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Ultrastructural studies of resistance mechanisms in *Pinus sylvestris* L. against *Melampsora pinitorqua* (Braun) Rostr. (pine twisting rust)

Finstrukturella undersökningar av resistensmekanismer mot knäckesjuka hos tall

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Abstract

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Seedlings of Pinus sylvestris *infected by* Melampsora pinitorqua *have been studied with regard to the ultrastructure of host and parasite. Septated and branched intercellular hyphae of the pycnial stage occurred mostly in the cortex but also in phloem and xylem. The haustoria, separated from their mother cells by septa, were elongated and sometimes coiled. In a few cases a papilla-shaped collar was observed at the penetration site. Cisternae of endoplasmic reticulum and organelles of the host cell were frequently observed accumulated around the haustoria.*

The haustorial sheath bordered by the invaginated host plasmalemma varied in thickness and structure. In young haustoria it was thin and filled with homogeneous material of moderate electron density. The sheath membrane was smooth. Those haustorial sheaths that probably represented later stages were thick and contained a lot of electron-dense grains. The associated sheath membranes were convoluted. Some haustoria were trapped in encasements, possibly originating from deposition of macromolecules in the sheath. These encasements could attain a considerable thickness and sometimes several zones of varying appearance were discernible. The encased haustoria were often more or less degenerated. The high frequency of necrotic host cells observed might indicate another type of defence reaction. Sometimes they contained dead haustoria.

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Contents

1 Introduction

The heteroecious rust fungus *Melampsora pinitorqua* develops pycnial and aecial stages on pine, and uredial and telial stages on aspen. In pine the fungus causes the disease known as pine twisting rust. The terminal shoots of young infected plants are quite severely damaged, which may result in the formation of several stems.

The literature on the pine twisting rust has been reviewed by Klingström (1963 and 1969) and Kurkela (1973). An electron microscopical study on the pycnio-aecial stage of *Melampsora pinitorqua* in *Pinus pinea* has been published by Longo and Naldini Longo (1975) and the ultrastructure of the uredial-telial stage in *Populus tremula* has been described by Longo and Naldini (1972).

The susceptibility to *Melampsora pinitorqua* varies between different clones and progenies of pine (Bergman 1954, Eklundh-Ehrenberg 1963, Gavris 1939, Klingström 1963 and 1969, Rennerfelt 1954). To investigate whether the susceptibility to the fungus in individual families is influenced by the genetical constitution of parental clones, extensive resistance tests on pine have been performed at the College of Forestry, Section of Botany at Umeå (cf. Martinsson 1975).

The present investigation has been carried out on material originating from those tests. The purpose has been to study the fine structure of the fungus and the host cells in infected pine seedlings in order to investigate whether resistance mechanisms are detectable by electron microscopy. This report concerns ultrastructural features which may represent such resistance mechanisms.

2 Material and methods

The material investigated was sampled from an experimental series of progeny tests for resistance to pine twisting rust, *Melampsora pinitorqua* (Braun) Rostr. Seedlings of *Pinus sylvestris* cultivated in a greenhouse were inoculated with *M. pinitorqua* at an age of 3 months. Aspen leaves with telia were placed on a wire-netting over the pine seedlings and air humidity was kept at a high level during the following day. The teliospores germinated forming basidia that released basidiospores. Four weeks later the susceptibility to the fungus was classified by a 0—5 range for each seedling and material could subsequently be selected for ultrastructural investigation.

Samples were collected from aecia-carrying segments of stems as well as from apparently unaffected shoot tissue, and were fixed in a mixture of formaldehyde and glutaraldehyde (Karnovsky 1965). They were submitted to vacuum treatment to remove air from intercellular spaces and stored in the fixative for several weeks at about +5°C. Material selected for investigation was postfixed in 1 % OsO_4 in 0.05 M phosphate buffer at +5°C for 2 hours. After dehydration in an acetone series the specimens were embedded in Spurr's epoxy medium (Spurr 1969). Sections were cut with a diamond knife on a LKB Ultrotome III, stained with 1 % uranyl acetate and 0.2 % lead citrate (Venable and Coggeshall 1965) and examined with a Hitachi HS-7S electron microscope.

3 Results

3.1 The intercellular mycelium

Tissue samples taken from different sites along the stem of infected plants showed that mycelial growth was relatively limited in space. The fungus grew in the form of intercellular hyphae, mainly in parenchymatic tissues and to some extent in phloem and xylem (Figs. 1 and 2). The hyphae were often attached to each other and to host cell walls by a material of moderate electron density (Fig. 3). Mostly the hyphae had distinct electron-dense walls, but in a few micrographs fungal profiles with electron-transparent walls were observed (Fig. 2).

The hyphae were septated and branched (Figs. 1, 2, 3 and 4). The septum consisted of two electron-dense layers separated by a thin electron-transparent middle lamella (Fig. 4). It possessed a narrow septal pore, which was occluded by a Woronin body. Several small spherical bodies of the same electron density as the Woronin body sometimes occurred in the neighbourhood of the pore (Fig. 4). Microbodies with crystalline inclusions were also present in the septal region. In addition, the fungal cell contained endoplasmic reticulum, mitochondria with numerous sheet-like cristae, dictyosomes, lomasomes and vesicles (Figs. 1, 4 and 5). Reserve material in the form of lipid droplets and glycogen granules was observed in most cells (Figs. 1 and 3). A single nucleus per cell showed that the hyphae belonged to the pycnial stage.

3.2 Intracellular fungal organs and host cell structure

The intercellular hyphae frequently penetrated the host cell wall and developed haustorial organs. Such haustoria contained the same structures and ergastic material as the intercellular hyphal cells (Figs. 6, 8, 9, 10 and 11). They were observed in parenchyma cells, sieve cells and tracheids.

At the site of penetration the haustorial mother cell narrowed to a short neck (Figs. 6 and 7). Inside the host cell the haustorium expanded, at least to the width of an intercellular hypha. A weakly developed collar containing material, similar in ultrastructural appearance to the host cell wall, was formed around the neck region (Fig. 6). Occasionally, the collar was quite prominent (Figs. 1 and 7). The haustorium was separated from its mother cell by a septum occurring at varying sites in the neck region, from the level of the host cell wall to the expanded part of the haustorium (Fig. 6). The structure of the septum was essentially the same as that of corresponding walls in intercellular hyphae. When the haustorium elongated, it occasionally became coiled and branched. The haustorial profiles were often observed near host organelles and in some cases a haustorium was even found in an invagination of the nucleus.

The haustorium was surrounded by the invaginated host plasmalemma constituting the sheath membrane (Figs. 8, 9 and 10). Cisternae of endoplasmic reticulum accumulated around the haustorium (Figs. 8 and 9). The surface of the rough endoplasmic reticulum that faced the sheath membrane was devoid of ribosomes (Fig. 8). The haustorial sheath, i.e. the space between the electron dense haustorial wall and the sheath membrane, varied in width and fine structure. In some cases the sheath membrane appeared smooth and the extrahaustorial space was almost filled with a

homogeneous material of moderate electron density (Fig. 8). In other cases the sheath membrane was convoluted and showed signs of exocytosis (Fig. 9). Electron-dense grains were accumulated in the extrahaustorial space (Figs. 9 and 10). At another stage such grains were discernible near the haustorial wall and electron-dense material coated the inside of the sheath membrane (Fig. 10).

Deposited material sometimes attained the characteristic structure of reaction material (cf. page 10): electron-dense granules of irregular shape embedded in a matrix of moderate electron density (Fig. 11). At later stages this deposit increased in thickness forming an encasement (Figs. 12 and 14), and a similar material was also accumulated on the host cell wall around the site of penetration. The peripheral region of this encasement appeared relatively homogeneous and electron-dense (Figs. 11 and 12). A narrow electron-transparent zone might envelop the fungal wall (Figs. 12 and 14). Finally, a layer of fibrillar material was deposited around the encasement and along a considerable part of the host cell wall (Figs. 13 and 14). In invaded, relatively unaffected host cells irregular granules of electron-dense material were frequently observed between the entire plasmalemma and the wall of the host cell (Figs. 6, 13 and 14).

Host cells could vary considerably as regards the amount of reaction material formed. Some of them possessed enormous deposits, whereas the adjacent invaded cells had no such material at all. When several profiles of haustoria occurred in a particular host cell, some of them might be encased, others not (Figs. 15 and 16). Figure 16 shows a haustorium enclosed by deposited host material which strikingly varies in thickness along the fungal cell. Sometimes the encased haustoria were necrotic with a dense structureless cytoplasm and large coalescing lipid bodies (Figs. 12, 14 and 16).

Host cells were frequently degenerated to varying degrees. In some cases the plasmalemma was convoluted. Cisternae of endoplasmic reticulum occurring in close proximity to the haustorial sheath were sometimes dilated. Alterations in the structure of chloroplasts as disorganizing of thylakoids were observed (Figs. 15 and 16). Advanced stages of degeneration were characterized by a destruction of host cell membranes, e.g. the tonoplast and finally also the plasmalemma. The disorganized cytoplasm and the vacuolar contents rich in tannins were mixed and normal structural elements were no longer discernible (Figs. 1, 11, 15 and 16). In some of these host cells haustoria were observed, several of which were associated with deposits of host material.

4 Discussion

Pycnial stage haustoria formed by *Melampsora pinitorqua* differ from the typical haustoria of the uredial stage of rusts by being separated from their mother cells by septa and having relatively wide necks without neckbands. The shape of these haustoria is filamentous, occasionally branched or coiled (cf. Longo and Naldini 1972, Longo and Naldini Longo 1975). The monokaryotic haustorium of *Peridermium pini* is also a separate cell and has no neckband (Walles 1974).

In accordance with Bracker's definitions (1968) the terms "sheath membrane" and "haustorial sheath" in the present paper refer respectively to the invaginated host plasmalemma and the extrahaustorial space separating it from the haustorial wall. In host cells invaded by *Melampsora pinitorqua* invaginations were developed in the sheath membrane (cf. Longo and Naldini Longo 1975) and were also observed at an early stage of haustorial formation by *Uromyces phaseoli* (Heath and Heath 1971). In pine cells infected by *Peridermium pini* the non-invaginated part of the plasmalemma may have its surface increased by proliferations which are absent in unaffected cells (Walles 1974). The non-invaginated plasmalemma in our material was convoluted, but not to the same extent as in *Peridermium* infections.

The reason for the host plasmalemma becoming convoluted has not been established. The surface of the invaginated host protoplast increases, with a subsequent need for new plasmalemma. Deposition of new membrane material can be assumed to occur by exocytosis. It is also possible that the haustorium induces the host cell to produce reaction material (cf. page 10), deposited by exocytosis. In both cases, this mechanism would give the plasmalemma the convoluted appearance. Another explanation might be an increase in surface area over which transport of substances could take place.

The main topic to be discussed in this paper is the occurrence of different resistance mechanisms in pine against *Melampsora* infections. These mechanisms act mostly after the fungus has penetrated into the host. Fungal growth may be inhibited by hypersensitivity reactions, where the host tissues around the hyphae die, or where the rapid death of the host cell causes the death of the haustoria. Another type of defence reaction is an incompatibility between host cell and haustorium, whereby the host cell survives. Finally, the haustorium may become encased in reaction material (cf. page 10).

Local reactions in host tissues resulting in rapid necrosis of cells adjacent to the site of infection, so-called hypersensitive reactions, are probably the most important cause of resistance to pathogenic fungi. The death of the host cells leads to the death of the fungus, probably because these cells no longer provide the specific metabolites required for fungal growth. Host cells adjacent to haustoria-invaded cells often became necrotic in incompatible interactions between *Puccinia sorghi* and *Zea mays*, as studied by Van Dyke and Hooker (1969). Various degrees of degeneration occurred in cells adjacent to *Erysiphe*-invaded cells in barley (Edwards 1975). Degenerative changes of host cells resembling those of a hypersensitive reaction could be induced by filtrates from cultures of the anthracnose fungus of French bean (Mercer et al. 1974). We have frequently observed necrotic cells in pine infected by *Melampsora*, but it was difficult to trace the causal factors involved.

Our observations of necrotic host cells containing dead haustoria indicate that the death of the host cell may cause the death of the haustorium. It is hardly possible to decide whether the haustorium entered the host cell before the death of the cell, but it seems unlikely that a haustorium would invade a dead cell. In a resistant variety of wheat, the subcellular structures of invaded cells were more rapidly broken down, and many of the haustoria formed there became necrotic, in contrast to haustoria observed in a susceptible variety (Shaw and Manocha 1965). This type of reaction plays an important role in host defence only if the death of the haustorium also affects the haustorial mother cell and the rest of the hypha. The fungal growth then ceases, probably due to starvation (Heath 1971 and 1972). In our investigation, some instances of haustoria remaining relatively unaffected by the necrosis of the host cell were also observed (cf. Mendgen 1975, Robb et al. 1975).

The kind of reaction in which the haustorium is killed and the host cell survives has not been observed in our material. In pine cells invaded by *Peridermium pini* a small fraction of the haustoria investigated were necrotic, probably due to an incompatibility reaction with the host protoplast, while the host cells were apparently healthy (Walles 1974).

One of the responses of host cells to the penetration of fungal hyphae is the formation of "reaction material". According to Mercer et al. (1974) this term is used for aggregates of material located between the plasmalemma and the wall of the host cell around the point of penetration and between the plasmalemma and the intracellular fungal structures. When deposited around the haustorial neck at the site of penetration, the material forms a papilla-like structure known as a collar (cf. Abu-Zinada et al. 1975, Coffey et al. 1972, Rijkenberg and Truter 1973, Robb et al. 1975, Stavely et al. 1969, Walles 1974). The pine cell invaded by a haustorium of *Melampsora pinitorqua* produces a fibrillar, wall-like collar around the site of penetration. In aspen infected by this fungus the host cell deposits a collar which is more or less electron-transparent with tubular inclusions (Holmvall, unpublished).

Material may also be deposited between the sheath membrane and the haustorium (cf. Coffey et al. 1972). In our investigation the reaction material seldom has the appearance of a typical papilla-shaped collar. Predominantly entire haustoria are encased. The reaction material consists of an amorphous matrix of moderate electron density containing electron-dense granules or tubular structures. In cowpea rust infections the material in encasements surrounding haustoria was found to consist mainly of callose (Heath 1971). Other authors report relatively electron-transparent reaction materials, often with electron-dense inclusions (Heath 1972 and 1974, Heath and Heath 1971, Mercer et al. 1974 and 1975). Zones of different appearance can often be distinguished (cf. Heath and Heath 1971). The peripheral layer of the reaction material may have a fibrillar character. Similar observations were made by Coffey et al. (1972), and by Mercer et al. (1974 and 1975).

The deposition of the collar material obviously starts at the site of penetration and it may keep pace with the growth of the penetrating fungus preventing it from reaching the host protoplast (Walles 1974). There are two possible ways for the reaction material in the sheath to be deposited. Either it is formed like a collar, successively spreading from the site of penetration towards the tip of the haustorium, or it is formed simultaneously around the whole haustorium. In Figure 15 in this paper the host cell contains several haustorial profiles, but only some of them are encased. This may represent a successive encasement of the same coiled haustorium, but another explanation may be that there are separate haustoria affected to varying degrees by encasement in the host cell.

Haustorial sheaths varying in thickness and structural appearance might represent different developmental stages of simultaneous deposition of material around the

haustorium. Similar variations in haustorial sheaths have been reported by other authors (Longo and Naldini 1972, Longo and Naldini Longo 1975), in some cases probably related to the age of the haustoria (Coffey et al. 1972, Ehrlich and Ehrlich 1971). In this connection it may be mentioned that granular sheaths developed faster in a resistant than in a susceptible variety of wheat (Shaw and Manocha 1965). The only case observed, where the thickness of the encasement varies along the haustorium is seen in Figure 16. This may be interpreted as implying that the encasement has not been completed, or that the haustorium grows through the reaction material. In French beans infected by *Colletotrichum lindemuthianum* virulent hyphae could usually grow through the reaction material while the avirulent hyphae could not (Mercer et al. 1974).

The reaction material encasing whole haustoria seems often to be a cause of their death. Several haustoria encased by reaction material as well as corresponding haustorial mother cells showed signs of necrosis in *Vicia faba* infected by *Uromyces fabae* (Abu-Zinada et al. 1975). Haustoria that were completely encased and necrotic occurred in approximately 5 per cent of invaded host cells of rust-infected flax (Littlefield and Bracker 1972). However, a high frequency of encased haustoria with intact cell structures have been reported by Walles (1974) in pine cells attacked by *Peridermium pini*.

The significance of this defence mechanism for the achievement of resistance probably depends more on the rate of encasement than on the presence or absence of it. We have found thick encasements also in the most susceptible family, which might be explained by the high age of the infection, provided that the deposition of reaction material increases with time. A callose-containing material was commonly observed around the site of penetration in immune cowpea cultivars but it was absent at early stages of infection in the susceptible cultivars (Heath 1971, Heath and Heath 1971). Moreover, encasement of haustoria did not result in their immediate death, nor that of the rest of infection hypha.

Several comparative ultrastructural studies have been carried out on plant material differing in susceptibility to pathogens (Heath 1972 and 1974, Heath and Heath 1971, Shaw and Manocha 1965, Stavely et al. 1969, Van Dyke and Hooker 1969). The results of these studies seem to indicate that different types of defence response are non-specific, occurring in both resistant and susceptible materials. The resistance mechanisms can occur at the cell level but may not always be efficient enough to reject the pathogen, with the result that the host plant will be more or less infected. The different defence reactions probably start at different stages of infection (cf. Heath 1974), occur simultaneously and differ in expression depending on different host-pathogen combinations.

This investigation shows the occurrence of ultrastructural changes reflecting defence reactions of the host cell. However, electron microscopical studies of variation in host response between resistant and susceptible materials require that the differences in susceptibility are qualitative. As regards *Pinus sylvestris* no plant material classified as being absolutely resistant to *Melampsora pinitorqua* is available. Only material varying in susceptibility to the pathogen is known.

5 Acknowledgements

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6 Sammanfattning

Strukturen av värd- och parasitceller hos tallplantor infekterade av knäckesjukesvampen (*Melampsora pinitorqua*) har undersökts elektronmikroskopiskt. Denna svamp är en värdväxlande rostsvamp, vars pyknidie- och aecidiestadier förekommer hos tall. Föreliggande undersökning behandlar pyknidiestadiet.

Svampen bildade septerade och förgrenade intercellulära hyfer i värdplantans cortex (parenkymatiska barkregion) men förekom också i dennas floem och xylem. I hyfspetsarna bildades avlånga och ibland skruvformade haustorier, dvs. speciella celler vilka inträngt i värdceller och torde representera näringsupptagande organ. På den plats där haustoriet vuxit genom värdcellens vägg iakttogs ibland en vårtlik "krage" runt haustoriets proximala del.

Haustorierna omgavs ofta av strukturelement tillhörande värdcellen, särskilt segment av det endoplasmatiska nätverket samt av olika organeller, inklusive cellkärnan. Haustoriet omslöts av en skida, vars ytterhölje ("skidhöljet") utgjordes av värdcellens inbuktade cellmembran. Haustorieskidan varierade i tjocklek och struktur. Hos vissa haustorier var den tunn och innehållet var måttligt elektrongenomsläppligt efter tungmetallfärgning. Skidhöljet var slätt. Andra haustorieskidor, sannolikt tillhörande senare utvecklingsstadier, var i stället tjocka och innehöll en mängd elektrontäta partiklar. Deras höljen var ytförstorade genom veckning. Vissa haustorier omgavs av s.k. inkapslingar vilka tycktes ha uppstått genom utfällning av makromolekyler i skidan. Dessa inkapslingar kunde uppnå en avsevärd tjocklek och ibland kunde man urskilja en uppdelning av det utfällda materialet i olika zoner av varierande elektrontäthet. De inkapslade haustorierna var ofta mer eller mindre degenererade. I värdvävnaderna iakttogs en hög frekvens av nekrotiska celler vilka ibland innehöll döda haustorier. Detta tyder på ytterligare en typ av försvarsmekanism, där värdcellernas död orsakar att haustorierna går under.

7 References

Abu-Zinada, A.-A. H., Cobb, A. and Boulter, D. 1975. An electron-microscopic study of the effects of parasite interaction between *Vicia faba* L. and *Uromyces fabae*. — Physiol. Plant Path. 5, 113—118.

Bergman, F. 1954. Om skogsträdens sjukdomsresistens och dess utnyttjande inom skogsträdsförädlingen. — Fören. för växtförädling av skogsträd. Årsberättelse 1953, 70—98.

Bracker, C. E. 1968. Ultrastructure of the haustorial apparatus of *Erysiphe graminis* and its relationship to the epidermal cell of barley. — Phytopathology 58, 12—30.

Coffey, M. D., Palevitz, B. A. and Allen, P. J. 1972. The fine structure of two rust fungi, *Puccinia helianthi* and *Melampsora lini*. — Can. J. Bot. 50, 231—240.

Edwards, H. H. 1975. The ultrastructure of M1-*a*-mediated resistance to powdery mildew infection in barley. — Can. J. Bot. 53, 2589—2597.

Ehrlich, M. A. and Ehrlich, H. G. 1971. Fine structure of the host-parasite interfaces in mycoparasitism. — Ann. Rev. Phytopathol. 9, 155—184.

Eklundh-Ehrenberg, C. 1963. Genetic variation in progeny tests of Scots Pine (*Pinus silvestris* L.). — Stud. For. Suec. 10, 1—135.

Gavris, V. P. 1939. Selection of immune forms of *common* pine. — Lesn. hoz. 8, 5—8. (Rev. Appl. mycol. 19, 375.).

Heath, M. C. 1971. Haustorial sheath formation in cowpea leaves immune to rust infection. — Phytopathology 61, 383—388.

— 1972. Ultrastructure of host and nonhost reactions to cowpea rust. — Ibid. 62, 27—38.

— 1974. Light and electron microscope studies of the interactions of host and non-host plants with cowpea rust — *Uromyces phaseoli* var. *vignae*. — Physiol. Plant Path. 4, 403—414.

Heath, M. C. and Heath, I. B. 1971. Ultrastructure of an immune and a susceptible reaction of cowpea leaves to rust infection. — Ibid. 1, 277—287.

Karnovsky, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. — J. Cell Biol. 27, 137 A.

Klingström, A. 1963. *Melampsora pinitorqua* (Braun) Rostr. — Pine twisting rust. Some experiments in resistance biology. — Stud. For. Suec. 6, 1—23.

— 1969. *Melampsora pinitorqua* (Braun) Rostr. on progenies of *Pinus silvestris* L. and in relation to growth regulating substances. — Ibid. 69, 1—76.

Kurkela, T. 1973. Epiphytology of *Melampsora* rusts of Scots pine (*Pinus sylvestris* L.) and aspen (*Populus tremula* L.). — Comm. Inst. For. Fenn. 79.4, 1—68.

Littlefield, L. J. and Bracker, C. E. 1972. Ultrastructural specialization at the host-pathogen interface in rust-infected flax. — Protoplasma 74, 271—305.

Longo, N. and Naldini, B. 1972. Osservazioni sull'ultrastruttura dell'austorio di *Melampsora pinitorqua* (A. Br.) Rostr. in cellule di *Populus tremula*. — Caryologia 25, 383—401.

Longo, N. and Naldini Longo, B. 1975. Observations on the fine structure of the haustorium of *Melampsora pinitorqua* (A. Br.) Rostr. in cells of *Pinus pinea* L. — Ibid. 28, 389—405.

Martinsson, O. 1975. Resistensprövning mot parasitsvampar. (Test of resistance against parasitic fungi on forest trees.) — Sv. Skogsvårdsförb. Tidskr. 1, 31—46.

Mendgen, K. 1975. Ultrastructural demonstration of different peroxidase activities during the bean rust infection process. — Physiol. Plant Path. 6, 275—282.

Mercer, P. C., Wood, R. K. S. and Greenwood, A. D. 1974. Resistance to anthracnose of French bean. — Physiol. Plant Path. 4, 291—306.

— 1975. Ultrastructure of the parasitism of *Phaseolus vulgaris* by *Colletotrichum lindemuthianum*. — Ibid. 5, 203—214.

Rennerfelt, E. 1954. Biologische Untersuchungen über den Kieferndreher *Melampsora pinitorqua* (Braun) Rostr. — XI IUFRO-Congress, Proc. 705—711.

Rijkenberg, F. H. J. and Truter, S. J. 1973. Haustoria and intracellular hyphae in the rusts. — Phytopathology 63, 281—286.

Robb, J., Harvey, A. E. and Shaw, M. 1975. Ultrastructure of tissue cultures of *Pinus monticola* infected by *Cronartium ribicola*. II. Penetration and post-penetration. — Physiol. Plant Path. 5, 9—18.

Shaw, M. and Manocha, M. S. 1965. The

physiology of host-parasite relations. XV. Fine structure in rust-infected wheat leaves. — Can. J. Bot. 43, 1285—1292.

Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. — J. Ultrastruct. Res. 26, 31—43.

Stavely, J. R., Pillai, A. and Hanson, E. W. 1969. Electron microscopy of the development of *Erysiphe polygoni* in resistant and susceptible *Trifolium pratense*. — Phytopathology 59, 1688—1693.

Van Dyke, C. G. and Hooker, A. L. 1969. Ultrastructure of host and parasite in interactions of *Zea mays* with *Puccinia sorghi*. — Phytopathology 59, 1934—1946.

Venable, J. H. and Coggeshall, R. 1965. A simplified lead citrate stain for use in electron microscopy. — J. Cell Biol. 25, 407—408.

Walles, B. 1974. Ultrastructure of the rust fungus *Peridermium pini* (Pers.) Lev. — Stud. For. Suec. 122, 1—30.

Abbreviations

Host cell constituents:

C = collar
ER = endoplasmic reticulum
FM = fibrillar material
H = host cell
RM = reaction material
SM = sheath membrane
W = cell wall

Fungal cell constituents:

er = endoplasmic reticulum
g = glycogen granule
h = haustorium
hy = hypha
l = lomasome
ld = lipid droplet
m = mitochondrion
n = nucleus
s = septum
wb = Woronin body

Figures

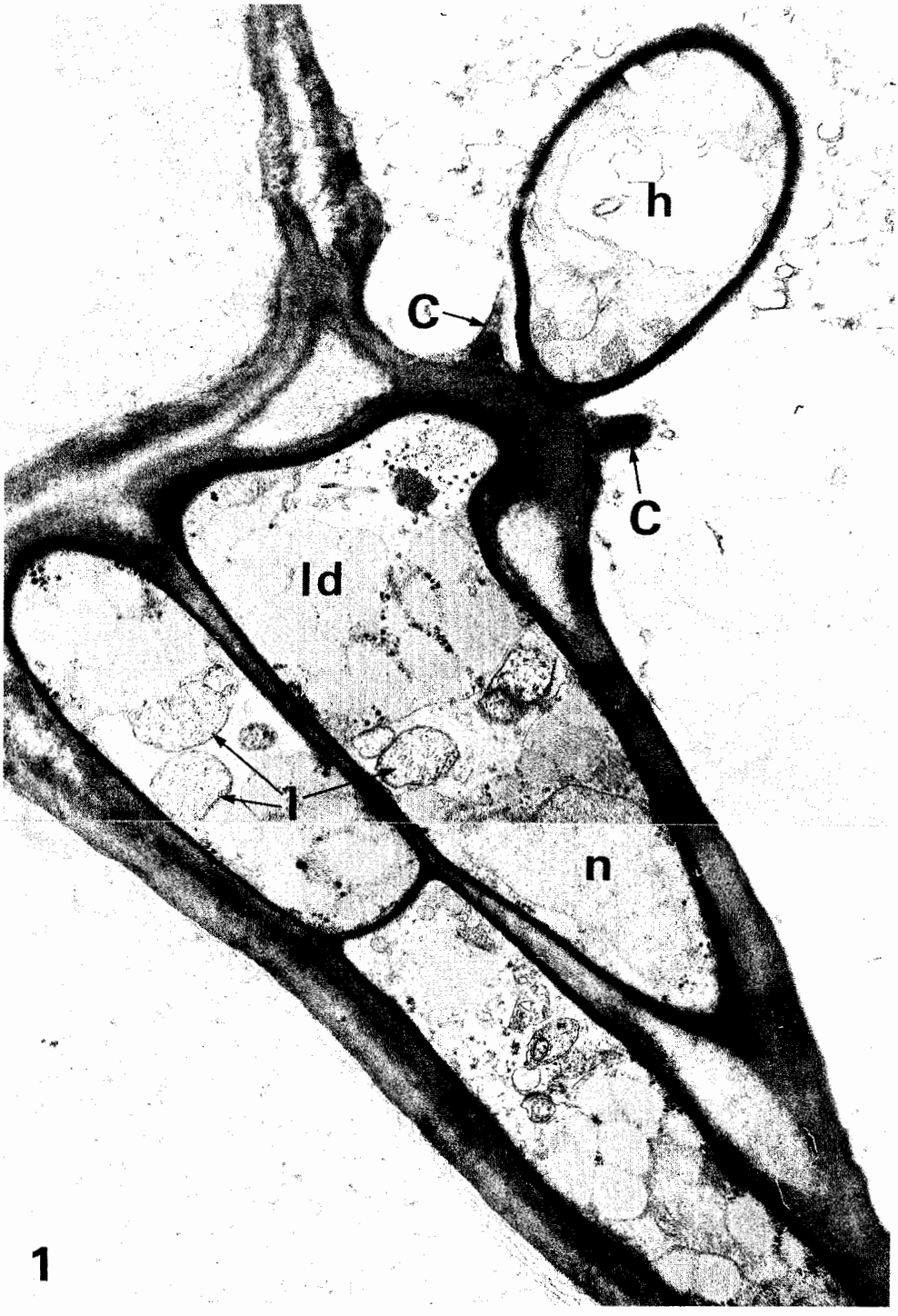


Figure 1. An intercellular hypha that has formed a haustorium. 14,000: 1.

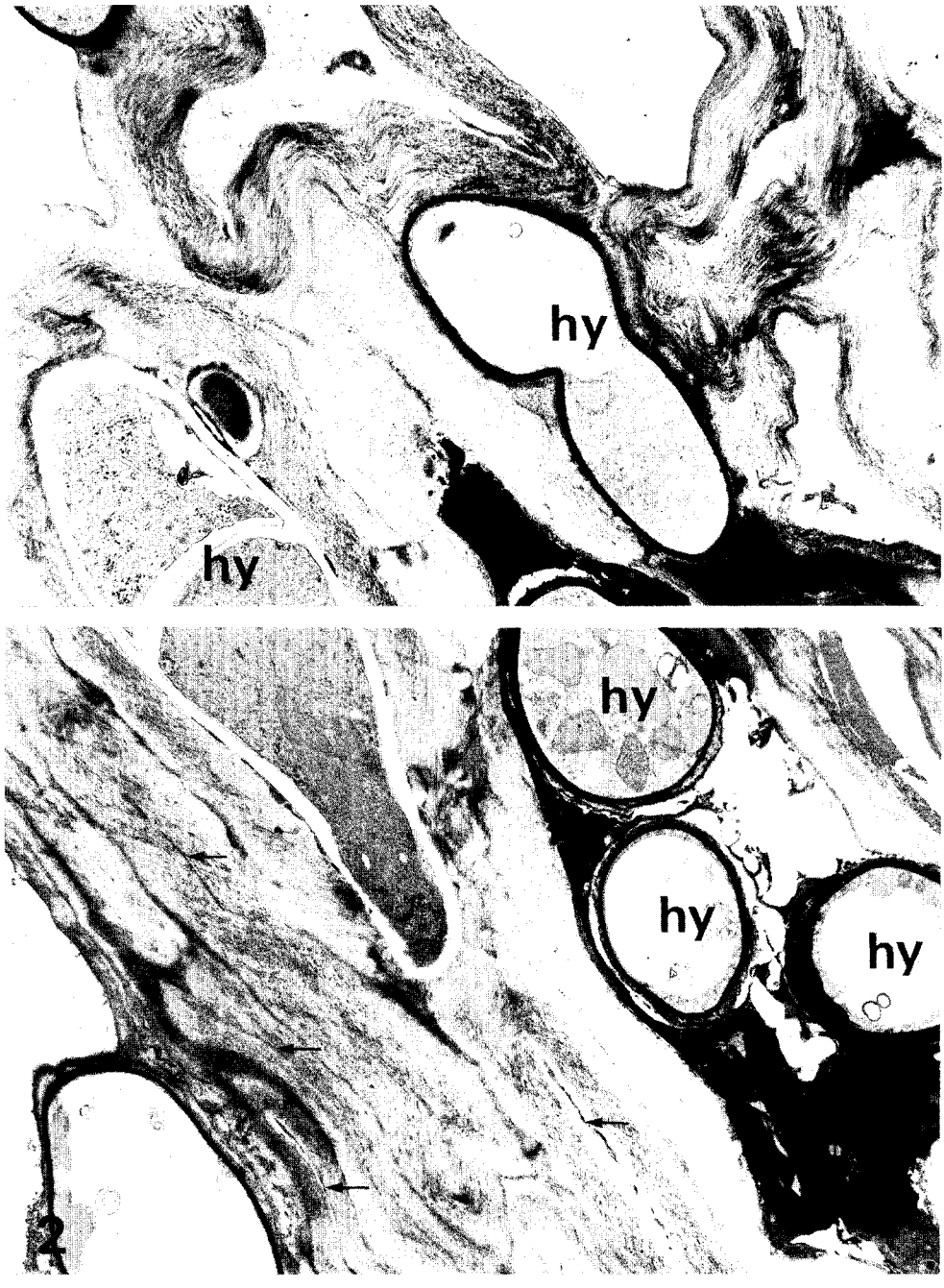


Figure 2. Hyphae growing in the phloem. The sieve cells are compressed so that their lumina are obliterated (indicated by arrows). 7,900:1.

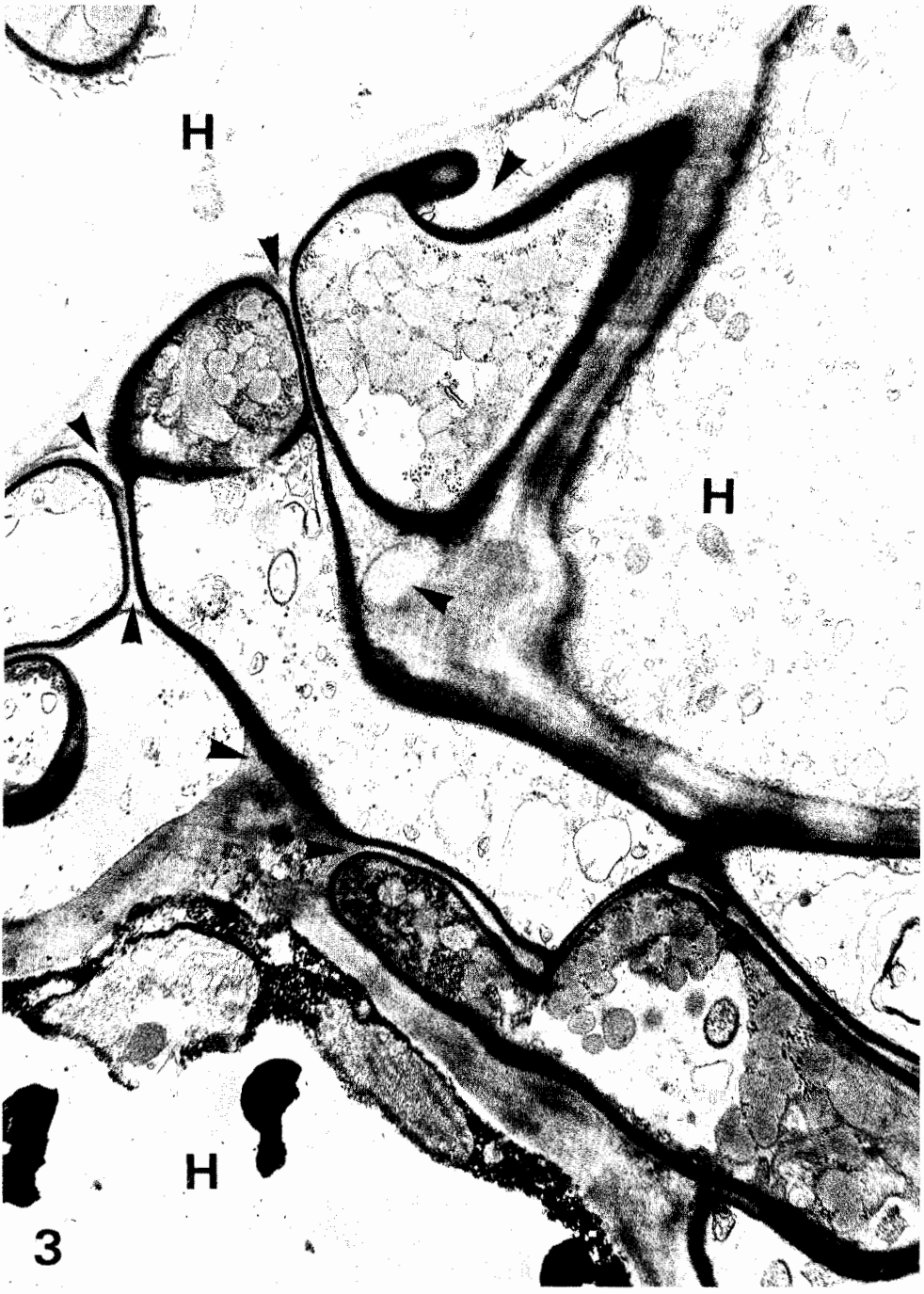
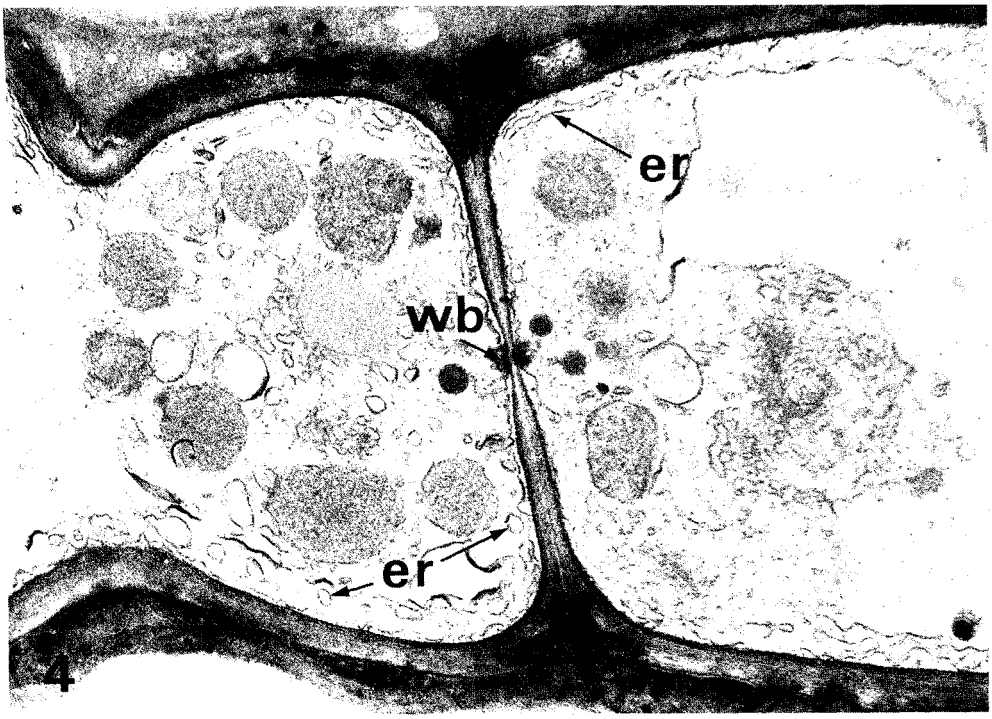


Figure 3. The intercellular hyphae are attached to each other and to the host cell wall by a sticky material of moderate electron density (arrow heads). 11,000:1.



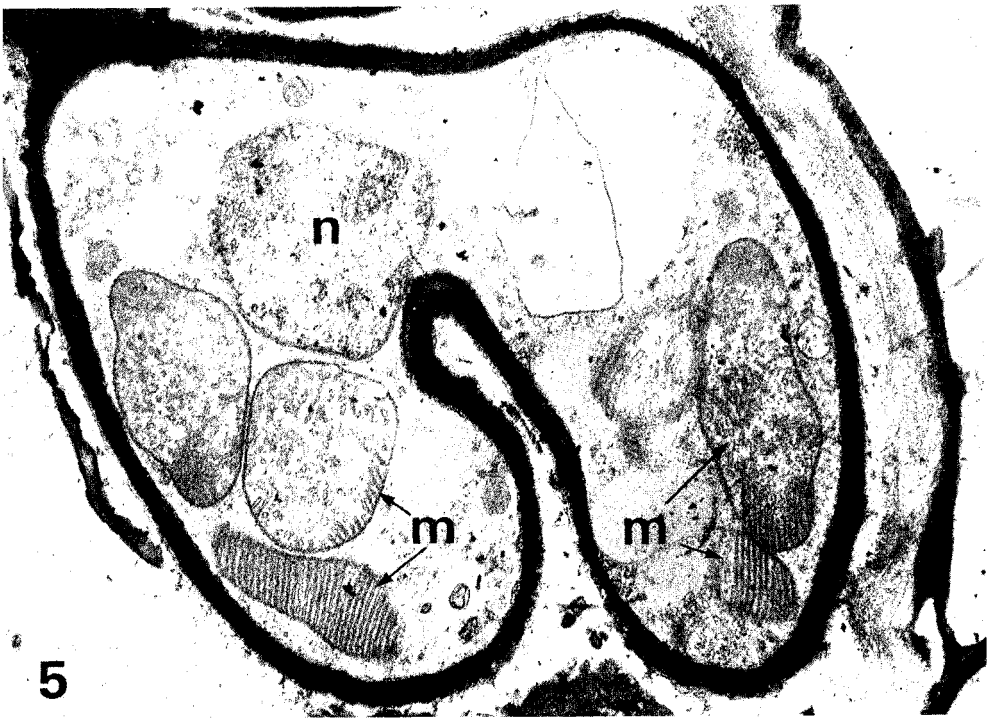


Figure 4. Part of a hypha with a septum in cross section. The septal pore is occluded by a Woronin body. 23,000:1.

Figure 5. Fungal cell with nucleus and mitochondria. 19,000:1.

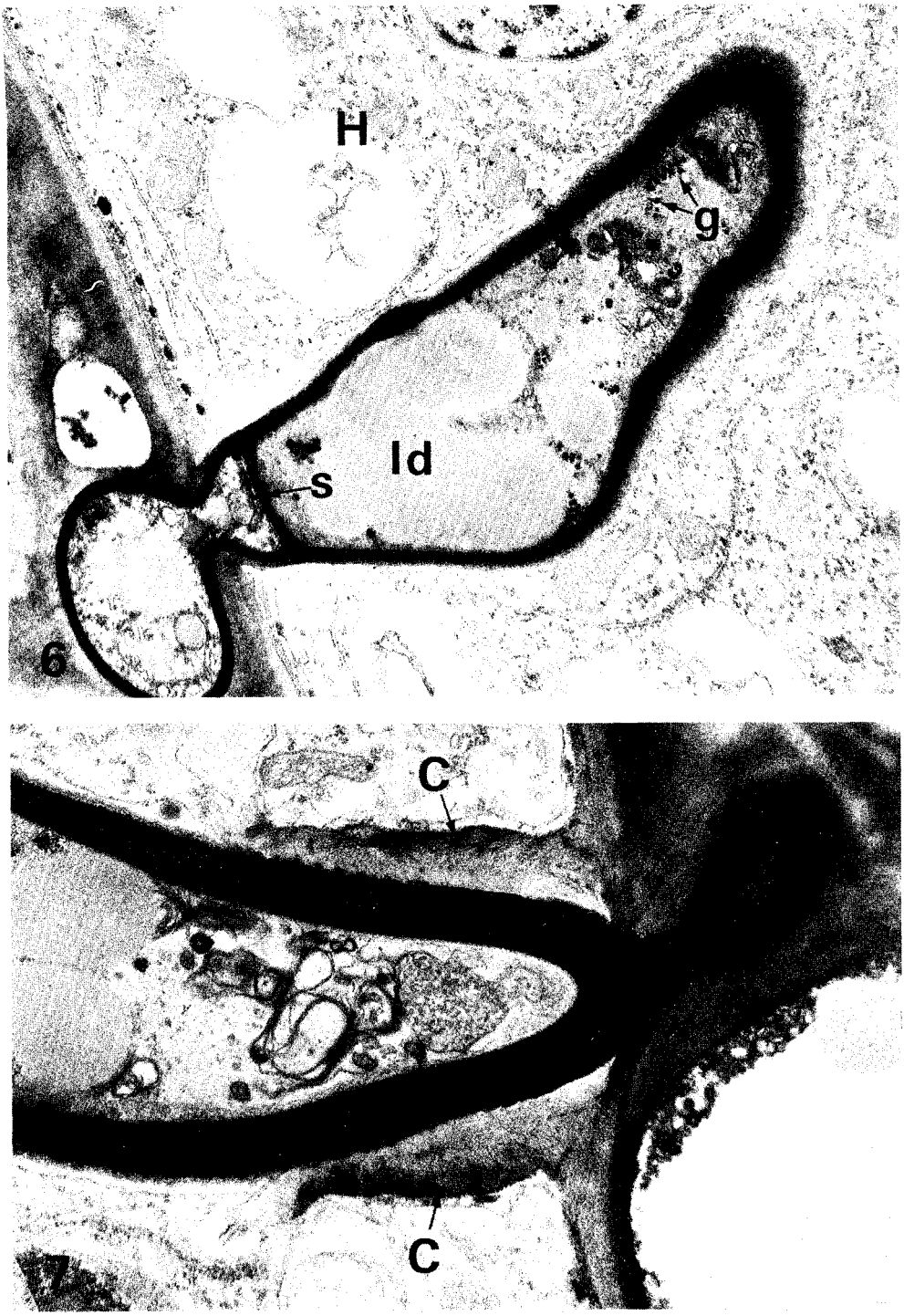


Figure 6. A haustorium separated from its intercellular mother cell by a septum. 16,000:1.

Figure 7. A collar deposited at the base of the haustorium. 20,000:1.

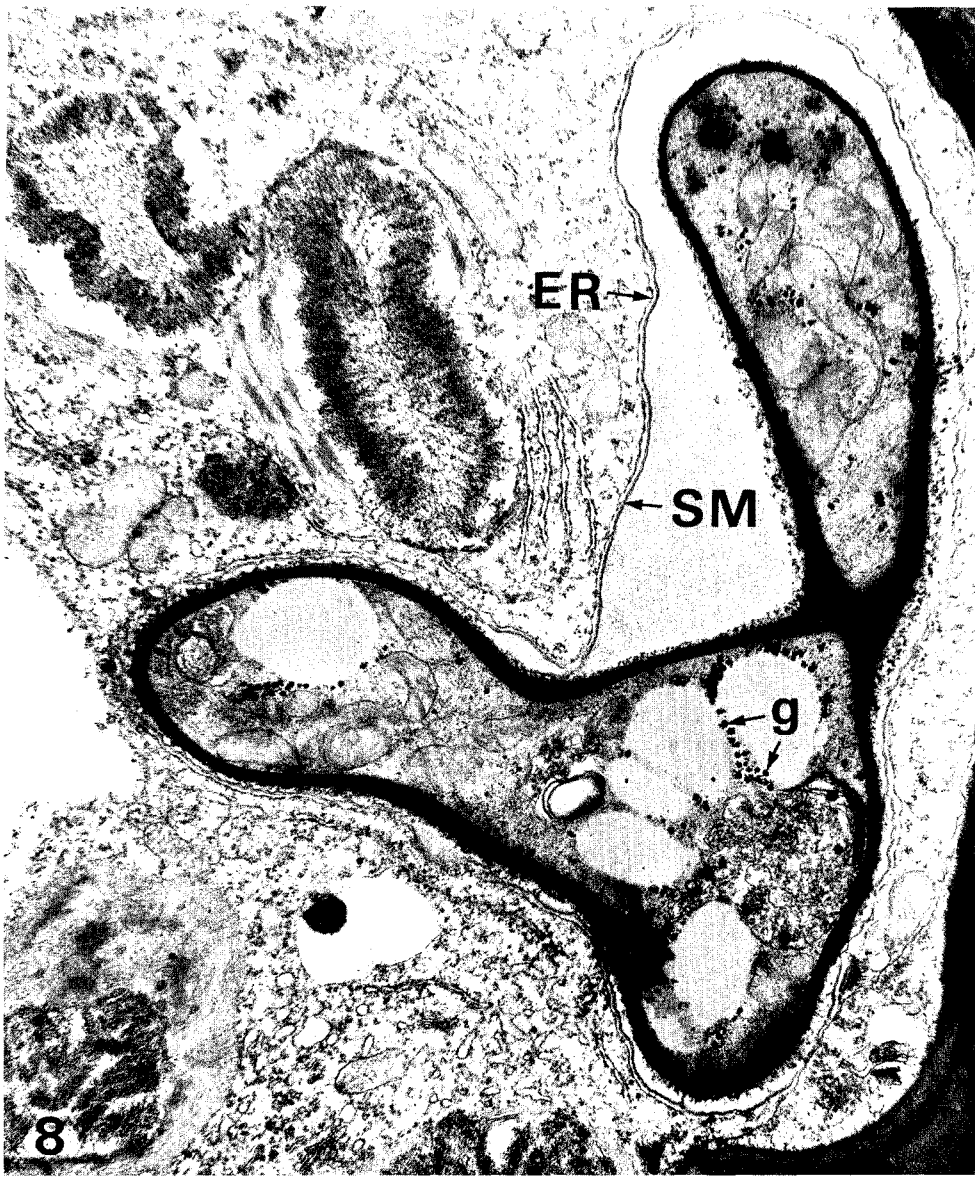


Figure 8. Material of moderate electron density accumulated in the extrahaustorial space. 12,000:1.

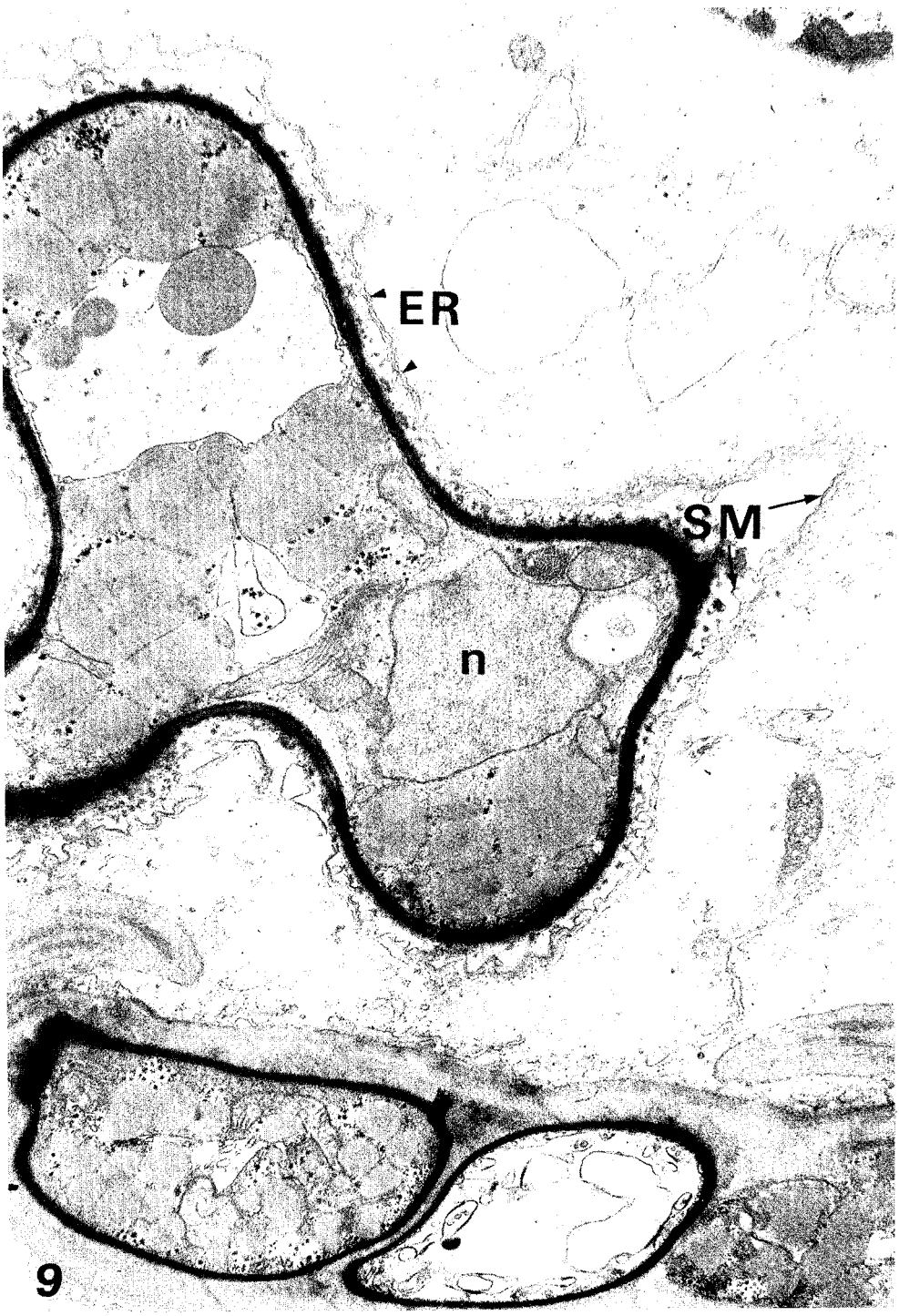


Figure 9. Haustorium surrounded by invaginated host plasmalemma (sheath membrane) that is convoluted. Small electron-dense grains are apparent between the sheath membrane and the haustorial wall. 17,000:1.

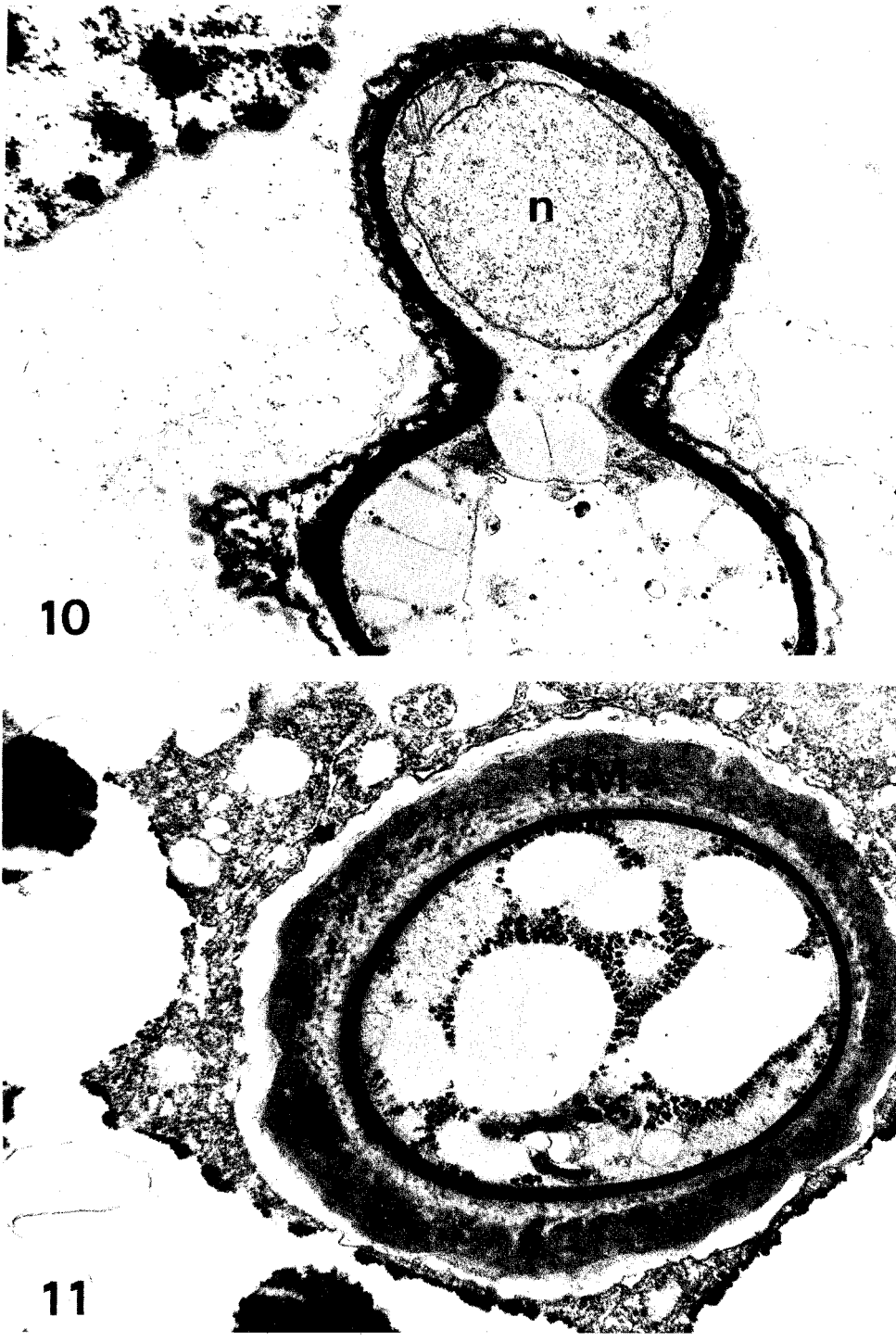
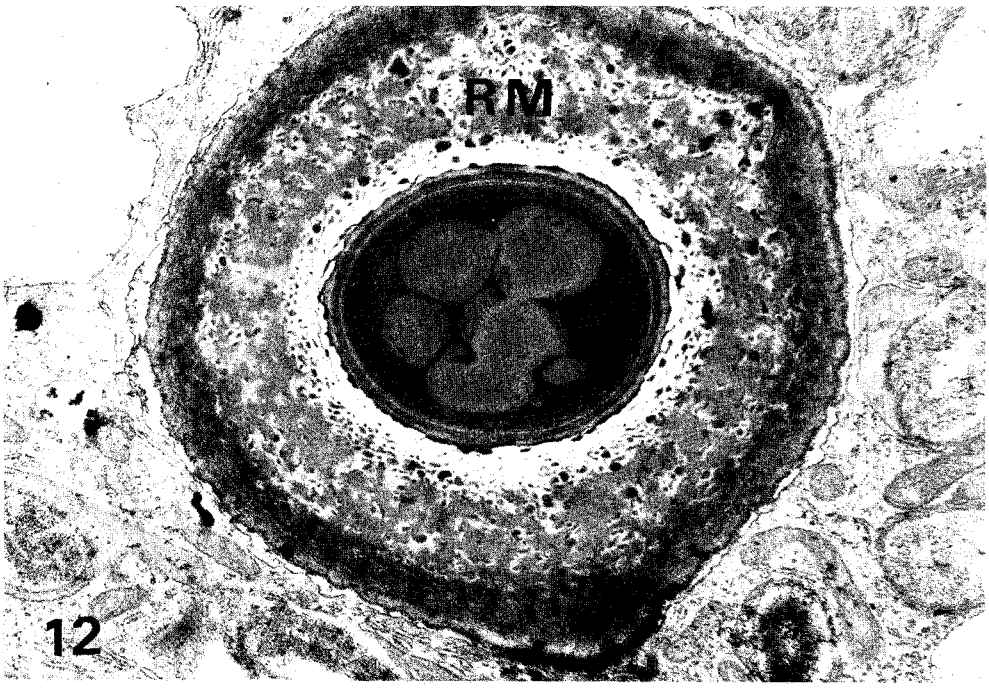


Figure 10. Electron-dense grains are discernible near the haustorial wall and the inside of the sheath membrane is coated by material of similar electron density. 15,000:1.

Figure 11. A haustorium encased in reaction material. 19,000:1.



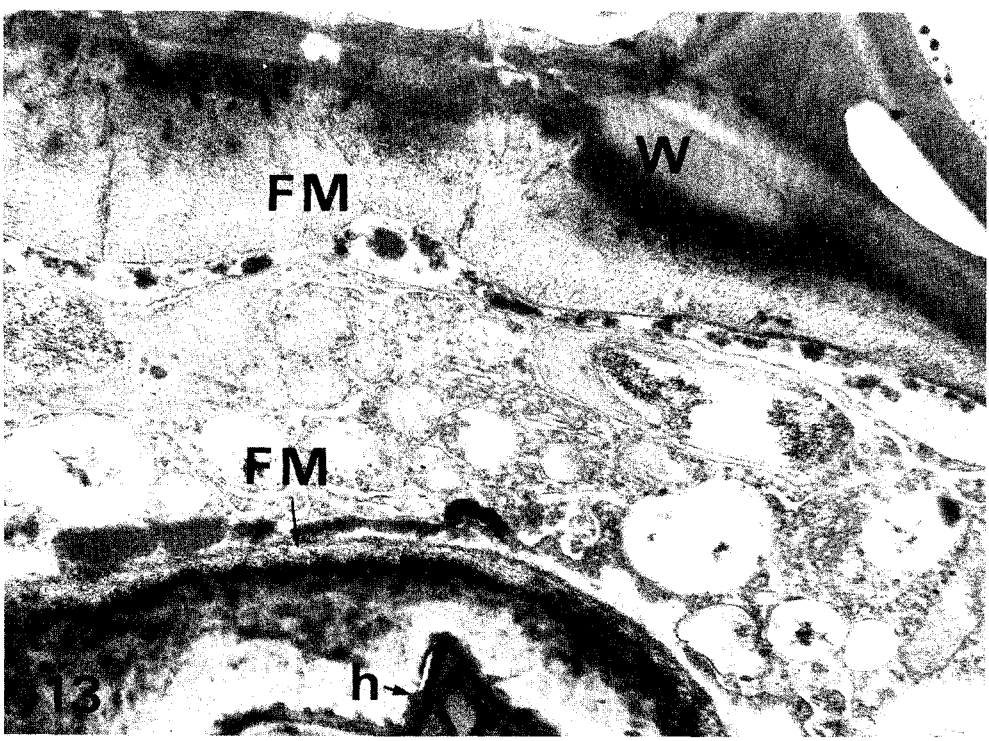


Figure 12. Reaction material forms a layer of considerable thickness around a necrotic haustorium. 14,000:1.

Figure 13. A fibrillar material is deposited around the encasement and along the host cell wall. Between that layer and the plasmalemma electron-dense aggregates appear. 16,000:1.

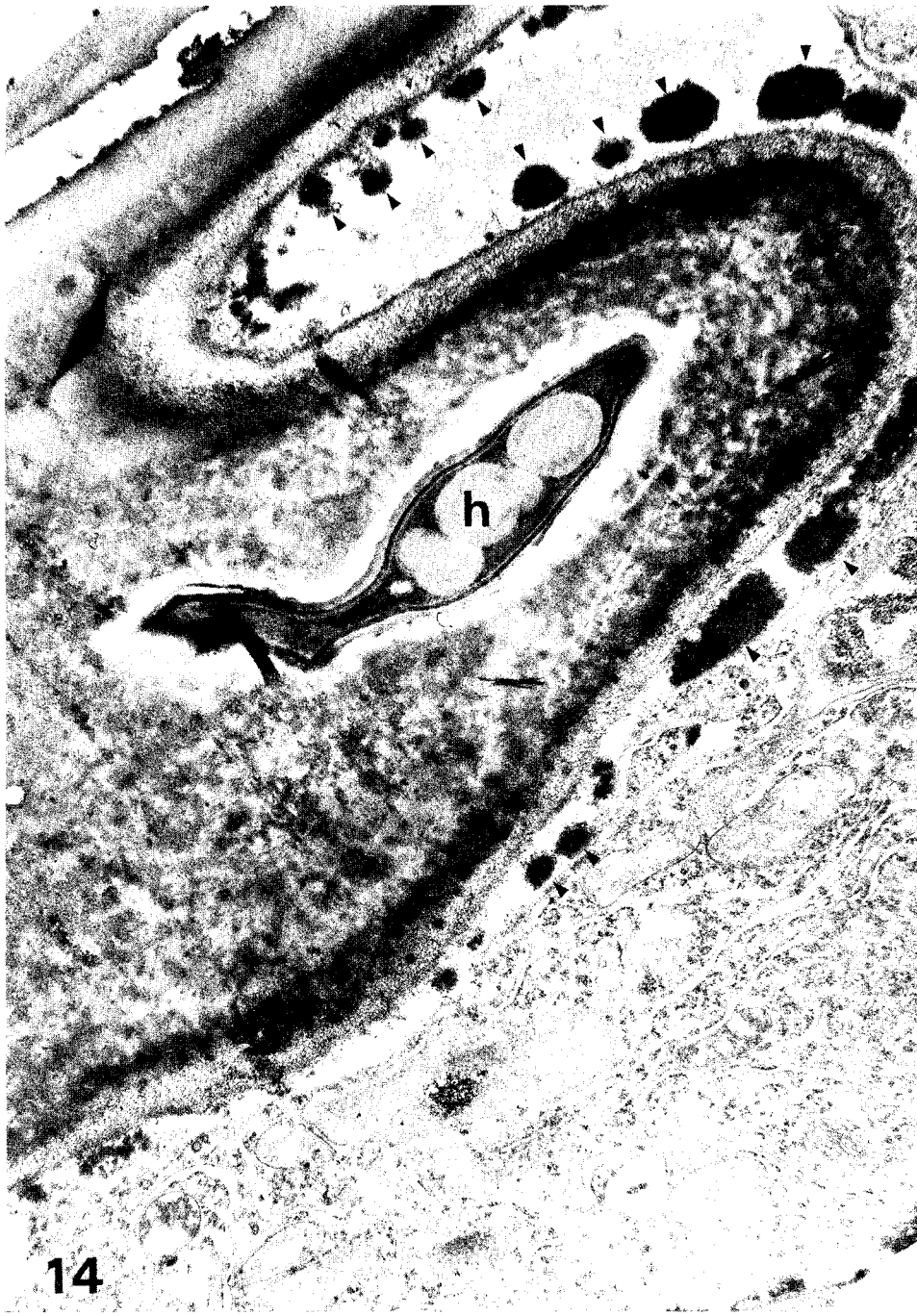
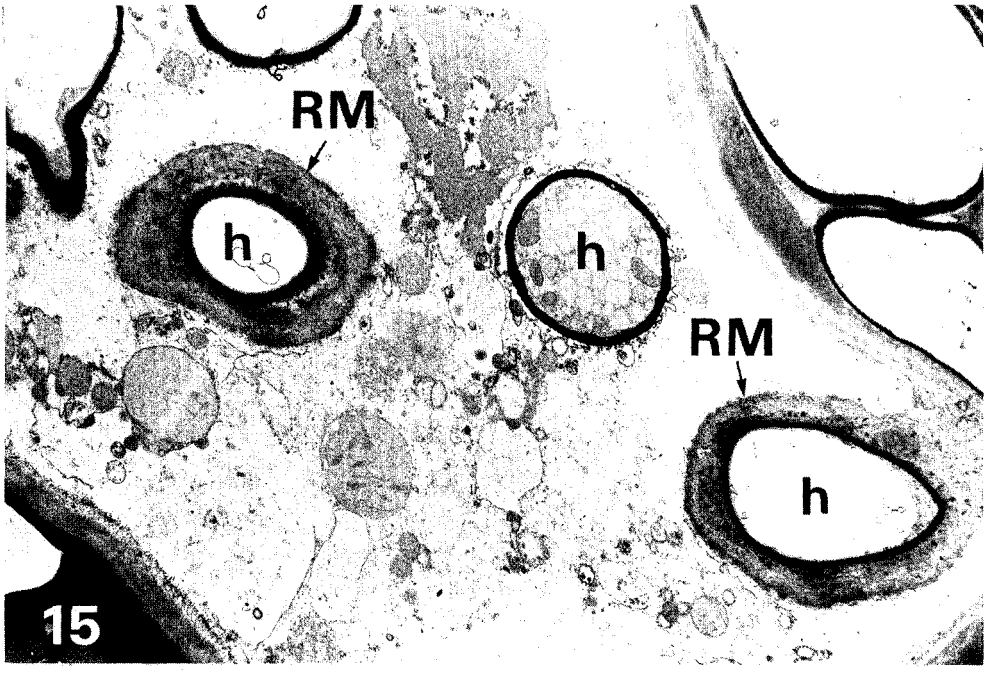


Figure 14. Encased haustorium. Electron-dense material (arrow heads) is deposited between the entire plasmalemma and the host cell wall. 16,000 : 1.



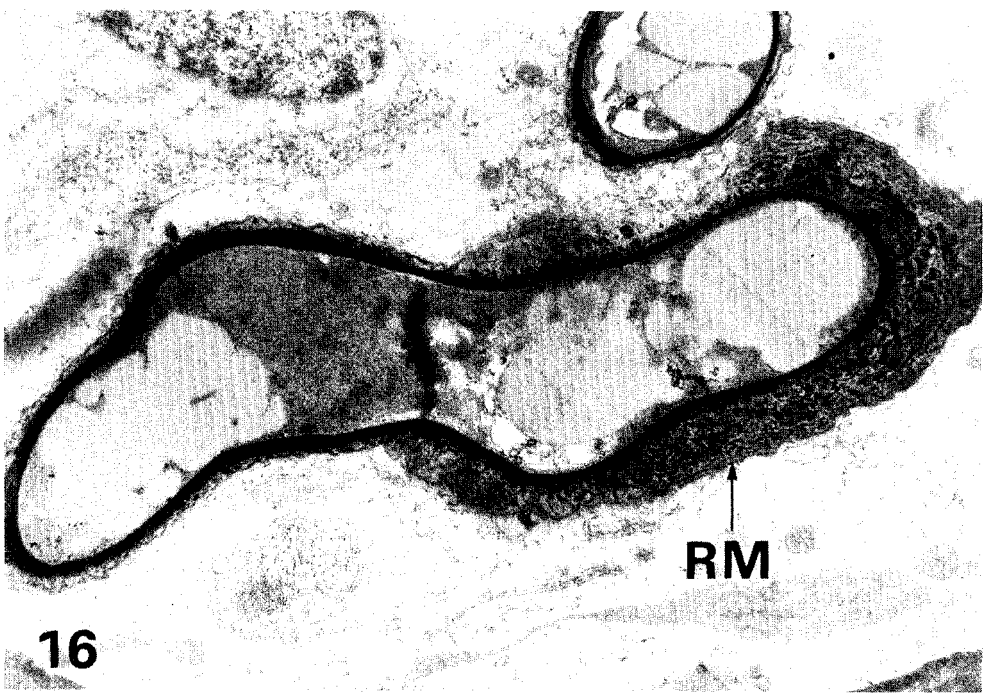


Figure 15. Haustorial profiles, some of which are encased. 9,200:1.

Figure 16. A haustorium enveloped by reaction material which varies in thickness along its body. 11,000:1.