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Ultrastructure of the rust fungus *Peridermium pini* (Pers.) Lev.

Ultrastrukturen hos rostsvampen Peridermium pini (Pers.) Lev.

BJÖRN WALLES
Institute of Botany, University of Stockholm, and
Department of Forest Genetics, Royal College of Forestry

SKOGSHÖGSKOLAN
ROYAL COLLEGE OF FORESTRY
STOCKHOLM

Abstract

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Peridermium pini *growing in the green, parenchymatous cortex of a young tree of pine* (Pinus sylvestris) *has been studied by electron microscopy.*

The extensively branched septate hyphae are spread in the intercellular spaces and in the middle part of walls separating contiguous host cells. The hyphal cells are uninucleate. The septa are simple and perforate. The septal pore is occluded by a Woronin body. This organization of the septum is similar to that of some Ascomycetes, as is the occurrence of a centriolar plaque on the nuclear envelope. P. pini *has in addition a structural configuration characteristic for rust fungi, namely an approximately hemispherical pore apparatus bordered by a layer of microbodies.*

The sac-like haustorium is separated from its extracellular mother cell by a cross-wall, situated in the short neck region. At the place of penetration, the host wall forms a papilla-shaped collar. Almost one third of the haustoria observed becomes encased by an extensively proliferating collar. A few per cent of the haustoria become necrotic after having entered host cells. The attacked parenchyma cells, which may each contain several haustoria, have generally an affected plasmalemma and more or less degenerated chloroplasts.

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1 Introduction

The parasite fungus *Peridermium (Cronartium) pini* causes the resin-top disease in the Scots pine (*Pinus sylvestris*). It is an autoecious rust, spread by aeciospores. The sporangia appear in lesions in the bark of infected trees. It has been suggested (Rennerfelt 1943, van der Kamp 1970) that under natural conditions infection generally occurs through needles, on which adhering spores could germinate and develop germ tubes which then may penetrate into stomata. An additional site of infection may be wounds on stems or needles. As a matter of fact successful artificial inoculations have involved such wounds (*vide* van der Kamp 1968, 1970 and literature cited there). When introduced into a non-resistant needle the fungus develops a mycelium which grows down the vascular bundles and eventually reaches the needle-carrying twig (van der Kamp 1970, cf. Rennerfelt 1943). The mycelium is reported not to spread from shoots into needles (van der Kamp 1970).

P. pini has been considered as an autoecious race of *Cronartium flaccidum*. Hiratsuka (1969) has suggested that *P. pini* has an endocyclic life cycle, i.e. the fungus has the gross morphology of an aecium but the spores, although they look like aeciospores, produce basidia upon germination. Hiratsuka (*loc. cit.*) found it therefore appropriate to rename this rust as *Endocronartium pini*. However, until the life cycle and taxonomy of this species has been unequivocally established it seems to be most practical to keep the old and in forestry research well-known name *P. pini*.

Light microscopic studies of hyphae and spores of *P. pini* were performed by van der Kamp (1969). He reported that the septate and uninucleate hyphae have an intercellular distribution in the host tissues with haustoria penetrating into host cells. So far the fine structure of *P. pini* has been unknown. Two papers have appeared that were based on electron miscroscopy of the American species *Cronartium ribicola*. Boyer and Isaac (1964) reported briefly on some ultrastructural features of this rust and of infected needle tissues of the host *Pinus strobus*. Recently Robb *et al.* (1973) have described hyphae of the same rust which were grown axenically in the presence of tissue cultures of the host plant *Pinus monticola*.

The ultrastructure of hyphal cells of haustoria of *P. pini* growing in pine bark has now been investigated and the results are presented here.

2 Material and methods

The material for the present investigation was collected in August 1973 from an about ten years old infected tree of pine (*Pinus sylvestris*), growing at Bogesund, 30 km north-east of Stockholm. This tree was in July 1969 inoculated with aeciospores of *Peridermium pini* according to a method involving stem wounds (Klingström 1967).

An about 5 cm long part of a small branch that had produced aeciospores in June—July 1973 was after collection immediately submersed in a mixture of formaldehyde and glutaraldehyde (Karnovsky 1965) and stored in a cold room at about +5°C. Material for analyses was removed at two occasions, after one day and after about three months. This material comprised small pieces of bark and adjacent parts of the vascular bundle and was cut out with a razor blade from the swollen lesion-region and within a region 2 cm external to the lesion. Pieces of healthy tissues were collected from small branches of young trees growing in the garden of the College of Forestry. The specimens were washed in 0.2 M phosphate buffer (ph = 7.0) and subsequently fixed in 1 per cent OsO_4 + 0.1 M phosphate buffer at +4°C for 2 hours. After dehydration with acetone the specimens were embedded in Spurr's medium (Spurr 1969). Sections were cut with diamond knives on a LKB UM I or UM III and stained with 1 per cent uranyl acetate and 0.2 per cent alkaline lead citrate (Venable and Coggeshall 1963). They were examined with a Hitachi HS-7S electron microscope.

3 Observations

3.1 Host cells

The cortex of the pine consists of thick-walled parenchyma cells of a more or less spherical shape. In the twig investigated these cells were green, i.e. they can be considered as chlorenchyma cells. Small groups of cork cells with a strongly electron dense content may be found scattered among the living cells. The intercellular system is well developed.

Most or all parenchyma cells are probably attacked by haustoria (see Section 3.3). In these cells the plasmalemma has usually a greatly increased surface area due to the development of numerous small inbuddings (Figs. 2, 10, 17, 18). The vacuoles are large and may contain dense aggregates of tannins (Figs. 2, 15). Lipid globules are spread in the cytoplasm. The chloroplasts are usually more or less irregular in shape and their lamellar system is swollen and deranged (Figs. 2, 11, 16). A layer of vesicles which might be arranged into a configuration similar to a peripheral reticulum (cf. Rosado-Alberio et al. 1968) occurs beneath the plastid envelope (Figs. 2, 11). The plastid stroma contains groups of plastoglobuli and large starch grains. Host cells killed by the parasite were not found.

Chlorenchyma cells from uninfected pine trees lack extensive plasmalemma proliferations, and their chloroplasts are of a regular shape with a well developed lamellar system without a reticulum (Fig. 1).

3.2 Intercellular hyphae

Short segments of intercellular hyphae can be observed in the thin sections of infected tissues (Figs. 2—8). Each such segment comprises the profile of a part of one cell or sometimes two contiguous ones separated by a cross-wall. The hyphae are extensively branched. They are distributed in the voluminous intercellular spaces of the pine cortex, where they mainly grow along the walls of the host cells framing the space, and further in the middle part of the wall separating contiguous host cells (Figs. 2, 4). Eventually the hyphal tip penetrates the wall of a host cell and develops a terminal haustorial cell (see Section 3.3).

The fungal cells are elongated and have a dense cytoplasm containing ribosomes, endoplasmic reticulum and mitochondria (Figs. 4, 5, 8). In the cytoplasm there are further a few vacuoles and large vesicles, filled with membraneous material, most of which forms narrow channels or tubes (Figs. 4, 7). These structures are similar to plasmalemmasomes, i.e. endocellular vesicles derived from the plasmalemma (Marchant and Robards 1968, Marchant and Moore 1973). Storage products occur in the form of large lipid globules and aggregates of glycogen particles (Figs. 4, 5).

The single nucleus is roughly spherical in shape, provided the diameter of the cell is sufficiently large (Fig. 4) but in constricted cells the nuclei are more or less tube-shaped (Fig. 3). Only in one instance has a cell been found which seems to contain two nuclei (Fig. 5). The chromosomes are electron-dense and appear sometimes contracted (Fig. 4). The nucleolus is prominent (Figs. 3, 5). Some sections reveal a dense fibrillar band-shaped structure adhering to the outside of the nuclear envelope (Fig. 5). It is similar to the centriolar plaque, which has been observed in a few other rusts and Ascomycetes (Coffey *et al.* 1972, Dunkle *et al.* 1970, Robinow and Marak 1966, Wells 1970).

The hyphal cell wall consists of an inner strongly stained part and an amorphous

outer layer. The electron dense part (the cell wall proper) is formed by several thin lamellae (Figs. 3, 8). The outer amorphous, moderately stained wall layer is of variable thickness in the free hyphae growing in the intercellular spaces of the host (Figs. 3, 5). This external layer appears plastic as it in some places fails to follow the contour of the walls, e.g. where branching of the hyphae occur or in the regions of contact between hyphae and host walls. When separate hyphal branches meet, coalescence of their external layers take place (Fig. 3). These observations indicate, that the amorphous wall layer consists of a mucous substance which may render the hyphae sticky and adhesive. In hyphae growing inside host cell walls the outer wall layer appears more or less fused with the host wall and its boundaries can consequently not be safely determined (Figs. 2, 4).

The individual cells of a hypha are separated from each other by crosswalls (septa). In the middle of the strongly stained septum is a thin unstained layer. The peripheral boundary of this middle layer of the septum lies halfway inside the longitudinal walls (Figs. 4—8).

The thickness of the septum declines towards its centrum, where a small opening (pore) is left (Fig. 6). This pore is occluded by a strongly stained body, which is drastically constricted at its middle where it is trapped into the narrow opening. On each side of the septum this body is expanded into incomplete spheres, which are flattened against the septum (Figs. 4—7). Apparently the plug just described represents a Woronin body. Such bodies are frequently associated with the septa of ascomycetous fungi (Bracker 1967, Wergin 1973). Small aggregates of the same electron density as the main body may be seen external to it along the septum (Fig. 7).

On each side the septal pore is usually surrounded by a faintly stained granular matrix, from which the common organelles are excluded (Figs. 4, 7, 8). Small microbodies with crystalline inclusions are found at the interface between this matrix and the rest of the cytoplasm (Figs. 6—8).

3.3 **Haustoria**

In the literature there is a regrettable confusion concerning the terminology of the structures formed by interactions between parasitic fungi and host cells. I have here chosen to follow the nomenclature proposed by Bracker (Bracker 1967, Littlefield and Bracker 1972).

In *P. pini* haustoria are developed at the tip of intercellular hyphae that change their direction of growth and proceed to grow through the host cell wall. At the site of penetration of a hyphal tip characteristic manifestations appear in the host cell wall. Normally this wall contains numerous layers of stained cellulose fibrils. In a zone around the penetrating part of the haustorial mother cell the cellulose fibrils disappear and leave an unstained (electron lucent) matrix containing scattered irregularly shaped particles (Figs. 9, 14, 15). This zone is probably formed by the action of enzymes (cellulases?) excreted by the fungus. As a reaction to the attempted invasion by the parasite, the host wall develops an apposition, the collar. The collar has a finely fibrillar-granular composition similar to that of the wall. In about 70 per cent of the cases (n = 29) the collar is papilla-shaped and penetrated by the terminal hyphal cell (= haustorium) which then establishes contact with the host protoplast (Figs. 9—10, 17—18). In the remaining cases the growth of the collar is so vigorous that the haustorium is completely encased by the host wall (Figs. 14—16). This phenomenon will be described further later in this chapter.

The haustorium of *P. pini* is separated by a cross-wall from its mother cell. The septum between these two cells is similar to other septa in the hyphae, i.e. it is perforated and plugged by a Woronin body (Fig. 10). The cytoplasmic pore apparatus with its microbodies is, however, poorly developed or absent in the haustorial cell.

The proximal part of the haustorial cell and the distal part of the mother cell are constricted (Figs. 9—10, 17—18) and form a stalk that corresponds to the haustorial neck described in other parasitic fungi. The

neck region is short and the main part of it is enclosed by the well-developed collar. Inside the host cell the bulk of the haustorial cell is expanded into a slender sac-like formation, the haustorial body (Figs. 9, 17, 18). External to the collar the haustorium is enveloped by the invaginated host plasmalemma, which in the literature often is called the sheath membrane (cf. Littlefield and Bracker 1972). This membrane has generally a considerably increased surface area due to numerous minute inbuddings (Fig. 11) just as that part of the plasmalemma of infected parenchyma cells which is in a normal position, adjacent to the cell wall (cf. Figs. 2, 10). Between the sheath membrane and the wall of the haustorial body a matrix occurs, which becomes successively thicker towards the end of the haustorium (Figs. 9, 10, 18). This matrix may be electron transparent (Fig. 11) but is in most cases filled with a fairly dense granular substance (Figs. 9, 10, 12, 13). The distal part of the haustorial body has a thickened wall with a more or less dissociated surface (Fig. 11). Sometimes electron dense particles, which appear to be derived from the fragmented wall surface, are found in the sheath matrix.

The haustorial cytoplasm contains large lipid bodies and spaces occupied by glycogen granules (Figs. 9—11). The same types of organelles and membraneous structures that occur in the intercellular hyphae can be found in the haustorium.

Haustoria are observed in a large proportion of the cells present in the sections through cortex tissues. Since each fine section only constitutes a very restricted part of each cell represented, it seems justified to conclude that all or most cells of the cortex are in fact attacked by haustoria. Sometimes more than one haustorium is seen inside an individual host cell (Figs. 9, 16).

Although it is mainly localized to the cortex, the mycelium is also found in the rays of the vascular tissues, and from these rays hyphae may spread to parenchymatous parts of the vascular bundle. Haustoria may also occur inside young sieve cells (Fig. 12) and tracheids of the outermost annual ring (Fig. 13).

As mentioned earlier about 30 per cent of the haustorial cells in the present material are encased by a capsule-shaped collar (Figs. 14—16). An amorphous, moderately stainable layer, probably of fungal origin, is inserted between the haustorial wall and the capsule. At the interface between this layer and the capsule, granules of a strongly stained material are deposited (Figs. 14, 15). Such granules may be spread a short distance into the capsule. In spite of being physically isolated from the host cell, the encased haustorium has a healthy-looking protoplast with normal organelles. The density and degree of permeability of the capsule is not known, however, and it can not be determined from the micrographs whether some nutrients from the host cell might diffuse through the capsule or whether the haustorium has to live on what it can receive from its mother cell.

A minor fraction of the haustoria (3 out of 29 observed) were necrotic. These degenerated haustoria have succeeded to penetrate the host wall and are surrounded by sheaths (Figs. 17, 18). Their intercellular mother cells appear normal (Figs. 17, 18). The death of the haustorial cells is thus selective and probably due to an incompatibility reaction with the host protoplasts.

4 Discussion

Whereas the ultrastructure of uredial thalli of rust fungi has been investigated extensively, only few and rather restricted studies have so far been devoted to the aecial stage of these fungi. Differences in ultrastructure between the different generations might occur in Uredinales, as suggested by e.g. Coffey *et al.* (1972) with regard to septal structures, and Rijkenberg and Truter (1973) with regard to the feeding intracellular proliferations of mycelia. The present study of the fine structure of a uninuclear rust mycelium has yielded some interesting results and might be of value for comparisons with other rusts, mainly of uredial stages, treated in the literature. I shall therefore in this section discuss some fine structural features of *P. pini* as compared with corresponding traits of other rusts investigated, and to some extent also of other basidiomycetous and ascomycetous fungi.

Septal pores are characteristic structures of the higher fungi and their organization can apparently be of some value for taxonomical work. In Ascomycetes and some Deuteromycetes, the typical septum is a simple plate with a central hole, which may stay open or be plugged by special structures (see review by Bracker 1967). The so-called dolipore septa in Homobasidiomycetidae and Tremellales are highly specialized formations with annular swellings circumscribing the septal opening (*loc. cit.*).

In Uredinales, the septa in filamentous hyphae have simple and narrow pores which usually are occluded by septal plugs. In uredial hyphae of *Melampsora* and *Puccinia,* the septal plug is shaped like a pulley-wheel (Littlefield and Bracker 1971, Coffey *et al.* 1972). This plug has been described as electron-dense (Littlefield and Bracker 1971) or electron lucent (Coffey *et al.* 1972). Possibly its staining ability increases with the age of the surrounding cells, as indicated by the micrographs of different ontogenetic stages published by Littlefield and Bracker (*loc. cit.*). Septal plugs in rusts were presumably reported for the first time by Ehrlich and Ehrlich (1969), who found them in the septa between developing uredospores and adjacent stalk cells in *Puccinia.* The present investigation reveals that *P. pini* has each septal pore occluded by a Woronin body, which is much larger than the opening and therefore noticeably constricted at its middle. The body thus has the appearance of a pair of incomplete spheres, united inside the septal pore. In aecial hyphae of *Cronartium ribicola* the occluding septal pores were reported to be electron dense, either pulley-wheel shaped or forming "a pair of large amorphous electron-dense bodies" (Robb *et al.* 1973).

Woronin bodies that function as septal plugs are known from some ascomycetous fungi (Bracker 1967, 1968, McKeen 1971, Reichle and Alexander 1965, Wergin 1973). Micrographs of septal plugs in some of these fungi show that they may assume a shape somewhat similar to a pulley-wheel. In *Erysiphe* Bracker (1968) found occluding Woronin bodies of the same shape as the corresponding bodies I have found in *P. pini.* In *Puccinia,* Coffey *et al.* (1972) noticed close to the septal plug one to several electron-dense bodies which usually had a spherical shape. A similar body appears in Fig. 4 in the paper on *Melampsora* septa by Littlefield and Bracker (1971). In my opinion these bodies might represent free Woronin bodies (cf. Wergin 1973). Also the characteristic occluding structure associated with the pores of uredial hyphae might well be a modified kind of Woronin bodies. In *Cronartium,* both this kind of plug and more typical Woronin bodies have

been reported from different septa of the same fungal culture (see Robb *et al.* 1973). Recently Dykstra (1974) reported the presence of an occluding septal plug in the parasitic Basidiomycete *Septobasidium*. His micrographs reveal that this plug is rather similar to that occurring in *P. pini* and some other rusts.

The origin of Woronin bodies in *Fusarium* has been investigated by Wergin (1973). He followed the development of microbodies in young hyphal cells. These organelles produced each a strongly grained inclusion that appeared to be gradually extruded from the parent organelle and transformed into a Woronin body. The Woronin bodies, several of which occurred in each cell, became distributed near the septa, where some of them came to act as septal plugs, a phenomenon previously reported for some other *Fusarium* strains (Reichle and Alexander 1965).

In *P. pini*, a granular matrix free from ribosomes and organelles can frequently be observed around the septal plug. This zone is generally surrounded by "vesicles" (microbodies) with crystalloid inclusions. A pore apparatus of this kind was previously described in other rust fungi (Ehrlich *et al.* 1968, Littlefield and Bracker 1971, Coffey *et al.* 1972, Robb *et al.* 1973) and is apparently a unique structure that characterises the order Uredinales.

Atypical septa, comprising partial septa (pseudosepta) and complete septa without pores, have been noticed in some rust fungi, mainly in pseudoparenchyma (Moore 1963, Ehrlich *et al.* 1968, Littlefield and Bracker 1971). They were not found in the *Peridermium* material examined, which entirely comprised typical hyphae.

The ultrastructure of the haustorial apparatus of uredial or thelial mycelia has been investigated in several species of the genera *Puccinia, Melampsora, Uromyces* and *Hemileia* (Bracker and Littlefield 1973, Coffey *et al.* 1972, Ehrlich and Ehrlich 1973, Ehrlich and Ehrlich 1971 a—b, Hardwick *et al.* 1971, Heath 1972, Heath and Heath 1971, Kajiwara 1971, Littlefield and Bracker 1970, 1972, Manocha and Shaw 1967, Mendgen 1973, Rijkenberg 1972, Rijkenberg and Truter 1973, Shaw and Manocha 1965, van Dyke and Hooker 1969, Zimmer 1970). Uredial haustoria are outgrowths from intercellular haustorial mother cells. At the site of future penetration of the host cell wall a characteristic thickening and reorganization of the haustorial mother cell wall is observed. At this place a penetration peg emerges from the fungal cell and grows into the host cell wall. During the subsequent penetration, the host and fungal walls appear to fuse. Inside the host cell the invading part of the parasitic cell elongates and differentiates into a narrow neck region and a voluminous haustorial body. A dark-staining ring occurs in the fungal wall midway along the haustorial neck. The nuclei and the bulk of the cytoplasm of the mother cell migrate into the haustorial body. The haustorium is surrounded by a sheath, the limiting membrane of which is formed by the invaginated plasmalemma of the host cell.

The haustorial apparatus of *P. pini* differs in essential respects from corresponding structural complex of the uredial rust stages. In *P. pini* the haustorium is separated from its mother cell by a perforated and plugged septum of the usual type, located in the neck region. With regard to wall structure and internal organization the haustorial mother cell is indistinguishable from other intercellular hyphal cells, but differs from these in shape. The distal part of this particular cell is narrow and bent to form an almost right angle to the bulk of the cell. This narrow end of the mother cell constitutes the proximal part of the haustorial neck. The neck is surrounded by an electron-lucent halo, probably produced through localized degradation of the host cell wall by fungal enzymes. The longitudinal walls of the hypha are continuous from their intercellular part to the haustorial body and do not fuse with the host cell wall. The host wall develops always a collar at the site of penetration. The haustorial neck, which has no visible wall ring, is short and may be restricted to that part of the fungal cells that remains inside the host cell wall

and the collar. The presence of a septum in the neck of the haustorial apparatus is an interesting feature that also occurs in the powdery mildew fungi (Bracker 1968).

Rijkenberg and Truter (1973) have studied the structural interactions between the pycnial stage of *Puccinia sorghi* and the host plant *Oxalis corniculata*. They reported that hyphal tips invading host cells are not significantly reduced in diameter when passing the host wall. In the lumen of the host cell the hypha frequently becomes septate and may coil extensively around host organelles. The micrographs included in the paper by Rijkenberg and Truter show that the septa do not occupy any fixed positions, and on two of the pictures an elongated fungal cell is partly inside, partly outside the host cell. The intracellular part of the hypha is enveloped by a layer in which a clear-cut distinction between a collar and a sheath hardly can be made. The authors claim that the proliferations formed by the invading hyphae of the pycnial mycelium are quite different from the true haustoria of dikaryont stages and therefore should be designated "intracellular hyphae".

Orcival (1969) has published a brief account for the aecial stage of some *Puccinia* species. Fig. 8 in his paper shows the intracellular distal part of a hyphal cell, the rest of which occupies an extracellular position. The invading part of the fungus has no neck region and might rather represent an "intracellular hypha" in the sense of Rijkenberg and Truter (1973) than a real haustorium. The rest of the figures in Orcival's paper are more or less cross-sectioned parts of intracellular fungal elements and their nature can therefore hardly be determined. This can be exemplified by his Fig. 1 which seemingly shows several segments of an extensively coiled "intracellular hypha" (or of a filamentous haustorium?).

According to the electron microscopic observations reviewed above the uredial and thelial stages of a number of rusts and the hyphae of *P. pini* have sac-like haustoria as feeding organs, although the haustorial apparatus formed by *P. pini* is differently organized than that of the first mentioned rusts. On the other hand, the pycnial (and aecial?) stages of *Puccinia* have as feeding elements filamentous, sometimes multicellular, intracellular hyphae.

The haustorium is normally enclosed by a special, probably liquid, layer, the sheath, which is covered by the invaginated plasmalemma of the host cell. At the place of penetration by a uredial haustorium the host cell wall may sometimes develop a swelling (an apposition), the collar. The host plasmalemma follows the contours of the collar and doubles back on itself at the base of the haustorial neck in the space present between the collar and the haustorial neck wall. In the *Peridermium/Pinus* association a papilla-shaped collar is formed, which is closely pressed against the fungal wall so that no space is left between them into which the host plasmalemma can protrude.

In the infected pine material that is described in this publication almost one third of the haustoria were encased by extensively developed collars. In this case it appears most likely that the host wall appositions were formed in advance of the penetrating fungus (cf. Bracker and Littlefield 1973). A somewhat similar situation has been illustrated as a rare event in a *Uromyces/Phaseolus* association (Hardwick *et al.* 1971). In that case the haustorium was, however, poorly developed and not differentiated into neck and body regions, whereas encased *P. pini* haustoria can be quite large and well developed. The encased *Uromyces* haustorium was not necrotic (Hardwick *et al.* 1971) and apparent necrosis was not found either in walled off haustoria of *P. pini*. In most of the host/rust interactions studied, in which collar formation occurs, this formation takes place after that the haustoria are established in the host cell (Coffey *et al.* 1972, Heath and Heath 1971, Littlefield and Bracker 1972). If deposition of collar material becomes very extensive, the whole haustorium can be encased together with the haustorial sheath and its limiting membrane (i.e. host plasmalemma) as well as some host cytoplasm. In such cases the protoplast of the trapped haustorium fre-

quently becomes necrotic, and the encasement of the fungus by growth of the host wall apparently represents a mechanism of defense. Necrotic haustoria are infrequent in the *Peridermium/Pinus* association studied by me, and those observed have been able to penetrate into the host cell lumen. These haustoria are presumably killed by an incompatibility reaction of irregular occurrence, since the haustorial mother cells, which are protected from direct contact with the host protoplasts, are not affected.

The pine cortex tissues investigated contain a widely spread and vigorous mycelium and most or all parenchyma cells are attacked by haustoria. From this observation it can be concluded, that although the host cells are able to wall off several haustoria and kill a few of those that do penetrate into the cell lumen, these potential ways of defense against the parasite are not very efficient in the pine individual studied. Only the last mentioned mechanism might be a real obstacle for the parasite, and if all or most of the cells of a host plant were able to kill the haustorial cells by producing some fungicidial metabolite the host should be able to stop the growth of the fungus and would then be resistant. Other mechanisms of resistance can be expected to occur in some trees, e.g. hypersensitivity reactions that cause rapid death of host tissues around hyphae. In the pine individual studied I have never observed death of host cells as the result of attack of the parasite.

To obtain a real insight into resistance mechanisms at the ultrastructural level it would be necessary to make a comparative study of several resistant and susceptible pine individuals. Since there are indications that the *Peridermium* infection in nature takes place through needles (cf. Introduction), newly infected needles would probably be the best material to investigate for getting information on resistance mechanisms. The ultrastructure of the normal pine needle has been described previously (Walles *et al.* 1973).

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6 Sammanfattning

Föreliggande arbete redovisar resultat från en elektronmikroskopisk undersökning av törskatesvampen (*Peridermium pini*), en icke värdväxlande rostsvamp, som angriper tall. Försöksmaterialet insamlades i augusti 1973 från en omkring tioårig infekterad tallplanta och utgjordes av en gren, som burit aecidier samma år. Från denna gren togs flera prover från den ansvällda delen och från områden högst 2 cm utanför denna. Proverna innehöll delar av den inre, gröna (klorofyllhaltiga) barken med angränsande floem och kambium jämte den yttersta veddelen.

I den infekterade barkvävnaden växer de rikt förgrenade svamphyferna i det välutvecklade intercellularsystemet samt i väggarna mellan intilliggande värdceller. Hyfer påträffas också i märgstrålarna, varifrån de i begränsad omfattning sprider sig till angränsande delar av floem och xylem (i yttersta årsringen). Hyferna har enkärniga celler, åtskilda av enkla, perforerade tvärväggar (septa). Septalporen är tillsluten av en s.k. Woronin-kropp. Tvärväggarnas byggnad och förekomsten av nämnda kroppar påminner om motsvarande företeelser hos många sporsäcksvampar (askomyceter). En annan likhet med askomyceter är förekomsten hos törskatesvampen (och andra rostsvampar) av en s.k. centriolar plaque på kärnhöljet. Törskatesvampen har dessutom ett strukturellt kännetecken, som torde vara unikt för rostsvamparna, nämligen ett mer eller mindre halvklotformat område på ömse sidor om septalporen, vilket är avgränsat från det övriga cellinnehållet genom ett skikt av små "microbodies" med kristallina inklusioner.

Som sugorgan utbildas haustorier. Haustoriet åstadkommer, åtminstone delvis med hjälp av enzymatisk nedbrytning, ett hål i värdcellens vägg och vidgar sig inne i denna cell till en säcklik kropp, som omges av en skida, vars ytterhölje utgörs av den intryckta cellmembranen. Haustoriemodercellen ligger utanför värdcellen och är avskild från haustoriet genom ett septum, beläget i haustoriets korta halsdel. På det ställe där svampen tränger in i värdcellen bildar dennas vägg en vårtlik ansvällning ("krage"). I närmare en tredjedel av de undersökta fallen reagerar cellväggen med en så kraftig kragbildning, att hela haustoriet blir inneslutet. Enstaka haustorier dör, sedan de trängt in i värdceller. Infekterade värdceller, vilka ibland kan innehålla flera haustorier, har i allmänhet sin cellmembran ytförstorad genom talrika små blåslika inbuktningar, och kloroplasterna är oregelbundna i formen med ett degenererat lamellsystem.

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Abbreviations

C = collar
CP = centriolar plaque
CW = cell wall (of host)
F = fungal cell
G = glycogen
H = haustorium
HMC = haustorial mother cell
HS = haustorial sheath
L = lipid body
MB = microbody
N = nucleus (of fungus)
P = plastid
PF = pit field with plasmodesmata
PL = plasmalemma (of host)
PS = plasmalemmasome
SER = sieve element reticulum
SP = sieve plate
V = vacuole
WB = Woronin body

The length of 1 μm is indicated by a scale line.

Figures

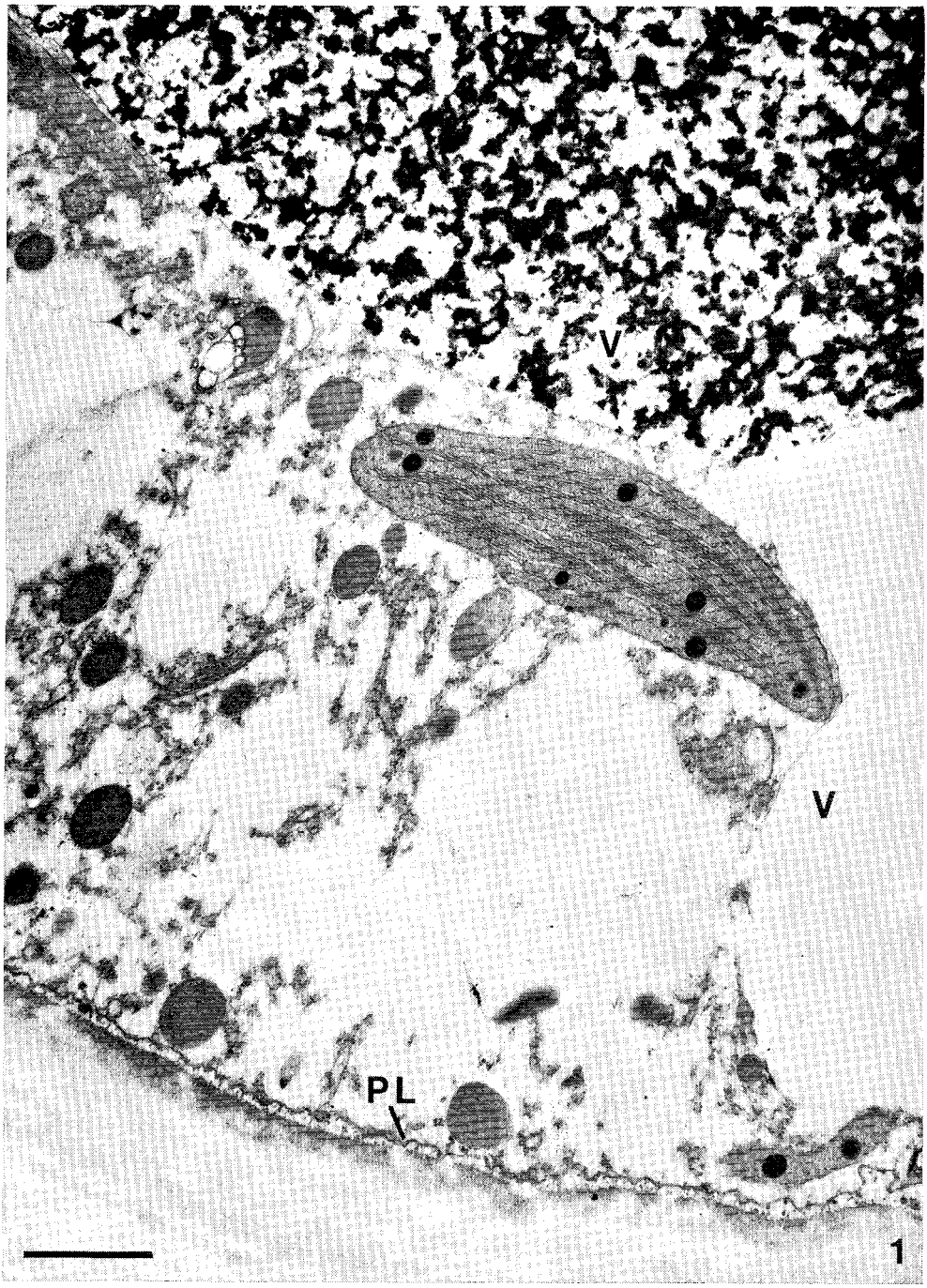


Figure 1 Part of a parenchyma cell from the green cortex of a young twig of a pine tree. Some small vacuoles and part of the central vacuole, which contains aggregated tannins, can be seen. Note the appearance of the chloroplast. Specimen collected in October 1973.

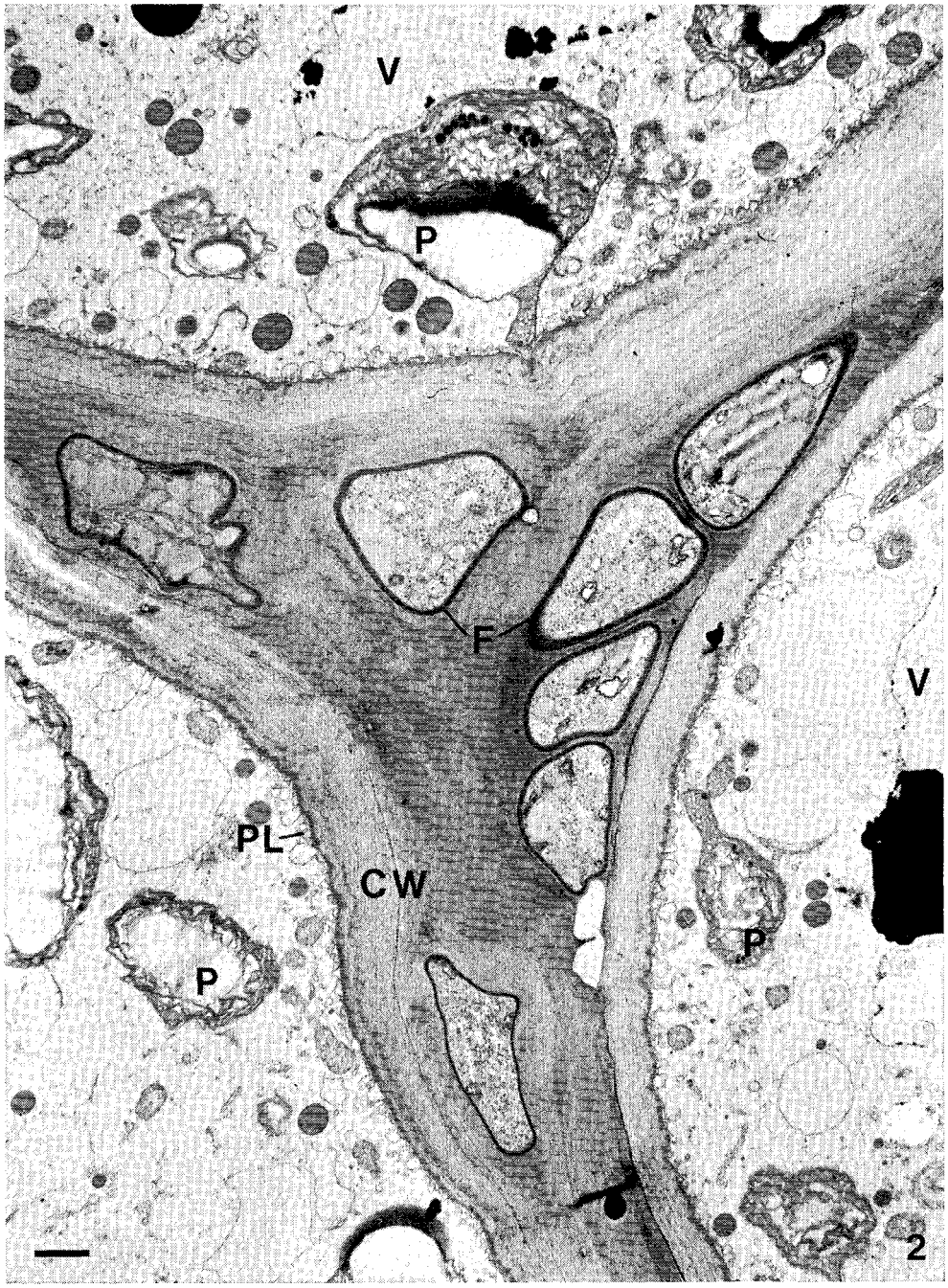
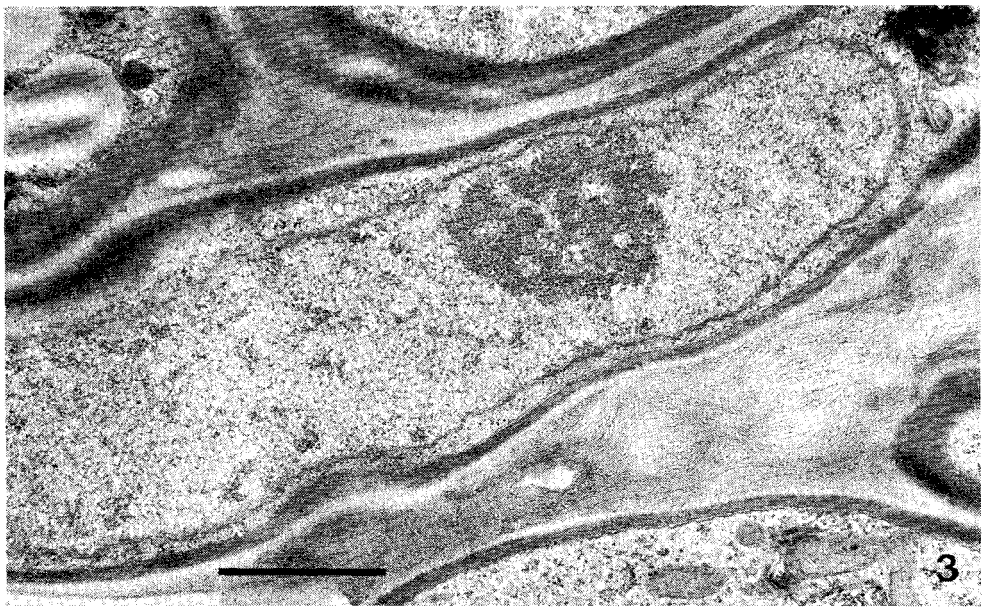


Figure 2 Parts of three adjacent parenchyma cells in the infected cortex tissue of pine. Several profiles of hyphal cells of *Peridermium* appear in the middle part of the cell walls. The surface area of the host plasmalemma is increased by numerous minute buds. The chloroplasts are of an amoeboid shape and have deranged lamellar systems and prominent starch grains.



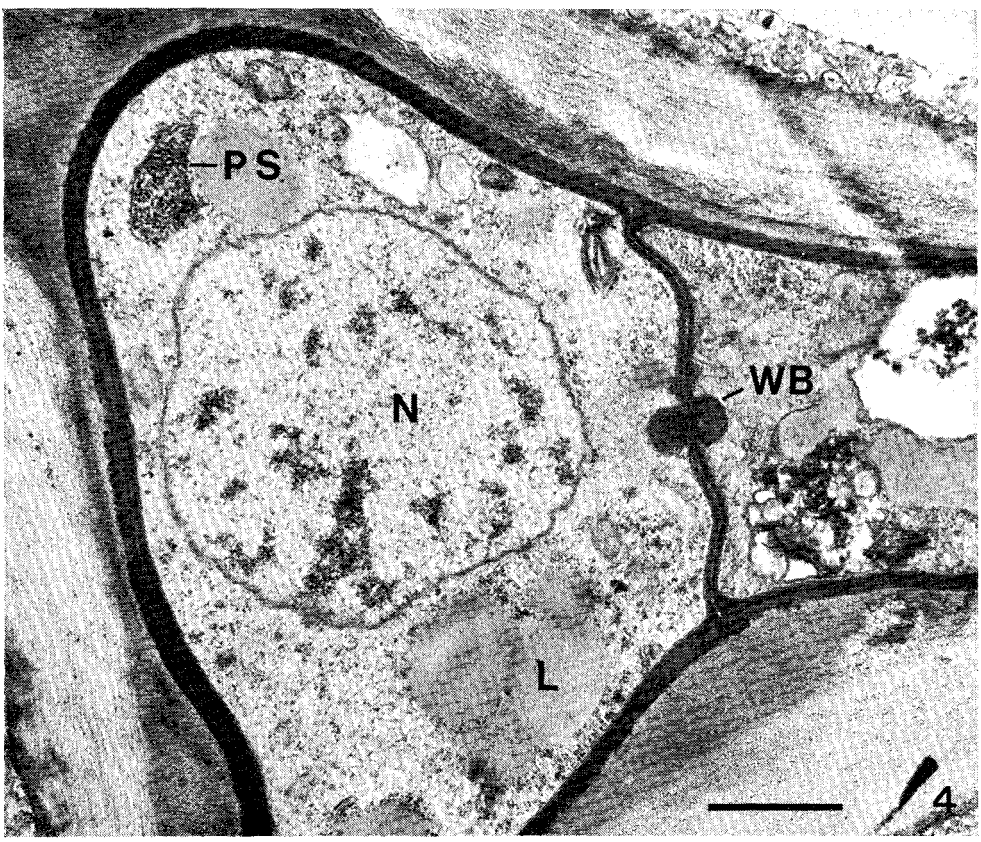


Figure 3 Part of a narrow hyphal cell with a tube-shaped nucleus.

Figure 4 Parts of two contiguous hyphal cells inside host cell walls. Note the perforated septum, plugged by a Woronin body, and the septal apparatus on each side of the septum.

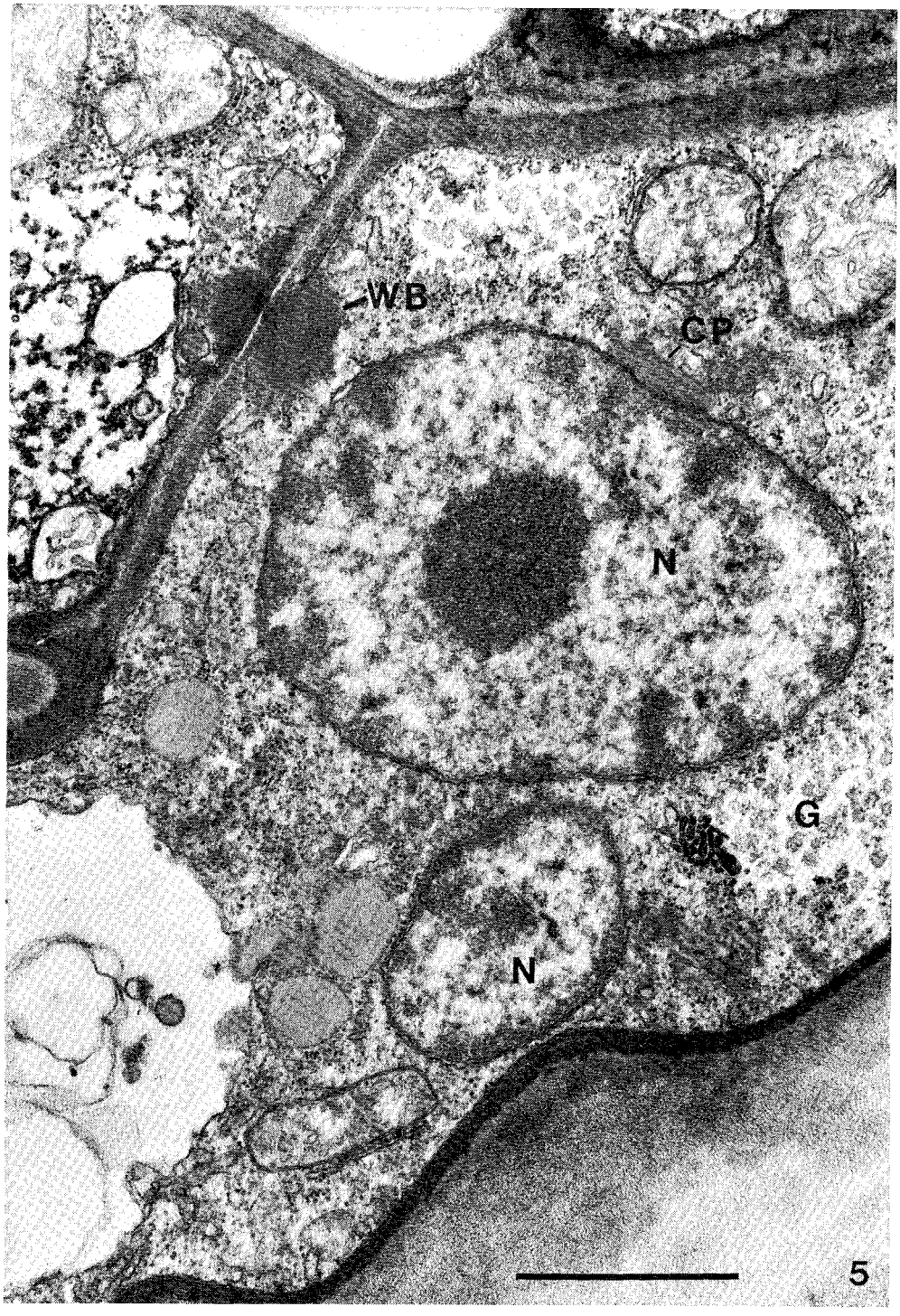
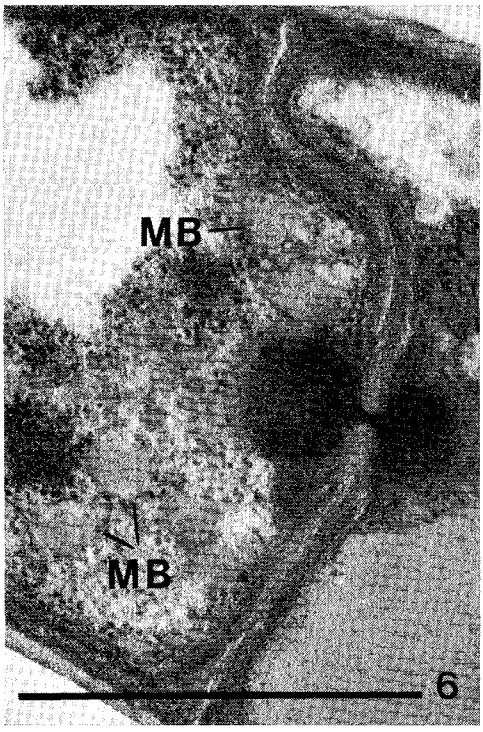
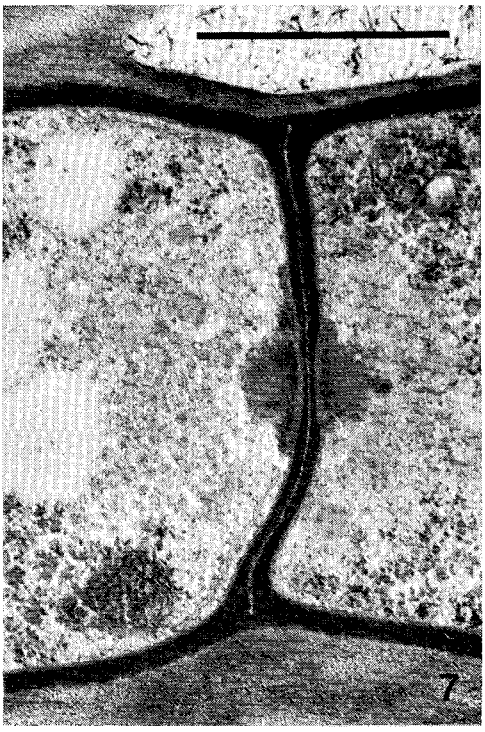


Figure 5 A hyphal cell with two nuclear profiles, presumably not connected. A fibrillar centriolar plaque is attached to the envelope of one of these profiles.





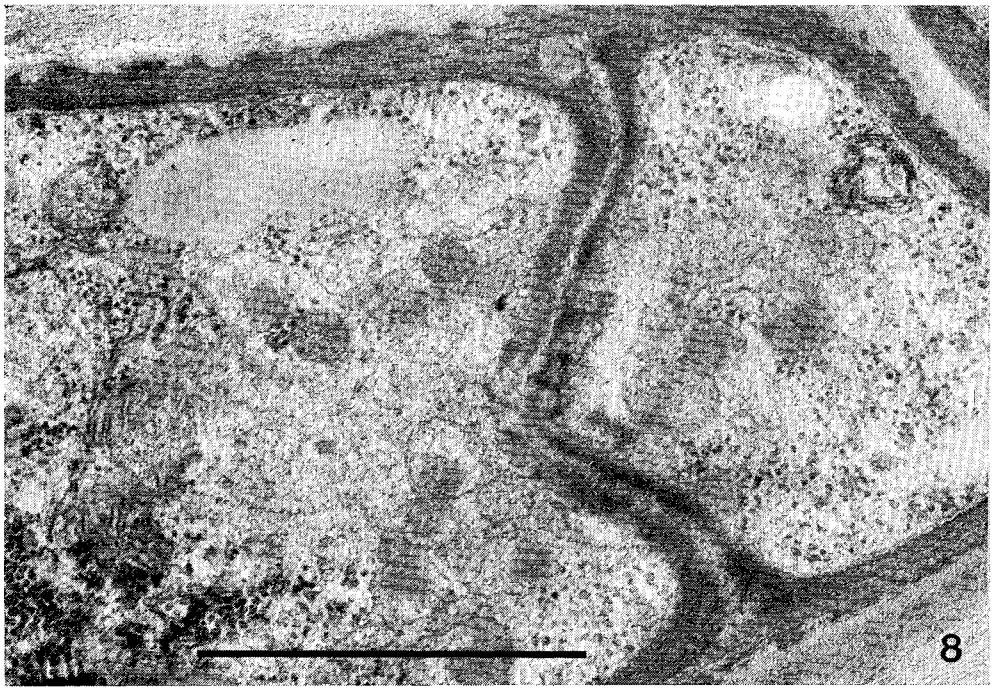


Figure 6 A section through the central part of a septal pore. Microbodies with crystalline inclusions surround the septal apparatus.
Figure 7 A septal apparatus, bordered by microbodies, is seen on both sides of the septum, the central pore of which is situated outside the section.
Figure 8 A peripheral part of a septum, external of the Woronin body. Numerous microbodies with inclusions are present at both sides of the septum.

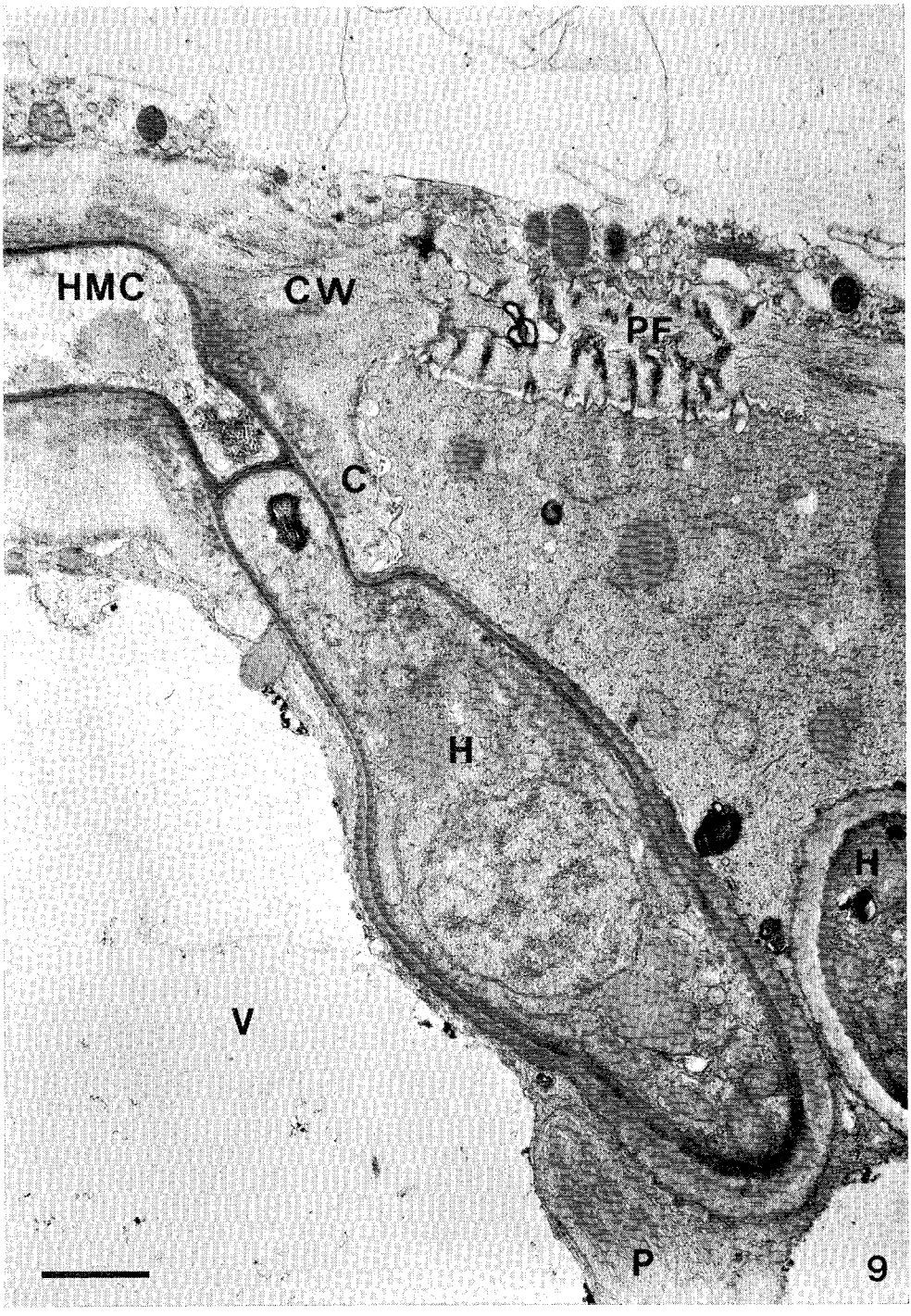
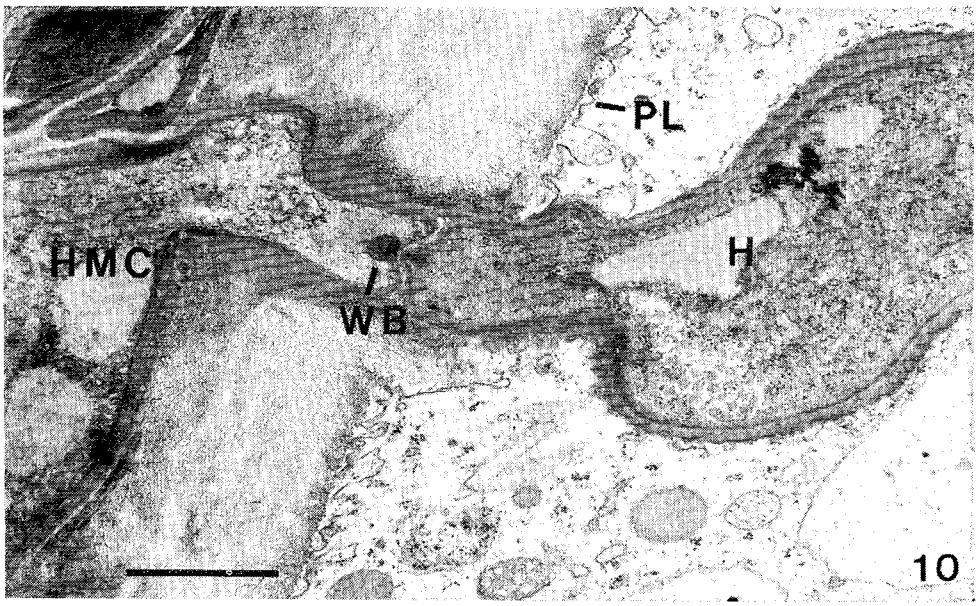


Figure 9 Haustoria of *Peridermium* inside a host parenchyma cell. Note the papilla-shaped collar and the zone of lysis in the host cell wall around the haustorial neck. The haustoria are rich in mitochondria.



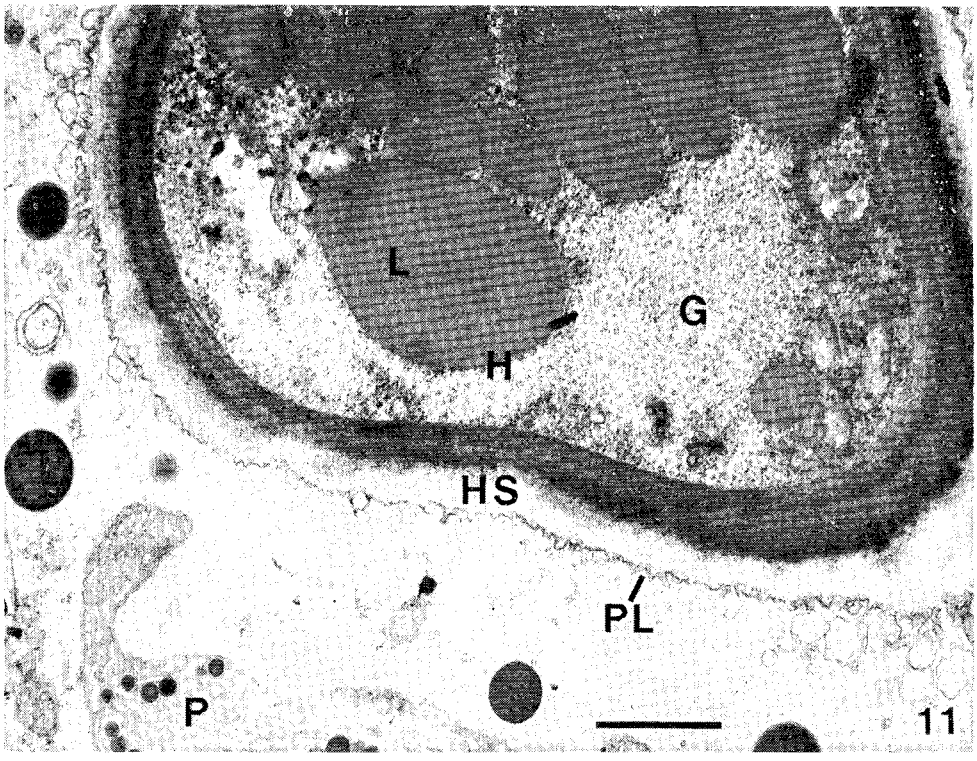
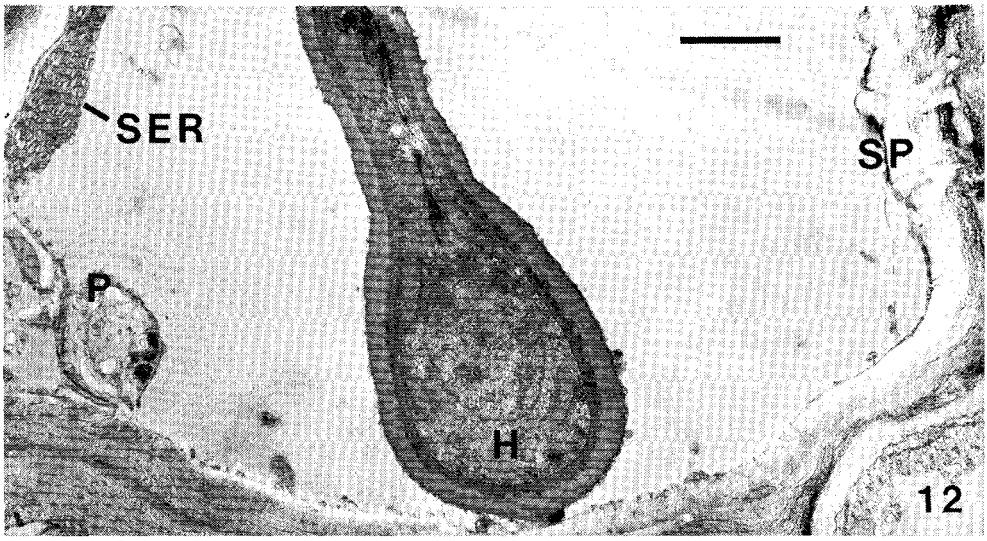


Figure 10 The haustorial neck region with the perforated, plugged septum separating the haustorium from its mother cell.

Figure 11 The distal part of a haustorium, showing the dissociated surface of its wall and the electron-lucent sheath, bordered by invaginated host plasmalemma with numerous inbuddings.



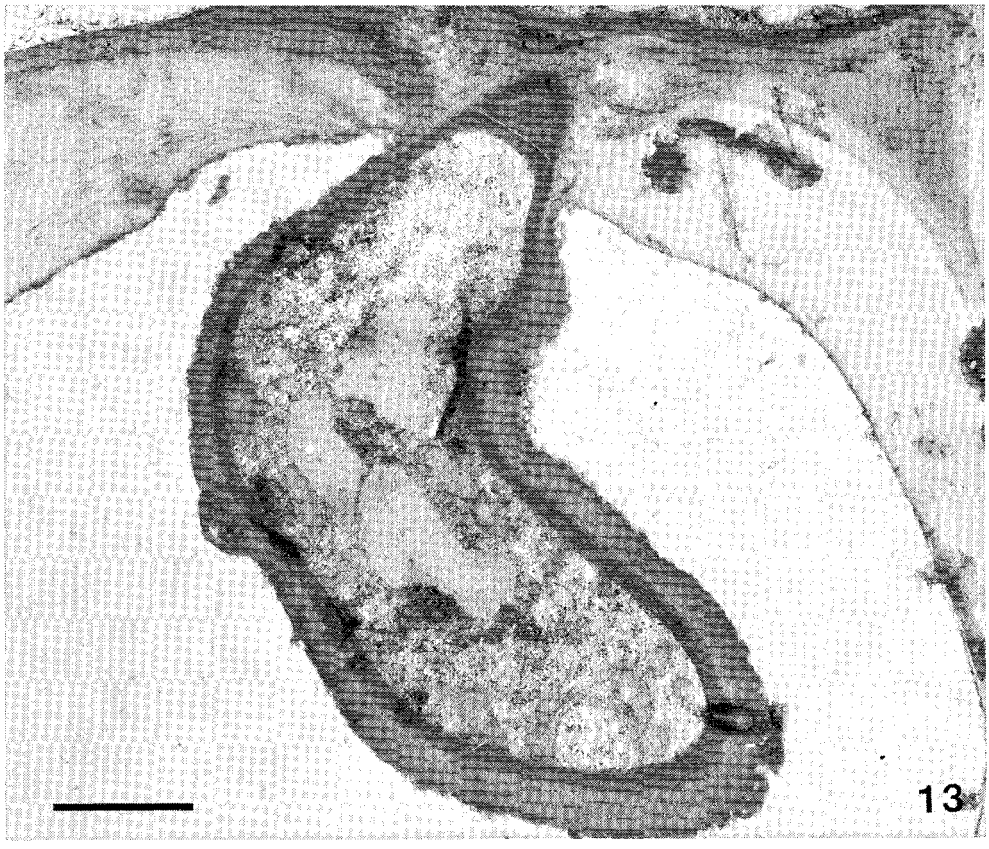
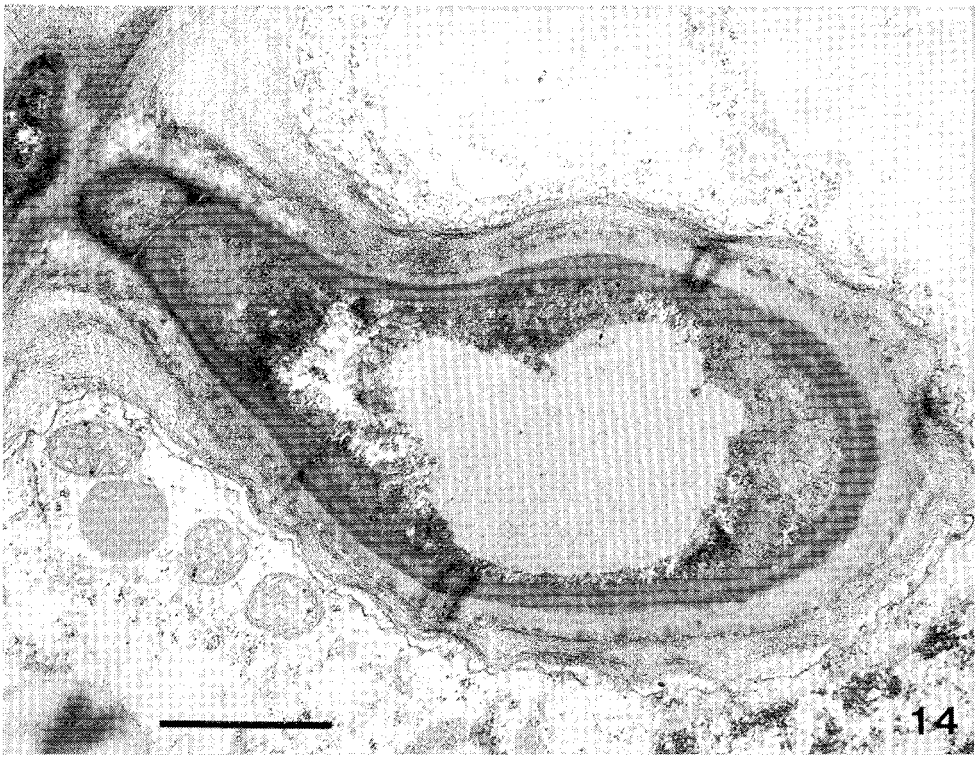
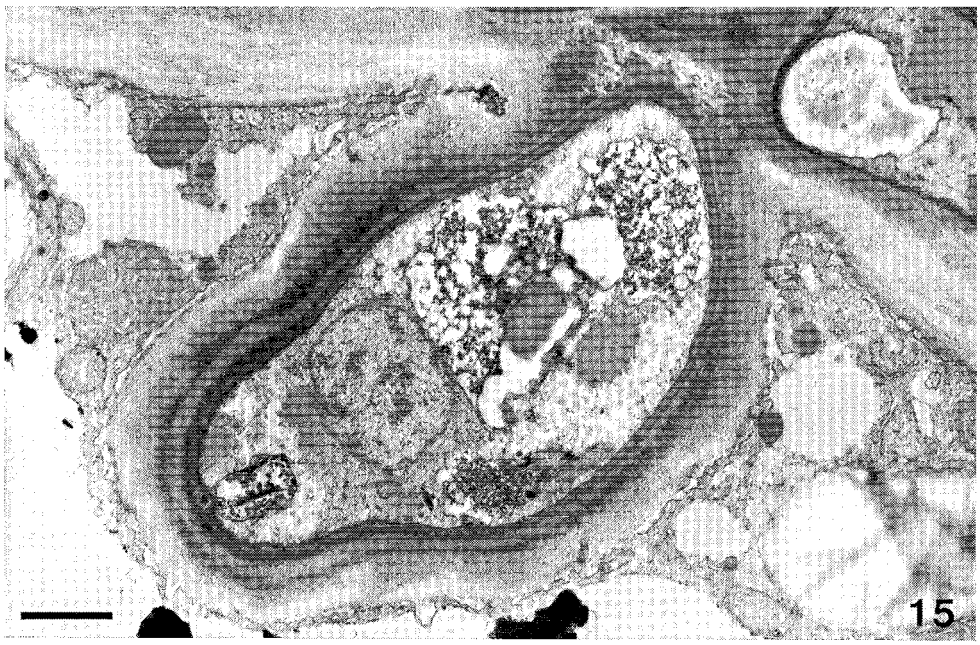


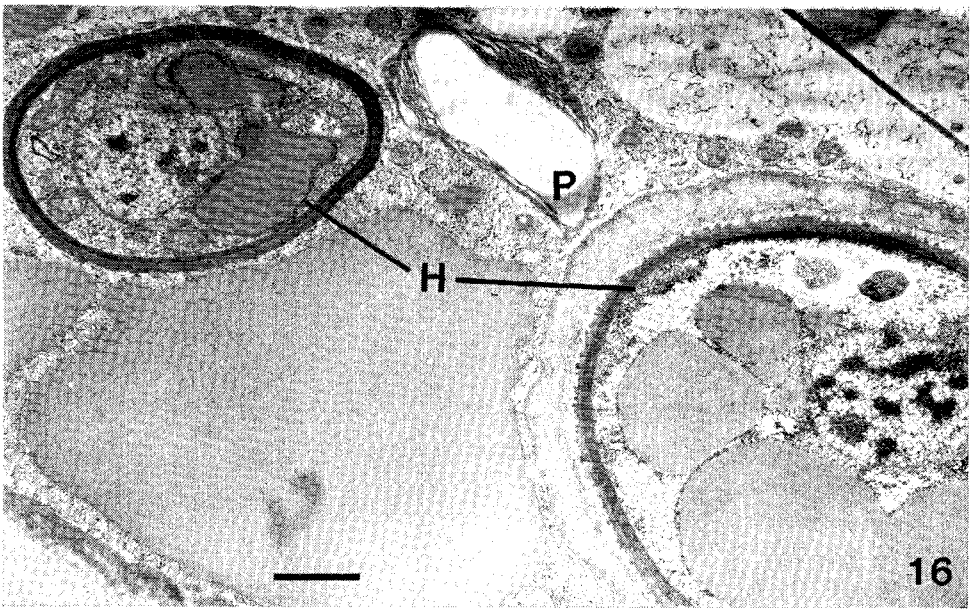
Figure 12 A haustorium inside a sieve cell. The host cell is characterized by a sieve plate, covered with callose, part of a sieve element reticulum and a special type of plastid with starch grains and electron dense inclusions (probably protein bodies).

Figure 13 A haustorium inside a tracheid.





Figures 14—15 Encapsulated haustoria. The haustorial neck region can be identified by the halo of lysis.



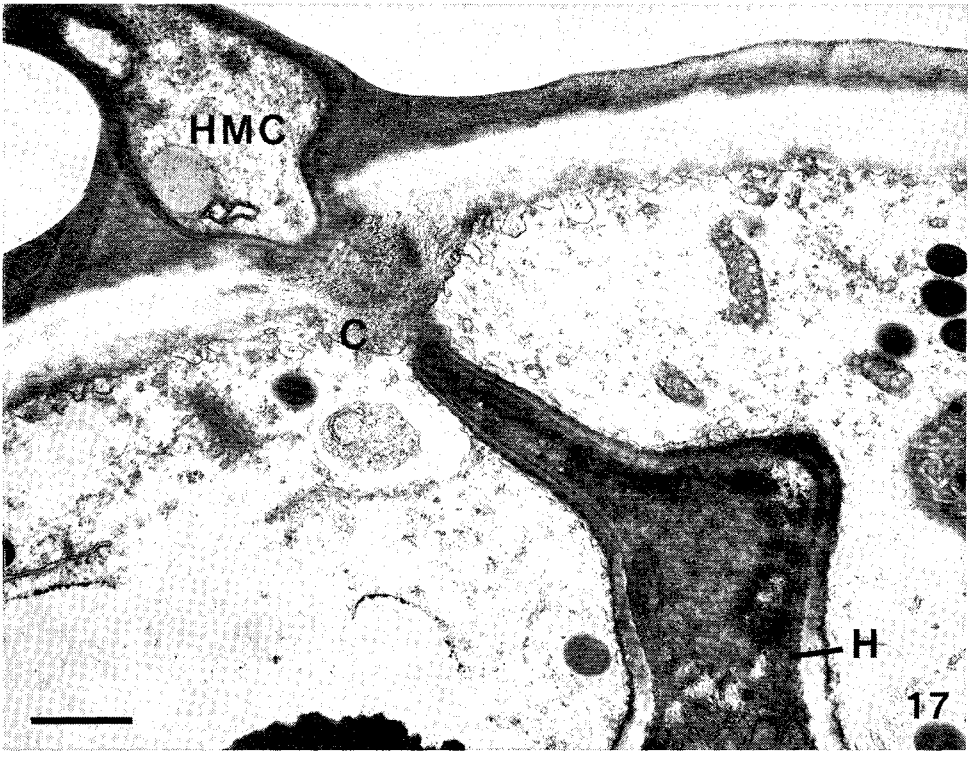


Figure 16 Two cross-sectioned haustoria inside a host parenchyma cell. One of the haustoria is encapsulated.

Figure 17 The proximal part of a necrotic haustorium, the mother cell of which appears healthy.

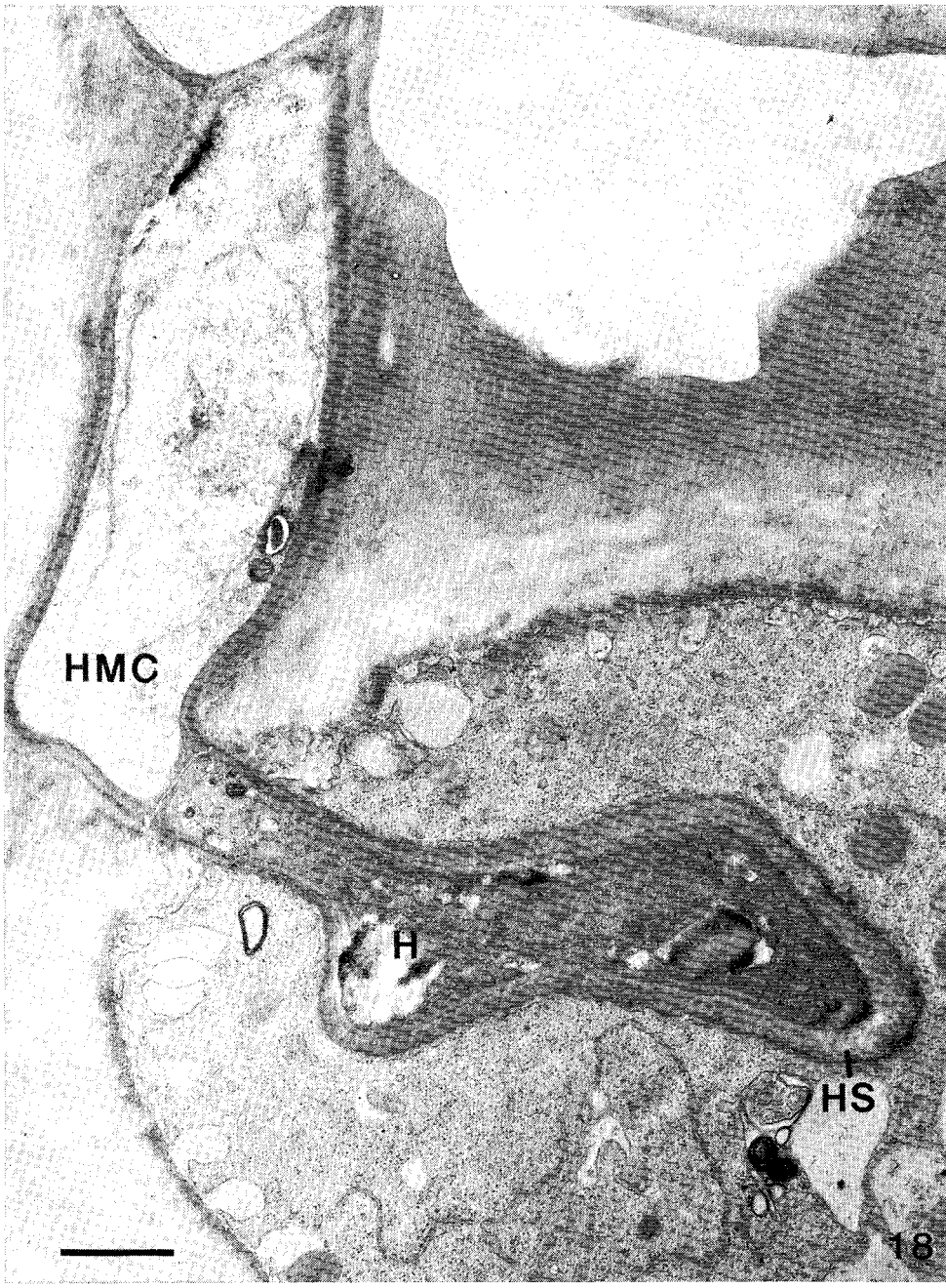


Figure 18 A necrotic haustorium with its apparently healthy mother cell. Note the surface extension of the plasmalemma.