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On the Ultrastructure of Needles of *Pinus silvestris* L.

Ultrastrukturen hos barr av tall (Pinus silvestris L.)

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Abstract

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The ultrastructure of the different cell types in mature needles of pine (Pinus silvestris *L.*) *is described. The epidermal cells are protoplasma-free with prominent secondary walls. The sunken guard cells have chloroplasts of a simple structure and many mitochondria. A hypodermal layer is formed by living vacuolated fiber cells. The mesophyll cells, which have tanniniferous vacuoles, are rich in chloroplasts and mitochondria and contain microbodies. The resin ducts have secretory cells with a unique kind of plastid, apparently engaged in resin production. The endodermal cells are characterized by strongly osmiophilic globules in the vacuoles. The transfusion tissue consists of thin-walled deformed tracheids with bordered pits and parenchyma cells with tanniniferous vacuoles of different types. Like endodermal cells these parenchyma cells have simple amoeboid chloroplasts. Albuminous cells with numerous small tannin-free vacuoles and localized wall thickenings traversed by bunches of plasmodesmata are found on the outer side of each bundle. The adaxial xylem contains protoxylem tracheids with spiral wall thickenings and metaxylem elements with evenly thickened walls. A fusiform cambium is inserted between the xylem and the phloem. The sieve cells have unique multilayered secondary walls. Plastids in these cells contain clusters of protein fibrils which become released into the protoplast.*

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Contents

1. Introduction

The present investigation is part of a research project concerning the physiological and structural background to resistance in pine *(Pinus silvestris)* against the rust fungus *Peridermium pini* (Nyman *et al.* 1973). One of the approaches to this project is to use electron microscopy for studies of cellular interactions between susceptible and resistant host needles on one hand and invading mycelia on the other hand. It was considered necessary to start this part of the project with a study of the fine structure of the different tissues in healthy pine needles and simultaneously evaluate various methods of preparation of this technically difficult material.

Since 1971 some descriptions of the ultrastructure of a few of the needle tissues in three different pine species *(P. nigra, P. strobus* and *P. silvestris)* have appeared (Campbell 1972 a; Harris 1971, 1972; Parameswaran 1971) and quite recently Campbell (1972 b) presented a richly illustrated account for his electron microscopical analyses of needle development in *P. nigra* var. *maritima*.

2. Material and methods

Small segments of *P. silvestris* needles up to about one year in age were used. Most of this material was collected in the garden of the Royal College of Forestry during the winter season but some samples were also taken from greenhouse plants.

Of the different methods of fixation tested the best structural preservation was achieved with the following two:

I A freshly prepared solution containing 1 % glutaraldehyde, 1 % OsO_4, 0.1 M sucrose and 0.1 M phosphate buffer of pH = 7.0.

II Karnovsky's glutaraldehyde-formaldehyde fixative (Karnovsky 1965) and as postfixative 1 % OsO_4 in phosphate buffer with 0.1 M sucrose.

The fixations were mostly carried out at about +4° C. After dehydration in an acetone series, propylene oxide was used as intermediate solvent and the specimens were embedded in the epoxy medium of Spurr (1969).

Large sections, about 0.1 μm in thickness, were cut with glass knives on a LKB Ultrotome I or Ultrotome III. After staining with toluidine blue (Trump *et al.* 1961) they were examined with a light microscope. Areas of interest were selected and the embeddings retrimmed for a subsequent production of thin sections cut with diamond knives. The thin sections were stained with 1 or 2 % uranyl acetate and 0.2 % alkaline lead citrate (Venable and Coggeshall 1965). They were examined with a Hitachi HS-7S electron microscope.

3. Results and discussion

3.1 Epidermis and hypodermis

The epidermal cells of the pine needle are rounded in cross-section (Fig. 1). The outer walls are covered by a faintly stained cuticle (cf. Fig. 2) which seems to continue downwards between the individual epidermal cells (Fig. 1). Leyton and Juniper (1963) have used the replica technique to demonstrate that the needle surface of *P. silvestris* is covered by tube-like and plate-like wax secretions. Wax tubes were also described from *P. nigra* needles examined with a scanning electron microscope (Campbell 1972 a). Such wax secretions are not seen in our micrographs as expected, since they should hardly be able to resist the organic solvents employed in conventional preparation methods for electron microscopy.

Most of the original cell lumen of the epidermal cell is filled out by the thick secondary wall so that only a narrow, protoplasma-free cavity of an irregular shape remains.

Stomata occur in rows along both the adaxial and the abaxial sides of the needle. The guard cells are sunken below the needle surface and overarched by a ring of subsidiary (accessory) cells. Sections passing through the central part of a stoma show one such subsidiary cell on top of each guard cell (Fig. 2). The walls of the guard cells are of different thickness in different regions (Figs. 2 and 3). Those parts of the cell that are in contact with subsidiary cells have the thickest walls but also those parts that face the intercellular space are thick-walled. An upper and a lower pair of ledges protude from the walls of the guard cells where these face the aperture in the bottom of the stomatal antechamber. The guard cell protoplast contains a large nucleus, many mitochondria and some chloroplasts which are poorly developed in comparison with mesophyll chloroplasts (Fig. 3). The vacuoles are small. Also the subsidiary cells have living protoplasts (Fig. 2). These cells are presumably less active in metabolism than the guard cells, as indicated by the fact that an appreciable part of their protoplasts may be filled with non-living material, *viz.* lipid globules and tanniniferous vacuoles. Their outer, cuticle covered walls are extremely thick-layered while those parts that are in contact with guard cells or other living cells are fairly thin.

Immediately below the epidermis follows a hypodermal layer of fiber cells (Fig. 1). These cells have protoplasts with large vacuoles, of which many contain tannin deposits.

3.2 Mesophyll

The large mesophyll (chlorenchyma) cells are lobated in shape and possess club-like wall invaginations (called "internal ridges" by Esau 1965). The largest of these ridges develop an internal hollow space which enters into connection with the intracellular system. The major part of the cell lumen is occupied by a large, central, more or less tanniniferous vacuole and in addition some small vacuoles with or without tannin deposits may be seen in the cytoplasm (Fig. 4). Lipid bodies are also found.

Numerous chloroplasts and mitochondria and some dictyosomes and microbodies are characteristically found in the protoplast. Other cytoplasmic constituents are groups of ribosomes and vesicles and tube-like structures belonging to the endoplasmic reticulum (Fig. 4).

The chloroplasts are generally located in the proximity of the cell wall and when adjacent to the plasmalemma flattened at the side facing the wall (Figs. 4 and 9). In the neighbourhood of this flattened side the interior of the chloroplast is devoid of thylakoids. Chloroplasts of this type, characterized by a plano-convex region from which the lamellar system is excluded are also found in mesophyll cells of spruce *(Picea abies)* (Walles, unpublished). Plastoglobuli occur in the chloroplasts of mesophyll cells and also in corresponding organelles of ther cell types (cf. Figs. 3 and 9). The chloroplasts survive the winter season without any apparent change in structural organization (cf. Parker and Philpott, 1961, 1963).

A characteristic kind of organelle enclosed by a single membrane occurs in the mesophyll cells (Figs. 4 and 5). It has a finely granular matrix which sometimes contains a dense amorphous nucleoid (Fig. 5). This organelle fulfills the morphological criteria of a plant microbody as defined by Mollenhauer *et al.* (1966) and Frederick *et al.* (1968).

Harris (1971) has published an ultrastructural study of mesophyll cells in *P. strobus* with special attention to the development of wall invaginations, vacuolar content and lipid globules. Tannins and lipids were shown to increase in amount in the autumn and winter.

3.3 **Resin ducts**

A varying number of resin ducts are embedded in the mesophyll tissue. There are two major ducts with a lateral position and these can be the only ones present in the distal part of the needle. A resin duct is lined by a secretory epithelium enclosed in a fiber sheath. A minor part of this sheath is usually continuous with the hypodermis at one side of the duct. The secretory cells are thin-walled and have large vacuoles. Mitochondria, dictyosomes and elements of the endoplasmic reticulum are common constituents of the secretory cells (Fig. 6). Notable is the occurrence of a special kind of plastid without thylakoids (Figs. 6 and 7). These plastids contain some fairly weakly stained globules of a lipid-like material and similar globules are attached to the plastid envelope and occur free in the cytoplasm. Other globules seem to be transported across the plasmalemma (Fig. 7). It is reasonable to assume that the various globules mentioned represent resin and/or resin precursors.

In a study of resin duct cells in shoots of *P. pinea* Wooding and Northcote (1965) observed numerous undifferentiated plastids closely sheathed with endoplasmic reticulum and small resin globules associated with the structures referred to or with the plasmalemma. The authors suggest that the resin is produced by the combined action of plastids and endoplasmic reticulum and subsequently secreted through the plasmalemma. In his study of needles of Corsican pine Campbell (1972 b) noticed plastids that appeared to be engaged in resin production and secretion. These plastids were each surrounded by a sheath of endoplasmic reticulum. Associations between plastids and the endoplasmic reticulum were not observed in the present investigation (cf. Figs. 6 and 7). Campbell (loc. cit.) found in the space of young ducts a material interpreted as resin whereas in older needles the canals appeared empty. It was not possible to see in the electron microscope resin passing across the cell walls into the space but the author suggests that the granular texture of these walls might be due to small particles of resin moving through them. We have noticed a similar electron scattering granularity in the walls of secretory cells (Fig. 6) and consider the explanation given by Campbell (loc. cit.) most plausible. However, in spite of the fact that the endothelial cells studied by us revealed resin secretion the space of the canals was always empty.

3.4 **Endodermis**

The two vascular bundles of the pine leaf are embedded in a transfusion tissue that is

separated from the mesophyll by an endodermal sheath.

The endodermal cells are rather thick-walled (Figs. 9 and 10). Numerous primary pit-fields occur in the radial walls between them (Fig. 10) but are far less common in the walls between endodermal cells and parenchyma cells of the mesophyll or the transfusion tissue (cf. Fig. 9). The endodermal cells have a characteristic kind of vacuolar inclusion consisting of strongly osmiophilic globules with a maximal diameter of 350—400 nm. These inclusions (Fig. 8) have in exceptional cases been observed in adjacent transfusion cells but seem nevertheless to deserve consideration as typical endodermal constituents in pine. In addition to the central vacuole several small vacuoles can occur (Fig. 10). The cytoplasm is also rich in vesicles belonging to the endoplasmic reticulum (Fig. 9). Noteworthy is further the occurrence in endodermal cells of slender, amoeboid chloroplasts with small grana (Fig. 9). Starch grains may be formed in these chloroplasts.

3.5 **Transfusion tissue**

The transfusion tissue consists of two different cell types, *viz.* tracheids and parenchyma cells (Fig. 11).

The tracheids are relatively thin-walled and deformed by the pressure created by other cells of the growing needle. The walls of the tracheids contain bordered pit-pairs. The torus suspended in each pit-chamber is in its central area covered by strongly electron scattering material (Fig. 11).

The parenchyma cells are large with a circular outline in crossections. They have a central tanniniferous vacuole and in addition several small vacuoles with or without tannins. The tannin particles are dispersed like in mesophyll cells or aggregated into large bodies (Fig. 12). Both types of tannin distribution can be noticed in different cells of the same ultrathin section, which excludes the possibility that the differences observed could be due to fixation artefacts. The cytoplasm contains numerous vesicles that are part of the endoplasmic reticulum and contains also well developed lipid bodies. The chloroplasts (Figs. 11 and 12) are comparable in shape and structure to corresponding organelles in the endodermis. They accumulate starch in some cases.

Adjacent to the phloem occurs on the outer flank of each bundle a group of specialized parenchyma cells known as albuminous cells or (in German literature) "Strasburger" cells. These cells (Fig. 13) have many small vacuoles, which lack tannin deposits, prominent nuclei and numerous mitochondria. The most peripheral of the albuminous cells (Fig. 12) have simple chloroplasts of the same type as those found in transfusion and endodermal cells while the cells closer to the phloem have poorly differentiated plastids without starch grains. The albuminous cells are further characterized by conspicuous local wall thickenings traversed by bunches of plasmodesmata. Such wall complexes occur also on walls adjoining sieve cells. The fine structure of the Strasburger cells in pine needles has been treated by Parameswaran and Liese (1970).

3.6 **Xylem and phloem**

In the collateral bundles the xylem points toward the adaxial side, the phloem toward the abaxial side.

The xylem consists of rows of tracheids and a few rays. The bundle tracheids (Figs. 16 and 17) have much smaller transections and thicker secondary walls than the transfusion tracheids and are not deformed like the latter ones. Only the protoxylem elements, localized at the adaxial side of the xylem, may be partly disrupted as the needle grows. They have spiral wall thickenings (Figs. 14 and 15). The later formed tracheids have secondary walls of uniform thickness (Figs. 16 and 17) except for the places where the bordered pits occur. The fine structure of bordered pits in pine xylem has been reviewed by Murmanis and Sachs (1969).

The parenchyma cells of the xylem accumulate lipids and contain large, elongated

starch grains, which are found inside plastids or sometimes free in the cytoplasm (Fig. 18).

A fusiform cambium is inserted between the xylem and the phloem (Fig. 17). The cambium cells are thin-walled and vacuolated. They contain those elements that are typical for meristematic cells, e.g. proplastids, mitochondria, dictyosomes, ribosomes, endoplasmic reticulum etc. and possess also lipid globules.

The abaxial side of the phloem is separated from the transfusion tissue by a fiber layer and some fiber cells are also embedded in the transfusion tissue between the two bundles. The fiber cells of the stele have much thicker secondary walls than other sclerenchyma cells in the needle and their protoplasts have been eliminated. The empty, greatly compressed sieve cells of the protophloem are pressed against this vascular sclerenchyma layer.

The phloem consists of rows of sieve cells and some interspersed rays, a few of which continue across the cambium into the xylem. With regard to development at the ultrastructural level the phloem is the most extensively studied tissue in pine. Detailed accounts for the fine structure of sieve cells in needles of *P. silvestris* and *P. strobus* have recently been published by Parameswaran (1971) and Harris (1972). Wooding (1968) has described needle callus phloem from *P. pinea*. In addition there are exhaustive structural descriptions concerning the secondary phloem in trunks of *P. strobus* and *P. pinea* (Murmanis and Evert 1966, Srivastava and O'Brien 1966, Wooding 1966). The report given here will therefore be rather short and concentrate on some selected aspects of special interest. In some details opinions differ among those authors that have studied pine phloem and more investigations are apparently needed.

The sieve cells of pine are characterized by a unique kind of secondary walls. As observed by Srivastava (1969) the organization of these walls is different from that of corresponding walls of most tracheids and fiber cells. The secondary walls of the sieve cells in question consist of several identical lamellae, in which the cellulose fibrils are arranged in two separate sets that intersect each other as in a "V" (cf. Figs. 19 and 22). The wall surface contains a strongly stained amorphous substance which at irregular intervals diffuses into underlying wall layers to produce a characteristic pattern (Figs. 17, 19, 20 and 22).

Sieve areas are scattered in the radial and tangential walls of the sieve cells (Figs. 20 and 23). Rows of sieve areas are frequently seen in longitudinal sections (Fig. 20). At the level of the middle lamella the sieve pores are expanded and connected with their neighbours to form cavities, so called median nodules (cf. Murmanis and Evert 1966, Srivastava and O'Brien 1966). The sieve pores are filled with a heavily stained material. Only small amounts of callose are seen. In most of the sieve cells studied callose was absent (Fig. 20). When deposited, callose was restricted to the surface of the cell walls at the position of sieve areas (Fig. 23). According to other authors relatively little callose occurs at sieve pores of living sieve cells of needles or needle callus (Harris 1972, Parameswaran 1971, Wooding 1968) whereas in secondary phloem of trunks considerable amounts of this compound have been found (Murmanis and Evert 1966, Srivastava and O'Brien 1966, Wooding 1966).

Mature sieve cells lack nuclei, and vacuolar content and cytoplasm are mixed into a "mictoplasm" (Engleman 1965) as a result of break-down of the tonoplast (Figs. 17, 19, 20 and 23). The plasmalemma persists until the cellular content has reached an advanced stage of degeneration. In some places a strongly stained conglomerate containing small vesicles is seen (Figs. 20 and 23). This complex, the so called sieve element reticulum (Srivastava and O'Brien 1966), is apparently derived from the degenerated endomembran system of the cell. Among the organelles mitochondria show the longest persistence (Figs. 17 and 19). The plastids are of a

special kind. Frequently they contain inclusions of fine fibrils arranged in concentric clusters (Fig. 21). In other sieve cells such fibrillar aggregates occur free in the mictoplasm (Fig. 23). Since some of these cells also contain free starch grains it can be concluded that their plastids have disintegrated and released their contents. Sieve element plastids with protein inclusions in the form of fibrils or crystalloids (P-type plastids) are known in several plant families (Behnke 1972). They were demonstrated in pine by a number of authors (Harris 1972, Murmanis and Evert 1966, Parameswaran 1971, Srivastava and O'Brien 1966, Wooding 1966, 1968) and have also been found in *Picea abies* (Behnke 1972). Release of plastid fibrils into the mictoplasm of needle sieve cells was reported by Parameswaran (1971) and Harris (1972).

In several plant species the sieve elements contain a proteinaceous component, previously known as slime and in modern literature generally referred to as P-protein. This protein occurs in tubular and fibrous forms and can constitute discrete bodies or be dispersed in the cell plasm (Cronshaw and Esau 1968). P-protein has been found in many dicotyledons but seems to be produced only in some monocotyledon species (cf. Behnke 1969, Singh and Srivastava 1972). Electron microscopical studies of pine have yielded conflicting interpretations. P-protein (or slime) bodies have been reported in secondary phloem of trunks (Murmanis and Evert 1966) and in needle phloem (Parameswaran 1971). On the other hand, Srivastava and O'Brien (1966) and Wooding (1966, 1968) did not find P-protein in secondary phloem or needle callus phloem. We have in some sieve cells seen large rounded or elongated bodies that are moderately stained (Fig. 21). They are not enclosed by any envelope. Sometimes several of these bodies coalesce to large irregular masses. The bodies just mentioned are with regard to size, internal structure and electron density similar to those bodies illustrated in the paper of Parameswaran (1971) that he interprets as P-protein bodies. However, we do not know enough about them to feel prepared to make any suggestion about their nature.

The parenchyma cells of the phloem are, except for their smaller size, rather similar to the albuminous cells on the outside of the vascular bundles. In the peripheral rays the cells are more or less degenerated, a phenomenon that was observed both in needles from greenhouse plants and from individuals growing in the field. On the outer side of the bundle the ray cells are less affected than on the inner side but have necrotic nuclei that resemble the nuclei in immature sieve cells. On the inner side of the bundle in the neighbourhood of the cambium the parenchyma cells are in an advanced stage of degeneration with a strongly stained groundplasm containing numerous vesicles (Fig. 24). At this stage perforations of the cell wall between contiguous cells is frequently observed. This results in a mixing of the contents of these cells. Of interest is that similar wall perforations have been reported by Murmanis and Evert (1967) in axial parenchyma cells of the secondary phloem in *P. strobus*. Nothing is known about the significance of this phenomenon, which apparently is due to an enzymatic dissolution of a confined part of the walls between adjacent cells.

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Sammanfattning

Ultrastrukturen hos barr av tall *(Pinus silvestris L.)*

Föreliggande arbete ger en beskrivning av de olika celltyperna i fullt utvecklade barr av tall *(Pinus silvestris L.)*.

De protoplasmafria epidermiscellerna har tjocka sekundärväggar, som tagit större delen av cellrummet i anspråk. Slutcellerna, som är försänkta under barrytan, har enkelt byggda kloroplaster och talrika mitokondrier. De är övertäckta av en ring av accessoriska celler, vilka innehåller lipider och tanniner (garvämnen). Hypodermisskiktet består av levande vakuolförande fiberceller. De stora mesofyllcellerna innehåller rikligt med tanniner i vakuolerna. Cytoplasman är rik på kloroplaster och mitokondrier och innehåller även "micro-bodies", vilkas finstruktur beskrivs i uppsatsen. Hartskanaler skyddade av en fiberskida passerar genom mesofyllvävnaden. Deras sekretceller har en unik typ av plastider som uppenbarligen deltar i hartsbildningen. Endodermiscellerna kännetecknas av starkt osmiofila globuli i vakuolerna. De två kärlsträngerna omges av en transfusionsvävnad. Denna består av tunnväggiga delvis sammantryckta trakeider med ringporer samt stora parenkymceller med olika sorters tanninförande vakuoler. Liksom endodermiscellerna har dessa parenkymceller enkla amöboida kloroplaster. Strasburgerceller ("äggviteceller") med talrika små tanninfria vakuoler och lokala väggförtjockningar genomdragna av buntar av plasmodesmata påträffas på floemets yttersida. Det adaxiala xylemet innehåller protoxylemtrakeider med spiralförtjockningar och metaxylemelement med jämntjocka väggar. Ett kambium är beläget mellan xylemet och floemet. Silcellerna har en unik typ av flerskiktade sekundärväggar. Endast begränsade mängder kallos bildas på silplattorna. Plastiderna i silcellerna innehåller aggregat av proteintrådar som släpps ut i protoplasten, när plastiderna faller sönder. I några celler påträffas kroppar som liknar vad andra författare påstår vara P-proteinkroppar. De perifera märgstrålarnas parenkymceller är mer eller mindre degenererade och väggenombrott uppträder mellan angränsande celler.

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Explanation of abbreviations:

AC = subsidiary (accessory) cell
C = cuticle
CP = chloroplast
EC = epidermal cell
ER = endoplasmic reticulum
FC = fiber cell
GC = guard cell
IC = intercellular space
L = ledges
M = mitochondria
MC = mesophyll cell
N = nucleus
P = plastid
PF = pit field
SA = sieve area
SC = sieve cell
SW = secondary wall
T = torus
TC = tracheid
TT = transfusion tracheid
V = vacuole

In each figure the length of 1 μm is indicated by a scale line.

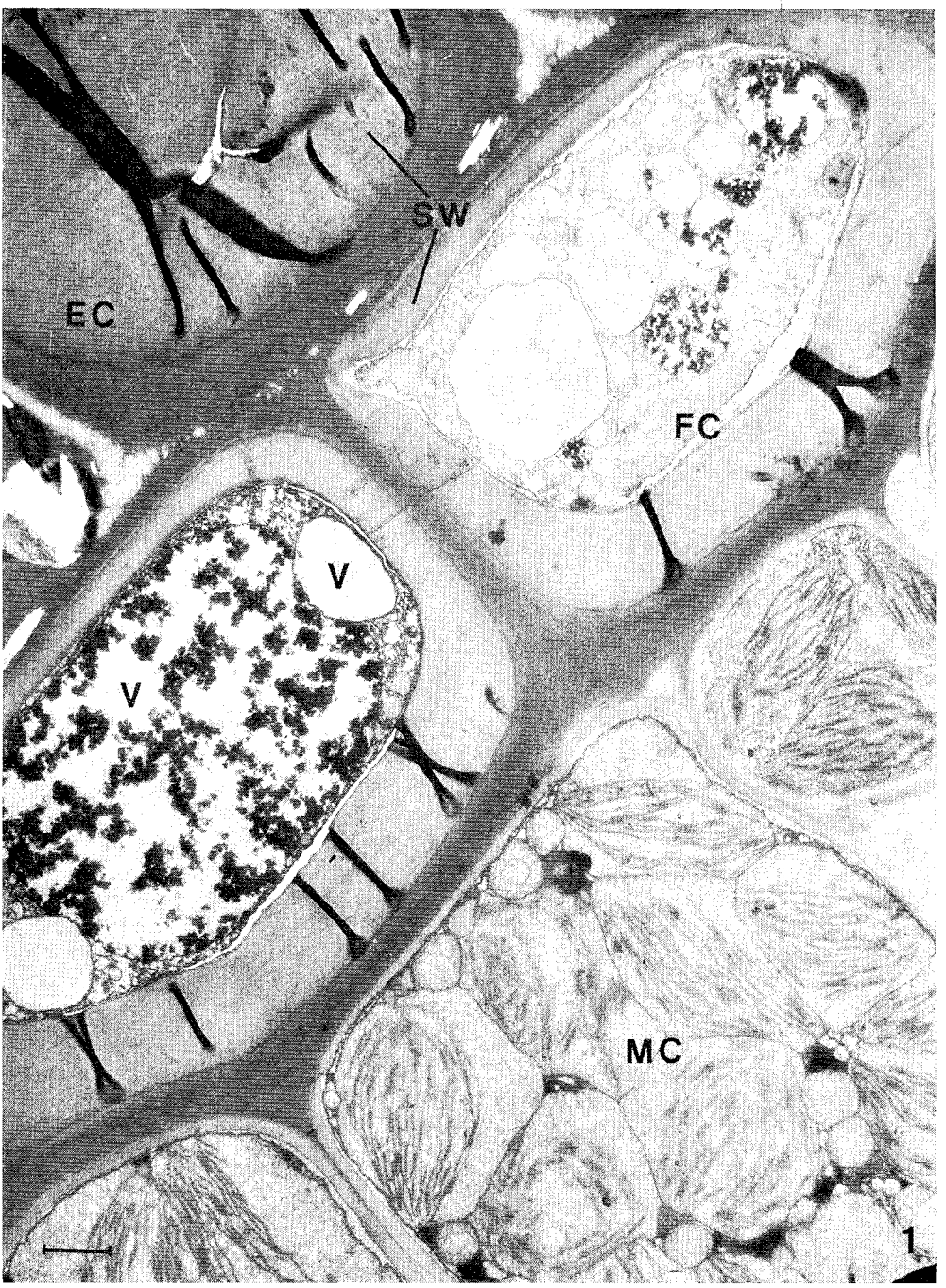
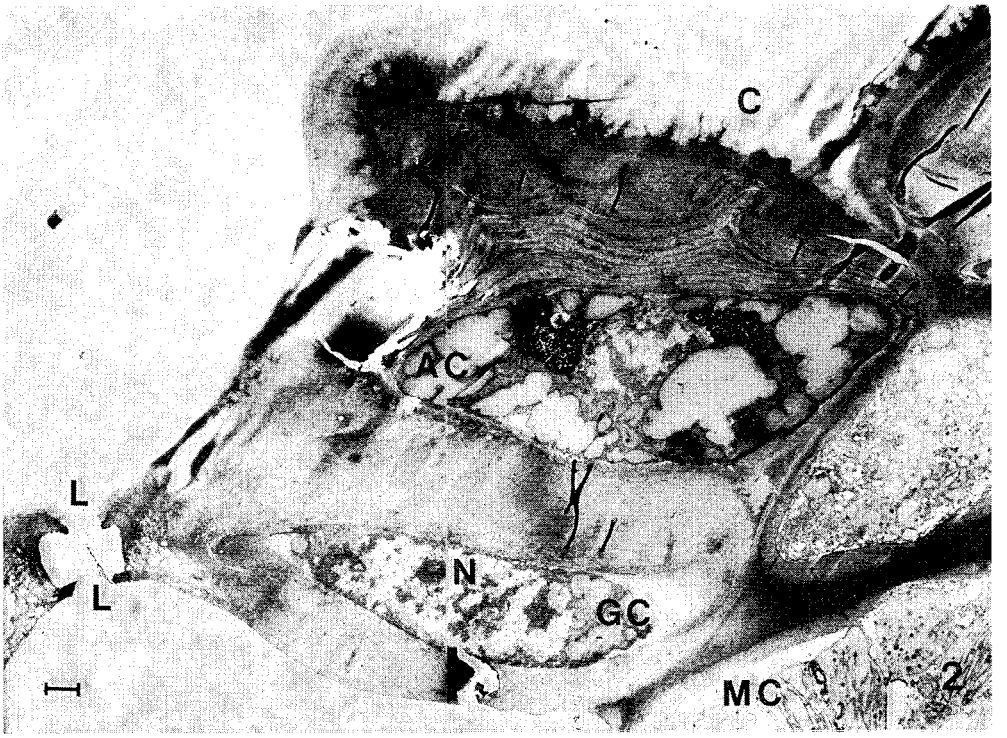
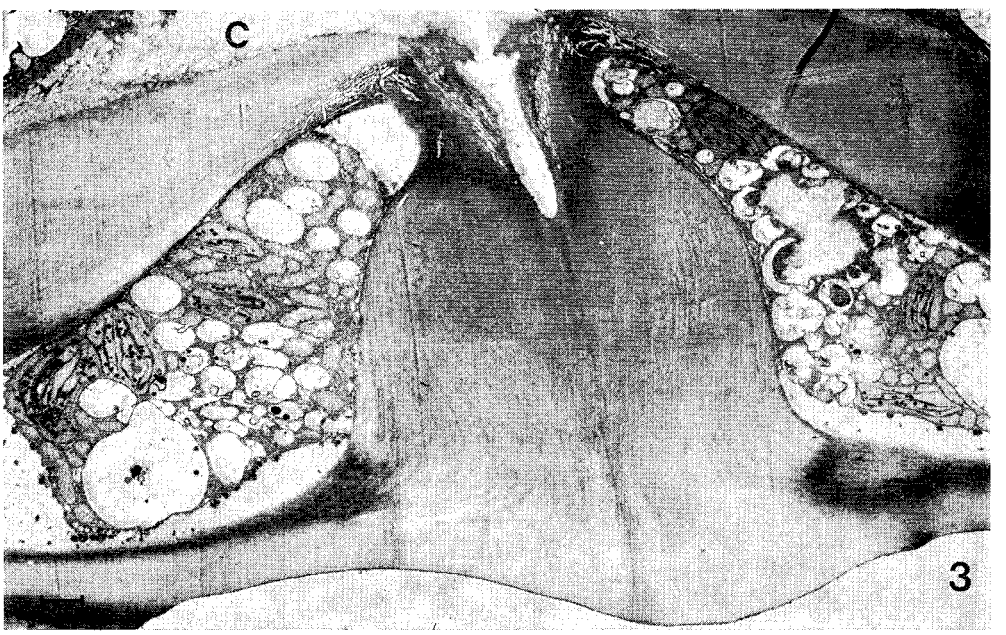


Fig. 1. A cross-section through the outer part of a pine needle. The epidermal cells (EC) have their lumena almost occluded by the thick secondary walls. The sclerenchymatic hypodermal cells (FC) have protoplasts with large vacuoles, some of which accumulate tannins. Mesophyll cells (MC) with chloroplasts are also shown. The black irregular lines in the secondary walls are artefacts (folds produced during the sectioning). The same phenomenon appears in some of the other figures. Collected in November. Fixation: glutaraldehyde — OsO_4.





Figs. 2—3. Stomata. *Fig. 2.* Section through the center of a stoma. The guard cells (GC) are sunken below the needle surface and covered by subsidiary cells (AC). Note the development of thicker and thinner parts in the walls and the lip-like ledges (L) projecting from the guard cell walls where these face the aperture. *Fig. 3.* Guard cells with chloroplasts. Collected in January (Fig. 2) and September (Fig. 3). Fixation: formaldehyde-glutaraldehyde. Postosmicated.

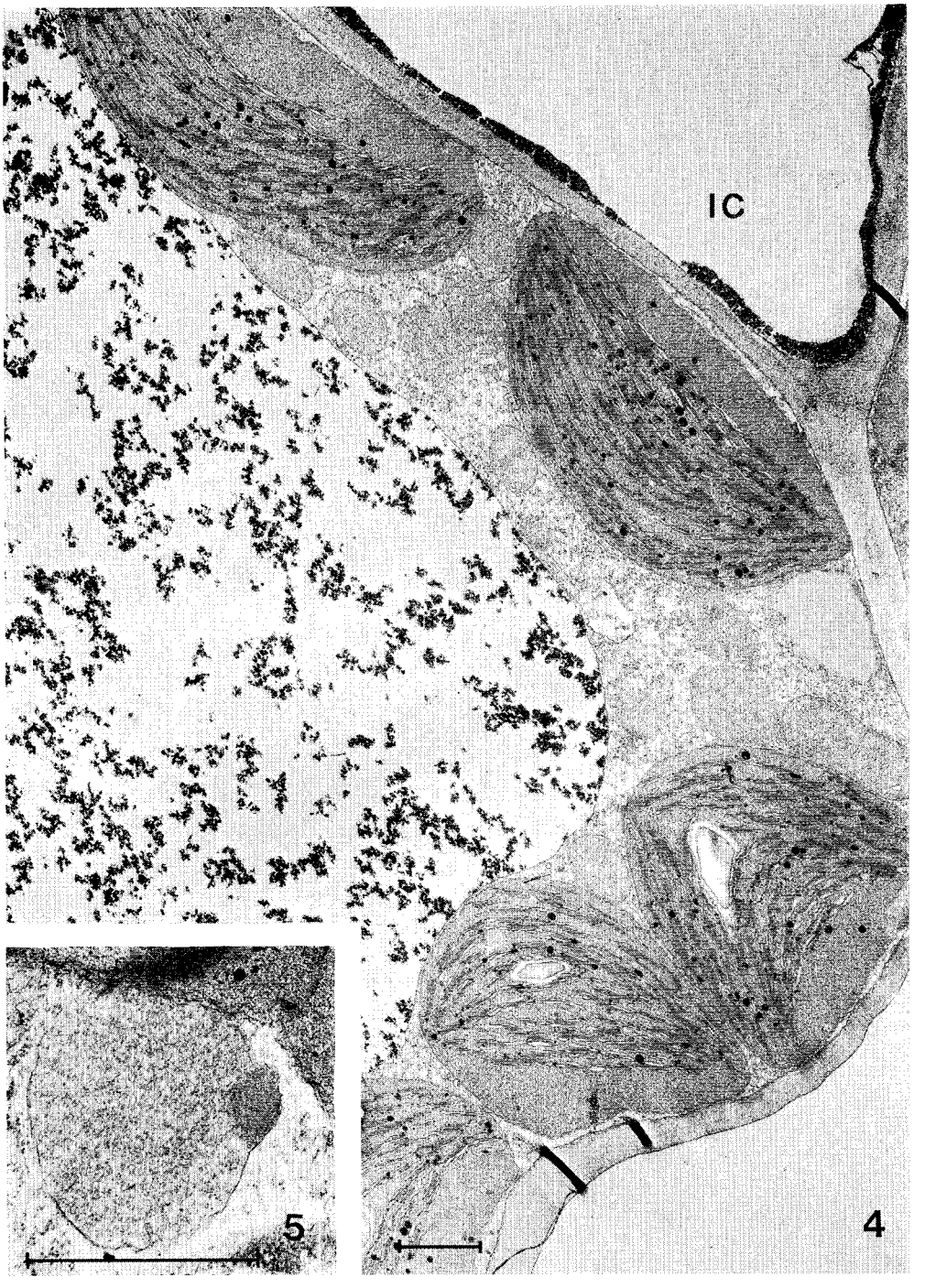
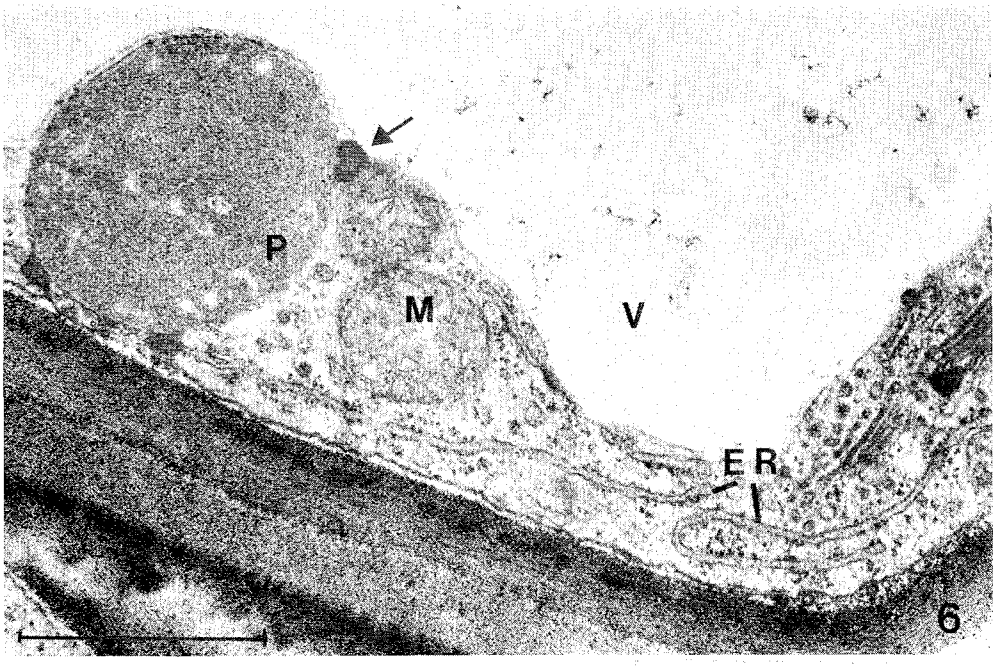
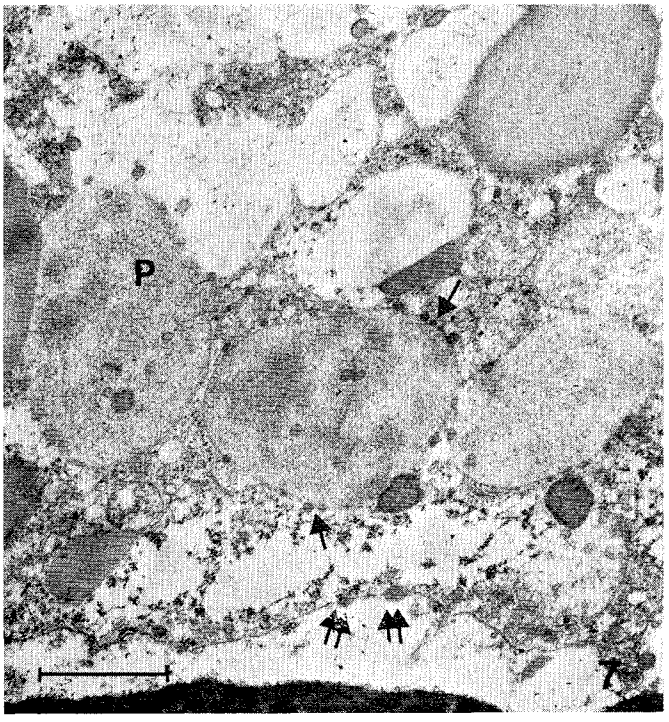
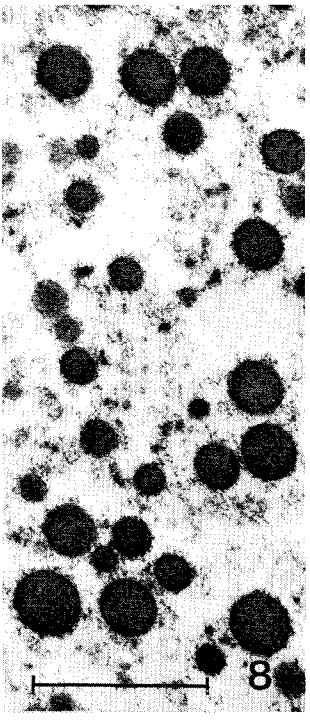


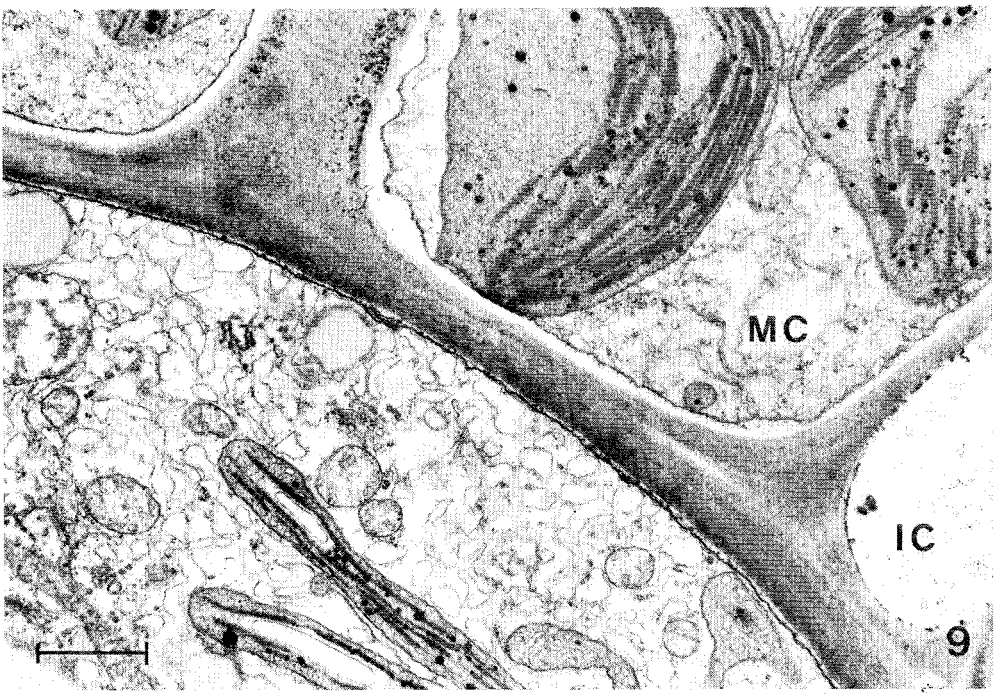
Fig. 4. Part of a mesophyll cell with a large tanniniferous vacuole. Chloroplasts and mitochondria are seen in the cytoplasm. *Fig. 5.* A microbody with a strongly stained inclusion. Figs. 4—5 are from greenhouse plants. Fixation: glutaraldehyde — OsO_4.







Figs. 6—7. Epithelial cells of resin ducts. The plastids lack thylakoids and appear involved in resin production. Arrows point to globules (presumably resin) excreted by the plastids. Similar material is apparently excreted through the plasmalemma (double arrows). Collected in July (Fig. 6) and January (Fig. 7). Fixation: formaldehyde-glutaraldehyde. Postosmicated. *Fig. 8.* Strongly osmiophilic globules that are characteristic vacuolar constituents in endodermal cells. From a greenhouse plant. Fixation: glutaraldehyde — OsO_4.



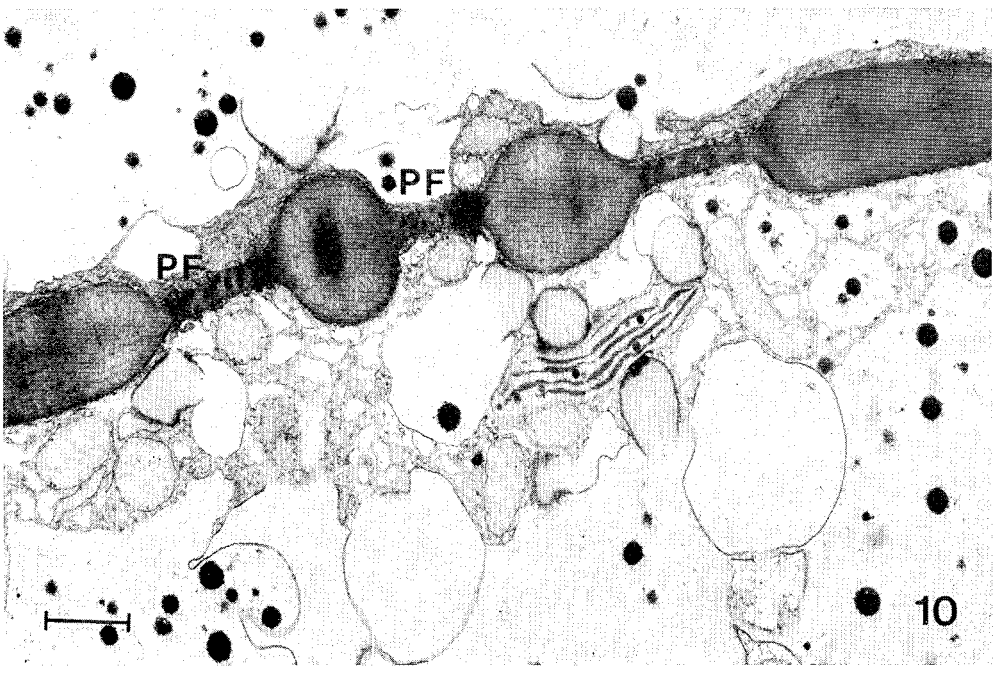
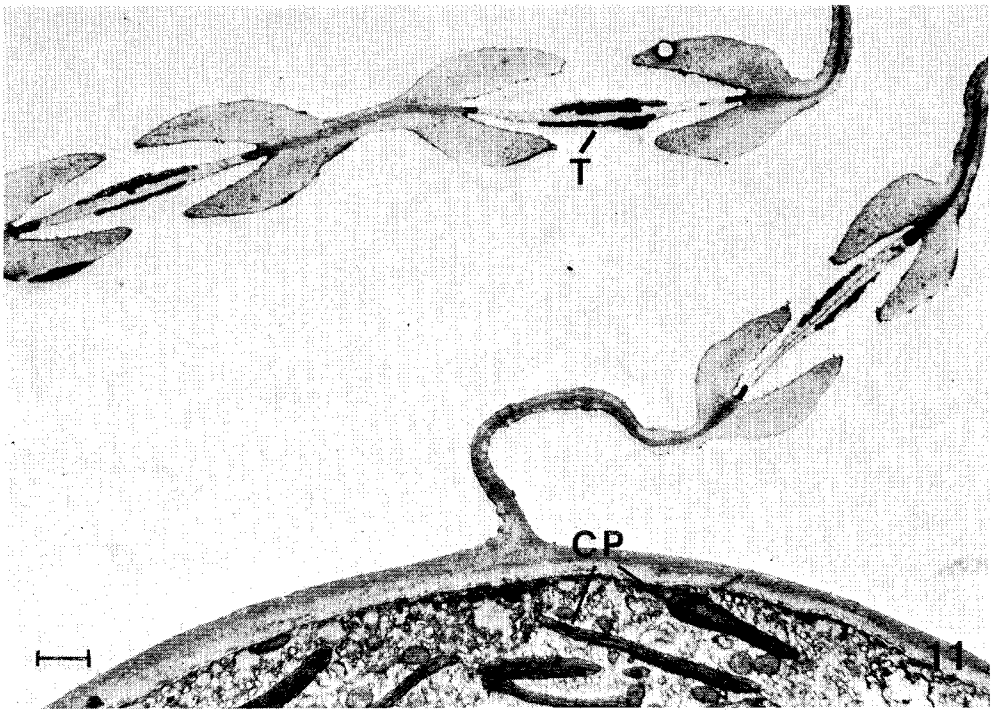


Fig. 9. Part of an endodermal cell with adjacent mesophyll cells. Note the difference in chloroplast structure between the two cell types. December. Fixation: glutaraldehyde — OsO_4. *Fig. 10.* Longitudinal section through two contiguous endodermal cells. The separating wall has several primary pitfields (PF). The vacuoles contain osmiophilic constituents of the kind shown in Fig. 8. January. Fixation: formaldehyde-glutaraldehyde. Postosmicated.



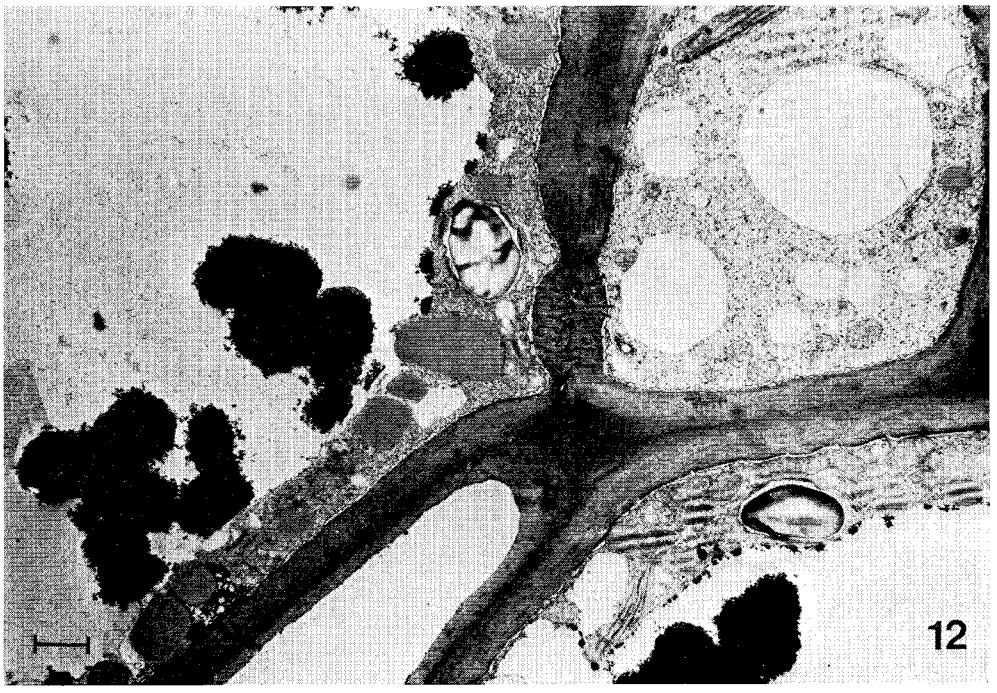
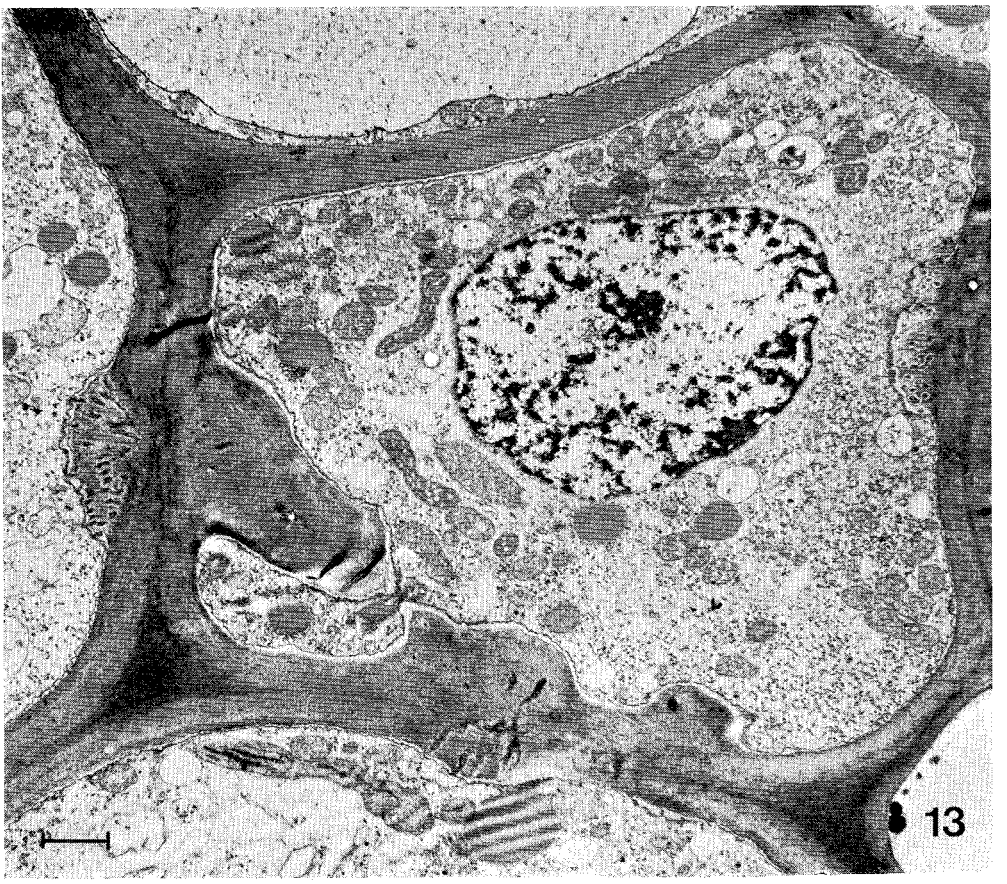


Fig. 11. Parenchyma cell and tracheids of the transfusion tissue. The parenchyma cell contains amoeboid chloroplasts, similar in appearance to those of endodermal cells (Fig. 9). Bordered pit pairs are seen in the tracheid walls. Each such pair contains a torus, formed by the middle lamella and associated parts of the primary walls. December. Fixation: glutaraldehyde — OsO_4. *Fig. 12.* Transfusion parenchyma cells with large vacuoles containing aggregates of tannin and a tannin-free albuminous cell. Collected in April and fixed in formaldehyde-glutaraldehyde. Postosmicated.



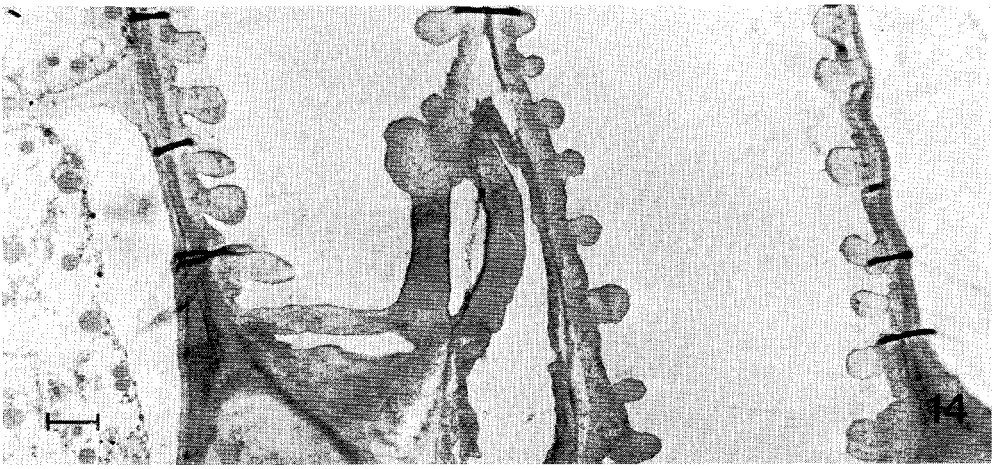
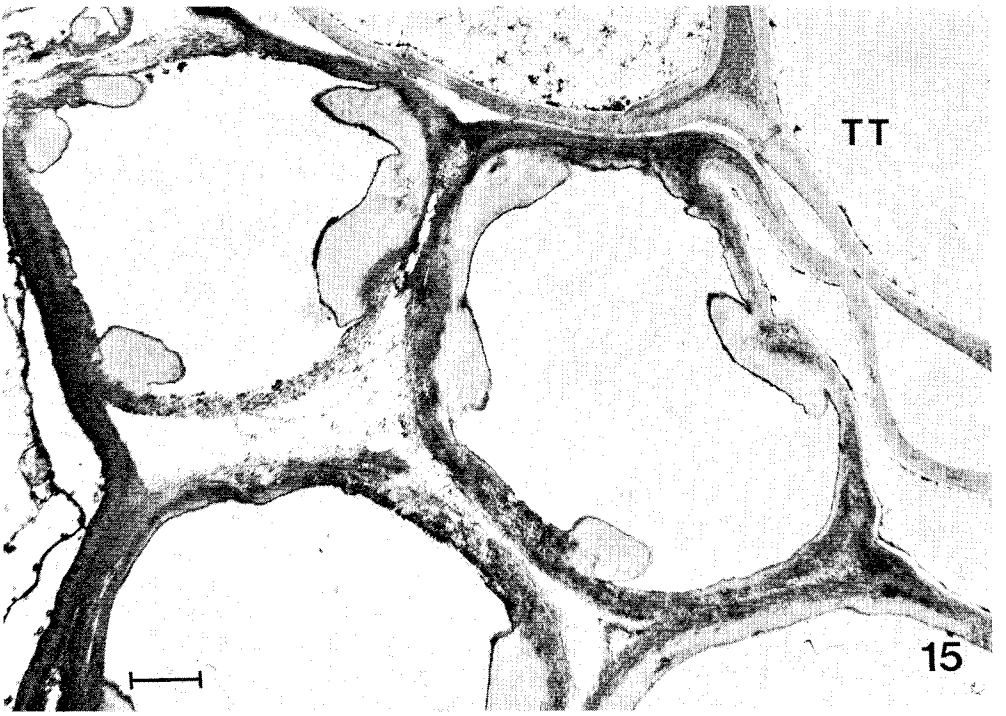


Fig. 13. Albuminous cell in the transfusion tissue close to the phloem. This cell has in some places prominent wall thickenings traversed by plasmodesmata. April. *Fig. 14.* Longitudinal section through protoxylem tracheids with spiral wall thickenings. To the left part of a ray cell. January. Both figures from material fixed in formaldehyde-glutaraldehyde and postosmicated.



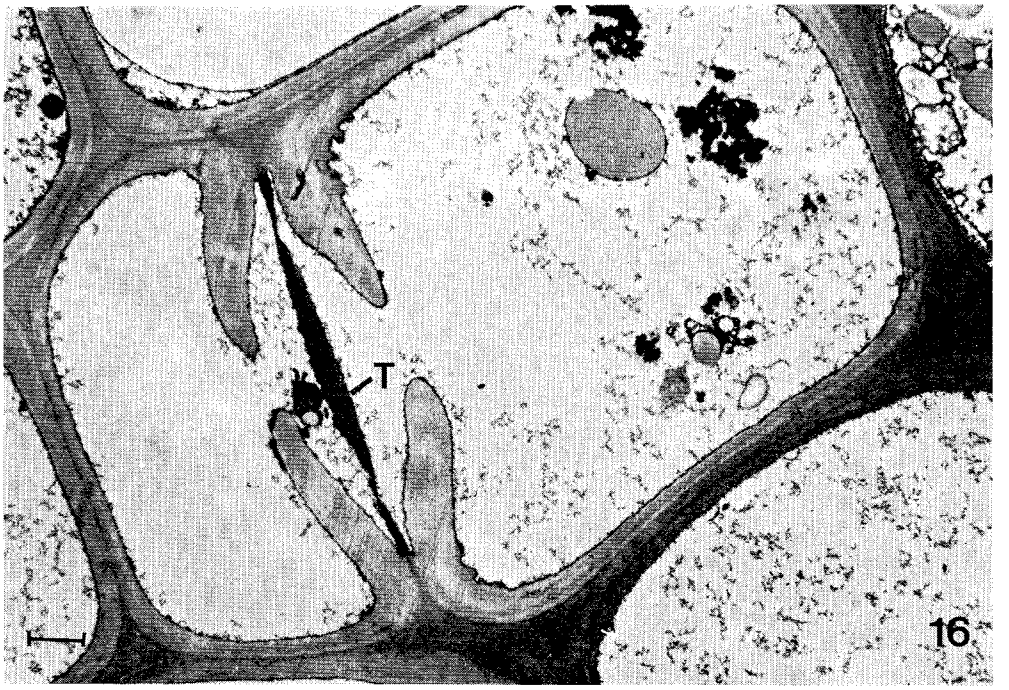
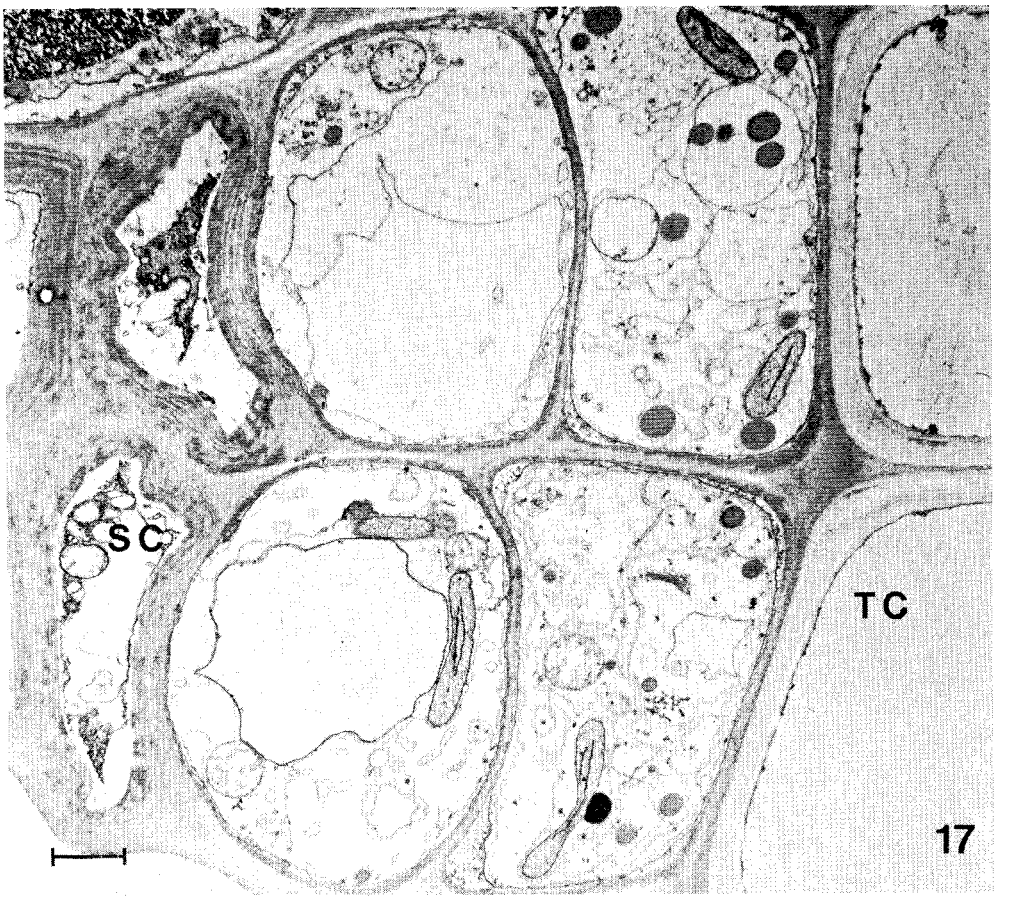


Fig. 15. Cross-section through protoxylem tracheids with spiral wall thickenings. From a greenhouse plant fixed in glutaraldehyde — OsO_4. *Fig. 16.* Cross-section of metaxylem tracheids with a bordered pit-pair. April. Fixation: formaldehyde-glutaraldehyde. Postosmicated.



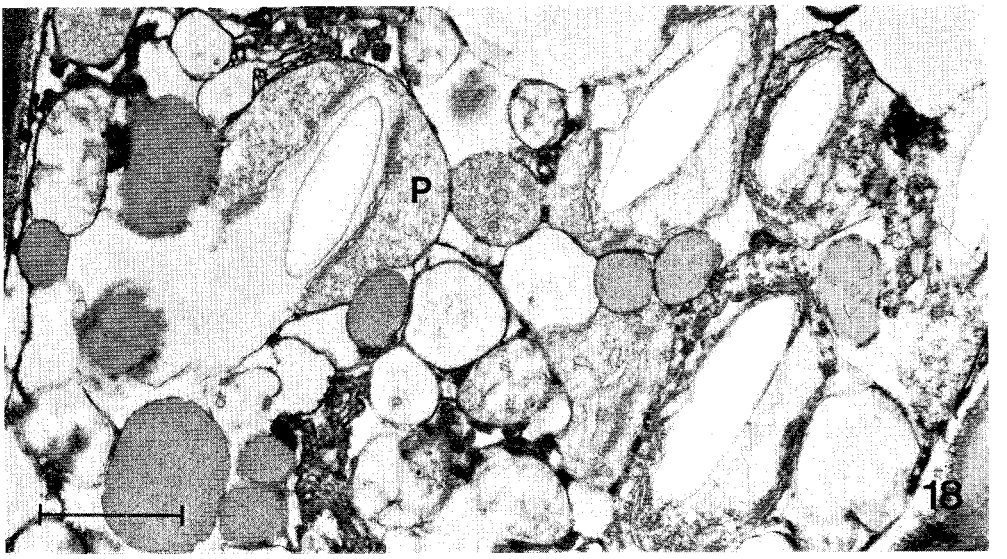


Fig. 17. Cambium cells inserted between phloem and xylem elements. December. Fixation: glutaraldehyde — OsO_4. *Fig. 18.* Part of a parenchyma cell in the xylem. April. Fixation: formaldehyde-glutaraldehyde. Postosmicated.

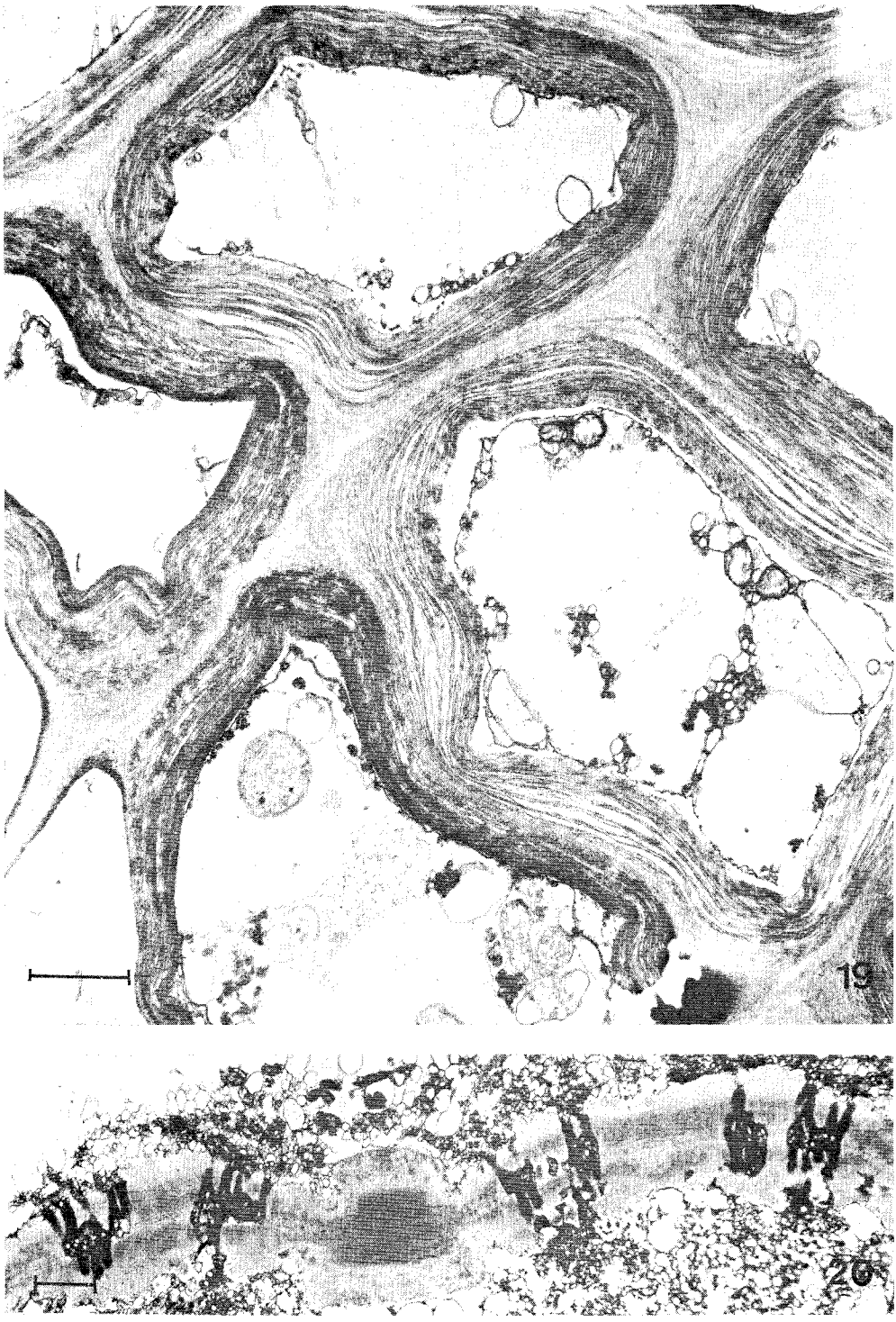
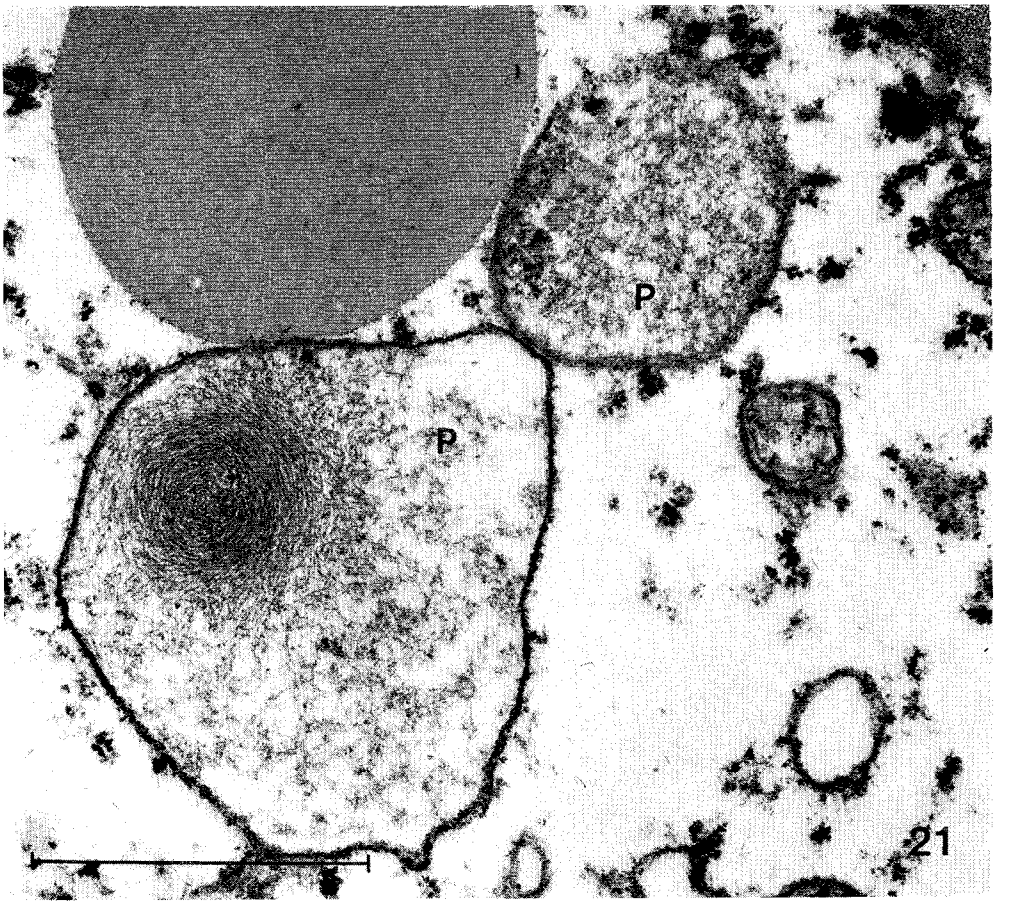


Fig. 19. Cross-section through sieve cells with their characteristic multilayered secondary walls. Each layer contains two sets of cellulose fibrils that cross each other at an acute angle. Inside the cells the tonoplast is largely broken down and vacuolar sap and cytoplasm mixed. Persisting plasmalemma and mitochondria are seen. From a greenhouse plant fixed in glutaraldehyde — OsO_4. *Fig. 20.* Longitudinal section through sieve cell wall with sieve areas and adjacent sieve element reticulum. April. Fixation: formaldehyde-glutaraldehyde. Postosmicated.



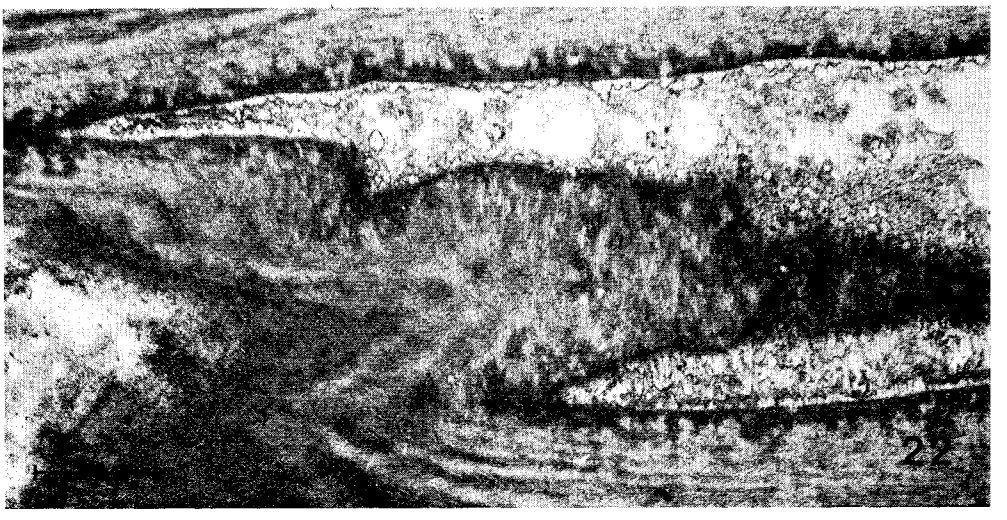
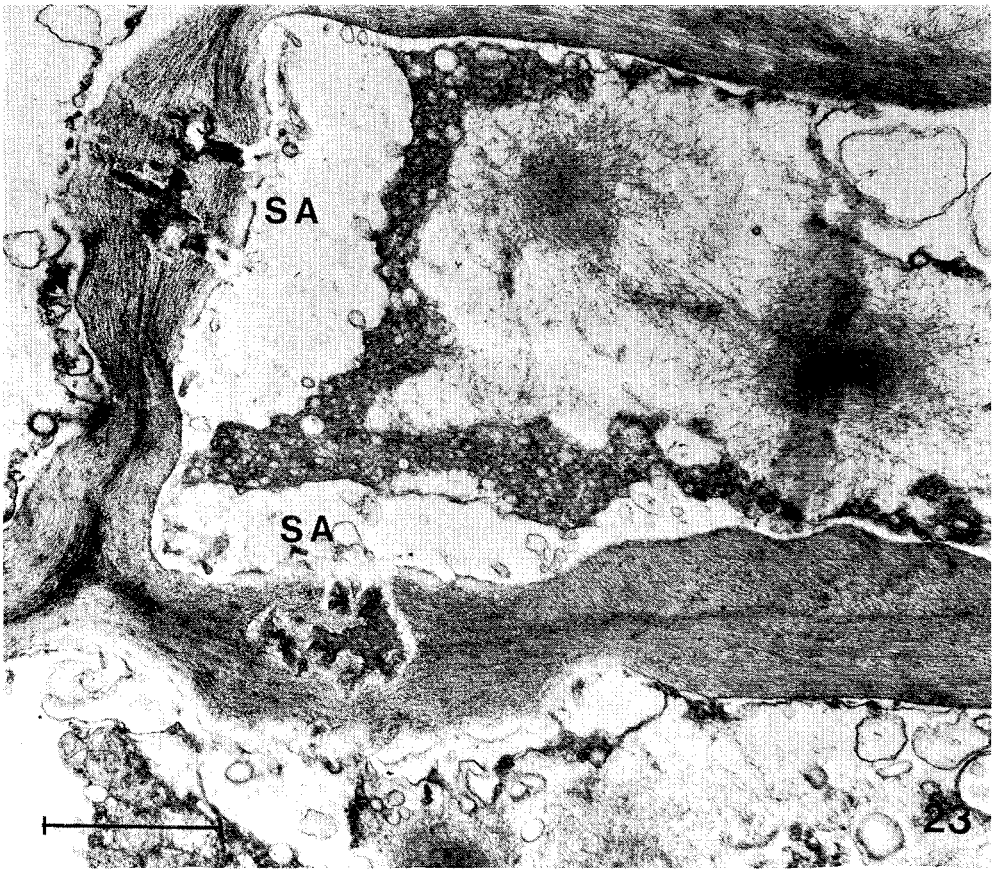


Fig. 21. Sieve cell plastids, one of which contains a cluster of concentrically arranged fibrils. A body of unknown nature is seen close to the plastids. It appears similar to the "P-protein bodies" described by other authors. Greenhouse material fixed in glutaraldehyde — OsO_4. *Fig. 22.* Longitudinal section through sieve cell walls with layers of cellulose fibrils as described in the legend of Fig. 19. A strongly stained matrix substance forms a characteristic pattern on the wall surface. January. Fixation: formaldehyde-glutaraldehyde. Postosmicated.



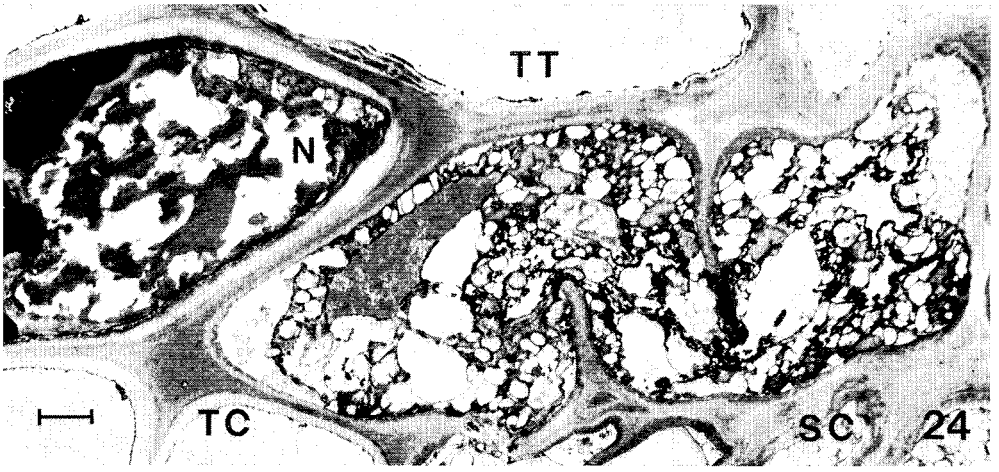


Fig. 23. Sieve cells having sieve areas covered with callose. Clusters of free fibrils occur in the mictoplasm (cf. Fig. 21). From greenhouse plant fixed in glutaraldehyde — OsO_4.
Fig. 24. Part of a ray on the border between a vascular bundle and the transfusion tissue. Note the necrotic nucleus in one cell and the large wall perforation between two other cells which has resulted in mixing of their contents. December. Fixation: glutaraldehyde — OsO_4.