Microsporogenesis in *Pinus sylvestris* — VII. Exine Expansion and Tapetal Development

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ABSTRACT: This paper deals with stages from the terminal portion of the microspore tetrad period and loss of the callose envelope through to extensive expansion of sacci (= ca. vacuolate stage of pollen grain cytoplasm). The alveoli of both cap and sacci expand greatly, circumferentially, without any separation of radial components. It is the distal portion of alveoli which elongates. Thickening of alveoli in cap and sacci is accompanied by many glycocalyx elements on, around or within the "walls" of alveoli. Tapetal cells go through many cycles with respect to dilation of rER, few or many vesicles and production of Ubisch bodies. They undergo mitosis at the beginning of vacuolation of microspores. Tapetal cells extend into locules surrounding many microspores during a stage of great vacuolation of microspore cytoplasm, expansion of sacci, and thickness increase of the exine.

KEY WORDS: Alveoli, Callose, Endexine, Exine foot layer, Microspores, *Pinus sylvestris*, Sacci, Sporopollenin Acceptor Particles (SAPs), Tapetum, Tetrad, Ubisch bodies.

INTRODUCTION

Microspore stages have been reported for *Pinus sylvestris* by Willemse (1971a, b) and Rowley *et al.* (in press). Dickinson (1970, 1976) and Dickinson and Bell (1970a, b, 1976) studied microspore development in *P. banksiana*. Studies of microspore development in other conifers include *Taxus baccata* (Rohr 1977, 1980; Pennell and Bell 1986); *Abies concolor, Tsuga canadensis, Taxodium distichium* and *Cunninghamia lanceolata* (Kurmann 1986, 1989, 1990a, b, c) and *Podocarpus macrophyllus* (Vasil and Aldrich 1970, 1971). One of the most complete studies of microspore development in conifers was by Lugardon (1995) on *Chamaecyparis lawsoniana*. Lugardon gave special attention to the initiation of the exine.

Willemse (1971a) found that in *Pinus sylvestris* the distal portion of alveoli was the first part of the exine to be thickened, presumably by sporopollenin. This was followed by the alveolar side walls and later by exine near the plasma membrane. Rowley *et al.* (in press) found that rod-shaped components of the plasma membrane-glycocalyx, which we call Sporopollenin Acceptor Particles (SAPs), are positioned between the plasma membrane and

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enveloping callose before there is any appreciable separation of the two. When separation occurs the plasma membrane and adjacent cytoplasm are cavated at sites that will be the central space of cup-shaped alveoli. These occur at both regions of future cap and sacci but not in the aperture region. SAPs and their fibrilliform cross-links fill the alveoli. Initially, the plasma membrane and cytoplasm remains in contact with smooth (not protruded) callose. Then the callose protrudes and plasma membrane/cytoplasm retracts. However, for a time the callosic/cytoplasmic contact is retained even across two or more micrometers of the expanding sacci. This was illustrated by Willemse (1971a) who considered the SAPs and their associated fibrils to be formed in Golgi vesicles. According to his interpretation the vesicles fuse with the plasma membrane and on the membrane surface become the cavities' contact to alveoli. While we have not offered a suggestion about the means of formation of the alveoli we show that at least some, if not all, endomembrane openings to the alveolar space through the plasma membrane result in transfer of SAPs into cytoplasmic vesicles where they appear to be digested in autophagic vesicles (Rowley et al. in press). Thus, our interpretation is that SAPs being plasma membrane-glycocalyx components, are assembled by the plasma membrane and bind molecules, as they did our tracer, and transfer the normally nutritional molecules into the cytoplasm. Later the trilamellar part of the plasma membrane retracts from the callose projections and SAPs become arranged in a file at the inner surface of alveoli. Then there is a file of SAPs at the surface of the plasma membrane that will become or is the endexine. After the endexine is in place there was no further evidence of the massive endocylosis as Willemse (1971a) reported of, for example, "SAPs". According to our interpretation SAPs are part of the plasma membrane surface coating and during the stages of exine formation, at least, they remain a part of the membrane even across a greatly expanded exine.

MATERIALS AND METHODS

Male strolobili of *Pinus sylvestris* were sampled each day from mid-May until dehiscence of microsporangia in mid-June. Collecting was rotated between six trees located at the Bergenian Botanical Garden, the site of the Botany Department of Stockholm University, and Björkudden on Lidingö, the most westerly island in the archipelago of Stockholm.

The fixations used are briefly described below. These fixations are noted in the illustration descriptions. The stains used are also noted by the abbreviations below in the figure descriptions.

GA-PF. Karnovsky (1965) high osmolality fixation mixture of glutaraldehyde (GA) and paraformaldehyde (PF) in 0.006 M phosphate buffer (pH 7.2).

GA-PF + 4% tannic acid. A mixture of 2% GA, 2% PF in 0.1 M sodium cacodylate buffer plus 4% tannic acid (Mallinckrodt) followed by osmium tetroxide.

RR. Luft ruthenium red (RR) method as used by Latta et al. (1975): male strobili were kept in 1% GA in 0.02 M cacodylate buffer for 3-10 days, then transferred to GA/cacodylate buffer with 500 ppm ruthenium red (pH 7.4) at 20°C for 1 hour, rinsed in three changes of buffer, and finally immersed in a mixture of 2% OsO$_4$ in 0.02 M buffer plus 500 ppm ruthenium red, pH 7.4, at 20°C for 3 hr.

Fe-CN. Hepler potassium ferrocyanate method (Hepler 1982). This consisted of fixing sectors of strobili in 2% GA in 0.005 M cacodylate buffer (pH 7.4) containing 5 mM $CaCl_2$, washed in buffer, post fixed in a mixture of 1% OsO_4 and 0.8% K $Fe(CN)_6$, and post stained *en bloc* with 2% uranyl acetate.

GA-C. 3% GA in 0.025 M Na cacodylate-HCl buffer, pH 7.4.

S-C. 1% glutaraldehyde (GA) in 0.075 or 0.05M s-collidine, pH 7.4.

In fixations GA-PF, GA-C and S-C microsporangia were transferred to 0.1% OsO_4 in water for 1 hour. Staining for PAS (Thiéry, 1967) carbohydrates was as follows: 0.2% aqueous periodic acid (PA) for 30 minutes, 0.3% thiocarbohydrazide (TCH) in 20% acetic acid, and then after thorough washing in water the sections on gold grids were immersed in 1% silver proteinate (SP) for 30 minutes. Controls for this PA-TCH-SP procedure followed the recommendations of Courtoy and Simar (1974).

General nonspecific contrasting was by aqueous (1%) uranyl acetate for 10 minutes and 4 minutes in a modification of Sato's lead stain (Hanaichi *et al.*, 1986).

For electron microscopy we used a Zeiss EM-10A at 60 or 100 kV.

RESULTS AND DISCUSSION

Growth of the exine

The alveolate exine was described by Van Campo (1971) as a type of ectexine structure characterized by partitions forming compartments of irregular size and shape. In *P. sylvestris* alveoli form over all of the microspore surface except the aperture.

In the late tetrad stages the alveolar walls at first are very thin (Fig. 1) and the endexine has relatively few lamellar components (Figs. 1, 3 and 16), but an increase in wall thickness and endexine components takes place before callose is completely lost from microspores. This growth coincides with appearance of Sporopollenin Acceptor Particles (SAPs) (Figs. 4 and 7). Because alveolar walls form on the inner surface of cylindrical protrusions of the callose, the alveoli are cylindrical with a distal "cap" against the callose surface. Alveoli are thus shaped like an inverted cup. Due to the callose protrusions around each alveolus thus, between alveoli, there is a space between the alveoli after callose is degraded. Willemse (1971a: Fig. 16) illustrates the cylindrical round protrusion before exine establishment. Our Fig. 2 is an oblique view of alveoli in the cap zone after exine has just been initiated. The space between alveoli is better seen after expansion of the exine as in Fig. 11. The circular form of alveoli is seen in oblique sections (Figs. 2, 4, 6, 22, 28 and 34). Oblique thin sections of the inverted cup shapes miss some side walls in Figs. 4, 6 and 22.

The callose envelope seems to be lost in three ways in our chemically fixed samples. In some the callose became very thin (Figs. 1 and 16), in others the callose appeared to be degraded (Figs. 4 and 42) and in others callose was detached (Figs. 19 and 21).

SAPs line the *callose template* for the exine and we consider that in *P. sylvestris* sporopollenin is assembled on SAPs (Rowley *et al.* in press). Then, after the exine is initiated, SAPs are not evident for a time (Figs. 1-3 and 16-18) but, during that interval,

Figs. 1-7. Microspores enveloped by callose (C). All fixations: GA-PF. Stain: UA-Pb. Bars= 1 μm (Figs. 1-6); Bar= 100 nm (Fig. 7). Fig. 1. The exine components are slender compared with later stages (e.g., Figs. 8 and 9). In the cap zone (arrows) the number of endexine components is far less than in slightly later stages (e.g., Figs. 8 and 13). Fig. 2. In this oblique section the cap zone alveoli near exine surface are generally circular in cross section (arrowheads). Fig. 3. Includes tapetal cells (T) with many Ubisch bodies (u) at their surface and three microspores showing exine components that are slender. Fig. 4. Later in development but still enveloped by callose (C) the microspore exine and Ubisch body (u) wall are thicker than in the stage in Fig. 3. The number of endexine components in the cap region across this figure is greater than in Fig. 1. SAPs are at only the inner surface of alveoli. There are SAPs (arrow) on the most distal endexine component where the foot layer is forming. Tapetum (T); callose (C). Fig. 5. Tapetal cells undergo mitosis during this stage. The tapetal cell (T) shows a mitotic figure (arrowheads). Ubisch body (u); callose (C); microspores (M). Microspore cytoplasm includes many vesicles during the stage represented in Figs. 4 and 5. Fig. 6. Oblique section otherwise similar to Fig. 4. The alveoli are circular in cross section shape (arrows). Fig. 7. Spaces, common in alveoli, show circular holes (arrows) in oblique section of an alveolar wall. The SAPs are ca. 20 nm in diameter (one is circled). Spaces in cross section (arrowheads); callose (C).

Figs. 8-12. Early or young free microspore stage. All fixations: S-C. Stain: UA-Pb. Bars= 1 μm (Figs. 8-11); Bar= 100 nm (Fig. 12). Fig. 8. A microspore free of callose sectioned through one saccus, the cap zone (star) and the aperture (asterisk). The endexine is thick and has many sites where components are separated by irregular channels. The adjacent tapetal cell (T) has several Ubisch bodies near its surface. Fig. 9. Margin of aperture region in Fig. 8. The irregular channels of the endexine show a fibrillar matrix (circular frame). Alveolar "walls" show dark glycocalyx SAPs (arrowhead). Fig. 10. A tapetal cell with Ubisch bodies near its surface. Mitochondria (m); numerous cisternae of rER (arrowhead). Fig. 11. This oblique section of the alveoli of a saccus shows that each alveolus is a separate entity. Alveoli are circular to ellipsoidal in the mid-part of the figure where alveolar "side" walls are most nearly perpendicular to the plane of section. Fig. 12. A micrograph of the aperture margin in Fig. 9 taken at a higher magnification. Dendritic-like cross links radiate from and between SAPs (arrowhead).

Figs. 13-18. Foot layer initiation (Figs. 13-16), alveolar wall components (Fig. 17) and Ubisch body relationship to the exine (Fig. 18). Fig. 13. Foot layer initiation in the apertural zone at the margin of a saccus (filled star). The outer portion of the distal-most endexine is decorated by a file of SAPs which are presumed to accumulate the sporopollenin of the foot layer (arrowheads). Granular material (circle) is prominent within irregular channels in endexine. Fixation: GA-PF. Stain: UA-Pb. Bar= 0.5 μm Fig. 14. An example of the initiation of the foot layer within a saccus (filled star). The precursors (SAPs) of the foot layer can be seen in favorable planes of section to be elevated (arrows) above the endexine. Ruthenium red staining of the granular material (circle) in irregular channels of the endexine suggests polysaccharide preservation. Fixation: RR. Stain: UA-Pb. Bar= 1 μm. Fig. 15. An example of the initiation of the foot layer in the cap region (open star). Loops in the alveolar walls are marked by arrowheads and there are two Ubisch bodies (u) between a tapetal cell (T) and the exine. Fixation, etc. same as Fig. 14. Fig. 16. Alveolar wall components have a circular core zone (arrows) ca. 40 nm in diameter that is rod-shaped in longitudinal views (arrowhead). Fixation: GA-PF. Stain: UA-Pb. Bar= 0.5 μm. Fig. 17. The obliquely cut alveolar wall shows circular sites (arrows) of low contrast like those in alveolar walls in Fig. 16. Fixation: GA-PF. Stain: UA-Pb. Bar= 1 μm. Fig. 18. Microspore and exine near a tapetal cell (T) with Ubisch bodies between them. The Ubisch body shows many low dense circular sites like those in the exine in Figs. 16 and 17. Fixation: GA-PF. Stain: UA-Pb. Bar= 1 μm.
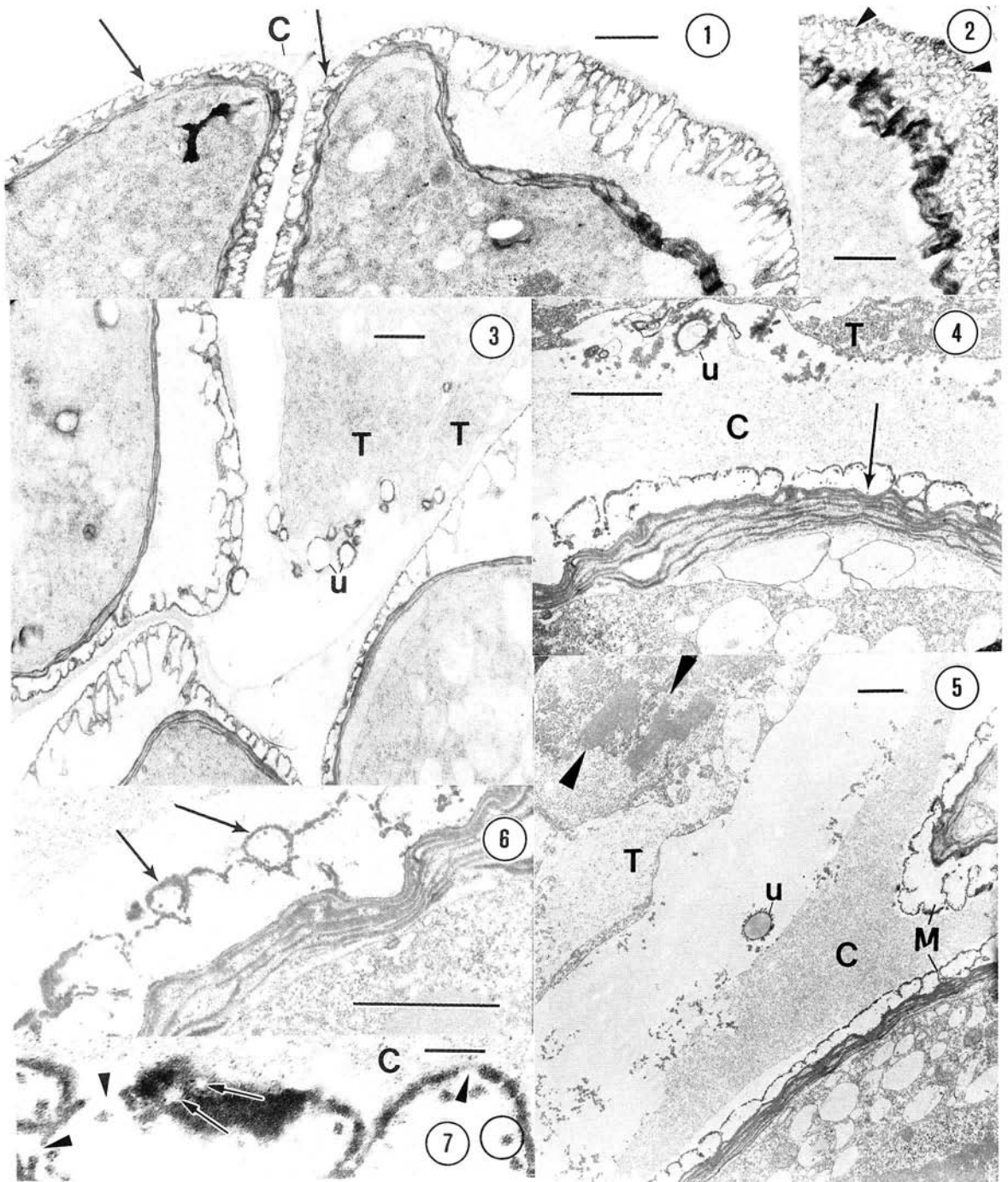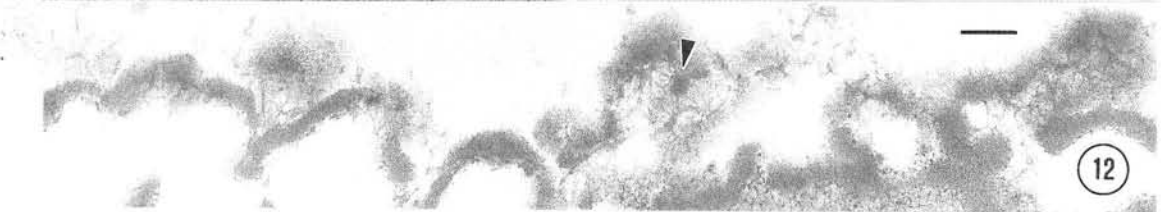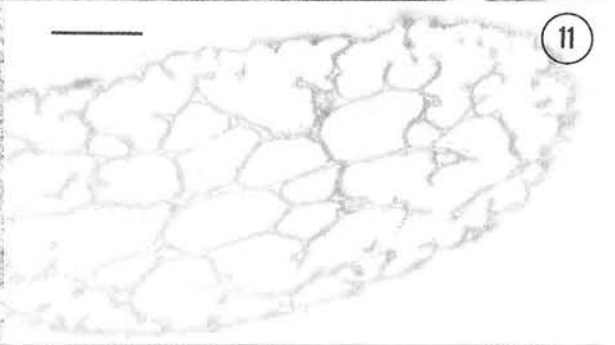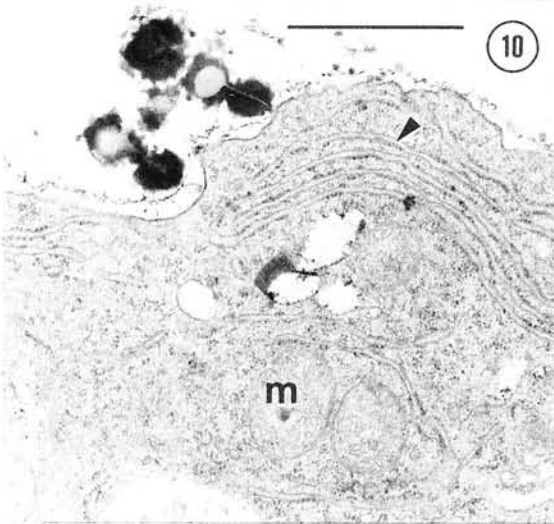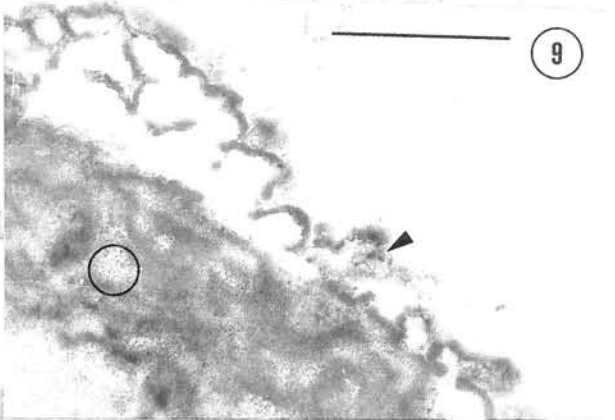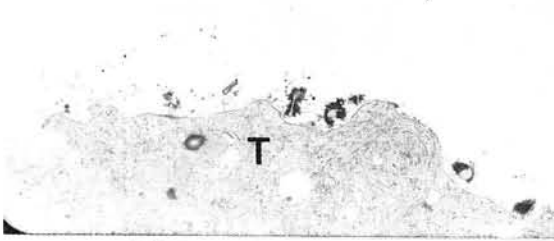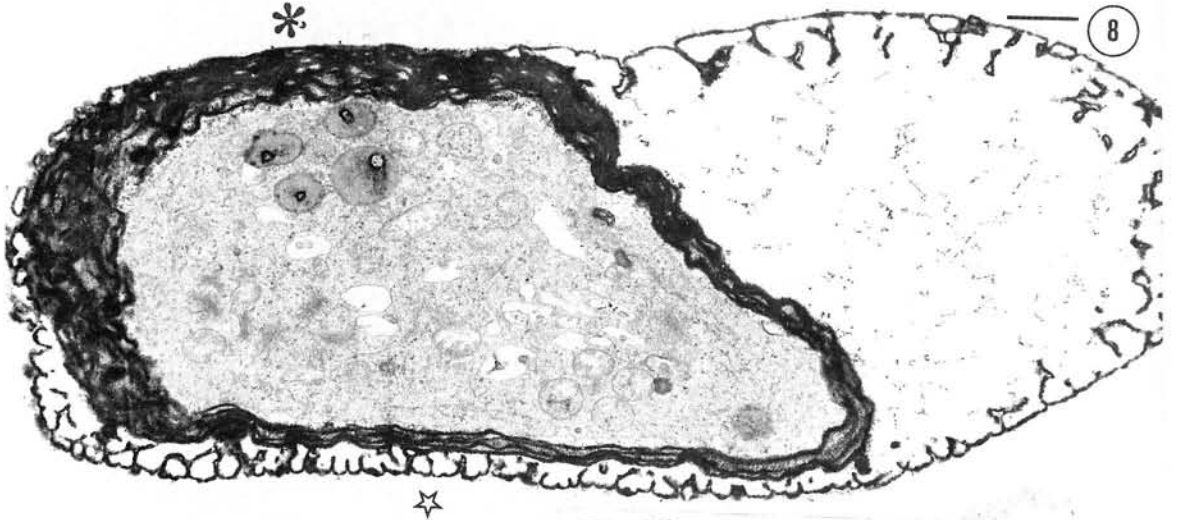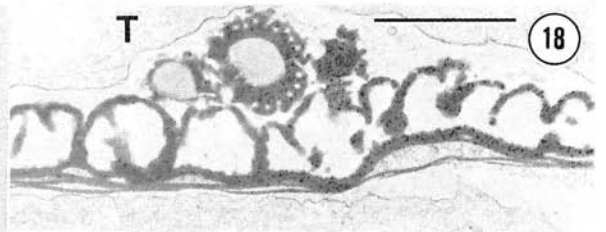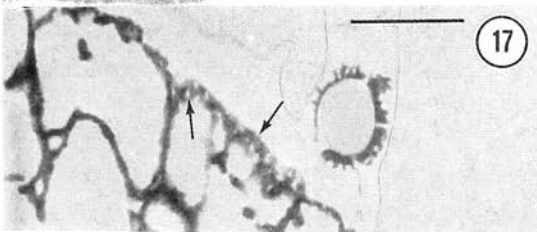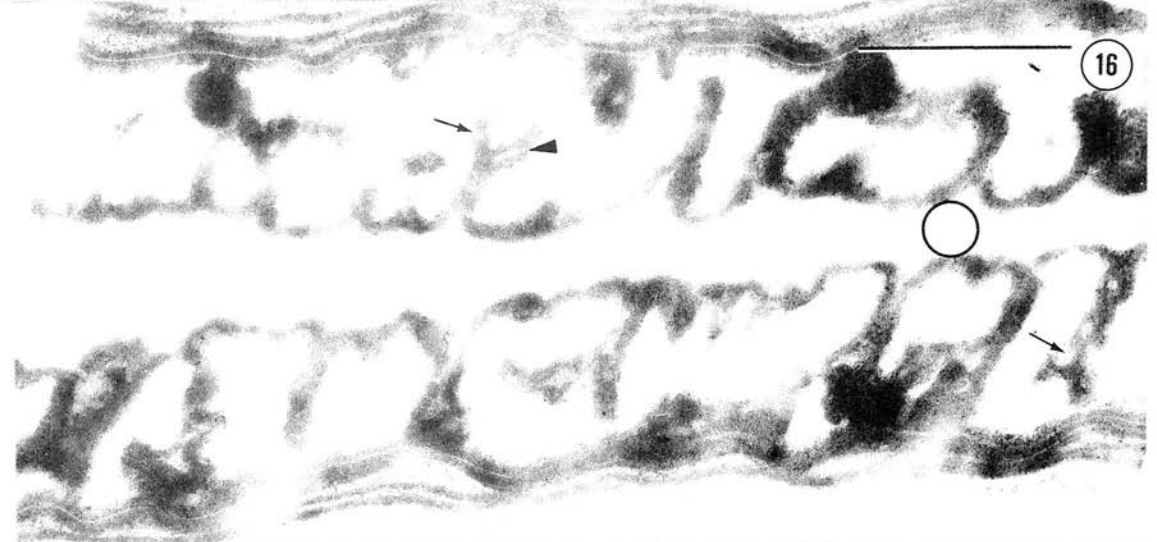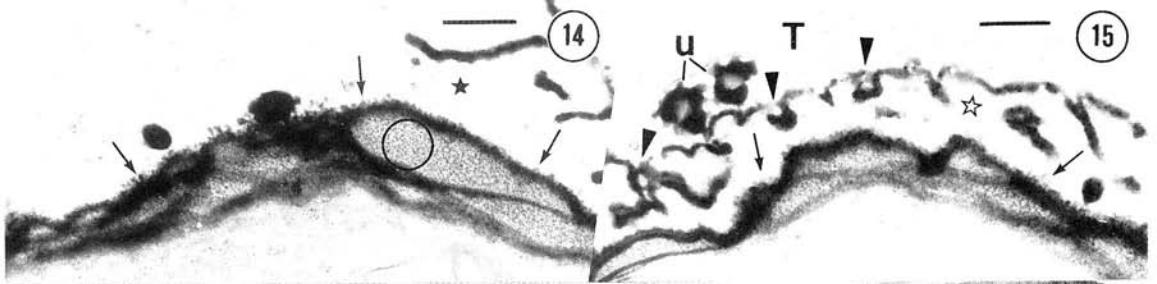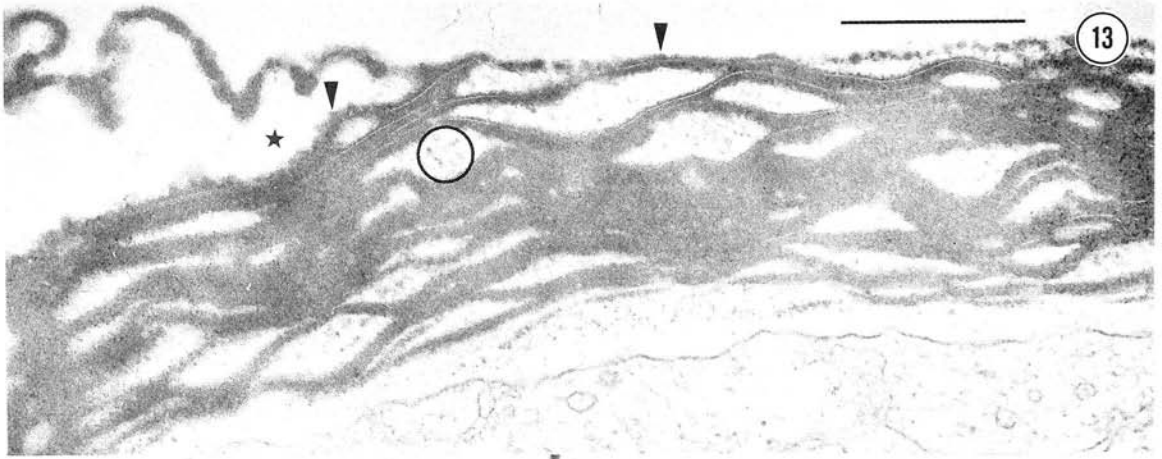
there are small, round, rod-shaped spaces in alveolar walls (Figs. 16 and 17) and also in the walls of Ubisch bodies (Fig. 18). These spaces are about the same diameter (ca. 20 nm) as the core zone of SAPs. There are also larger spaces, for example, those in Fig. 7 range from ca. 20 nm to ca. 40 nm. During the second phase of development when the exine thickens and expands such holes in alveolar walls are no longer evident. There are, however, sites where SAPs or their components cross the distal wall of alveoli (e.g., Fig. 29). Although less clear, and thus unmarked, there appear to be many sites of SAP components crossing the distal wall in Fig. 27 and in Fig. 28. Using ruthenium red or s-collidine assisted fixation the core is surrounded by weakly contrasted material through which there are filaments that radiate out from the SAP core (Figs. 23, 24, 28, 29, 32 and 34). Fig. 12 shows that SAPs early in exine development fit the description above. Depending upon stage and the effectiveness of stabilization by fixation SAPs were seen as spheres and rods (Figs. 30 and 31) or as part of a complex dendritic-complex radiating three-dimensionally around the SAPs (e.g., Figs. 23 and 29). We refer to a *core zone* of SAPs to suggest that there is more to them than the darkly contrasted component prominent in many micrographs.

The space between alveoli is filled during the stages when SAPs cover most parts of the exine. While SAPs do not occur or are rare on the distal surface of alveoli they do occur between the side walls (Fig. 28). The figure illustrates a SAP or several SAPs between walls for a distance of about a micrometer. In mature *P. sylvestris* pollen Rowley (1990-91; Fig. 4) shows that while the space between walls is filled the central part had a different contrast from the original wall sporopollenin.

In side views SAPs appear ellipsoidal or as rods. In end views SAPs are ca 40 nm in diameter and round. One is circled in Fig. 7. In end views SAPs are separated by 20 to 40 nm (e.g., Fig. 31) and when the alveolar wall is obliquely cut SAPs may be seen to be in a hexagonal arrangement (Fig. 33). Side views of SAPs showing their rod profile are specially marked in several figures (Figs. 28, 30-33), but actually, such views are common although less easily seen than the round on-end orientations.

In early free microspore stages the sacci greatly expand. Blackmore and Crane (1988) proposed that the sacci form in two stages: first polysaccharide deposition on sites on the distal wall following primexine deposition, then just prior to dehiscence, expansion of polysaccharide to inflate sacci. We find that in *P. sylvestris* glycocalyx (polysaccharide and protein) components do build up in early stages of formation of sacci (Rowley *et al.* in press), as Blackmore and Crane (1988) suggested. Substantial inflation occurs, however, early in the free microspore stage, even before the callose of the tetrad is entirely lost.

SAPs are not evident on the exine during the early free microspore stage (Figs. 1-3). The stage is also characterized by expansion of sacci and a vacuolated microspore cytoplasm (Fig. 1). This latter suggests that expansion during the earliest free microspore time does not involve exine growth (thickening). In all subsequent stages covered in this report SAPs are extremely prominent and the exine becomes thickened.

The invasive tapetum is a primary feature of the stage showing the greatest exine expansion during the period covered. The tapetum fills the locular space between microspores and it is highly vesiculated, like the microspores (Figs. 35-37, 39 and 40).

We have previously referred to fibrillar assemblies in plastids. They are shown in Fig. 41 at sufficient magnification to appreciate their fibrillar composition. Rowley and Walles (1993) described them and showed that they originate from starch grains.

Figs. 19-24. Early free microspores. Fixations: RR except Fig. 21 (S-C). Stain: UA-Pb (Figs. 19-21 and 23). No stain: Figs. 22 and 24. Bars= 1 µm (Figs. 19-21 and 23); Bars= 100 nm (Figs. 22 and 24). Fig. 19. Microspore with associated but not enveloping callose (C). Fig. 20. SAPs are on the inner surface of alveoli in the saccus (filled star) and cap (open star). The incipient foot layer is coated by SAPs (arrows). Fig. 21. Two tapetal cells (T) bordered by Ubisch bodies. The microspore has closely associated but not enveloping callose (C). Fig. 22. The circular construction (asterisk) of cap exine alveoli is evident in this oblique section. The contrast is due to ruthenium red and osmium tetroxide. The incipient foot layer (arrows) is coated by SAPs. The endexine appears to be composed of small dark-centered circles (arrowheads). Plasma membrane (Pm). Fig. 23. Cap exine showing SAPs on the alveoli and thin foot layer (arrowheads). Cross-linking substructures radiate from SAPs (circular frame). The exine shows many SAPs on inner surfaces; they are rare on distal surfaces. The white line called a junction plane is marked by an arrow. Fig. 24. An unstained section serial to the section in Fig. 23. This figure is centered upon the circled region in Fig. 23. There are dendritic-like cross links around and between SAPs. Since the section had no section stain, the contrast is due to osmium tetroxide and suggests the presence of lipid and/or protein in SAPs and their cross links. The white line termed the *junction plane* between the foot layer and endexine is marked by an arrow.

Figs. 25-29. Exine thickening and SAPs. All fixations: GA-SC. Stain: UA-Pb. Bars= 1 µm (Figs. 25-28); Bar= 100 nm (Fig. 29). Fig. 25. The appearance of SAPs (arrow) begins while there is still some callose (C) on but not enveloping microspores. Tapetal cell cytoplasm is rich in rER (circular frame). Ubisch body (u). Fig. 26. Orientation at an origin of a saccus in support of Fig. 29. Fig. 27. Transverse section of a saccus at the same stage seen in Figs. 32-34. Alveoli sectioned transversely (asterisks) are circular or ellipsoidal. The walls of adjacent alveoli appear to be mostly fused, not separate as in the earlier stage in Fig 11. SAPs are darkly contrasted at the surface of alveoli and on either side of the walls of alveoli (Fig. 28). Fig. 28. SAPs appear to join (between arrowheads) adjacent walls of alveoli. SAPs are rare at the distal surface of alveoli but their substructures extend across alveolar walls (arrows; see also Fig. 27). Fig. 29. The complexity of the SAPs and their interconnections is apparent. Since the alveolar walls (asterisks) are continuous cup-shaped sheets the SAPs and their cross connections pass through the alveolar wall during this stage. Examples of SAP components across the distal wall are marked by arrowheads. SAPs and their cross connections are part of the foot layer (F) during its formation. Endexine (En).

Figs. 30-34. SAP relationship with the exine. Fixations: Fig. 30=GA-PF; Fig. 31=GA-C; Figs. 32-34=S-C. Stain: UA-Pb. Bars= 0.5 µm. Figs. 30 and 31. SAPs appear to be discrete after fixation in GA-PF in Fig. 30 and GA-C in Fig. 31. After s-collidine fixation there are many fine radiating connections between SAPs (Figs. 32-34). The images in Figs. 32-34 show that the SAPs seen in Figs. 30 and 31 are only the core of a larger sporopollenin acceptor component. Fig. 32. Obliquely cut alveolar walls show some SAPs in end views (arrows) with connections radiating from the core. Many of the SAPs are shown to be elongate (arrowheads). SAP cross links are most prominent in the inner part of obliquely cut portions of the distal wall (asterisks) of alveolae. Fig. 33. In surface views of alveolar walls SAPs are hexagonally arranged (half circled area). The region between SAPs is crossed by many filaments which radiate from and cross connect SAPs (Figs. 32 and 34). SAPs which are not arranged perpendicular to the plane of section show as rods (arrowheads; Figs. 30 and 33). Fig. 34. The micrograph is serial with the one in Fig. 29 and thus gives further evidence that the cross connections associated with SAPs are not simply on the surface of alveoli (asterisks) but pass through them. Endexine (En).
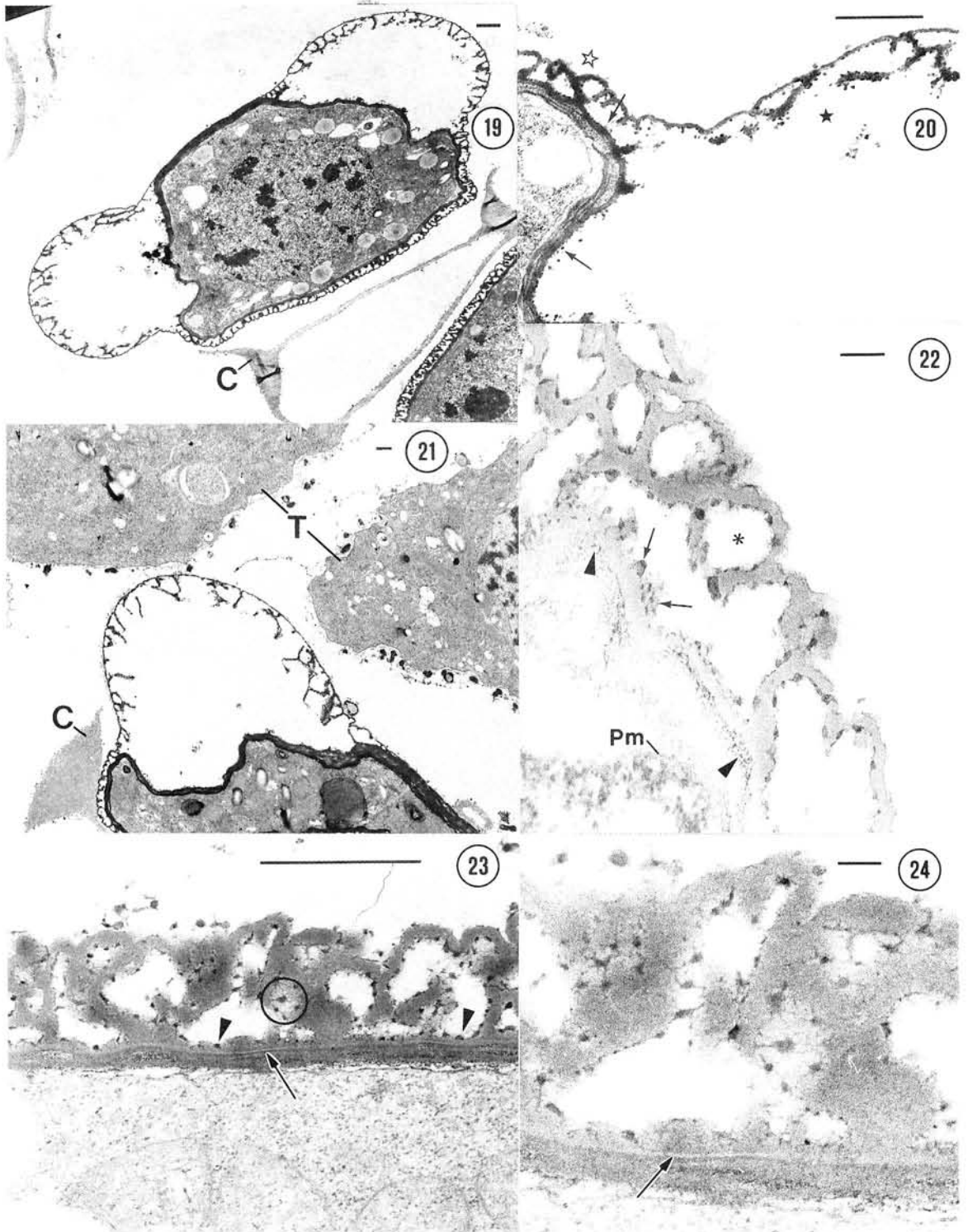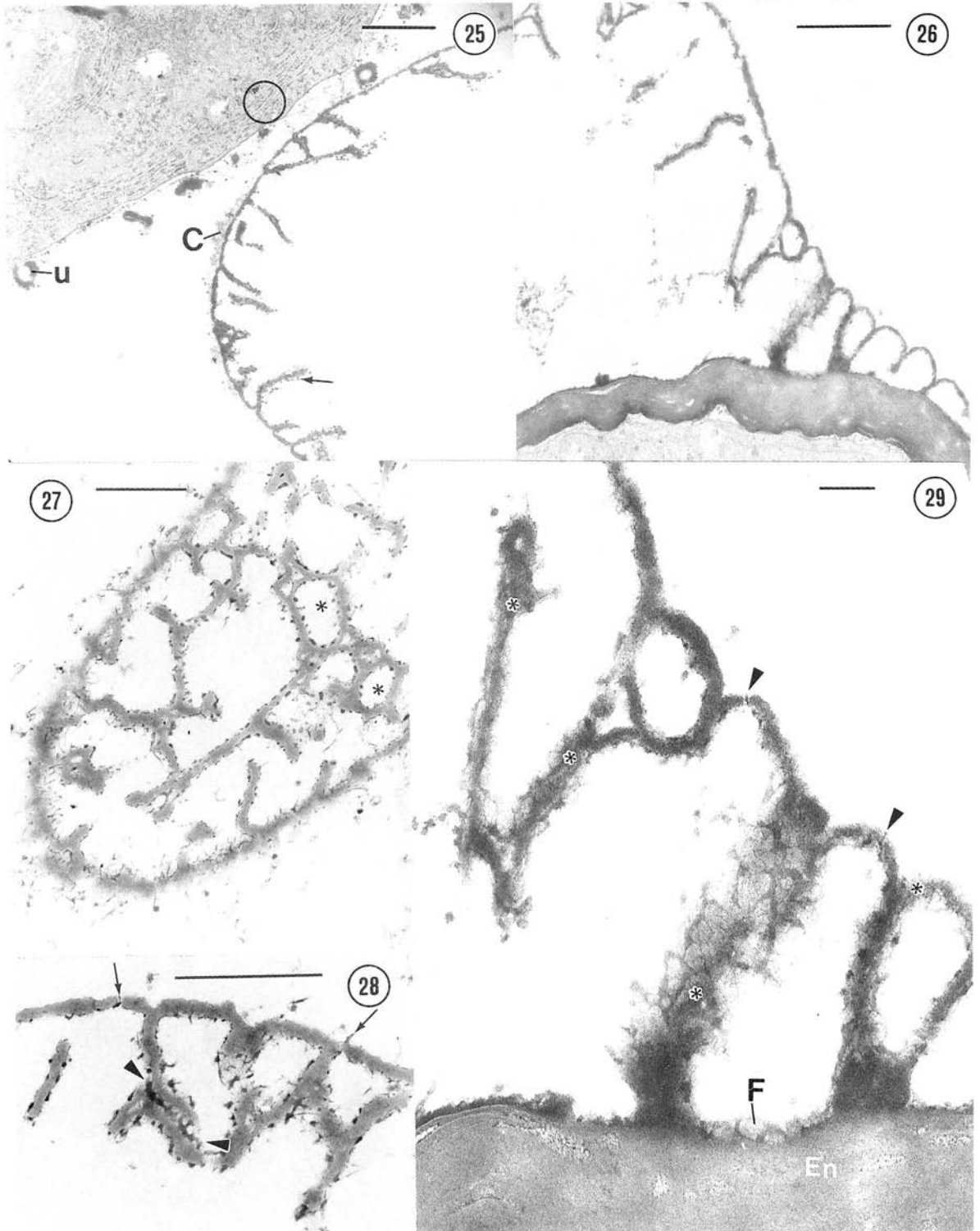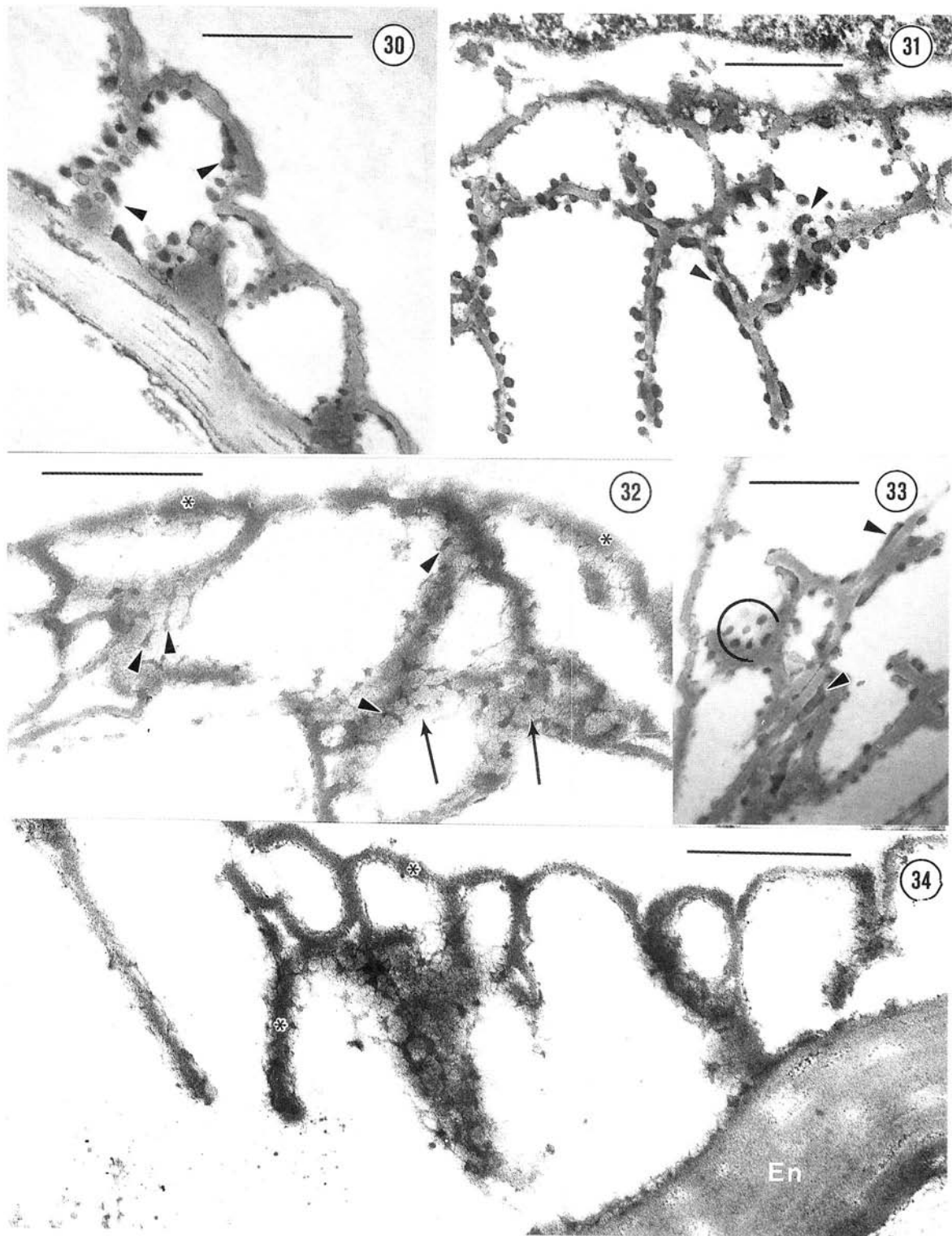
Initiation of the foot layer

The foot layer forms on the outer-most endexine component and begins as a file of SAPs (Fig. 13). Early foot layer formation is illustrated within the saccus and in the cap zone in Figs. 14 and 15, respectively.

The foot layer distal surface has a hemispheroidal (bumpy) form (Figs. 20 and 22-24). Its inner (proximal) surface is a white line shared by the underlying endexine (Figs. 23 and 24). Rowley (1995) has referred to the white line as a junction plane (between foot layer and endexine). Rowley (1995: Pl. 3) suggests that the white line of the junction plane and white lines in general are produced by loops in exine subunits that are ca 30 nm in diameter. The oblique section in Fig. 22 shows tubule-like loops of about the above diameter located proximal to the foot layer.

Pettitt (1966) illustrated early stages in formation of the foot layer in *P. sylvestris*. In one of his stages the foot layer is composed of more-or-less discrete SAP-like particles. This is like our early stage of foot layer formation (e.g. Fig. 13). Chen *et al.* (1990) show the foot layer, at a later stage than above, as does Pettitt (1966), with hemispheroidal form at its distal surface. Chen *et al.* (1990) also note that there is a prominent white line between the foot layer and the endexine.

Tapetum

Collections and fixations were repeated for nine years with similar results between microspores and tapetum each year although sometimes at quite different times of the months of spring due to weather fluctuations. It is difficult to adequately fix both tapetal and microspore cells to show them together to illustrate their condition at each stage. In some years all the stages of this report were separated by only three days when the day time temperature had been +6°C or more. Growth and development of these stages can occur rapidly. The tapetal cells in Figs. 3, 8, 10, 25 and 35-41 do not show any indication of hyperactivity as has been evident many times in earlier stages of development.

During the late tetrad time of callosic envelopment tapetal cell nuclei undergo a mitosis (Fig. 5). During the stages with SAPs on the exine the tapetum had a great deal of rER (Figs. 10 and 25). In the material we refer to as an early free microspore stage, tapetal cells expand into the loculus surrounding or contacting many microspores (Figs. 35-40).

Willemse (1971b), however, reported that in his *P. sylvestris* material tapetal cells quickly degenerate after the breakdown of the callose wall around the tetrad. His descriptions of the tapetal cells during earlier stages, before callose degeneration, are much like those Rowley and Walles (1982, 1985a, b, 1988 and 1993) have reported for repeated intervals of hyperactivity followed by mitosis and return to normal activity. Willemse (1971b) also reported that the plasma membrane of tapetal cells remained intact and that all cell organelles were swollen during these pre-free microspore stages. In his material, however, tapetal cells disappeared completely after the young free microspore stage. This is very different from *P. sylvestris* collected in the Stockholm region that showed an intact tapetum (e.g. Fig. 35) as seen in similar form during nine years of collecting.

Ubisch Bodies

Rowley and Walles (1987) found that in *P. sylvestris* Ubisch bodies are produced many times and that many of the productions are distinctive. For example the bodies found during the stages represented in Figs. 3-5 differ from those represented in Figs. 8, 10, 15 and 42.

Rowley and Walles (1987, 1993) have illustrated aspects of Ubisch body formation in *Pinus*. The central zone that will become the Ubisch body core is filled from ER cisternae within tapetal cells, then this future Ubisch body core is positioned in a cytoplasmic invagination (a crypt). Then the pro Ubisch body is expelled from the cytoplasmic crypt becoming, in passage, coated by plasma membrane and glycocalyx (Rowley and Walles 1987: Figs. 1, 2 and 18; 1993: Figs. 18 and 19). The last part to be released has a beak-like process underlain by a small area without glycocalyx (Fig. 42). Both the beak and area free of glycocalyx just below the beak are well shown in Fig. 42. The plasma membrane and its glycocalyx that encompases these Ubisch bodies provide the template for the Ubisch body wall just as they do for the exine of microspores (Rowley and Skvarla 1974).

Ubisch body ornamentation is often much like exine form at each stage of development. The Ubisch body in Fig. 5 has a cog-wheel appearance like the SAP covered inner surface of alveoli (Fig. 5). The Ubisch body in Fig. 18 has holes (ca. 20 nm in diameter) in its wall like the holes in the alveolar walls in Figs. 16 and 17 at a similar stage. The Ubisch body in Fig. 38 is ornamented like that on the tapetal markers of the peritapetal membrane as is described below.

Oryol and Golubeva (1982) showed many different stages in development of *P. sylvestris*. One of these stages illustrates thin wall Ubisch bodies with a cog-like surface ornamentation like those in our Figs. 3 and 4. Also like our Fig. 3 their micrographs, as do those of Pettitt (1966), show Ubisch bodies partly within tapetal cell surface crypts. Oryol and Golubeva (1982) show a stage in which Ubisch bodies have a thick and irregular surface as in our Figs. 39-41.

Figs. 35-37. An early free microspore stage of great sacci expansion and also great invasion of tapetal cells into the loculus. All fixations: GA-PF + 4% tannic acid. Stain: UA-Pb. Bars= 1 μm. Fig. 35. Tapetal cells more-or-less fill the space in loculi between microspores. Ubisch bodies (u) are common at tapetal cell surface. The cytoplasm of both tapetal cells and microspores includes many vesicles (V). Lipoidal globules (L) and plastid-containing fibrillar assemblies (asterisks) encircle the microspore nucleus. Fig. 36. This micrograph shows how microspores (asterisks) are surrounded by the tapetum during this stage. The peritapetal membrane (arrows) between tapetum and parietal cells (stars) is sporopolleninous and stains like the exine. Fig. 37. The microspore at the right and saccus of a microspore at the left (asterisk) are boardered by a tapetal cell.

Figs. 38-42. Figs. 38-41. The same stage, fixation and staining as in Figs. 35-37; Bars= 1 μm (Figs. 38 and 41): Bars= 2 μm (Figs. 39 and 40). Fig. 42. Ubisch body shape. Fixation: GA-S. Bar= 0.5 μm. Fig. 38. The peritapetal membrane (arrowheads) is thick, dark staining and ornamented. The ornamentation is in special evidence on the tapetal marker (arrow) and is like the Ubisch body (u) ornamentation. Tapetal cells (T); parietal cell (star). Fig. 39. Tapetal cell (T) cytoplasm in contact with two sacci (stars) and the aperture region (asterisk) of a microspore. Both the tapetal cell and microspore are strongly vacuolate. Fig. 40. Two tapetal cells (T) adjacent to the microspore saccus. There are many Ubisch bodies between and at the locular face of the tapetal cells. Several shapes and wall ornamentations are represented in these bodies. One Ubisch body (arrow) is characteristic of the stages in Fig. 2 and Fig. 5. Fig. 41. A tapetal cell (T) near the aperture endexine (En) with an Ubisch body (u) sandwiched between them. There are two plastids in the figure with fibrillar assemblies (asterisks). Fig. 42. Ubisch bodies produced during the stage represented by Figs. 8 and 10. They show the beak which in *Pinus* is part of all of these bodies but in early stages the beaks are longer than later in development. They also have a region lacking on all Ubisch bodies of *Pinus* that is poor in glycocalyx covering (arrows). Tapetum (T); callose (C).
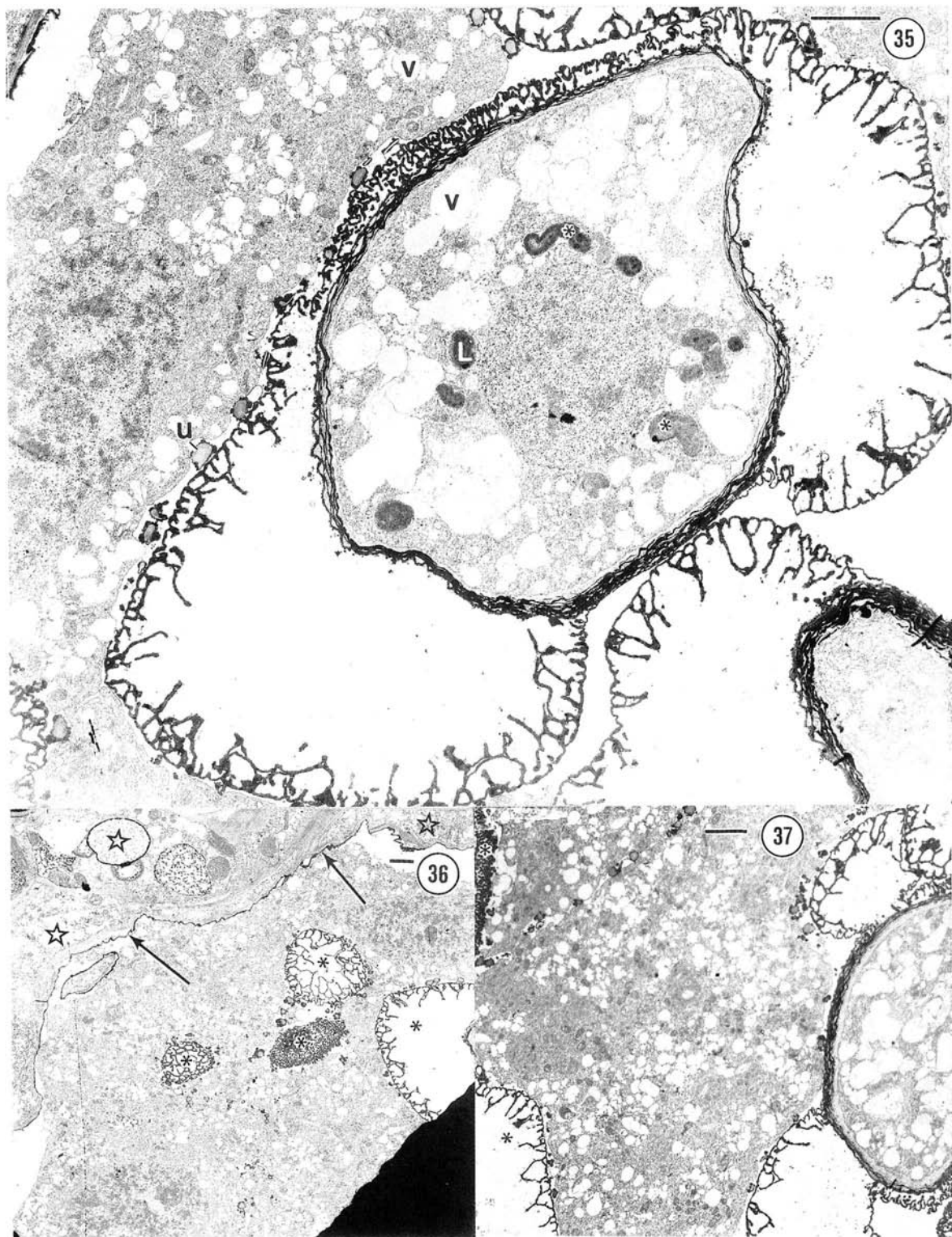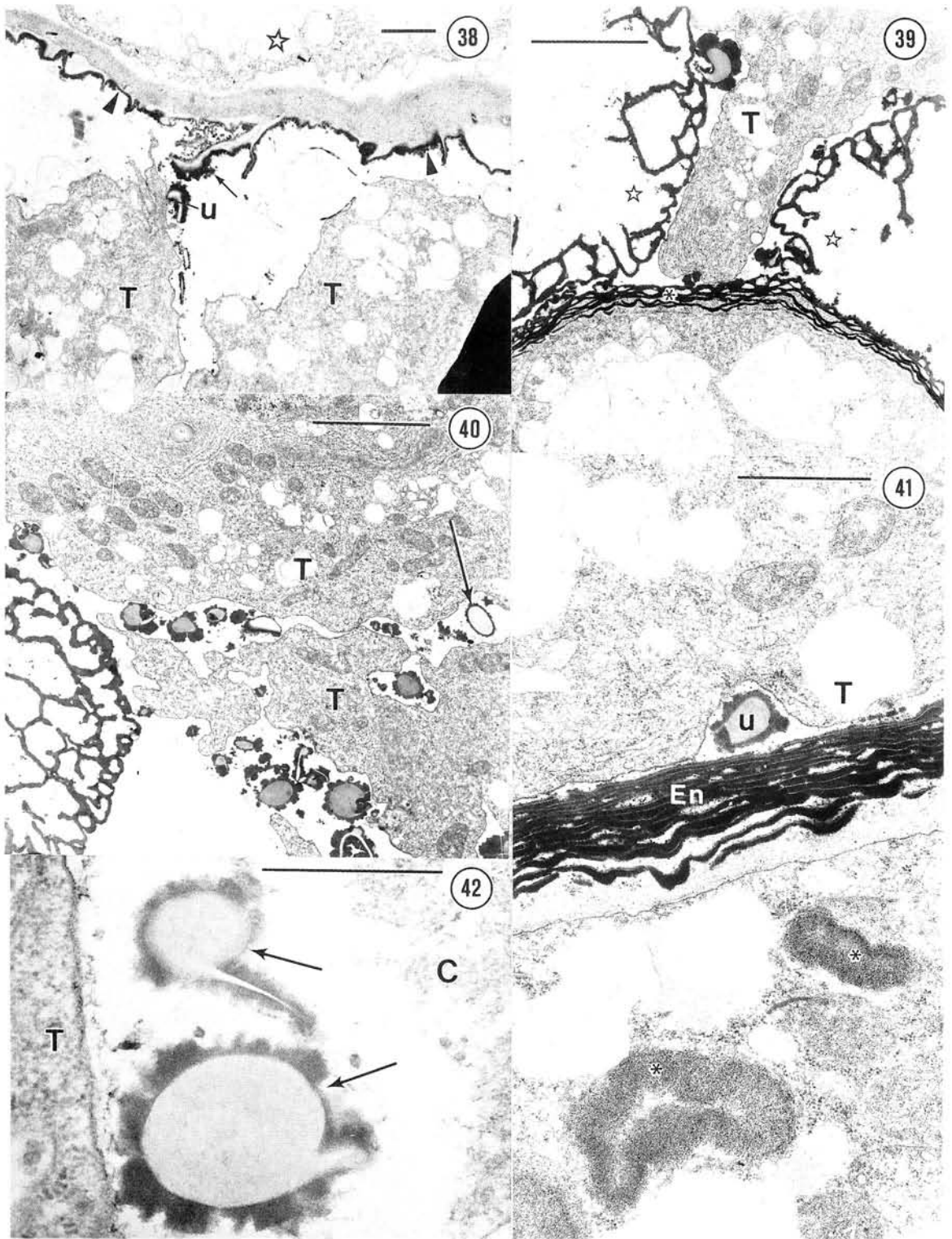
The peritapetal membrane between tapetal and parietal cells

During the period of exine formation the peritapetal membrane is intensely contrasted as is the exine. The term "peritapetal membrane" was suggested by Dickinson (1970) with respect to his work on microspore development in *Pinus banksiana*. In early development, at least, this peritapetal membrane makes an excursion between each tapetal cell. For this reason Rowley and Walles (1988) called these excursions "tapetal markers".

The peritapetal membrane is thin and smooth until the early free microspore stage when it becomes relatively thick and ornamented (Fig. 38). Ornamentation is notable at the tapetal marker and is like that on the adjacent Ubisch body.

Oryol and Golubeva (1982) illustrate a *P. sylvestris* stage having a thick and highly ornamented peritapetal membrane. Both this and their Ubisch bodies are like those in our Figs. 38-41.

Based upon what we illustrate here and observations reported by Rowley and Walles (1987, 1993), the wall of each production of Ubisch bodies is similar to the then current state of the exine with regard to thickening and ornamentation. Since the glycocalyx that determines the Ubisch body wall comes from the plasma membrane of tapetal cells we conclude that the microspore plasma membrane that "controls" exine formation and development, is similar at each stage with that of the tapetum.

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歐洲松 (*Pinus sylvestris*) 的小孢子形成－VII. 花粉外壁的膨脹和營養層的發育

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摘　　要

　　本文研究歐洲松 (*Pinus sylvestris*) 由小孢子四分體末期和胼脂質壁喪失一直到花粉氣囊膨脹這階段之發育，此發育階段約相當於花粉粒之液胞期。在發育中，花粉帽 (近心部分) 和氣囊的小泡皆大大的膨脹，但無任何放射狀成分的分離。氣囊小泡的遠心部分延長。在花粉帽和氣囊小泡進行加厚之同時，在小泡的壁上或附近有許多臘梅糖。營養層的粗糙內質網有多次的膨脹，囊胞忽少忽多，產生烏氏體。在小孢子的液胞期一開始，營養層細胞即行有絲分裂。在小孢子的液胞期的全盛時期。營養層細胞伸入花粉腔，並圍繞在小孢子周圍。此時氣囊膨脹，花粉外壁亦加厚。

關鍵詞：氣囊小泡，胼脂質，花粉外壁內層，外壁底層，小孢子，歐洲松，氣囊，孢粉質接受粒，營養層，四分體，烏氏體。

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