PERSOONIA
Published by the Rijksherbarium, Leiden
Volume 8, Part. 1, 1–22 (1974)

ULTRASTRUCTURE OF THE ASCOSPORE WALL IN PEZIZALES (ASCOMYCETES)—II

Pyronemataceae sensu Eckblad

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(With Plates 1–14)

The development of wall layers and ornamentation of ascospores is studied with the electron microscope in members of the Pyronemataceae. In all the species primary and secondary walls are formed successively. The primary wall appears to differentiate into two layers, an inner endospore and an outer epispore. The secondary wall is homogeneous in appearance and by redistribution and differentiation gives rise to patterns of ornamentation, which in *Lamprospora crec'hqueraultii* alone show an internal structure. The epiplasm appears to be involved in the formation of the secondary wall.

INTRODUCTION

In classifying the Pezizales the ornamentation patterns of ascospores have often been used as a character for determining species and genera. In particular Le Gal's light microscopy (1947, 1949) has been of importance to this subject; her detailed and extensive study of the structure and development of the ornamentation patterns of the ascospores of the Pezizales is of great value.

An earlier study of mine (Merkus, 1973) summarized both Le Gal's work and the more recent electron microscopy of others. The discrepancies between Le Gal's observations and the results so far obtained by electron microscopy were pointed out and the importance of new studies was emphasized. My own study started with the ornamentation patterns of the ascospores of *Ascodesmis microscopica* (Crouan) Seaver and *A. nigricans* van Tiegh. It confirmed my conjecture that fewer complications in the development of ascospore ornamentation are present than Le Gal thought likely. The differences she reported in the development of the two species were not found either. My results at that time indicated that further studies on this subject were necessary.

The present paper makes it possible to present new information on the development of the ornamentation patterns of the ascospores of the Pezizales. The species studied belong to the Pyronemataceae sensu Eckblad (1968), which are practically identical with the Humariaceae of Le Gal (1947: 285). *Boudiera*, according to Eck-

blad a member of the Ascobolaceae, is added because the development of its spore ornamentation is similar to that of the other species.

REVIEW OF EARLIER WORK

Le Gal's light microscopy of the ornamentation patterns of the ascospores of the Pyronemataceae is reviewed in order to facilitate the present interpretation of it. Where necessary the names used by Le Gal (see footnotes) have been changed according to the rules of the international code of botanical nomenclature (Stafleu *& al.*, 1972).

According to Le Gal the species belonging to this family may vary widely in their mode of development of spore ornamentation. In all her species a primary wall, on which ornamentation arises, is present; the ornamentation consists of callose and pectine formations and is of sporal origin.

In *Trichophaea paludosa* (Boud.) Boud. the ornamentation is very simple and arises directly on the primary wall, extra wall layers not forming. In the other species the development of ornamentation is more complicated, their primary wall being covered by an "assise sous-périsporique" and a "pellicule membranaire". The "assise sous-périsporique" is formed before ornamentation develops. The "pellicule membranaire" is termed "tunique externe de l'assise" if it is formed before ornamentation develops and the substance of the ornamentation does not penetrate it. The "pellicule membranaire" is termed "coque interpérisporique" if it is formed at the same time as the ornamentation and the substance of the ornamentation does penetrate it; the "coque interpérisporique" and the substance of the ornamentation both grow into one, the "coque interpérisporique" also consisting of callose and pectine.

The genera *Lamprospora* and *Boudiera* are very much alike; both develop simple ornamentation. In *Lamprospora ascoboloides* Seaver, *L. areolata* Seaver, *L. crouani* (Cooke) Seaver,[1] *L. dictydiola* Boud., *L. polytrichi* (Schum. per Fr.) Le Gal, *Boudiera areolata* Cooke & Phill. apud Cooke, and *B. echinulata* Seaver the ornamentation is formed between the primary wall and its covering layers; *Lamprospora crec'hqueraultii* (Crouan) Boud. differs, the substance of the ornamentation penetrating the covering layers of the primary wall and the ornamentation developing on the "coque interpérisporique".

Scutellinia asperior (Nyl.) Dennis[2] and nearly all the other species of the genus *Scutellinia* develop simple ornamentation that penetrates the covering layers of the primary wall and develops on the "coque interpérisporique". During the development of the ornamentation a "périspore" is present on the outside of the spores but in a later stage it disappears.

[1] *Lamprospora miniata* (Crouan) Boud.
[2] *Ciliaria asperior* (Nyl.) Boud.

Melastiza chateri (W. G. Smith) Boud.,[3] *Aleuria aurantia* (Pers. per Hook.) Fckl.,[4] *Scutellinia pseudotrechispora* (Schroet.) Le Gal,[5] *Melastiza chateri* (W. G. Smith) Boud. sensu Grelet,[6] and *Aleuria bicucullata* (Boud.) Boud.[7] develop complex ornamentation. Apart from the "assise sous-périsporique" and the "pellicule membranaire" a "périspore" as well as "masses globuleuses" are present; the ornamentation is formed on the "coque interpérisporique". In the first three species the "masses globuleuses" are epiplasmic in origin, while in a later stage they disappear; in the other two species they are of sporal origin and disappear only in *Aleuria bicucullata*. In a later stage the "périspore" disappears in all these species.

In recent light microscopy of the spore ornamentation in *Boudiera echinulata* Seaver, Dissing (1974) described the presence of a hyaline layer around the spores during spore development, which ought to have disappeared when the spores have matured.

In electron microscopy of *Pyronema domesticum* (Sow. per S. F. Gray) Sacc., Reeves (1967) described the first stages of spore development. As wall layers he found an endospore that becomes surrounded by a smooth electron-transparent spore matrix.

MATERIALS AND METHODS

The material of the species in the present study was collected in the Netherlands and in Switzerland; the following list gives some details about the specimens and their localities: *Aleuria aurantia* (Pers. per Hook.) Fckl. — *Piepenbroek 721*, on soil, Wilp, Gelderland, The Netherlands, 11.XI.1973 (L); *Anthracobia melaloma* (Alb. & Schw. per Pers.) Boud.; *Boudiera echinulata* Seaver — *Piepenbroek 665*, in foot-print of horse on soil, Duursche Waarden between Olst and Wijhe, Overijsel, The Netherlands, 23.IX.1973 (L); *Cheilymenia pulcherrima* (Crouan) Boud. — *Bas*, on cow dung, "Het Oerd", Ameland, Friesland, The Netherlands, 27.X.1973 (L); *Lamprospora crec'hqueraultii* (Crouan) Boud. — *Piepenbroek 559*, on soil, "'t Woold", Winterswijk, Gelderland, The Netherlands, 17.VI.1973 (L); *L. dictydiola* Boud. — *van Brummelen 4105*, on burnt ground among mosses, "de Bannink", Colmschate, Overijsel, The Netherlands, 12.VI.1973 (L); *Melastiza chateri* (W. G. Smith) Boud. — *Piepenbroek 722*, on sandy soil, Wilp, Gelderland, The Netherlands, 11.XI.1973 (L); "*Neotiella*" *ithacaensis* (Rehm) Schweers[8] — *van Brummelen & Piepenbroek 4101*, on clay soil, Wilp, Gelderland, The Netherlands, 3.VI.1973 (L); *Scutellinia armatospora* Denison — *Piepenbroek 575a*, on damp soil, Duursche Waarden between Olst and Wijhe, Overijsel, The Netherlands, 19.VIII. 1973 (L); *S. scutellata* (L. per St-Amans) Lamb. —

[3] *Melastiza miniata* (Fckl.) Boud.
[4] *Peziza aurantia* Pers. per Hook.
[5] *Ciliaria pseudotrechispora* (Schroet.) Boud.
[6] Le Gal 1947: 206
[7] *Peziza bicucullata* Boud.
[8] correct name not available

van Brummelen 4070, on burnt wood, Nederhorst den Berg, North-Holland, The Netherlands, 12.V.1973 (L); *van Brummelen 4006*, on dead wood, Axalp, Tiefental near Brienz, alt. 1150 m, Switzerland, 9.IX.1972 (L); *Sepultaria arenosa* (Fckl.) Rehm — *Daams & van Brummelen 4071*, on sandy soil, Nederhorst den Berg, North-Holland, The Netherlands, 12.V.1973 (L); *S. tenuis* (Fckl.) Boud. — *Piepenbroek 620*, on damp soil, Hengforder Waarden, Olst, Overijsel, The Netherlands, 9.IX.1973 (L); *Trichophaea abundans* (P. Karst.) Boud. — *van Brummelen 4106*, on burnt ground, Twello, Gelderland, The Netherlands, 12.VI.1973 (L); and *T. woolhopeia* (Cooke & Phill. apud Cooke) Boud. — *Piepenbroek 564c*, on sandy soil amongst mosses, "Frieswijk", Diepenveen, Overijsel, The Netherlands, 5.VIII.1973 (L).

The apothecia were collected on their substratum in the field. In the laboratory they were taken from the substratum and placed in the fixative. Several types of fixative were applied.

Apothecia were fixed for 30 minutes at room temperature in 1.5% $KMnO_4$ in distilled water, to which one drop of Invadine (Geigy) was added; after several washings in pure distilled water it was postfixed for 60 minutes at room temperature in 1% OsO_4 in cacodylate buffer. Other material was fixed for 3 or 4 hours at 4 °C in 3–3.25% glutaraldehyde in cacodylate buffer; after several washings in pure cacodylate buffer it was postfixed for 60 or 90 minutes at 4 °C in 1% OsO_4. Moreover some material was fixed for several days at 20 °C in 3% glutaraldehyde and 3% acrolein in cacodylate buffer; after several washings in pure cacodylate buffer it was postfixed for 60 minutes at room temperature in 1% OsO_4.

The $KMnO_4$-OsO_4-fixed material was washed in pure cacodylate buffer and dehydrated at room temperature in ethanol, as was the glutaraldehyde-acrolein-OsO_4-fixed material. The glutaraldehyde-OsO_4-fixed material was washed at 4 °C in pure cacodylate buffer, then dehydrated at 4 °C in ethanol. During dehydration the material was stained in 1% uranyl acetate in 30%, 50%, 70%, and 96% ethanol in distilled water or in the 70% and 96% solution or in the 96% solution only. In a few instances staining occurred in a series of dehydration steps, each consisting of a mixture of 1% uranyl acetate in 96% ethanol and a corresponding amount of distilled water.

The material was then transferred at room temperature to the usual Epon embedding medium (Luft, 1961), via 100% ethanol, propylene oxide, and mixtures of propylene oxide and Epon.

The Epon components were used at a rate of 6.1 g Epikote 812, 1.9 g dodecenylsuccinic anhydride, 3.3 g methylnadic anhydride, and 0.15 g 2, 4, 6-tri(dimethylaminomethyl)phenol.

Sections were cut with a glass knife or a Du Pont diamond knife on an LKB Ultrotome III, occasionally stained with various combinations of uranyl acetate (Glauert, 1967) and Reynolds' lead citrate (1963), and examined with a Philips EM 300 electron microscope.

OBSERVATIONS

The ultrastructure of these species accords fairly closely with the ultrastructure of *Ascodesmis microscopica* and *A. nigricans*. A general description will therefore be given first. This is followed by the characteristics of each species separately.

Like with *Ascodesmis microscopica* and *A. nigricans*, in the species in this study the development of the ascospores starts by a delimitation of the spores in the ascoplasm. When delimitation has been completed the two delimiting unit membranes surround sporoplasm in the middle of which a nucleus is situated. The remaining part of the ascoplasm is called the epiplasm.

At these very early stages of spore development epiplasm and sporoplasm are not essentially different from each other. Because of their common origin they have the same electron density and contain the same type of organels, like in *Ascodesmis microscopica* and *A. nigricans*. The form and structure of the organels also answer to the description of these two species. Not until the spores have evolved do the epiplasm and the sporoplasm develop an appearance characteristic of each of them.

In all the species studied here a first spore wall develops between the two delimiting unit membranes. The mode of development and the general structure of the first spore wall is the same as that described for *Ascodesmis microscopica* and *A. nigricans*. Like in those species this wall, here also called primary wall, consists of homogeneous electron-transparent material; it varies from 200–1400 nm in thickness. At a later stage two layers are present, an outer epispore and an inner endospore. In *Ascodesmis microscopica* and *A. nigricans* these two layers developed through redistribution of the primary wall material. This may also occur in the species studied here, but since the origin of the two layers could not be easily traced, I do not wish to state this without comment.

The epispore is 30–100 nm thick and at first consists of only one fairly electron-dense layer. In a later stage three layers are often found. In material fixed in permanganate-OsO_4 the outer and inner layers are electron-dense, while the middle layer is electron-transparent; the outer layer may further increase in thickness. In material fixed in glutaraldehyde-acrolein-OsO_4 or in glutaraldehyde-OsO_4 the outer and inner layers are only fairly electron-transparent, while the middle layer is electron-dense. In a few cases only two layers are found; the outer layer then seems to correspond with the afore-mentioned outer layer. Ultimately with all three fixatives still finer striation, which may alter this original pattern considerably, often becomes visible in all the layers.

The endospore varies in thickness from 150–2000 nm and has the same structure with all the fixatives. In the younger stages it is electron-transparent. Unlike the endospore in *Ascodesmis microscopica* and *A. nigricans*, in the present species the structure may vary in the later stages; its outer part often increases in electron density, sometimes developing a layered structure that joins with the epispore.

Like in *Ascodesmis microscopica* and *A. nigricans*, the inner delimiting unit membrane becomes the sporoplasmalemma and the outer delimiting unit membrane the investing membrane.

After formation of the primary wall new wall material is deposited between the primary wall and the investing membrane. In this way a second spore wall is formed. This occurs in the species that develop ornamented spores as well as in those that develop smooth spores. The very first stages in the development of this spore wall are the same as in *Ascodesmis microscopica* and *A. nigricans*. Like in those species the investing membrane separates from the primary wall and the resulting space fills up with new wall material. In the older stages however all these species show their own characteristic development, which will be described in detail for each of them. The second spore wall will be called secondary wall, as in the two species of *Ascodesmis*.

As the primary and secondary walls evolve, the epiplasm and the sporoplasm each develops its own characteristic appearance. The structural changes in the epiplasm are complete. Almost all the organels disappear, the endoplasmic reticulum probably remaining longest; in the later stages of development the organels are often found in a thin layer just inside the ascoplasmalemma. The structure of the remaining epiplasm varies according to the species; when the spores finally mature almost all the epiplasm in the upper part of the ascus disintegrates. The appearance of the sporoplasm scarcely changes, except for a general increase in electron density; in nearly all the species oil drops develop.

ALEURIA AURANTIA

Fixatives: glutaraldehyde-acrolein-OsO_4, glutaraldehyde-OsO_4, and permanganate-OsO_4. Incipient spores have been seen from the moment at which formation of the primary wall takes place.

After use of the two second fixatives the appearance of the epiplasm and the sporoplasm is in line with the general description. After use of the glutaraldehyde-acrolein-OsO_4-fixative however it is different; because of the irregular and freakish aspect of all the membranes and the presence of extensive clusters of membranes both look rather chaotic. These clusters occur along the ascus wall, where the membranes mostly run parallel to the wall and join with lomasomes; more centrally in the ascoplasm and in the sporoplasm the clusters consist of more or less regularly arranged concentric membranes, which may be associated with electron-transparent vesicles and electron-dense granules. Another frequent phenomenon after use of this fixative is bulbing of the nucleus in the sporoplasm. The bulbs are often associated with sporoplasmic membranes that occur close to the nuclear membrane.

The first structural changes in the epiplasm are found during the formation of the primary wall. In this stage the epiplasmic vacuoles increase in size and number; after fixing in permanganate-OsO_4 they contain some flocky material. The sporoplasm becomes more electron-dense but maintains its original structure. The primary wall develops regularly, becoming 300–400 nm thick.

Deposition of secondary wall material occurs regularly along the whole primary wall, but separation of the investing membrane is more conspicuous at some places

than at others. After use of the two aldehyde-OsO_4-fixatives the investing membrane runs irregularly, after fixing in permanganate-OsO_4 straighter; this difference is found in all the stages of spore development. The secondary wall material is fairly electron-dense; in the permanganate-OsO_4-fixed material it is homogeneous, in the aldehyde-OsO_4-fixed material perhaps slightly more granular (Pl. 1A, B).

When the secondary wall has joined together around the whole primary wall and widens, the structural changes in the plasm increase. The sporoplasm becomes far more electron-dense and develops two oil droplets. The epiplasm undergoes extensive vacuolation whereby the organels originally present as well as the contents of the vacuoles disappear. In the glutaraldehyde-OsO_4-fixed material the remaining epiplasm between the vacuoles is quite normal in appearance; in the permanganate-OsO_4-fixed material it seems to have about the same structure as the secondary wall, whereby it sometimes appears like more or less globularly separated parts (Pl. 1B).

The epispore and the endospore develop regularly. The epispore forms the usual complex of layers of alternating electron density, 30–50 nm thick. The distribution of electron-dense and electron-transparent material in the endospore, which is 250–350 nm thick, is less regular but fairly constant in appearance; it is especially visible after use of the aldehyde-OsO_4-fixatives (Pl. 1C, D).

During the development of the epispore and the endospore the secondary wall increases in thickness and its appearance varies according to the fixatives used. In the permanganate-OsO_4-fixed material the secondary wall material at first becomes slightly more electron-dense; the increase in electron density then occurs in special areas that are often found in the form of bands along the outer border of the secondary wall, or spread more granularly (Pl. 1B, C). In the aldehyde-OsO_4-fixed material electron-dense globules arise throughout the secondary wall; these globules expand and the intermediate material also becomes more electron-dense and granular (Pl. 1D).

During the last stages of the maturation of the spores the secondary wall material, which has further increased in electron density, appears to concentrate on the epispore, where ornamentation is formed. Meanwhile the epiplasm loses its structure completely and disappears (Pl. 1D). The ornamentation consists of a high and coarse reticulum of crests and ridges about 1000 nm thick and connected by a smooth layer about 100 nm thick covering the epispore in the meshes of the net.

ANTHRACOBIA MELALOMA

Fixative: glutaraldehyde-OsO_4. The youngest stages present show fairly advanced spore development. Not only have an epispore and an endospore developed but a secondary wall is also present.

Epiplasm, sporoplasm, and endospore are regular in appearance; the sporoplasm is more electron-dense than the epiplasm, where vacuolation has started. The epispore shows a striated structure parallel to the spore surface and striation perpendicu-

lar to it. The secondary wall has electron-dense and somewhat granular contents; the bounding investing membrane runs slightly undulating (Pl. 2A).

In a following stage the vacuolation in the epiplasm continues. The striated structure in the epispore parallel to the spore surface becomes clearly visible but the striation perpendicular to it has disappeared; the epispore is 60–80 nm thick. The endospore (300–400 nm thick) now shows a differentiated pattern of varying electron density, with largely dominant electron-dense material perpendicular to the epispore; an electron-dense layer in the innermost part of the endospore may be present, too (Pl. 2C, D). The secondary wall has also developed further and the granular contents appear to increase in electron density at several places on the epispore. In this way one or more big electron-dense mass develops on each spore (Pl. 2A).

By the latest stages of spore development the epiplasm has almost totally disappeared, while the sporoplasm has become almost electron-dense and developed several oil drops. The electron-dense masses in the secondary wall have loosened from the epispore and, together with the rest of the secondary wall, appear to be lost in the remnants of the epiplasm (Pl. 2C). No ornamentation is formed; the mature spores are smooth.

BOUDIERA ECHINULATA

Fixative: permanganate-OsO_4. The aspect of the primary wall and the sporoplasm agrees with the general description; the primary wall measures 600–1000 nm; in the sporoplasm small areas with electron-dense granular material are present.

By an early stage of spore development the structural changes have already made great progress in the epiplasm. Where the development of the secondary wall has not yet started, nearly all the organels have disappeared; those remaining are mostly found close to the ascoplasmalemma, where the epiplasm now consists of a broad layer of electron-dense granular material. More centrally in the ascus the epiplasm looks rather empty, except for numerous globules that vary in size and consist of the same granular material as that in the outer layer of the epiplasm; areas with electron-dense flocky material are also found (Pl. 3A).

The development of the secondary wall starts with the separation of the investing membrane from the primary wall. This separation occurs along the whole spore but tends to become fairly conspicuous in some places. In this stage the same flocky material and the same globular structures as in the epiplasm are present in the secondary wall. The globular structures are often seen in association with the investing membrane (Pl. 3A, B).

When the secondary wall develops further it increases in size and remains freakish in outline; it no longer contains the globular structures but consists of the flocky material only. The epiplasm does not change its appearance; in the sporoplasm several oil drops develop and the electron density increases (Pl. 3B).

During the development of the secondary wall an epispore and an endospore

evolve; the epispore varies in thickness from 30–45 nm, the endospore from 600–2000 nm. As the result of a vaguely layered structure sometimes present in the outer part of the endospore, the epispore and the endospore are not clearly separated from each other (Pl. 3C, D).

When the spores mature the flocky material in the secondary wall appears to concentrate on the epispore, where ornamentation is formed (Pl. 3B, C, D). The epiplasm disintegrates completely, as does the rest of the secondary wall. The ornamentation is spinose; the spines vary in height from 250–4000 nm; they are mostly regular in form but sometimes also conical or pin-shaped, or even with a split top (Pl. 3C). A rather smooth layer of about 100 nm thick covers the epispore between the spines (Pl. 3D).

CHEILYMENIA PULCHERRIMA

Fixative: permanganate-OsO_4. In the youngest stages an incipient formation of the secondary wall is present.

Both the primary wall and the sporoplasm are normal in appearance, agreeing with the general description; the primary wall is 500–700 nm thick. By an early stage of development the structural changes in the epiplasm have made great progress, like in *Boudiera echinulata*; it contains only a few organels and consists mainly of fairly electron-dense flocky material. Separation of the investing membrane has occurred along the whole primary wall; the secondary wall varies considerably in thickness and consists of the same flocky material as that in the epiplasm. The investing membrane runs straight but is often interrupted; the free endings then spread locally into the epiplasm, so that the secondary wall and the epiplasm merge into each other (Pl. 4A).

In a somewhat later stage of spore development the appearance of the primary wall and the sporoplasm has scarcely changed; the sporoplasm increases in electron density but no oil droplets are formed. The alterations in the appearance of the secondary wall and the epiplasm are more striking. The secondary wall has widened and increased in electron density; it has also become homogeneous and now contains epiplasmic inclusions. In the epiplasm the flocky material has become more homogeneous and vacuoles with flocky contents have developed. The investing membrane is no longer interrupted as it was before but now shows numerous associations with the epiplasm; the epiplasmic material seems to concentrate into globules that are incorporated in the secondary wall; numerous small vesicular and membranous structures are also found close to the secondary wall (Pl. 4B, C, D).

In a following stage an epispore (40–60 nm thick) and an endospore (600–2500 nm thick) develop (Pl. 4B, D) and at various places on the epispore diffuse and electron-dense, vaguely layered spots are found. Both the secondary wall and the homogeneous material in the epiplasm sometimes still increase in electron density; the associations of the investing membrane with the epiplasm seem to disappear, as do

the vacuoles in the epiplasm. On the epispore a thin electron-dense layer of about 50 nm develops; although nearly everywhere on the spores this layer is smooth, here and there very tiny warts may be present (Pl. 4B, D).

Still later stages were not present in the material so that ornamentation on the mature spores could not be demonstrated; in the literature the mature spores are described as smooth.

LAMPROSPORA DICTYDIOLA

Fixative: permanganate-OsO_4. In this material the spores could be examined in a very early stage of development when the primary wall had not yet reached its ultimate thickness of 200–350 nm. In this stage the epiplasm and the sporoplasm still have the same aspect, which does not differ very much from the general description; both contain areas with electron-dense granules.

When the primary wall is completed the development of the secondary wall starts. Separation of the investing membrane from the primary wall occurs fairly regularly along the whole surface of the spore; the investing membrane runs straight but is often difficult to distinguish. The secondary wall has rather electron-dense homogeneous contents and finally reaches an average thickness of 500 nm (Pl. 5A, B).

During the development of the secondary wall an epispore of 30–40 nm thick and an endospore of 150–300 nm thick are formed (Pl. 5A, B, C) and changes in the epiplasm and the sporoplasm occur. In the epiplasm the areas with electron-dense material at first seem to enlarge (Pl. 5A) but in a somewhat later stage only less electron-dense flocky material is found; close to the investing membrane it seems to be packed into globules of varying size. The electron density of this flocky material and the globules in the epiplasm corresponds with the electron density of the secondary wall material (Pl. 5B, C). In a somewhat later stage vacuolation starts in the epiplasm, whereby nearly all the other organels disappear; the vacuoles contain some flocky material (Pl. 5B, C). Further changes occur in the sporoplasm, where the electron density increases and one large oil drop is formed.

Simultaneously with the continuous changes in the epiplasm and the sporoplasm the appearance of the secondary wall alters further. On the epispore a diffuse layer with increased electron density is formed (Pl. 5C). Locally just after its formation, this layer may show very fine striation running parallel to the spore surface; in a later stage it becomes more homogeneous and about 15 nm thick. In the originally striated parts flattened globules about 50–150 nm across showing the same increased electron density and homogeneous appearance develop. The globules are distributed regularly over the spore surface and grow to rounded spines and ridges of max. 200 nm (Pl. 5D); these finally form reticulate ornamentation on the epispore. The epispore now measures 50–70 nm in thickness (Pl. 5D). Together with the epiplasm the rest of the secondary wall decreases and seems to disappear.

LAMPROSPORA CREG'HQUERAULTII

Fixatives: permanganate-OsO_4 and glutaraldehyde-OsO_4. This species closely resembles *L. dictydiola* in the stages where the primary wall (800–1100 nm thick), the epispore (60–80 nm thick), and the endospore (500–1000 nm thick) develop and the first formations of the secondary wall are found.

During the further development of the secondary wall the appearance of the epiplasm remains the same as in *L. dictydiola*. In the sporoplasm the only difference between the two species is that here instead of one large oil drop several smaller drops develop.

A marked difference between this species and *L. dictydiola* is found in the further development of the secondary wall. At first the secondary wall of this species may widen to 2500 nm (Pl. 6A). In a later stage a number of globules with a granular structure and about 300–500 nm across arise distributed regularly on the epispore (Pl. 6B). The secondary wall material then seems to shape into packets of striated structures. At first these structures are found only around the globules but in a later stage they are evident throughout the secondary wall; they start by running in all directions (Pl. 6C), then seem to pile up and finally unite into large spines of 500–3500 nm, which form complete ornamentation on the epispore (Pl. 6D). Together with the epiplasm the rest of the secondary wall disappears.

MELASTIZA CHATERI

Fixatives: permanganate-OsO_4 and glutaraldehyde-OsO_4. In the youngest stages an incipient formation of the secondary wall is found.

The structures of the primary wall (300–700 nm thick), the sporoplasm, and the epiplasm largely agree with the general description; in the sporoplasm the electron density has increased, in the epiplasm the endoplasmic reticulum is fairly abundant and in the permanganate-OsO_4-fixed material areas with electron-dense granules are found.

In the permanganate-OsO_4-fixed material vacuolation in the epiplasm is found in a somewhat later stage; the vacuoles are completely filled up with slightly electron-dense flocky material, which however disappears in the later stages. In the remaining epiplasm most of the other organels disappear and fairly electron-dense homogeneous areas are found; the granular areas increase in size (Pl. 7B, C, D). In the glutaraldehyde-OsO_4-fixed material vacuolation in the epiplasm has often started in a somewhat earlier stage of development and the vacuoles look empty. The aspect of the remaining epiplasm continues to be normal (Pl. 7A).

The epiplasmic vacuoles are all about the same size and occur against the spores. Separation of the investing membrane has started along the whole spore, but the secondary wall develops particularly between the vacuoles. The investing membrane runs straight; the secondary wall has a fairly electron-dense homogeneous structure.

Together with the development of the secondary wall an epispore (40–60 nm thick) and an endospore (300–600 nm thick) are formed. In the sporoplasm the electron density increases further in this stage and several oil drops develop (Pl. 7C, D).

In later stages the contents of the secondary wall increase in electron density locally and become fairly granular (Pl. 7B, C), ultimately forming reticulate ornamentation 700–1000 nm thick. At the two poles of the spores an apiculus of about 2000 nm may be found and in the meshes of the net the epispore is covered by a thin layer about 50 nm thick (Pl. 7D). The rest of the secondary wall disappears, as does the epiplasm.

"NEOTIELLA" ITHACAENSIS

Fixative: permanganate-OsO_4. The primary wall (300–900 nm thick) as well as the epiplasm and the sporoplasm are regular in appearance and agree with the general description; in the epiplasm small areas with electron-dense granules are present; in the sporoplasm the electron density has slightly increased.

When the secondary wall develops, separation of the investing membrane from the primary wall occurs over nearly the whole spore but locally it is very conspicuous; the investing membrane runs somewhat irregularly. The secondary wall then increases in thickness and its outline becomes undulating; its contents are homogeneous and rather electron-dense. At the same time the appearance of the epiplasm and the sporoplasm changes. In the epiplasm vacuoles with flocky contents develop; the areas with electron-dense granules enlarge but most of the organels originally present remain; in a later stage the epiplasm also contains areas with less electron-dense homogeneous material. In the sporoplasm the electron density increases and several oil drops develop (Pl. 8A, B, C).

During the development of an epispore (30–50 nm thick) and an endospore (300–500 nm thick) (Pl. 8B, C, D) a thin electron-dense layer arises about 40 nm from the epispore and at the same time large diffuse areas with increased electron density are found throughout the secondary wall (Pl. 8C). In the following stage almost globular structures, measuring about 400 nm across and sometimes looking a bit granular, and two or three extra electron-dense layers, which mostly appear to run through the globular structures, develop on the epispore (Pl. 8D). The epiplasm and the rest of the secondary wall seem to disappear. Still later stages could not be studied; it is known from the literature that the mature spores have warty ornamentation.

SCUTELLINIA ARMATOSPORA

Fixatives: permanganate-OsO_4 and glutaraldehyde-OsO_4. In the youngest stages discernible the development of the secondary wall has already started.

The primary wall (450–850 nm thick), the epiplasm, and the sporoplasm have a normal aspect. Separation of the investing membrane has occurred along the whole primary wall; the investing membrane itself mostly runs slightly undulating. The secondary wall material is homogeneous and rather electron-dense.

In the following stage of development the secondary wall at first increases only in thickness, then important changes are found. In the permanganate-OsO_4-fixed material the investing membrane is interrupted; this was also found in *Cheilymenia pulcherrima* and will be described for *Scutellinia scutellata*, *Sepultaria arenosa*, *S. tenuis*, and *Trichophaea woolhopeia*. Like in these species, the free endings of the investing membrane spread into the epiplasm, so that the secondary wall and the epiplasm merge into each other (Pl. 9A). It is not yet clear whether this phenomenon also occurs in glutaraldehyde-OsO_4-fixed material. In the epiplasm the existing organels slowly disappear and vacuolation starts. In the permanganate-OsO_4-fixed material the vacuoles remain rather small and contain flocky material; the rest of the epiplasm becomes homogeneous and fairly electron-dense; in the glutaraldehyde-OsO_4-fixed material the vacuoles may enlarge considerably and look empty, while the rest of the epiplasm looks somewhat granular. The secondary wall itself remains homogeneous and fairly electron-dense; both fixatives show the presence of epiplasmic membranous fragments and vesicles, but after fixing in permanganate-OsO_4 small electron-dense spots that seem to consist of a compact mass of membranes also occur (Pl. 9A). In the sporoplasm the electron density increases and several oil drops are formed.

In the following stage an epispore (70–80 nm thick) and an endospore (550–800 nm thick) develop (Pl. 9B, C, D, E). At the same time an increase in electron density, giving the secondary wall a somewhat more granular look, is found particularly near the epispore, on which electron-dense warts and spines arise (Pl. 9B, C, D).

When the spores mature the epiplasm disappears almost completely, just as the rest of the secondary wall appears to do. The mature spores have warty-spinose ornamentation; the warts and rounded spines are about 500–2000 nm high and may have grown together or else be connected by a continuous layer of 20–50 nm thick and covering the epispore in between completely.

SCUTELLINIA SCUTELLATA

Fixatives: permanganate-OsO_4 and glutaraldehyde-acrolein-OsO_4. The first stages of development of the spores are exactly the same as in *S. armatospora*; the primary wall measures 600–850 nm, the epispore 90–100 nm, and the endospore 500–2000 nm. The glutaraldehyde-acrolein-OsO_4-fixative gives this species a somewhat different look, like in *Aleuria aurantia*; in the epiplasm as well as in the sporoplasm the same irregular and freakish aspect of the membranes and the same clusters of membranes are found.

Differences between the two species of *Scutellinia* occur only in the last stages of development of the secondary wall (Pl. 10A, B, C, D). In the mature spores of *S.*

scutellata the distribution of electron-dense material results in ornamentation of small warts of about 200–300 nm; these may fuse, to some extent forming an irregular network; all over the epispore a thin layer of about 10 nm appears to be present in the meshes of the net. The epiplasm and the rest of the secondary wall disappear, like in *S. armatospora*.

SEPULTARIA ARENOSA

Fixatives: permanganate-OsO_4 and glutaraldehyde-OsO_4. The structures of the primary wall (250–600 nm thick), the sporoplasm, and the epiplasm do not differ from the general description; in the epiplasm and the sporoplasm small areas with electron-dense granules are found after fixing in permanganate-OsO_4.

In the following stage the investing membrane separates along the whole primary wall and a secondary wall with fairly electron-dense contents develops. In the glutaraldehyde-OsO_4-fixed material the investing membrane may run somewhat irregularly; the secondary wall material has a flocky-granular appearance; locally small homogeneously structured globules and parts of layers are then found on the primary wall (Pl. 11B). Where the investing membrane runs less irregularly the secondary wall material is more homogeneous and forms a more continuous layer on the primary wall. In a few rare cases the secondary wall thickens considerably and very large homogeneous electron-dense masses are found on the primary wall (Pl. 11B). In the permanganate-OsO_4-fixed material the investing membrane runs straight; the secondary wall material has a homogeneous aspect and shows many thickenings in which the same electron-dense spots as in *Scutellinia armatospora* and *S. scutellata* are found; further the same interruptions in the investing membrane as described earlier also occur. The electron-dense masses on the primary wall are not present (Pl. 11A).

In the epiplasm most of the organels disappear and vacuolation starts; the vacuoles remain very small and in the permanganate-OsO_4-fixed material they have flocky contents. After use of the glutaraldehyde-OsO_4-fixative the remaining part of the epiplasm obtains the same appearance as the secondary wall. After use of the permanganate-OsO_4-fixative the areas with electron-dense material in the epiplasm enlarge and large areas with the same homogeneous aspect as in the secondary wall are also present (Pl. 11C, D).

By this advanced state of spore development the sporoplasm has increased in electron density; per spore one large oil drop, occasionally accompanied by several smaller drops, is found. Simultaneously an epispore (30–40 nm thick) and an endospore (200–300 nm thick) have evolved. At first the endospore shows a rather broad electron-dense outer part; the electron-dense material then occurs for the most part as a fairly thin and sometimes interrupted layer in the innermore parts (Pls. 11C, D; 12A, B, C).

During maturation of the spores both the epiplasm and the complete secondary wall disappear; the mature spores are smooth.

SEPULTARIA TENUIS

Fixatives: permanganate-OsO_4 and glutaraldehyde-OsO_4. The development of the spores is very much the same as in *S. arenosa*. The structures of the primary wall (200–500 nm thick), the sporoplasm, and the epiplasm agree with the general description. When the secondary wall develops, the investing membrane separates along the whole primary wall. In the glutaraldehyde-OsO_4-fixed material the investing membrane may run a bit irregularly, in which case the secondary wall material is also flocky-granular in appearance; in a later stage an increase in electron density is found especially in the middle of the secondary wall. Like in *S. arenosa*, in a few rare cases the secondary wall thickens considerably and the same large homogeneously structured masses are then present on the primary wall. The permanganate-OsO_4-fixed material has the same appearance as in *S. arenosa*.

During the further development of the spores this species closely resembles *S. arenosa* (Pl. 12D, E), though the sporoplasm develops several smaller oil drops instead of one large drop. Like in *S. arenosa* both the epiplasm and the complete secondary wall disappear when the spores mature; the mature spores are smooth.

TRICHOPHAEA ABUNDANS

Fixatives: permanganate-OsO_4 and glutaraldehyde-OsO_4. The youngest stages present show an incipient formation of the secondary wall.

The structures of the primary wall (400–500 nm thick), the epiplasm, and the sporoplasm do not differ from the general description; in the epiplasm small areas with electron-dense material are found after fixing in permanganate-OsO_4. The investing membrane, which runs fairly straight, has separated from the primary wall at some places and the secondary wall formed locally in this way consists of homogeneous and rather electron-dense material. In the following stages this process of secondary wall formation proceeds along the whole primary wall (Pl. 13A).

At the same time an epispore (35–45 nm thick) and an endospore (350–450 nm thick) develop and changes in the epiplasm and the sporoplasm take place. In the epiplasm some larger vacuoles, which in the permanganate-OsO_4-fixed material have flocky contents, arise and the organels slowly disappear. In the glutaraldehyde-OsO_4-fixed material the remaining epiplasm is normal in appearance; in the permanganate-OsO_4-fixed material the electron-dense granules remain present and larger homogeneous and rather electron-dense areas are also found. In the sporoplasm the electron density increases and two oil drops develop. In the inner part of the secondary wall itself the electron density increases and a continuous electron-dense layer with a wavy outline is finally formed on the epispore (Pl. 13B, C, D).

In the mature spores the epiplasm has disintegrated completely and disappeared, together with the rest of the secondary wall; meanwhile warty ornamentation, which covers the epispore completely and varies in thickness from 50–200 nm, has developed

TRICHOPHAEA WOOLHOPEIA

Fixatives: permanganate-OsO_4 and glutaraldehyde-OsO_4. The glutaraldehyde-OsO_4-fixed material could be studied only in the latest stages of spore development.

This species at first develops the same as *T. abundans*; the primary wall is 650–1400 nm thick. At a later stage however important differences between the two species occur. The secondary wall material appears more flocky and contains the same electron-dense spots as were found in *Scutellinia armatospora*, *S. scutellata*, *Sepultaria arenosa*, and *S. tenuis*; besides epiplasmic membranous fragments, which were also present in the two species of *Scutellinia*, and the interruptions of the investing membrane described above occur (Pl. 14A, B, C). In the sporoplasm the electron density increases and one or two oil drops develop; occasionally several smaller drops are also present.

In this stage an epispore (35–40 nm thick) and an endospore (450–650 nm thick) develop (Pl. 14B, C, D). The endospore at first has a broad and electron-dense outer part, in which striation may be distinguished. In the following stage additional wall material is formed between the sporoplasmalemma and the endospore; it is electron-dense and appears to occur as a continuous layer or as globular and lens-shaped structures; it does not distort the epispore and the endospore (Pl. 14D). The occurrence of this additional wall material is very uncommon and is only found in *Trichophaea woolhopeia*.

No further changes take place until the spores mature and the epiplasm and the secondary wall disappear; the mature spores are smooth.

DISCUSSION

Like in my earlier study of *Ascodesmis microscopica* and *A. nigricans* (Merkus, 1973), the usual methods of preparing material for electron microscopy led to confirmation of the general ultrastructure given thus far for the Ascomycetes (o.a. Hawker, 1965; Bracker, 1967). Different and too aggressive fixatives may have been the cause of the deviations from it found in *Aleuria aurantia* and *Scutellinia scutellata*.

For *Ascodesmis microscopica* and *A. nigricans* the generally accepted hypothesis about the first stages of spore development could be confirmed; in my investigations the delimitation of the spores in the ascoplasm by two unit membranes and the formation of spore walls between these two unit membranes is also found in the species belonging to the Pyronemataceae. Therefore I repeat my theory that this may be seen as a very common process that possibly occurs in all Ascomycetes.

The successive formation of primary and secondary walls has been described for *Ascodesmis microscopica* and *A. nigricans*, the two wall layers are evidently different in structure. In the Pyronemataceae the two different stages in the formation of wall layers are again found. The aspect of the first wall does not differ essentially from that of the primary wall in the two species of *Ascodesmis* so that this first wall is here

also called primary wall. Though the second wall is quite different in appearance from the secondary wall in *Ascodesmis microscopica* and *A. nigricans*, it is also called secondary wall because it has exactly the same position as in the two species of *Ascodesmis* and also develops after the primary wall has been completely formed.

In my discussion on the origin of the spore walls I supposed that lomasomes do not play an important role in the formation of the primary wall in *Ascodesmis microscopica* and *A. nigricans*; this also appears to hold for the Pyronemataceae, where it seems improbable that lomasomes play an important role in the formation of both primary and secondary walls. Special activity of the sporoplasm during the formation of the secondary wall could not be found either. Again, no evidence has been obtained for Le Gal's assumption that the secondary wall is of sporal origin. On the contrary, the present study shows that emphasis should be laid on the possibility that the epiplasm plays an important role in the formation of the secondary wall. This I also emphasized with the two species of *Ascodesmis*, whereby I suggested that, according to the results obtained thus far "the way in which the epiplasm is involved possibly determines the appearance of the secondary wall".

My results on the Pyronemataceae warrant confirmation of this last statement. When spore development starts, the epiplasm has a common structure; this structure and the changes it undergoes as soon as a secondary wall begins to form differ in aspect according to the fixative applied. With both fixatives most of the organels disappear and associations of the epiplasm with the secondary wall are found. After fixing in permanganate-OsO_4 however vacuolation of the epiplasm is not so frequent or so extensive as after fixing in glutaraldehyde-OsO_4, while the epiplasm sometimes also contains electron-dense granules. These granules either remain present during the development of the spores, sometimes even increasing in number, or else they have disappeared beforehand; sometimes they are present in the sporoplasm and may be compared with the glycogen granules in *Ascodesmis microscopica* and *A. nigricans*. Together with less electron-dense homogeneous or flocky material, which often arises in the epiplasm during the development of the spores and is possibly related to the granules, they may finally form the ground mass of the epiplasm. Between the vacuoles the epiplasm often develops the same appearance as the secondary wall, especially after use of the permanganate-OsO_4-fixative; it is therefore not improbable that the epiplasm and the secondary wall both contain identical substances.

In its first stages of development the secondary wall material has the same appearance throughout the entire secondary wall. In later stages however important changes that completely alter the appearance of the secondary wall occur; in nearly all the species of the Pyronemataceae studied a local increase or decrease of the electron density of the secondary wall is found, so that it seems as though the secondary wall material condenses at certain places. A continuing increase in the amount of secondary wall material during this condensation process should not be excluded. The starting-point, the further course of the condensation process, and the ultimate ornamentation pattern on the epispore that finally results from the condensed material

differ from species to species but they are all quite regular and characteristic for each species separately.

At those places where the electron density of the secondary wall decreases the secondary wall material finally disappears completely; it is not yet clear whether it all joins the condensed areas or that part of it disintegrates. Like in the study of *Ascodesmis microscopica* and *A. nigricans* it is also uncertain whether the investing membrane remains present or disappears.

All the species with ornamented spores, viz. *Aleuria aurantia*, *Boudiera echinulata*, *Cheilymenia pulcherrima*, *Lamprospora dictydiola*, *L. crec'hqueraultii*, *Melastiza chateri*, "*Neotiella*" *ithacaensis*, *Scutellinia armatospora*, *S. scutellata*, and *Trichophaea abundans* develop their ornamentation in this way. In *Lamprospora crec'hqueraultii* the ornamentation shows a very peculiar internal structure that arises in an originally homogeneous secondary wall. In the other species the ornamentation does not show any special internal structure, although it sometimes has a somewhat granular appearance.

Apart from the species with ornamented spores several species with smooth spores have been studied. Although in these species the development of the spores is not essentially different, the smooth spores may arise in different ways. In *Anthracobia melaloma*, *Sepultaria arenosa*, and *S. tenuis* an incipient condensation disappears in a later stage, so that smooth spores rather than ornamented spores develop; it is striking that in these species the condensation may be fairly extensive but it is then restricted to one or a few spots on the epispore. In *Trichophaea woolhopeia* no condensation at all is found, there the secondary wall material in its original form disappears.

In comparing the development of the ornamentation patterns in *Ascodesmis microscopica* and *A. nigricans* and in the species that belong to the Pyronemataceae it should be stressed that in *A. microscopica* and *A. nigricans* the secondary wall builds up the ornamentation patterns in a final form immediately after it is formed; the ornamentation patterns in the Pyronemataceae however arise only through redistribution of already present secondary wall material.

In a certain stage of spore development all the species in this study show an epispore that consists of a fairly constant pattern of layers of alternating electron density. If however in a later stage extra layers arise between the existing layers, each species develops a more characteristic epispore. This was also found in *Ascodesmis microscopica* and *A. nigricans*.

Both in *Ascodesmis microscopica* and *A. nigricans* and in the species in this study the endospore starts as a homogeneous electron-transparent layer. In later stages of spore development it may become internally structured, in contrast to the endospore in the two species of *Ascodesmis* that remains homogeneous; this internal structure is especially apparent in the aldehyde-OsO_4-fixed material. The outer part of the endospore often becomes fairly electron-dense, perhaps with a layered structure joining the epispore.

For *Ascodesmis microscopica* and *A. nigricans* it appeared as though the epispore and the endospore both developed in the primary wall by a redistribution and differentiation of the primary wall material itself. Since my findings were not conclusive however

I do not wish to state this without further comment for the species belonging to the Pyronemataceae. In discussing the origin of the epispore and the endospore, it is evident that the endospore must consist of primary wall material. On the other hand the epispore may have developed from either primary wall material, secondary wall material, or primary and secondary wall material. The fact that there is a strong similarity in structure between the epispore of the present species and the epispore of *Ascodesmis microscopica* and *A. nigricans*, that the boundary between the epispore and the endospore is often not distinct but gradual, and that the secondary wall material is totally different in appearance from the epispore (only the layered structure of the innermost part of the secondary wall in "*Neotiella*" *ithacaensis* disagrees) and the endospore however confirms my theory that in the species belonging to the Pyronemataceae both the epispore and the endospore also differentiate within the primary wall.

The variation in thickness of the primary wall, the epispore, and the endospore must for a large part be ascribed to the possibility of swelling during fixation; as was also found in *Ascodesmis microscopica* and *A. nigricans* the glutaraldehyde-OsO_4-fixative gives low and fairly constant values, while the permanganate-OsO_4-fixative gives high and variable values.

Like for *Ascodesmis microscopica* and *A. nigricans*, the results of the present study do not entirely accord with Le Gal's observations (1947, 1949). In all the species the wall layers and membranes involved in the development of ornamentation have the same position, in contrast to Le Gal's descriptions of the occurrence of ornamentation on the inner or outer side of the "assise sous-périsporique" and the "pellicule membranaire". If the "pellicule membranaire" is described as "tunique externe de l'assise" the secondary wall may possibly be compared with the "assise sous-périsporique" and the investing membrane with the "pellicule membranaire". On the other hand if the "pellicule membranaire" is described as "coque interpérisporique" it should be compared with the innermost layer of the ornamentation but a layer comparable with the "assise sous-périsporique" is then absent; in that case the secondary wall should be seen as the "périspore". No evidence has been obtained for the presence of "masses globuleuses" or for sporal origin of the substance of the ornamentation constituting the secondary wall. The hyaline layer Dissing (1974) described for *Boudiera echinulata* and the spore matrix Reeves (1967) indicated for *Pyronema domesticum* are probably identical with the secondary wall.

ACKNOWLEDGEMENTS

The author is much indebted to Professor Dr. C. Kalkman, Director of the Rijksherbarium (Leiden), for his stimulating interest and to Dr. J. van Brummelen for critical discussions. Thanks are also due to Miss B. A. D. Dam and Mrs. F. P. Boers-van der Sluijs for their skilled technical assistance, to Mrs. G. Piepenbroek-Groters for sending valuable living material of many species, and to Mrs. E. Helmer van

Maanen Ph. D. for suggestions about the manuscript. This study could not have been carried out without the financial support of the Foundation for Fundamental Biological Research (BION), which is subsidized by the Netherlands Organization for Advancement of Pure Research (ZWO).

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EXPLANATION OF PLATES 1–14

ABBREVIATIONS USED IN PLATES. — AW, ascus wall; CM, condensed material; E, epiplasm; En, endospore; Ep, epispore; Gr, granules; IM, investing membrane; PI, plasmatic includings; PW, primary wall; S, sporoplasm; SW, secondary wall; T, tonoplast; Va, vacuole.

PLATE 1

Figs. A–D. *Aleuria aurantia*, spore development: Fig. A. beginning of secondary wall formation, fixed in 3% glutaraldehyde and 1% OsO_4 and stained with lead citrate, × 33,300; Fig. B. condensation of secondary wall material, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with lead citrate, × 30,900; Fig. C. id. also showing development of the epispore and the endospore, × 23,800; Fig. D. id. fixed in 3% glutaraldehyde and 1% OsO_4 and stained with uranyl acetate and lead citrate.

PLATE 2

Figs. A–D. *Anthracobia melaloma*, spore development, fixed in 3.25% glutaraldehyde and 1% OsO_4 and stained with uranyl acetate and lead citrate: Fig. A. condensation of secondary wall material, × 36,300; Fig. B. development of the epispore and the endospore, × 47,800; Fig. C. id. × 23,100; Fig. D. id. detail of the epispore and the endospore, × 36,300.

PLATE 3

Figs. A–D. *Boudiera echinulata*, spore development, fixed in 1.5% $KMnO_4$ and 1% OsO_4: Fig. A. development of the secondary wall, stained with lead citrate, × 11,700; Fig. B. condensation of secondary wall material, stained with lead citrate, × 10,100; Fig. C. id. also showing development of the epispore and the endospore, stained with uranyl acetate and lead citrate; Fig. D. id. detail of the epispore and the endospore, × 39,700.

PLATE 4

Figs. A–D. *Cheilymenia pulcherrima*, spore development, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate: Fig. A. beginning of secondary wall formation, × 22,000; Fig. B. condensation of secondary wall material and development of the epispore and the endospore, × 22,000; Fig. C. detail of the epiplasm and the secondary wall, × 22,000; Fig. D. condensation of secondary wall material and development of the epispore and the endospore, × 13,400.

PLATE 5

Figs. A–D. *Lamprospora dictydiola*, spore development, fixed in 1.5 % $KMnO_4$ and 1% OsO_4: Fig. A. development of the secondary wall, the epispore, and the endospore, stained with uranyl acetate and lead citrate, × 17,100; Fig. B. id. stained with lead citrate, × 31,200; Fig. C. condensation of secondary wall material, stained with lead citrate, × 25,500; Fig. D. id. stained with uranyl acetate and lead citrate, × 31,200.

PLATE 6

Figs. A–D. *Lamprospora crec'hqueraultii*, spore development, stained with lead citrate: Fig. A. development of the secondary wall, the epispore, and the endospore, fixed in 1.5% $KMnO_4$ and 1% OsO_4, × 14,500; Fig. B. condensation of secondary wall material, fixed in 1.5% $KMnO_4$ and 1% OsO_4, × 26,300; Fig. C. id. also showing the internal structure in the secondary wall, × 23,100; Fig. D. id. fixed in 3.25% glutaraldehyde and 1% OsO_4.

PLATE 7

Figs. A–D. *Melastiza chateri*, spore development, stained with uranyl acetate and lead citrate: Fig. A. development of the secondary wall, fixed in 3% glutaraldehyde and 1% OsO_4, × 31,200; Fig. B. condensation of secondary wall material, fixed in 1.5% $KMnO_4$ and 1% OsO_4, × 25,500; Fig. C. id. also showing development of the epispore and the endospore, × 23,200; Fig. D. id. × 14,300.

PLATE 8

Figs. A–D. "*Neotiella*" *ithacaensis*, spore development, fixed in 1.5% $KMnO_4$ and 1% OsO_4: Fig. A. development of the secondary wall, stained with lead citrate, × 39,700; Fig. B. id. also showing development of the epispore and the endospore, stained with uranyl acetate and lead citrate, × 24,800; Fig. C. condensation of secondary wall material, stained with uranyl acetate and lead citrate, × 27,300; Fig. D. id. × 55,700.

PLATE 9

Figs. A–E. *Scutellinia armatospora*, spore development, stained with uranyl acetate and lead citrate: Fig. A. development of the secondary wall, fixed in 1.5% $KMnO_4$ and 1% OsO_4, × 25,500; Fig. B. id. also showing condensation of secondary wall material and development of the epispore and the endospore, × 22,000; Fig. C. id. fixed in 3.25% glutaraldehyde and 1% OsO_4, × 26,900; Fig. D. id. × 17,100; Fig. E. detail of the epispore, fixed in 1.5% $KMnO_4$ and 1% OsO_4, × 65,800.

PLATE 10

Figs. A–E. *Scutellinia scutellata*, spore development: Fig. A. condensation of secondary wall material, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate, × 29,800; Fig. B. id. fixed in 3.25% glutaraldehyde and 1% OsO_4 and stained with lead citrate; Figs. C, D. subsequent stages in the development of the epispore and the secondary wall, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate, × 52,000; Fig. E. id. fixed in 3.25% glutaraldehyde and 1% OsO_4 and stained with lead citrate.

PLATE 11

Figs. A–D. *Sepultaria arenosa*, spore development: Fig. A. beginning of secondary wall formation, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with lead citrate, × 22,000; Fig. B. condensation of secondary wall material, fixed in 3.25% glutaraldehyde and 1% OsO_4 and stained with uranyl acetate and lead citrate, × 30,500; Fig. C. development of the epispore and the endospore, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate, × 26,900; Fig. D. id. × 17,100.

PLATE 12

Figs. A–C. *Sepultaria arenosa*, subsequent stages in the development of the epispore and the endospore, fixed in 1.5% $KMnO_4$ and 1% OsO_4: Fig. A. stained with lead citrate, × 70,000; Fig. B. stained with uranyl acetate and lead citrate, × 70,000; Fig. C. id. × 70,000.

Figs. D, E. *Sepultaria tenuis*, spore development, stained with uranyl acetate and lead citrate: Fig. D. development of the secondary wall, the epispore, and the endospore, fixed in 1.5% $KMnO_4$ and 1% OsO_4, × 18,000; Fig. E. detail of the epispore and the endospore, fixed in 3.25% glutaraldehyde and 1% OsO_4, × 63,800.

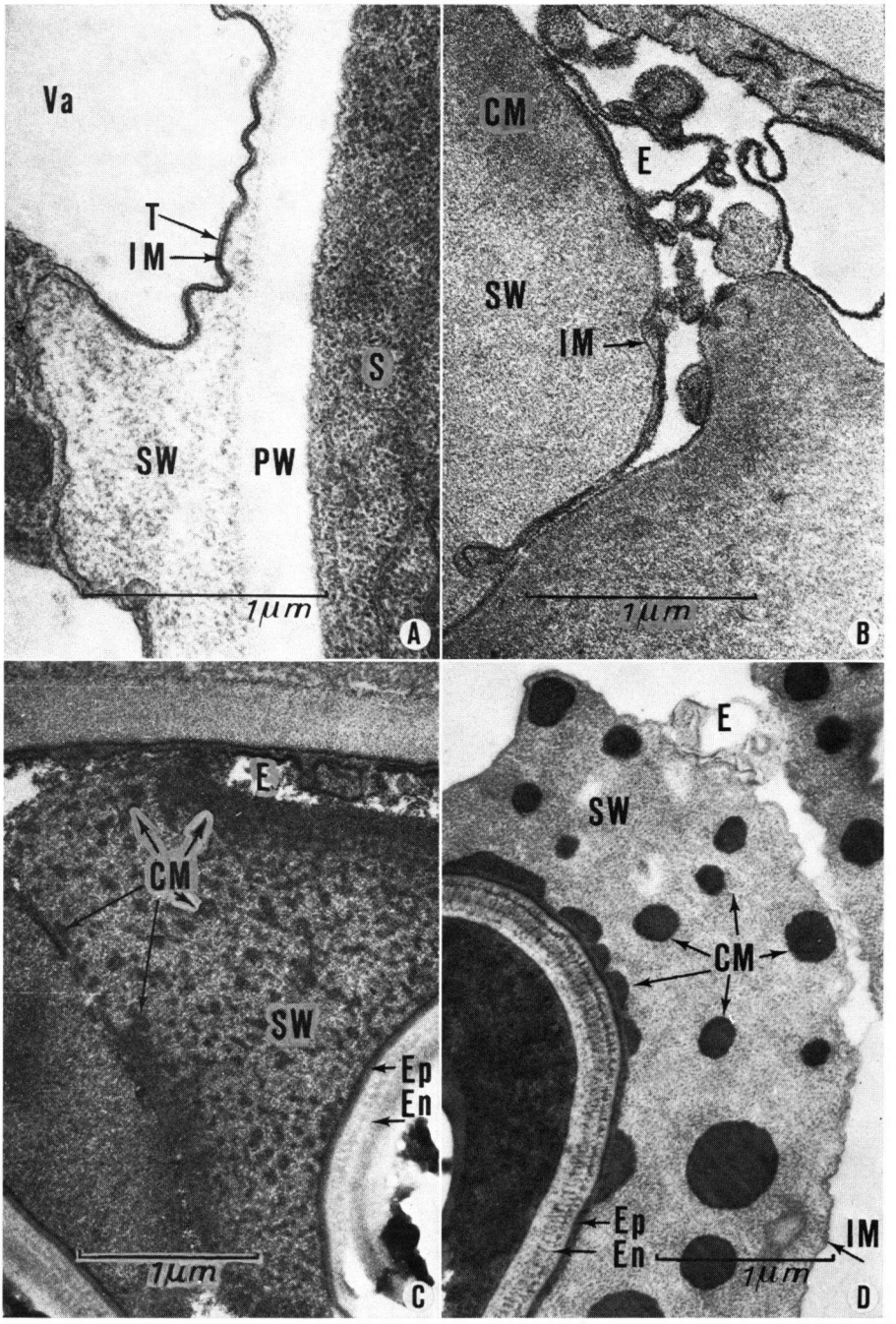
PLATE 13

Figs. A–D. *Trichophaea abundans*, spore development: Fig. A. development of the secondary wall, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with lead citrate, × 36,100; Fig. B. condensation of secondary wall material and development of the epispore and the endospore, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with uranyl acetate and lead citrate, × 28,300; Fig. C. id.; Fig. D. id. fixed in 3.25% glutaraldehyde and 1% OsO_4, × 37,800.

PLATE 14

Figs. A–D. *Trichophaea woolhopeia*, spore development: Fig. A. development of the secondary wall, fixed in 1.5% $KMnO_4$ and 1% OsO_4 and stained with lead citrate, × 15,600; Fig. B. id. also showing development of the epispore and the endospore, stained with uranyl acetate and lead citrate, × 19,900; Fig. C. id. not stained, × 29,700; Fig. D. detail of the epispore and the endospore, fixed in 3.25% glutaraldehyde and 1% OsO_4 and stained with uranyl acetate and lead citrate, × 45,000.

PLATES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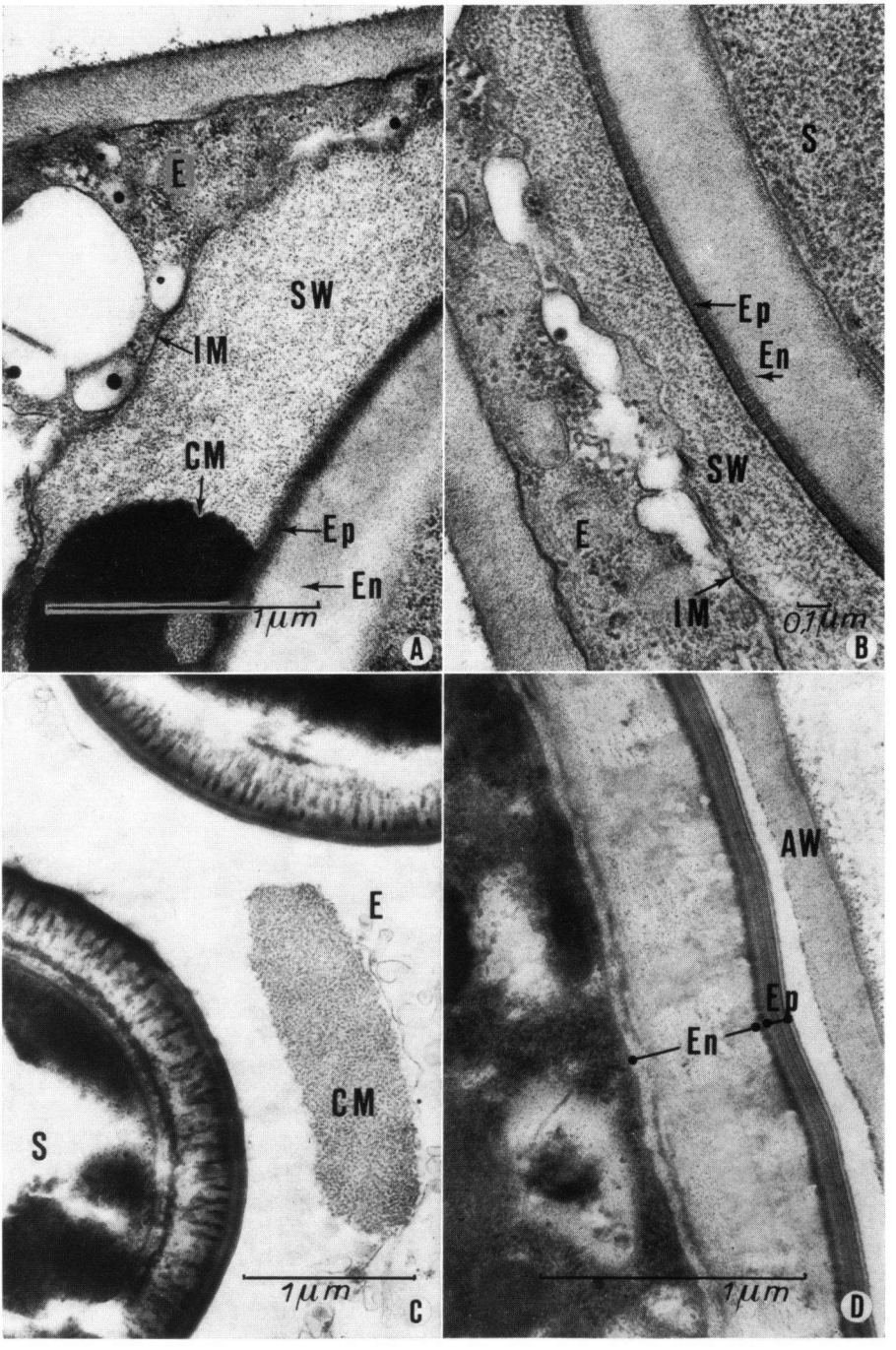
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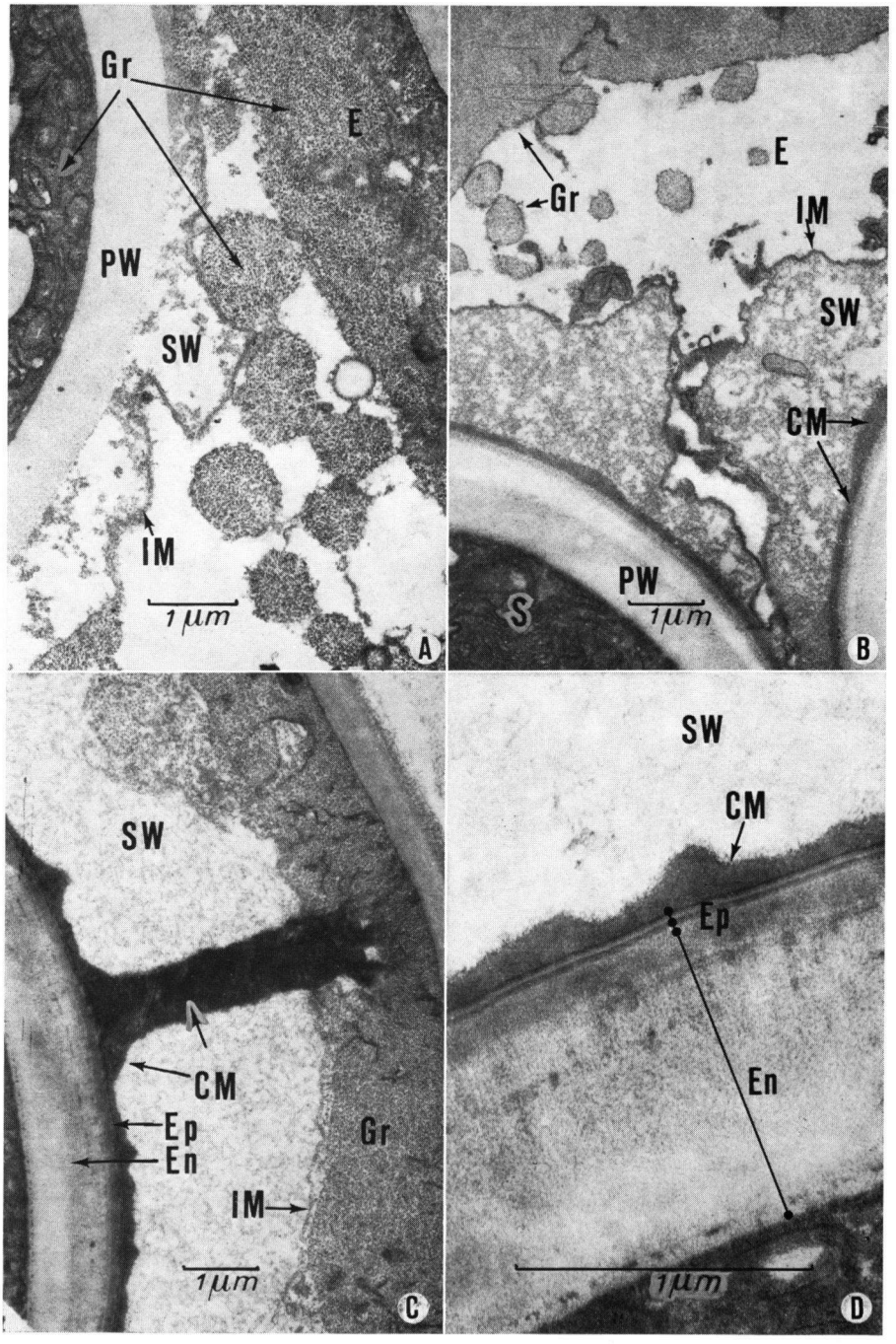
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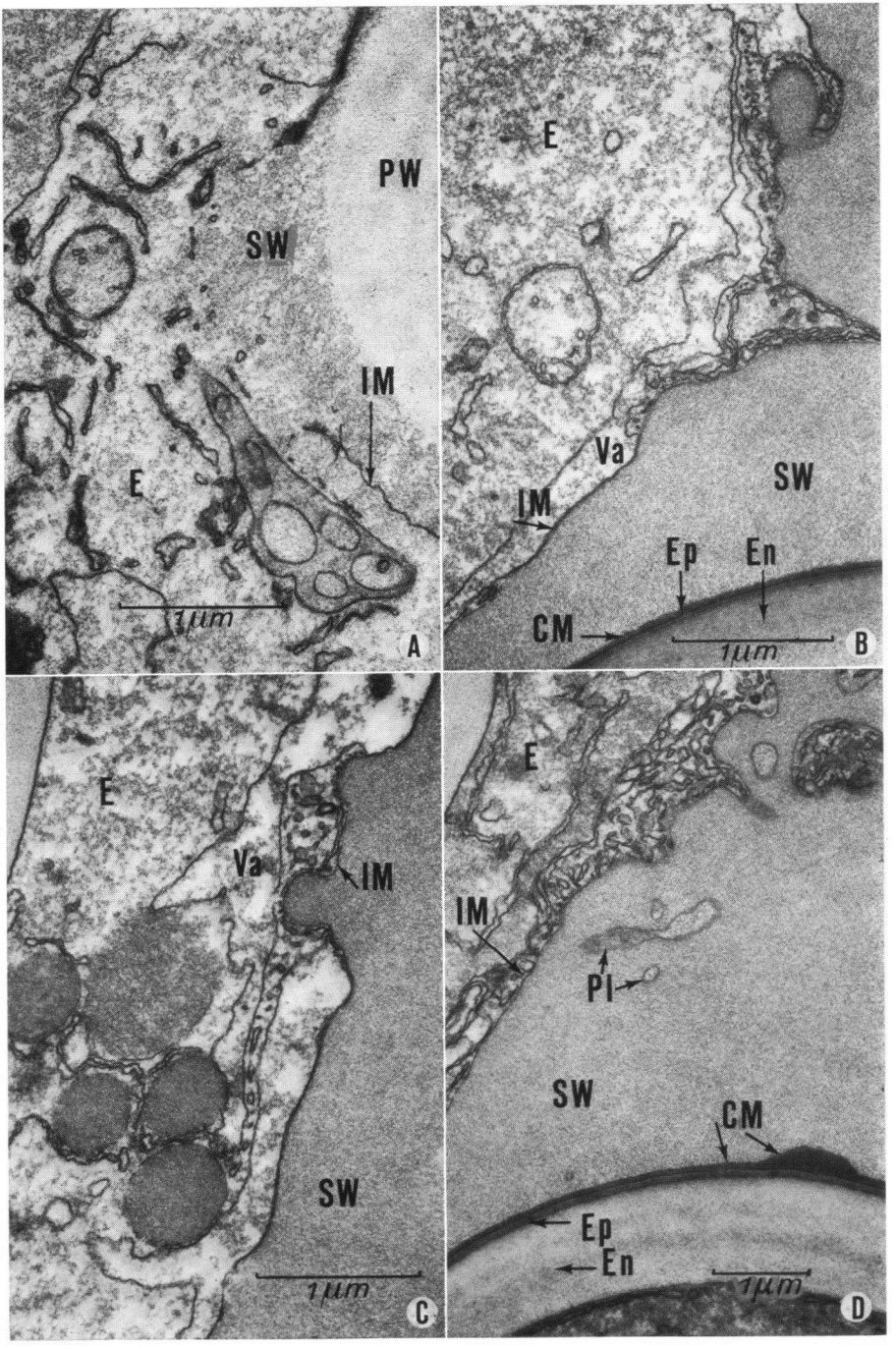
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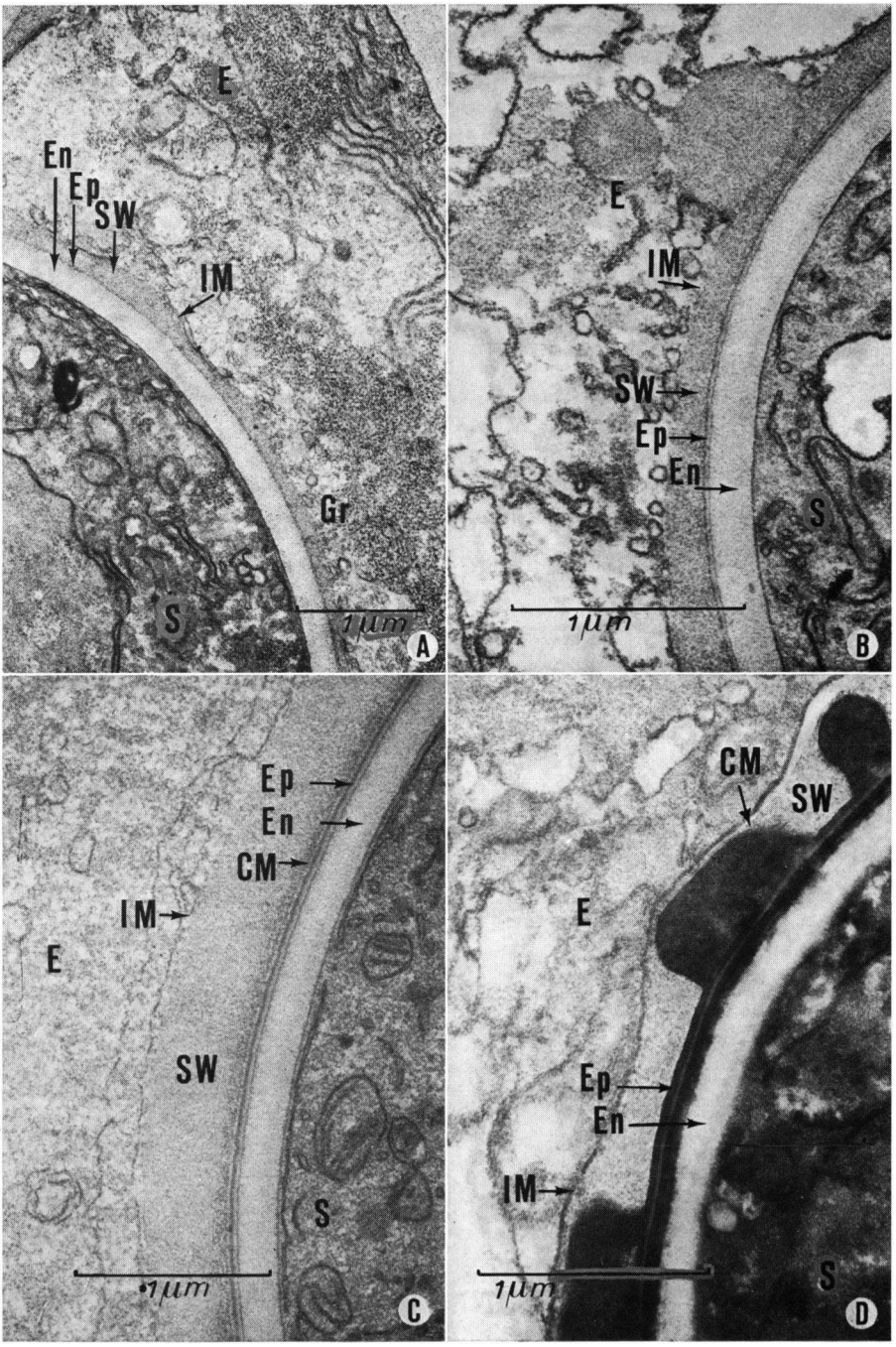
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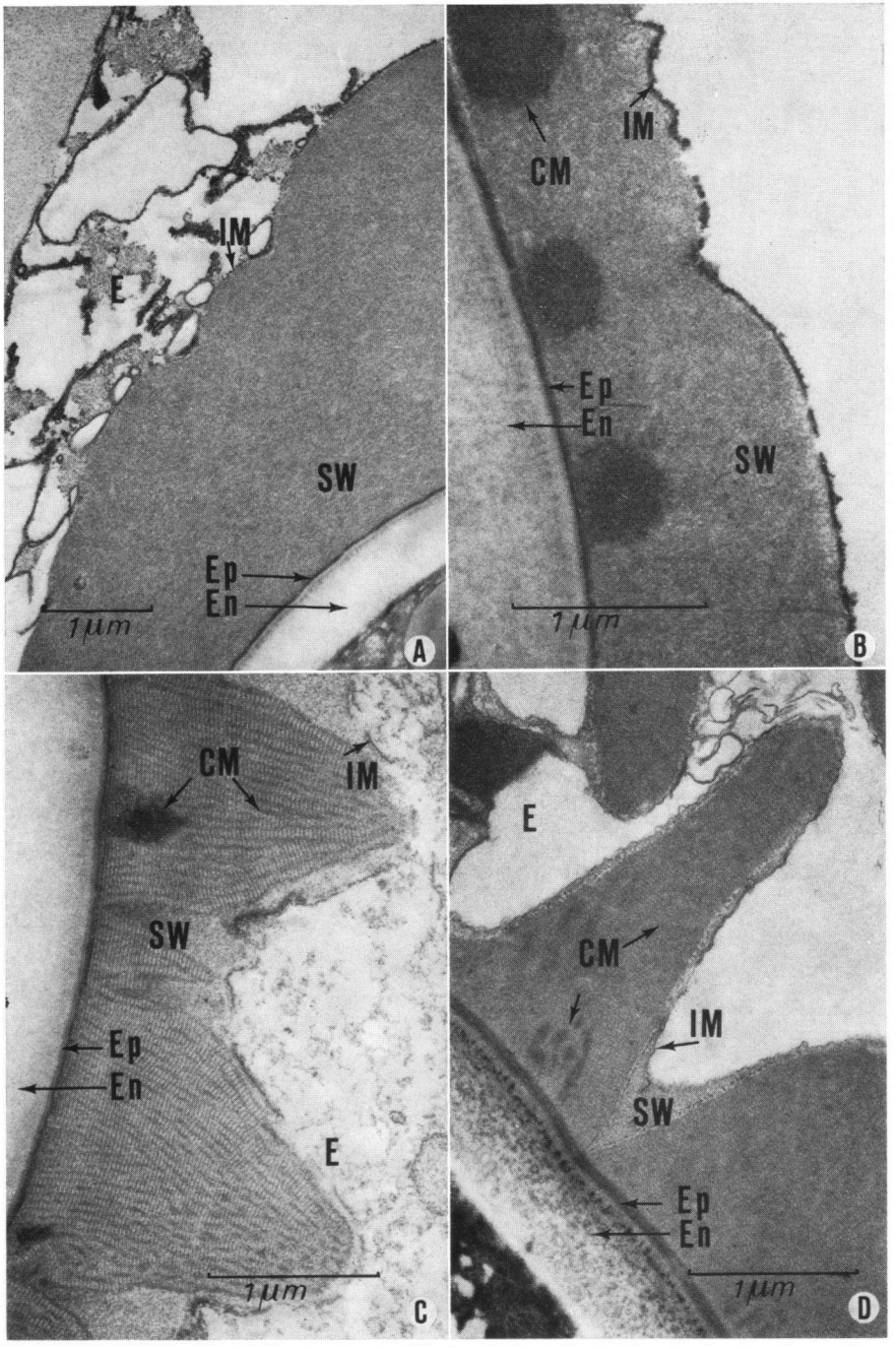
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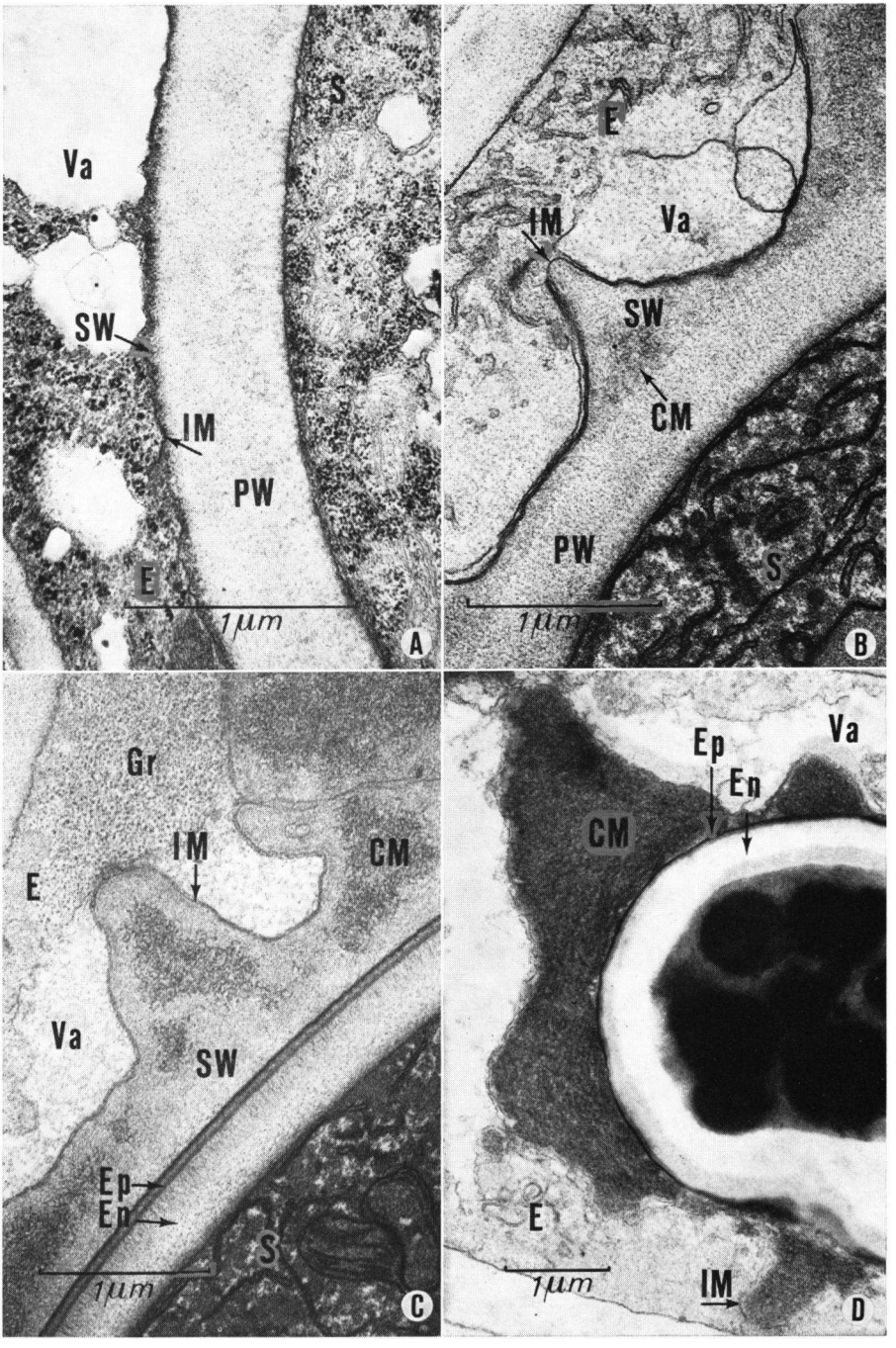
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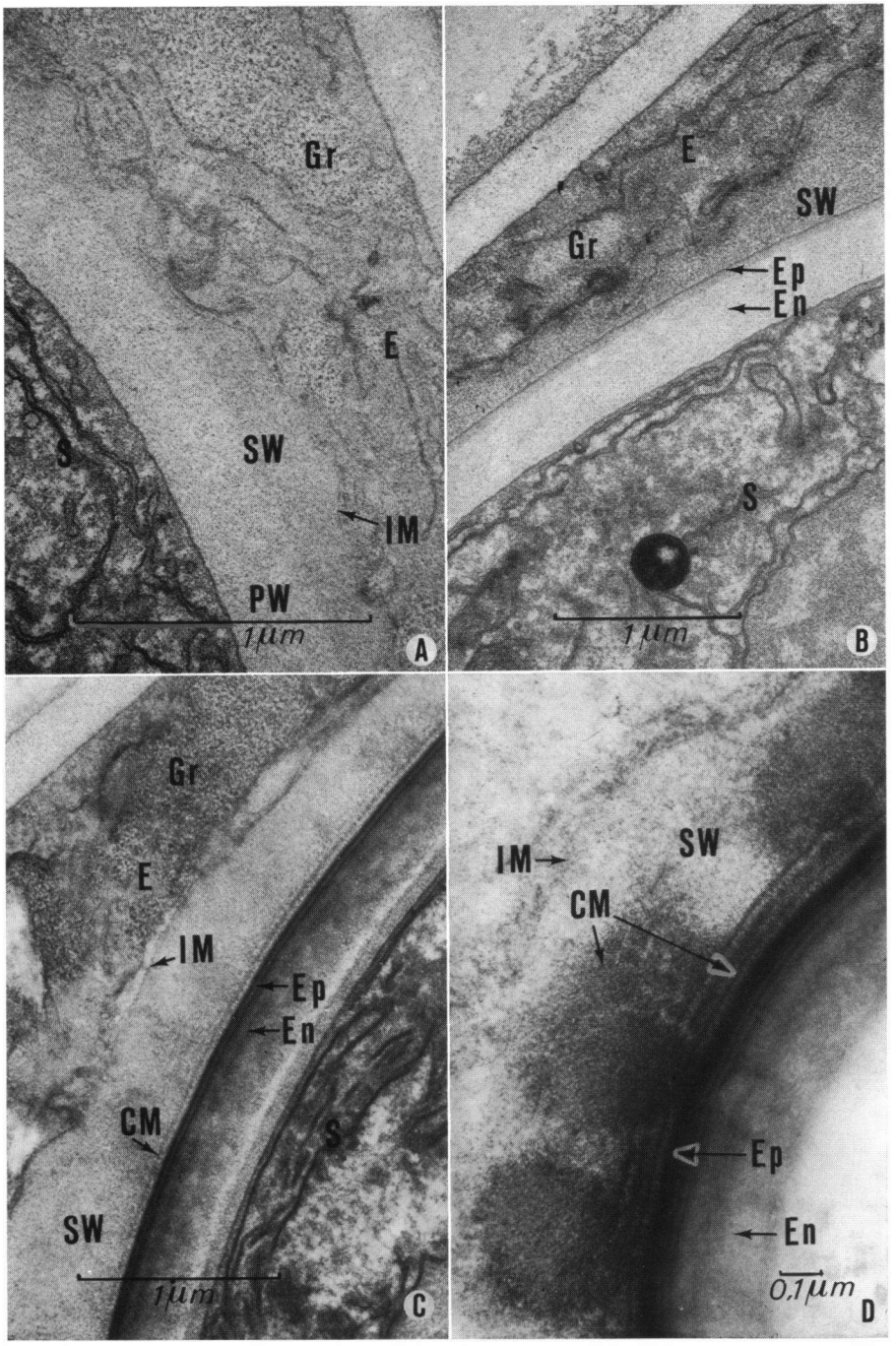
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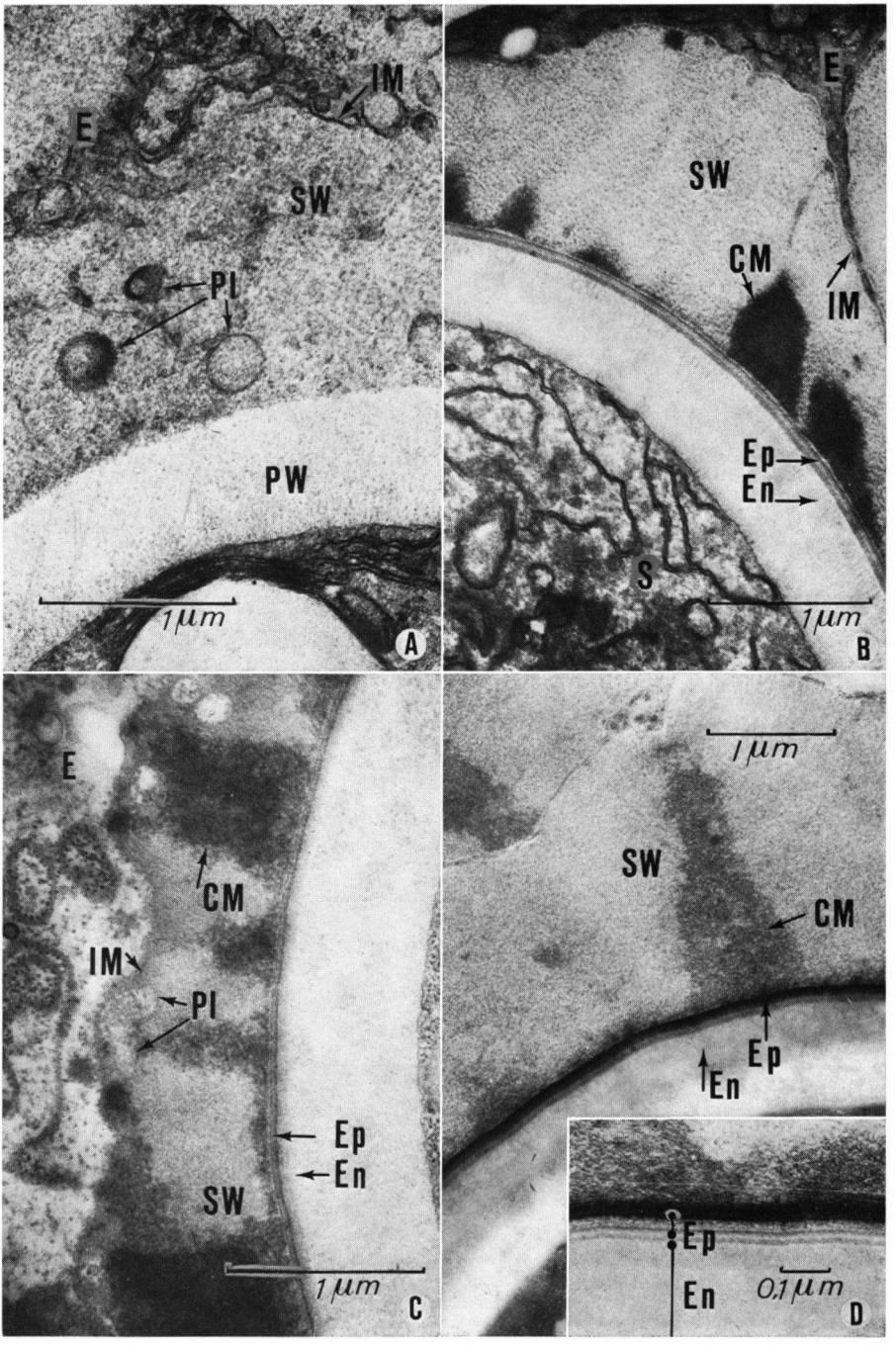
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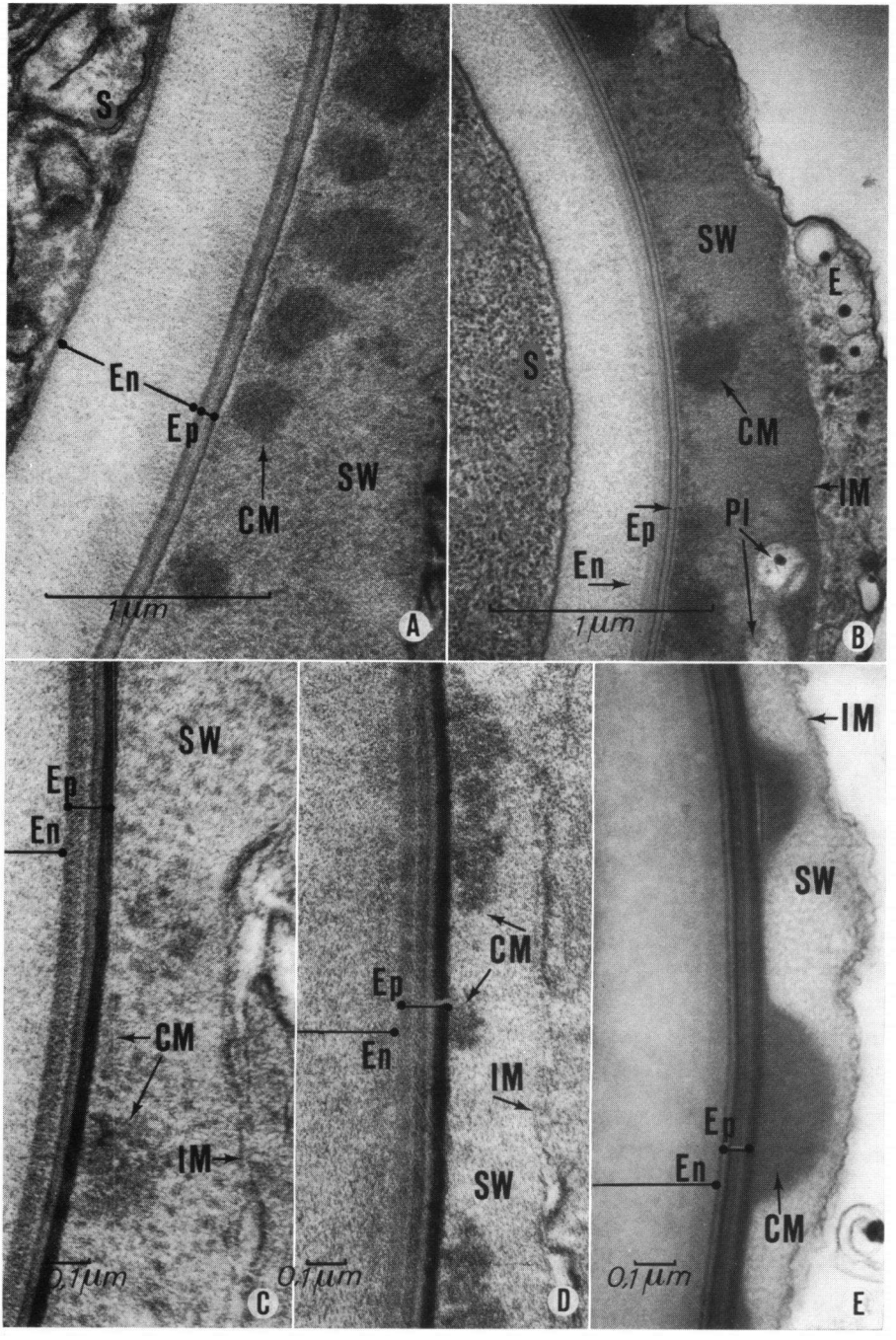
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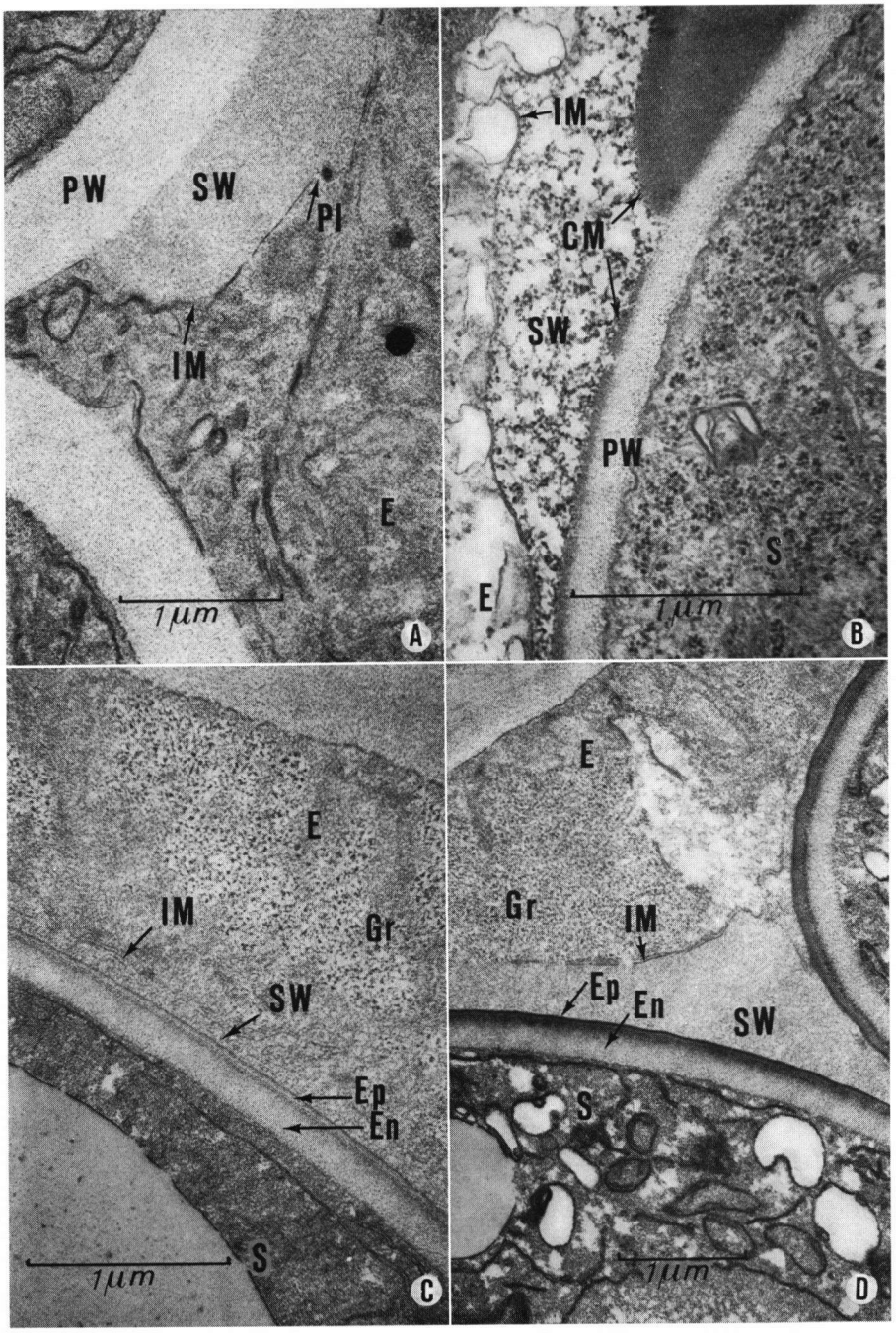
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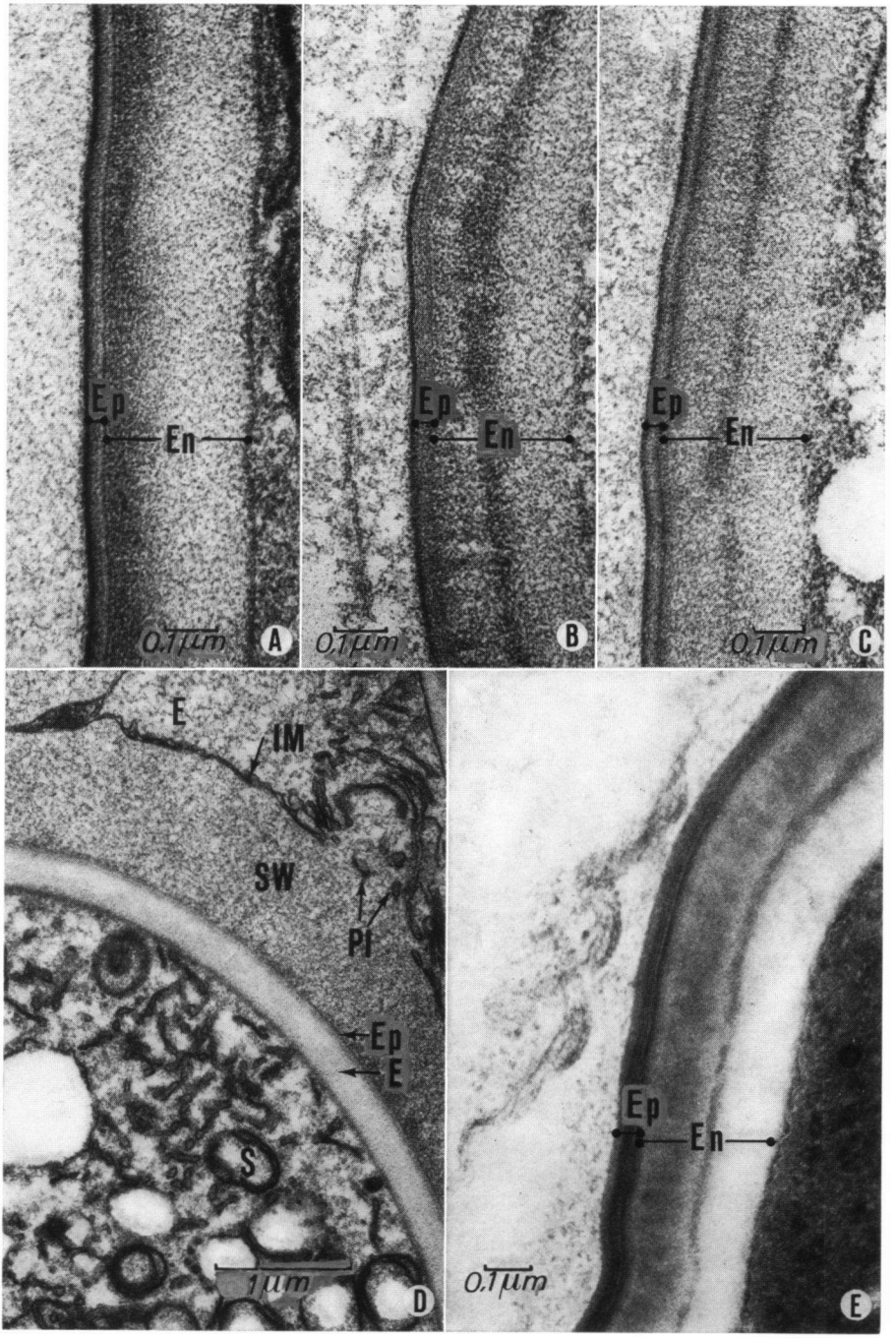
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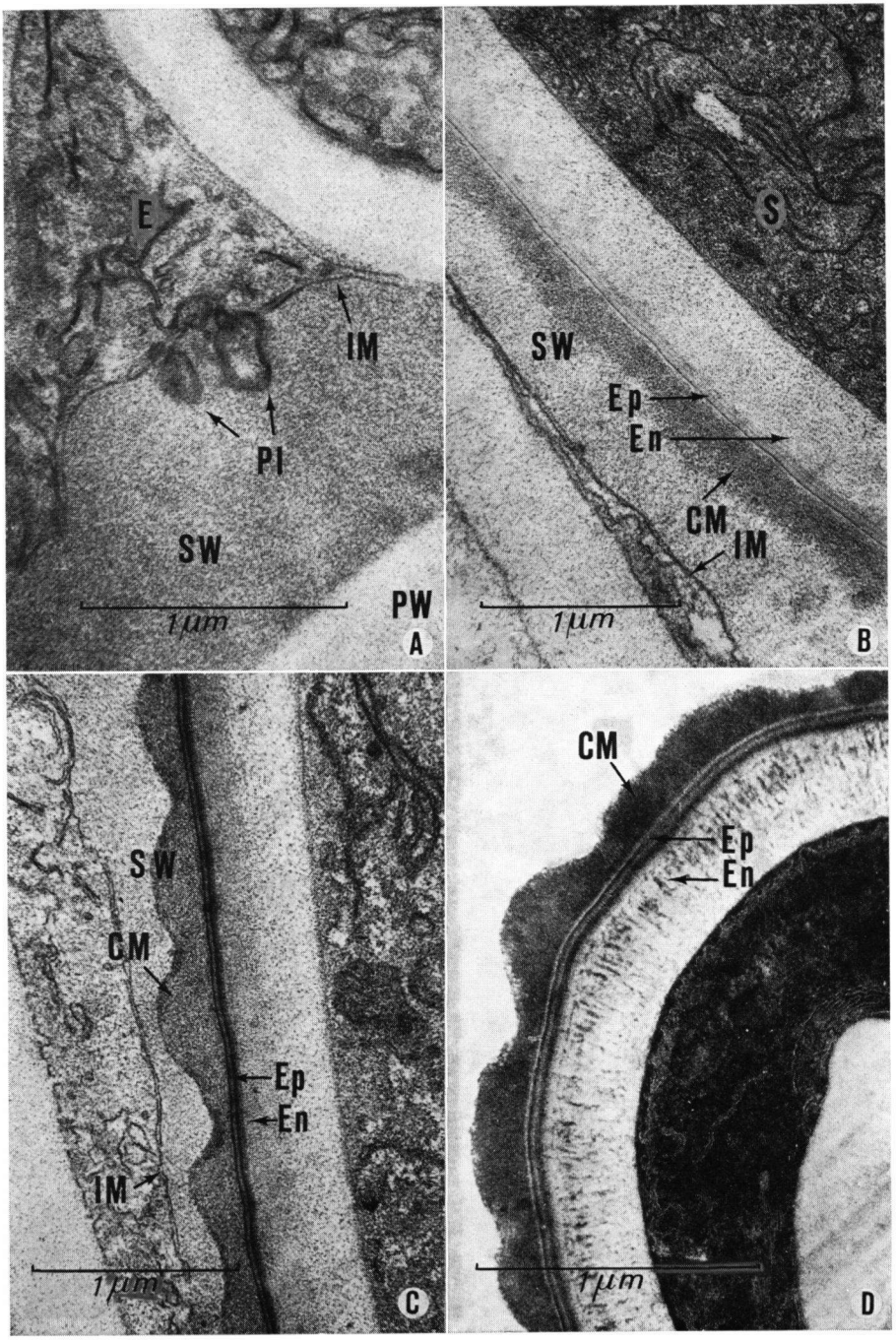
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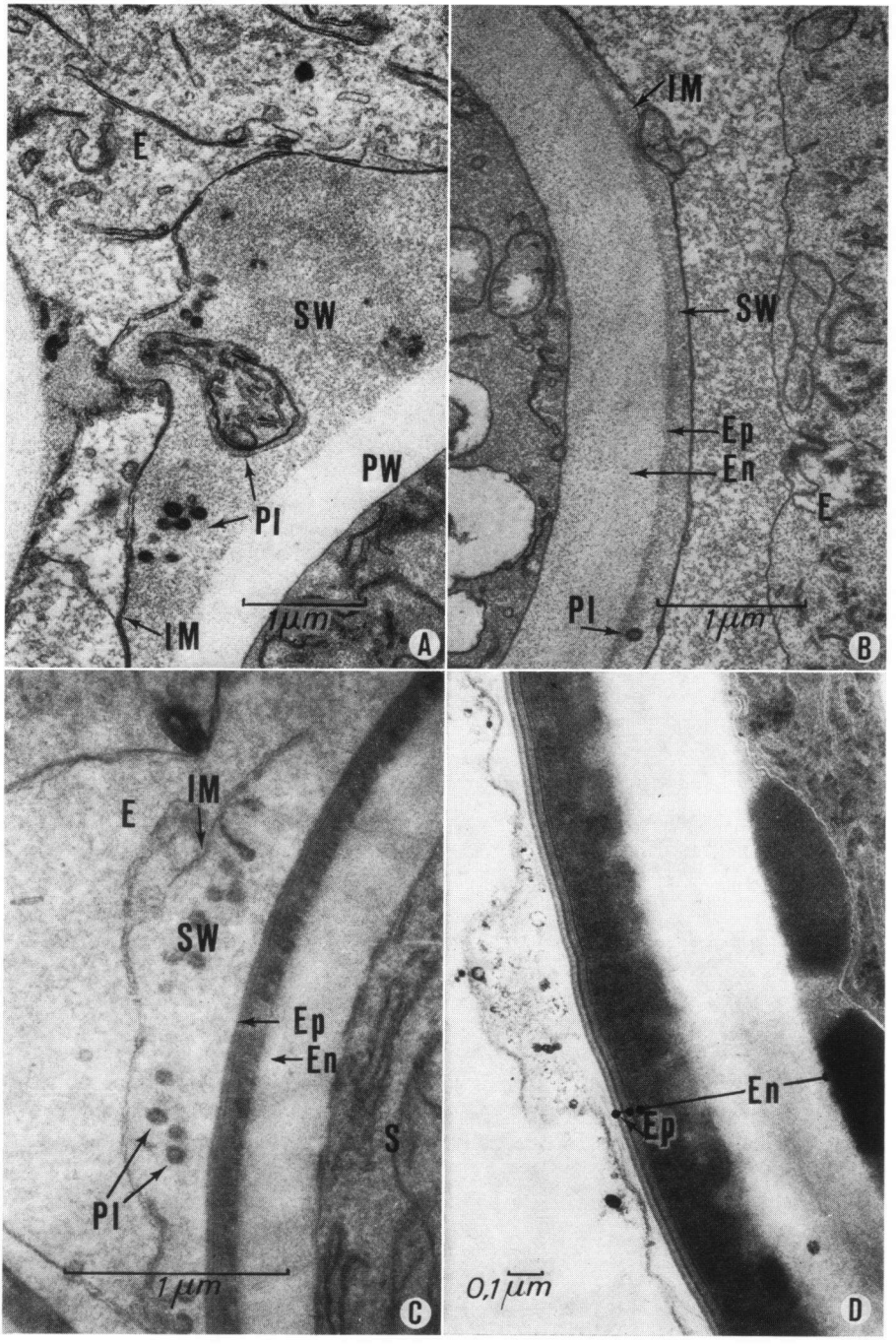
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