus top and the mechanism of spore liberation.

The ascospore wall

The ultrastructure of these species of *Fimaria* and *Pseudombrophila*, with respect to the development of the ascospores, closely accords with the general process as described by earlier students of representatives of this group of fungi (e.g. Hawker, 1965; Bracker, 1967; Reeves, 1967; Wells, 1972; Merkus, 1973, 1974, 1975, 1976).

In the very young ascus, directly after nuclear division, each nucleus becomes surrounded by a double membrane separating the nuclei with some sporoplasm from the epiplasm. The wall of a young ascospore develops as a homogeneous electron-transparent substance between both parts of this double ascospore delimiting membrane. This primary wall is of rather constant thickness and remains the most constant part of the ascospore wall.

On further ripening an extra layer, the secondary wall, develops between the primary wall and the outer spore delimiting membrane. The aspect of this new wall material is slightly granular and more electron-dense than that of the primary wall.

At first the substance of the secondary wall is homogeneous, but during further development more electron-dense material condenses and accumulates locally in this matrix. The continuous addition of new wall material results in the formation of a distinct ornamentation pattern over the ascospore.

Where new material is formed the outer investing membrane is lifted up. In the end this membrane is often indistinct or fragmentary.

Simultaneously with the formation of the secondary wall, differentiation of the primary wall takes place. In the outer zone of the primary wall a more electron-dense band is formed. On further ripening of the ascospores two or more electron-dense layers become visible. The whole complex of thin layers is called the epispore. The remaining inner part of the primary wall is called the endospore. At times, especially after post-staining with uranyl and lead salts, also a sublayering of the endospore can be made visible (cf. Figs. 4 B–D, 5 B, C).

During ripening of the ascospores the epiplasm and the sporoplasm undergo changes. Especially in the end the epiplasm disintegrates almost completely, losing its original organels, forming a few very large vacuoles, and remaining only as a thin layer just inside the ascoplasmalemma and in the tip.

In the sporoplasm the organels remain present and increase in size and electron-density. Oil-drops are not formed.

Fimaria theioleuca—Figs. 3C–E, 4

In material fixed both in permanganate-OsO_4 and in glutaraldehyde-OsO_4, the primary wall is of rather constant thickness (290–310 nm). The investing membrane separates along the whole surface of the primary wall and a secondary wall of strongly varying thickness with fairly electron-dense contents develops. The investing membrane may run rather irregularly. Often the secondary wall thickens considerably (up to 500 or even 1100 nm) and large homogeneous electron-dense masses are formed on the primary wall (Figs. 3C, E, 4A, B).

During the development of the secondary wall an epispore of about 60 nm thick and an endospore of 180–225 nm thick are formed (Figs. 3E, 4A–F). In the epispore usually two electron-dense layers can be observed, whereas the endospore may show four or five zones of slightly higher electron-density alternating with more electron-transparent ones. Simultaneously with changes in the epiplasm and the sporoplasm the secondary wall modifies. Within the secondary wall, on the outside of the epispore, a rather sharply delimited layer with increased electron-density is formed (Fig. 4B). Gradually this layer grows to form a layer 120–250 nm thick, while the rest of the secondary wall disappears gradually. At maturity a very fine fibrillar structure can be recognized in this layer. Sometimes remnants of the investing membrane can be found on its outside (Fig. 4E). The mature ascospores are smooth.

With light microscopy the secondary wall of mature ascospores stains intensely with methyl blue, showing a thin uninterrupted smooth layer.

Fimaria cervaria—Figs. 5F–J

At first the ascospores in this species develop in the same way as in *F. theioleuca*. In the permanganate-OsO_4-fixed material, the primary wall is homogeneously electron-transparent, 330–370 nm thick. The investing membrane separates from the primary wall and the secondary wall is formed in between, consisting of homogeneous and fairly electron-dense material. The process of secondary wall formation proceeds along the whole primary wall. Locally the secondary wall thickens enormously, up to 1300 or

sometimes even 1700 nm. At the same time an epispore (40–60 nm thick) and an endospore (290–310 nm thick) develop and changes in the epiplasm and the sporoplasm take place. The epispore shows two thin electron-dense layers, the endospore remains homogeneous.

In the homogeneous matrix of the secondary wall locally and close to the surface of the epispore areas of slightly higher electron-density appear (Fig. 5G). Gradually these areas become more electron-dense and grow together to form a continuous layer with a wavy outer boundary, 90–336 nm thick (Figs. 5F, H). During further maturation an electron-dense layer of constant thickness (165–200 nm) with a fine fibrillar structure is formed, while the rest of the secondary wall and most of the epiplasm disappear; the mature spores are smooth (Fig. 5J).

Also with light microscopy, after staining with methyl blue or with interference contrast optics, the thin secondary wall in mature spores shows as an uninterrupted and smooth layer.

Pseudombrophila obliquerimosa—Figs. 5A–E

The glutaraldehyde-OsO_4-fixed material of this species has especially been studied during the last stages of spore development. The early development is the same as in *Fimaria theioleuca* and *F. cervaria*. The primary wall is 380–400 nm thick and has the normal aspect. Separation of the investing membrane from the primary wall has made formation of the secondary wall possible; this is composed of homogeneous and fairly electron-dense material. In the following development a two- or multi-layered epispore (65–70 nm thick) and a sublayered endospore (290–330 nm thick) differentiate within the primary wall. At the same time the secondary wall material concentrates as a continuous, rather uniform layer (120–230 nm thick) of electron-dense material on the epispore (Figs. 5A, B). Together with the main part of the epiplasm, the rest of the secondary wall disappears.

During the last stage of maturation the surface of the rather uniform secondary wall breaks up to form a series of more or less oblique ridges over the surface of the spore

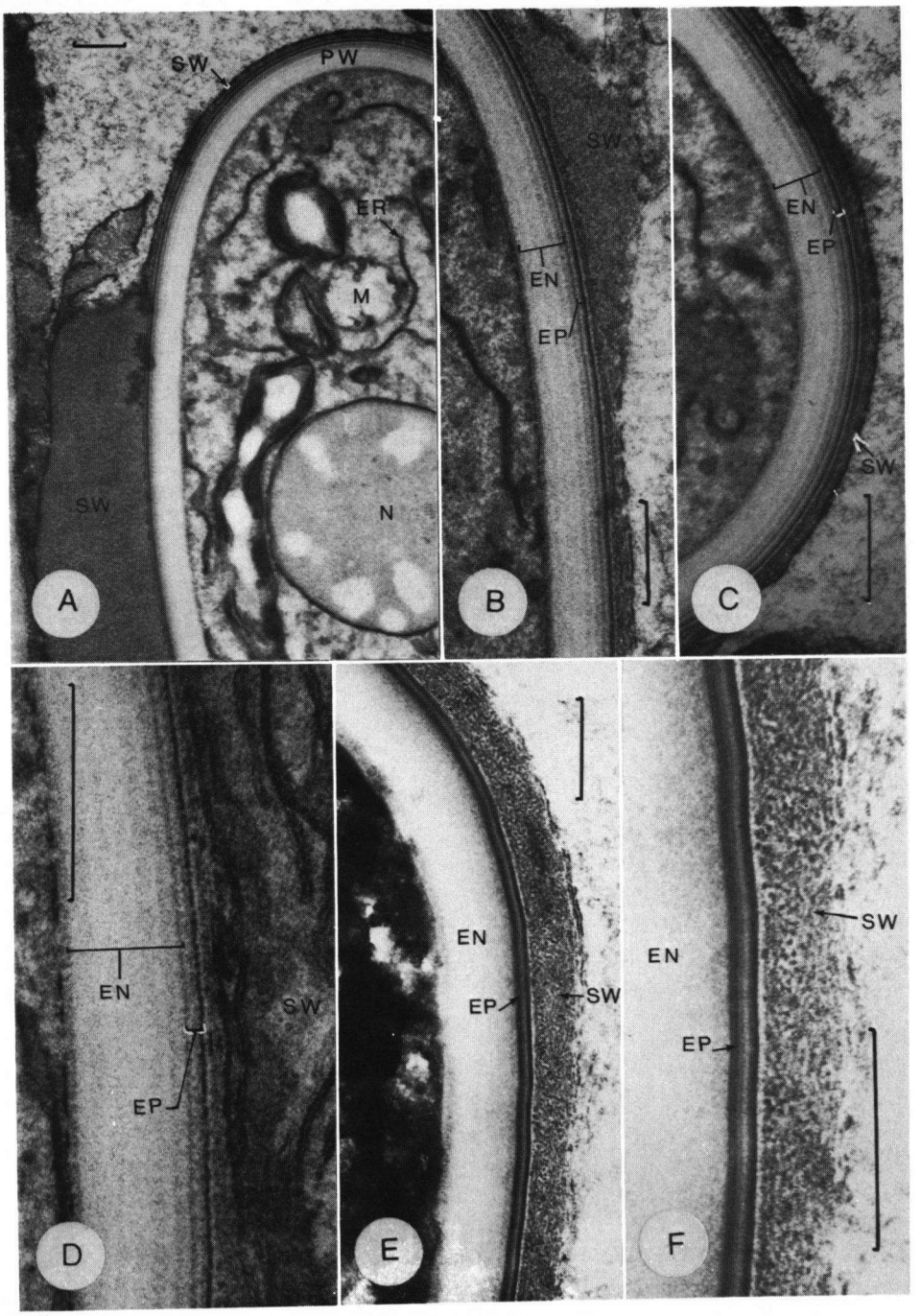
Fig. 4. *Fimaria theioleuca*, electron micrographs of ascospore development, fixed in 1% $KMnO_4$ and 1% OsO_4. — A–C. Condensation of secondary wall material. — D. Detail of condensed material. — E–F. Advanced state of ascospore development.

Figs. 5A–E. *Pseudombrophila obliquerimosa*, electron micrographs of ascospore development, fixed in 1% glutaraldehyde and 1% OsO_4. — A. Development of the secondary wall. — B, C. Id., also showing development of the endospore and the epispore. — D, E. Advanced state in development of ornamentation, showing the fibrillar structure of secondary wall material.

Figs. 5F–J. *Fimaria cervaria*, electron micrographs of ascospore development, fixed in 1% $KMnO_4$ and 1% OsO_4. — F–H. Development of the endospore and the epispore and condensation of secondary wall material. — I. Advanced state of ascospore development, showing a smooth layer of secondary wall material.

Figs. 6A, B. *Pseudombrophila deerata*, electron micrographs of an advanced state of ascospore development, fixed in 1% glutaraldehyde and 1% OsO_4.

Figs. 6C–F. *Fimaria theioleuca*, electron micrographs of septa. — C, D. Septa of excipular cells, fixed in 1% glutaraldehyde and 1% OsO_4. — E, F. Plugged septa in paraphyses, fixed in 1% $KMnO_4$ and 1% OsO_4.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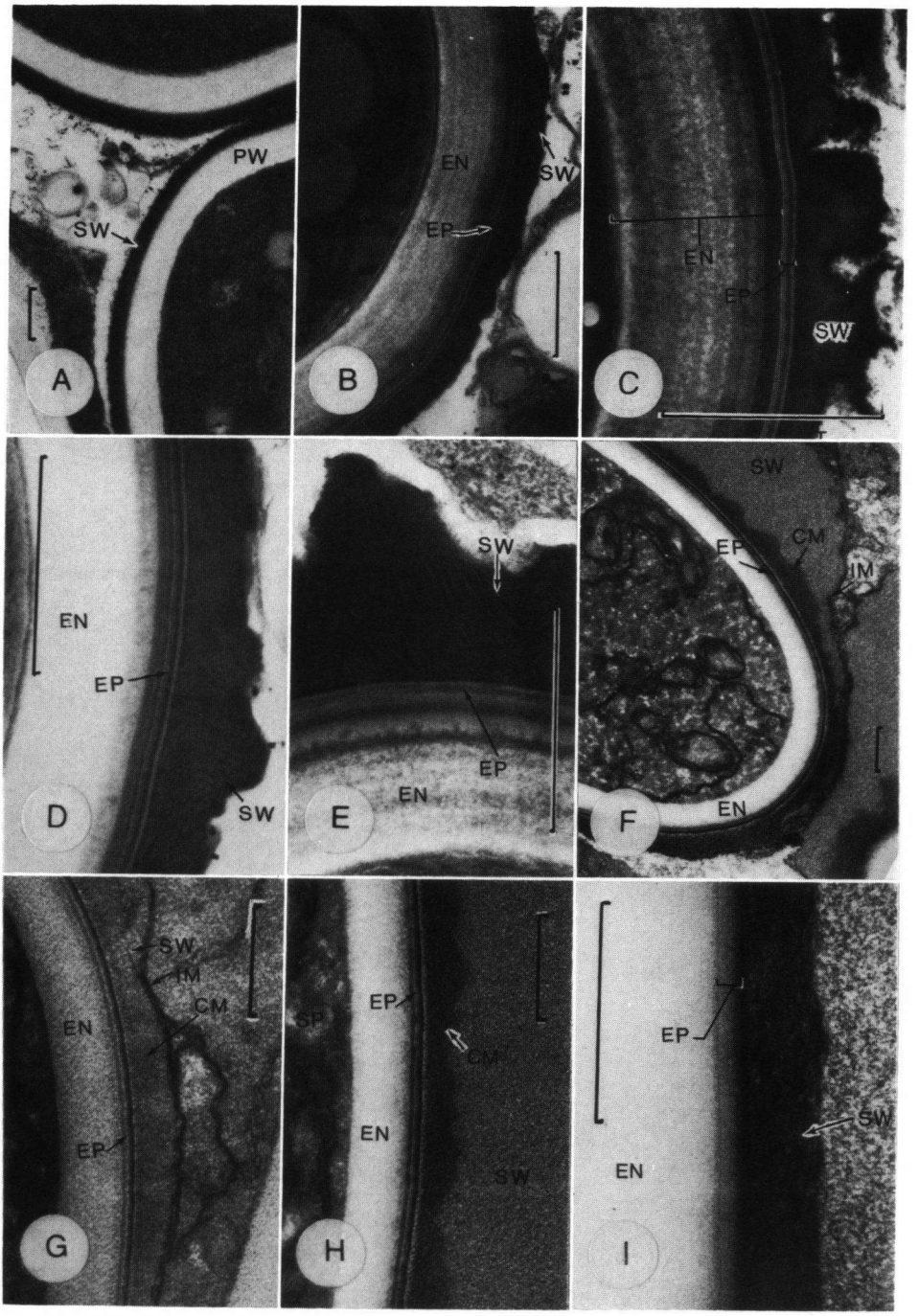
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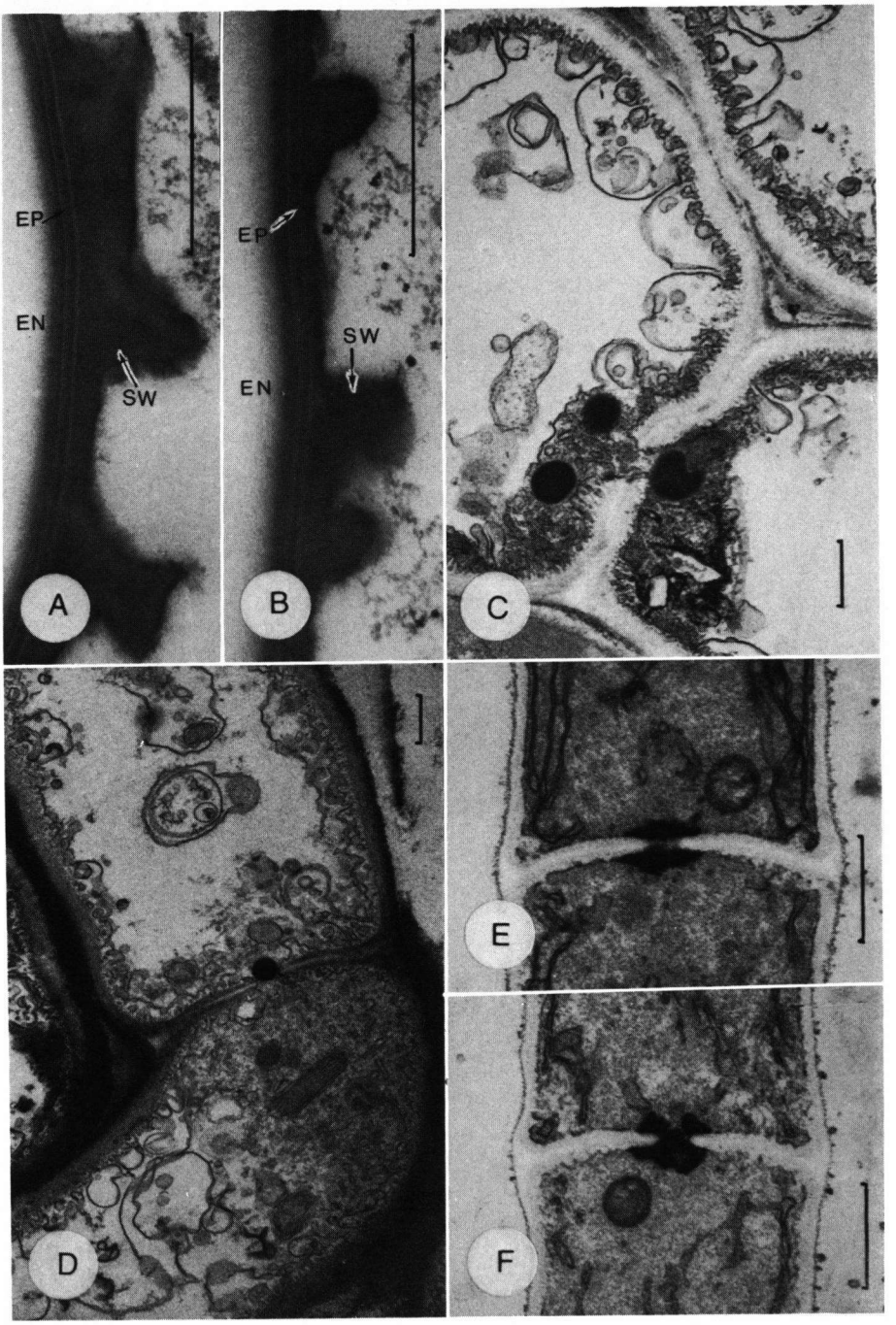
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(Fig. 5C). In the mature spores an ornamentation of irregular ridges (130–490 nm high) of a fine fibrous structure and with an irregularly eroded surface can be observed. Usually the furrows between the ridges do not reach the base of the secondary wall.

With light microscopy and methyl blue staining the ornamentation, consisting of occasionally anastomosing oblique striae, can be observed in the major part of the mature ascospores.

Pseudombrophila deerata—Figs. 6A, B

The glutaraldehyde-OsO_4-fixed material of this species shows that the structure of the primary wall (380–400 nm thick), the epiplasm, and the sporoplasm resemble those in the other species of this study. At an early stage an epispore (55–60 nm thick) and an endospore (330 nm thick) arise. At first the endospore is homogeneous and electron-transparent. Later the outer part increases in electron-density and forms a complete extra layer (25–30 nm thick) adjoining the epispore (Figs. 6A, B). The secondary wall material is fairly electron-dense and homogeneous at the moment when it is deposited between the primary wall and the investing membrane. At later stages the secondary wall material partly condenses on the epispore as a homogeneous and compact, rather smooth electron-dense layer (35–100 nm thick). Finally this layer often breaks up to form local protrusions or warts (160–280 nm high) over the surface of the ascospore (Figs. 5A, B). The rest of the secondary wall disappears together with the main part of the epiplasm. The structure of the ornamentation is not quite clear and the surface is irregular eroded and somewhat fibrous.

With light microscopy the fine warts can just be observed under optimal conditions with methyl blue staining or with interference contrast optics.

The septa

Especially in *Fimaria theioleuca* septa have been studied. In glutaraldehyde-OsO_4-fixed material, cells of the cortical part of the excipulum show strongly thickened cell walls and septa with a simple septal plate; each with a single central pore.

Apparently the thickened walls are densely clothed at their inner side with protuberances that are often densely and minutely diverticulate. Also the septum may be thickened in the same way (Figs. 6C, D). One or more spherical, electron-dense Woronin bodies accompany the septum. The diameter of the Woronin bodies is larger than the septal pores and often one of them can be found to occlude the poral opening.

This is considered the 'typical' ascomycete septal type by Gull (1978). It has been described from a great number of Ascomycetes and their anamorphs. Even in cells where the main part of the cytoplasm has already disappeared, Woronin bodies can be found active at the septal pores (Fig. 6C).

In permanganate-OsO_4-fixed material of the same species septa have been studied in the paraphyses. Here also septal plates with a central pore are formed, but no accompanying Woronin bodies are found. The septal pore is closed by a tightly fitting electron-dense plug (Figs. 6E, F). In a section grazing the edge of the septal pore (Fig. 6E)

electron-dense flattened sides can be observed at each side of the pore. No central opening in the plug is found.

The septal plug in the paraphyses of *Fimaria theioleuca* resembles that of *Chaetomium brasiliensis* Batista & Pontual (Rosing, 1981), *Chaetomidium arxii* Benny (Benny & Samuelson, 1980), and *Neurospora crassa* Shear & B. Dodge (Trinci & Collinge, 1973). It is called the 'solid pulley-shaped plug' by Rosing (l.c.) or 'diabolo-shaped plug' by Chadefaud (1973).

DISCUSSION

The structure of the ascus top in the species of *Fimaria* and *Pseudombrophila* studied is very similar. This structure is summarised in a diagrammatic scheme (Fig. 7).

This type of ascus top with a rather roughly delimited operculum and ascostome, without internal indentation or a prominent ring shows great affinity to the '*Octospora* type' of van Brummelen (1978) or with the 'apical apparatuses' described by Samuelson (1978b) for representatives of the '*Otidea-Aleuria* complex'. This type is known from species of the genera *Pyronema, Anthracobia, Aleuria, Otidea, Coprobia, Cheilymenia, Scutellinia, Octospora, Sowerbyella, Jafnea, Humaria*, and *Sphaerosporella*; all genera belonging to the family Pyronemataceae. So the structure of the ascus top in *Fimaria* and *Pseudombrophila* underlines a close affinity with the members of this family.

In several species with this type of ascus a subapical swelling can be observed at the inner side of the wall at some distance behind the tip. This swelling has the shape of a more or less constant and regular ring. It is composed of material of the rather thin inner ascal layer and additional material precipitated from the surrounding ascosplasm, as may be concluded from the observed local concentration of endoplasmatic reticulum and the activity of lomasomes.

These rings differ clearly from the thick ring found in the subapical ascal wall in species of *Ascozonus*, which is composed of material of deeper wall layers (cf. van Brummelen, 1974; Samuelson, 1978b). The ring in *Ascozonus* is of a different origin and not homologous with the subapical ring found in some genera of the Pyronemataceae as suggested by Samuelson (l.c.).

The strong change in affinity of the operculum and the ascal walls in the subapical region to stains like toluidine blue may be due to local physical changes in the ascal wall at the moment of the forcible discharge of the spores. The walls in this region are strongly overstretched and deformed during dehiscence and often swollen afterwards.

The structure in the asci of several species of Pezizales, described as 'bourrelet sous-apical' by Chadefaud (1942, 1946), corresponds exactly with this area of swelling. From his descriptions it is clear that this 'bourrelet' is rather inconstant and often unequally developed at both sides of the ascus top. Sometimes after strong swelling the constituent layers or strata become partly loose one from the other. This agrees well with earlier observations on representatives of the '*Octospora* type' (van Brummelen, 1978), where this cleavage of the ascal wall is often observed in the swollen region near the ascostome.

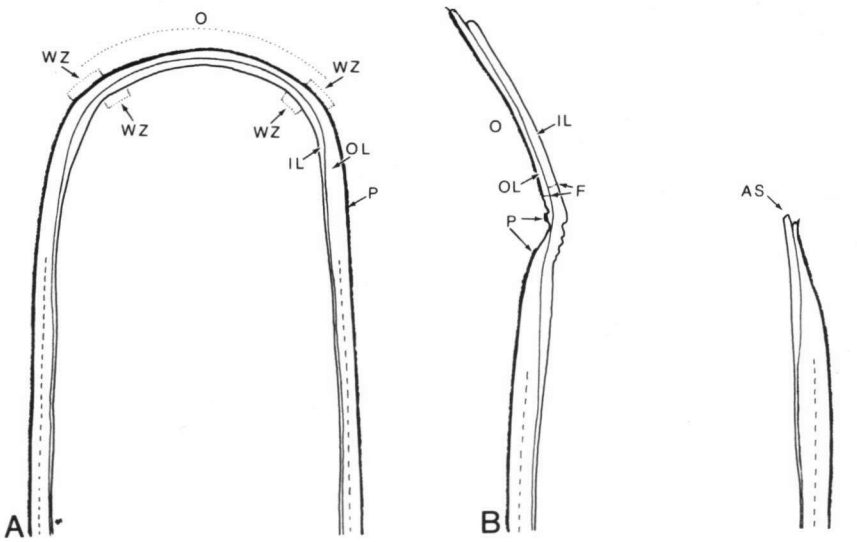

Fig. 7. Diagrammatic sections of ascus tops, as seen with electron microscopy. — A. Almost mature ascus. — B. Ascus after spore discharge.

The 'projecting border' described by Boedijn (1933) as part of the ascus top of *Cookeina sulcipes* (fam. Sarcoscyphaceae) relates to the same structure. In the Sarcoscyphaceae this subapical region is particularly obvious, because of the very strong swelling of the inner layer of the ascal wall. The strongly eccentrically placed operculum in species of *Cookeina* and some related genera make a comparison with symmetrical forms difficult (cf. Eckblad, 1968, 1972; van Brummelen, 1975; Samuelson, 1975; Samuelson & al., 1980).

For the same subapical region of the ascus Samuelson (1975) introduced the term 'suboperculum', defining 'the area of the ascus wall immediately below the line of dehiscence in which transitions in the wall layers are notable.' The upper boundary is well defined by the (future) ascostome, but the lower boundary is more variable, since the transition in wall layers is often rather gradual at the proximal side, especially when a subapical ring is not present.

The term 'suboperculum' for a certain part of the ascal wall is unfortunate, since the same term was more or less implied by Le Gal's (1946a, 1946b) introduction of the 'Suboperculés' for discomycetes with a certain type of operculum. Fully parallel with Le Gal's terminology, Chadefaud (1946) introduced simultaneously his 'para-opercule' for the same operculum model. Moreover the suboperculum of Samuelson is not restricted to the suboperculates of Le Gal, as he might have expected at first (cf. Samuelson, 1975; Samuelson & al. 1980). The prevent further confusion the terms 'subapical region', or 'projecting border' can better be used.

With regard to the development of the ascospore wall, considerable agreement is found between the species studied. The development of the primary wall and its differentiation into an epispore and an endospore reveal a strong resemblance to the general process described in other Pezizales (e.g. Wells, 1972; Merkus, 1973, 1974, 1975, 1976). The perceptibility of the sublayering of the epispore and the endospore depends much upon the methods of fixation and staining used.

A secondary wall of strongly varying thickness is always formed between the primary wall and the investing membrane. In *Fimaria cervaria* local areas of condensed secondary wall material are formed before this concentrates as a uniform smooth layer on the epispore. In the other species studied this local condensation is not observed and the secondary wall material seems to concentrate directly as a smooth layer.

In all cases a uniform smooth layer of electron-dense material is formed that on further ripening shows more or less clearly a fine fibrous structure. During final maturation the outer surface of the smooth layer may break up to form a pattern of ornamentation in all or, at least, a part of the spores. Since this ornamentation arises secondarily from a smooth layer, there is no fundamental difference between both types of spores. The formation of rough or ornamented ascospores in *Pseudombrophila obliquerimosa* and *P. deerata* can be considered as a process of final ripening that is not always completed before their discharge.

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