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Ultrastructure of American Licorice (Glycyrrhiza lepidota pursh) Leaf Cells and an Associated Rust Pathogen (Uromyces Glycyrrhizae (rab.) Magn.)

Richard Wynia

ULTRASTRUCTURE OF AMERICAN LICORICE (*GLYCYRRHIZA LEPIDOTA* PURSH) LEAF CELLS AND AN ASSOCIATED RUST PATHOGEN (*UROMYCES GLYCYRRHIZAE* (RAB.) MAGN.)

BY

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AND AN ASSOCIATED RUST PATHOGEN
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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ULTRASTRUCTURE OF AMERICAN LICORICE (*GLYCYRRHIZA LEPIDOTA* PURSH) LEAF CELLS AND AN ASSOCIATED RUST PATHOGEN (*UROMYCES GLYCYRRHIZAE* (RAB.) MAGN.)

ABSTRACT

by

Richard Wynia

Under the supervision of Professor Wayne S. Gardner

Amercian licorice is a herbaceous, perennial, native legume common to disturbed areas, draws, woods, and depressions in prairies and pastures over much of temperate North America. It is utilized by livestock in the Great Plains and produces nutritious and highly digestible forage. However, forage value or use reports for this species have been inconsistent. Reports ranged from "relished by livestock" to "withstands excessive use" depending on location of utilization and author cited. *Glycyrrhiza* contains tannic acid and palatability of some forage legumes has been influenced by their tannic acid content.

Severe natural infections of rust were noted in a licorice ecotype nursery in June 1984 at Brookings, South Dakota. Severe infections normally induce leaf loss and create a subsequent reduction in forage quality.

Our objectives in undertaking this ultrastructural examination of G. lepidota and its associated rust pathogen U. glycyrrhizae were to study the fine structure of the host-pathogen interaction and to examine the pathogen and its legume host for unique cell types, cellular organelles, and other cellular inclusions, such as tannin.

Rust-infected leaves were collected in June, fixed in glutaraldehyde and osmic acid, dehydrated, and then embedded in epoxy plastic. Polymerized epoxy blocks were sectioned with an ultramicrotome. Sections were mounted on copper grids, stained with uranyl acetate and lead citrate, and subsequently viewed with the transmission elecron microscope.

Electron micrographs of rust hyphae in intercellular spaces of host vascular parenchyma and mesophyll cells elucidated the development of U. glycyrrhizae. The typical dikaryotic nuclear condition and fungal organelles were observed in intercellular hypha. Intercellular hyphae formed haustorial mother cells that penetrated host cell walls and formed dikaryotic intracellular haustoria. Haustoria were surrounded by extrahaustorial sheaths which delimited intimate contact of haustoria to host cytoplasm.

Micrographs of intact host mesophyll cells showed lens-shaped chloroplasts with short grana stacks, nuclei, nucleoli, mitochondria, and microbodies. Vascular parenchyma cells contained numerous small vacuoles, mitochondria, dictyosomes, chloroplasts, and invaginations of cellular plasmalemmas. Two types of specialized parenchyma cells were also observed for the first time in Glycyrrhiza. A-type transfer cells, which specialize in short distance solute transport, were located in minor leaf veins. Tannin cells, which package and store tannins, were found in leaf mesophyll cells.

INTRODUCTION

American licorice (*Glycyrrhiza lepidota* Pursh) is a native perennial legume common to disturbed areas, draws, woods, and depressions in prairies and pastures over much of temperate North America (Duke, 1981). Forage value reports for this species have been inconsistent. Weaver (1954) and Johnson and Nichols (1982) stated that it is relished by livestock in the Great Plains, and may be excluded in heavily grazed areas. Johnson and Nichols (1982) reported that further west it withstands excessive use and Allen and Allen (1981) describe it as having low palatability as a forage. Fransen and Boe (1981) found *in vitro* dry matter disappearance (IVDMD) of *G. lepidota* at early pod to be slightly greater than alfalfa at late bud.

Allen and Allen (1981) and Duke (1981) recognized *G. lepidota* for its good soil binding characteristics and vigorous rhizomatous growth habit. Rhizome characteristic studies of 9 South Dakota ecotypes indicated that *G. lepidota* has tremendous vegetative reproduction potential (Boe and Wynia, 1985). Whitman (1979) reported that it showed exceptionally vigorous growth on mine spoil material at Dickinson, North Dakota, and was one of the best native forbs he evaluated for providing dense cover on that substrate.

While native legumes offer tremendous potential for enhancing the productivity of pastures and rangelands where introduced species are not adapted or desired (Davis, 1982), there are some inherent difficulties which native species must overcome. Many native plants have unique disease, insect, and antiquality problems which have never been addressed. In the case of G. lepidota, the following fungi are known pathogens: Cylindrosporium glycyrrhizae, Erysiphe polygoni, Microsphaera diffusa, Septoria glycyrrhizae, and Uromyces glycyrrhizae (Duke, 1981). In addition to these disease-causing organisms a seed beetle, Acanthoscelides fraterculus, reduces viable seed production and the foliage is known to contain tannic acid (Duke, 1981). A high percentage of chlorophyll deficient seedlings were also observed in certain ecotypes (Wynia et al., 1981).

The life cycle of Uromyces glycyrrhizae (licorice leaf rust) may be termed macrocyclic or demicyclic depending on the presence or absence of urediospores (Cummins, 1978). Macrocyclic species produce repetitive infections via urediospores and demicyclic species delete the uredial stage from an otherwise macrocyclic form (Petersen, 1974). Cummins (1978) stated that uredia were uncertain and not distinguishable from aecia if systemic in U. glycyrrhizae.

U. glycyrrhizae is an autoecious rust, thus restricting all its spore stages to G. lepidota (Cummins, 1978).

A generalized life cycle of rust as presented by Agrios (1969) was utilized since so very little is known about the life cycle of U. glycyrrhizae. The haploid monokaryotic stage is usually initiated by basidiospore germination and host penetration in the spring (Petersen, 1974). Infectious haploid hypha quickly expands into a network of intercellular mycelium, which produces spermogonia of two distinct mating types. Dikaryotization usually takes place by nuclear fusion between a spermatium of one mating type and a receptive hypha of the spermogonium of the opposite mating type. Resulting dikaryotic hyphae proliferate into a mycelium distinct from either parent, and produce aecial sori containing aeciospores. Aeciospores germinate into a dikaryotic hypha which usually penetrates the host via stomatal openings, forming a dikaryotic mycelium which eventually produces uredial sori and urediospores. Urediospores are also dikaryotic and serve to reinfect host plants. Aeciospores and urediospores, in some species are morphologically similar and very difficult to differentiate (Petersen, 1974). Later in the season, uredia produce teliospores along with urediospores.

Teliospores are usually thick-walled and serve as overwintering structures. It is within the teliospore that fusion of the two nuclei occurs, resulting in the diploid stage of the life cycle. Teliospores germinate into a short tube, the promycelium, into which the diploid nucleus migrates and undergoes meiosis. The four daughter nuclei are separated by cross-walls and on each basidial cell a sterigma is formed, surmounted by a basidiospore. Basidiospores formed are of two distinct mating types.

Janzen (1969) reported that seed beetles prey on numerous wild and cultivated legumes and Baker (1895) observed heavy infestations by Acanthoscelides fraterculus (Horn) on G. lepidota near Ft. Collins, Colorado. Boe and Wynia (1985) reported A. fraterculus beetle infestations on G. lepidota seeds from 37 collections made in the Dakotas. Infestation rates ranged from 7% to 71% with an overall mean of 41%.

Duke (1981) stated that G. lepidota contains tannic acid. Tannins are acetogenins, which are biosynthetically classified as secondary metabolites (Walton, 1983). In plant cells, tannins occur within the cell wall (Esau, 1965) and more commonly as inclusions in cytoplasmic vacuoles (Esau, 1963). Some plant cells may even specialize in tannin production (Esau, 1965; Swain, 1965).

In 1939, Clarke et al. speculated that tannin content of Lespedeza sericea made this legume less palatable due to its bitter taste and astringency. Other studies with Lespedeza indicated that tannin content lowered forage intake by livestock (Wilkins et al., 1953). Donnelly (1959) concluded that tannin content of Lespedeza cuneata increased with plant maturity and concurrently with increased temperature and decreased rainfall. In 1946, Stitt et al. reported that tannin content of leaves from a replicated clonal experiment utilizing Lespedeza cuneata varied significantly with soil type and between cuttings made at different dates on the same soil.

Levels and types of tannins vary between species and between different tissues and organs of the same plant (Swain, 1965). Stitt (1943) showed a high leaf tannin concentration with genetic variation among inbred lines of Lespedeza cuneata. Cope and Moll (1969) produced a low tannin strain of Lespedeza via incorporation of a simply inherited low tannin character. Donnelly and Anthony (1970) found differences in forage digestibility between low and high tannin strains of Lespedeza. A negative association existed between tannin content and dry matter digestibility, indicating that as tannin increased dry matter digestibility decreased.

Research indicates that the rumen limits the metabolic potential of the animal with respect to quantitative and qualitative amino acid supply (McDonald, 1968). McDonald (1948) showed that some proteins are readily degraded by rumen microorganisms to ammonia and then excreted as urea. Proteolysis and excretion of dietary proteins can be decreased by protecting the protein from microbial degradation. Tannins are polyphenolic compounds which have strong protein-binding properties (Walton, 1983). Drieger and Hatfield (1972) conducted a study to investigate the extent of digestion of tannin-treated soybean meal and the subsequent effect of the meal on lamb nitrogen balance. They found that average daily gain, feed efficiency, and nitrogen balance were all increased in lambs fed soybean meal treated with 10% tara tannin. Efficiency of nitrogen utilization also appeared to be enhanced by the treatment.

McArthur and Miltimore (1966) presented evidence that indicated that bloat was associated with high levels of 18S protein in a number of legume species. Studies of 18S protein indicated that these spherical, insoluble proteins are the major factor in generating bloat-causing stable foam in ruminant animals (McArthur, et al., 1964). Walton (1983) stated that certain tannins are capable of

precipitating proteins found in bloat-causing foam. Jones and Lyttleton (1971) found that legumes which do not cause bloat generally contain more protein precipitating tannins and less soluble proteins than do bloat inducing legumes.

Our objectives in undertaking this ultrastructural examination of G. lepidota and its associated rust pathogen U. glycyrrhizae were to study the fine structure of the host-pathogen interaction and to examine the pathogen and its legume host for unique cell types, cellular organelles and other cellular inclusions, such as tannin.

MATERIALS AND METHODS

Leaves of G. lepidota, which were naturally infected by U. glycyrrhizae, were collected on June 12, 1984 from a licorice ecotype nursery located at Brookings, South Dakota. Leaves of uninfected G. lepidota were also collected for ultrastructural examination.

Leaves of both types were cut into small sections (1x 5 mm) in a drop of 2.5% glutaraldehyde (GA), in 0.1 M (pH 7.1) potassium phosphate buffer placed on the cover of a plastic petri plate filled with crushed ice. Leaf sections were transferred into glass vials containing 6 ml of the buffered GA, and kept in an ice bath for 60 minutes. One milliliter (ml) of 1% phosphate-buffered osmic acid (OsO_4) was added to each vial, allowed to stand for 60 minutes at 3C, and then removed. Plant tissue sections were dehydrated using 25% followed by 50% cold acetone treatments. Each treatment was repeated twice for 15 minute intervals. At room temperature four ml of 2, 2 - Dimethoxypropane (DMP) (acidified with one drop of concentrated hydrochloric acid per 100 ml) was added to each vial. The 4 ml DMP was discarded and new added for an additional 15 minutes. Tissues were then soaked for two hours in an epoxy plastic medium mixture which was frequently stirred (Gardner, personal communication).

Tissue sections were transferred into "micron" molds containing fresh epoxy plastic medium. Molds were subjected to 60 C temperature for 24 hours in order to polymerize the epoxy plastic. Blocks were trimmed manually prior to thin sectioning with a Porter-Blum MT-2 ultramicrotome. Thin sections were cut utilizing either a glass or a diamond knife. Thin sections were mounted on 200 mesh copper grids. Mounted sections were stained in 2% uranyl acetate (2 hours), rinsed in glass distilled water, and stained in lead citrate (1 minute). Stained sections were viewed with an Hitachi HU-12 transmission electron microscope operated at 75 KV.

RESULTS AND DISCUSSION

Ultrastructure of Glycyrrhiza lepidota.

Examination of electron-micrographs of Glycyrrhiza cells revealed some ultrastructurally important characterisitics of this plant (Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11). Figures 1 and 2 show mesophyll cells containing large central vacuoles, several lense-shaped chloroplasts, mitrochondria, nuclei, nucleoli, and microbodies. Enlarged segments of mesophyll cells (Figs. 3 and 4) allow a more detailed examination of individual chlorplasts. Chloroplasts are bound by an envelope consisting of two unit membranes (Racker, 1970). Internally, chloroplasts are made up of the embedding matrix or stroma and the internal membrane system (O'Brien and McCully, 1969). The membrane system consists of a number of membrane-rich stacks, the grana, which are connected to one another by stroma lamellae (O'Brien and McCully, 1969). Glycyrrhiza chloroplasts have short stacked grana which are connected by conspicuous stroma lamellae. Starch grains and lipid globules are present in abundance in the stroma of chloroplasts of many plant species (Esau, 1977). However, Glycyrrhiza chloroplasts contained very limited starch (Fig. 17) and lipid (Fig. 22) deposits. In addition to the chloroplasts the

enlargements offer a more detailed view of cytoplasmic ribosomes. Ribosomes were observed scattered in Glycyrrhiza cell cytoplasm (Figs. 3 and 4), and also united to form polyribosomes (Fig. 11).

Vacuoles of many Glycyrrhiza cells contained inclusion bodies of a dark circular to irregularly shaped amorphous substance (Figs. 1, 2, 5, 6, 8, 9, and 10) considered to be tannin. The exclusive vacuolar nature of these deposits, within cells, allowed dismissal of the idea that an artifact of preparation was involved rather than a natural cellular phenomenon. Tannin inclusions within the vacuoles of other plant species have been observed utilizing the electron microscope (Chafe and Durzan, 1973; Parham and Kaustinen, 1977). Parham and Kaustinen (1977) hypothesized that minute tannin filled vacuoles arise from the endoplasmic reticulum. These minute vacuoles enlarge and fuse to form larger vacuoles while moving toward the central vacuole of the cell where they ultimately deposit their contents. This appears to be the case in Glycyrrhiza as well. Figure 2 shows several small vacuoles containing inclusions located within the cytoplasm of the leaf mesophyll cell and a larger tannin inclusion in the cells central vacuole. Form and dimension of tannin inclusions were variable. Size varied

from tiny electron dense bodies in small vacuoles to deposits which occupied the major portion of the central vacuole of a cell (Figs. 5 and 6). Such cells may be specialized in the production of tannin (Esau, 1965). Total occlusion of these cells resulted from massive tannin deposits in the central vacuole of highly vacuolated cells (Fig. 6). The highly vacuolated cytoplasm which does exist in these cells is compressed between the vacuolar tonoplast and the plasmalemma next to the cell wall. Mitochondria are the major cellular organelles observed within the cytoplasm.

The metabolism of carbohydrates and tannins is interrelated, and deposition of starch and tannins may be mutually exclusive within the cell (Esau, 1965). Wardrop and Cronshaw (1962) stated that cells containing large quantities of phenolic substances contain few or no starch grains. Ultrastructural evidence concerning the paucity of starch grains within the chloroplasts of *Glycyrrhiza* cells and the large amount of tannin located within vacuoles, supports the suggested interrelationship between tannins and starch.

Tannin production by *G. lepidota* may account for some of the inconsistencies in reported forage values of this species. There may be instances of ecotypic

variability in tannin production levels depending on soil type, weather conditions, and maturity of the plant. Since G. lepidota has such a broad ecological amplitude there may be opportunities to select an ecotype which has low tannin production. However, ecotypes with high tannin production may also be useful as a high protein roughage to reduce the incidence of bloat or to prevent proteolysis in the rumen.

Figures 7 - 11 are typical cell types located in the vascular tissue of G. lepidota. A vascular parenchyma cell depicted in Figure 7 includes numerous smaller vacuoles instead of a large central vacuole commonly seen in mesophyll cells. Other cellular contents include a mitochondrion, dictyosome, chloroplast, and three invaginations of the cells plasmalemma. Invaginations of plasmalemma, which often form pockets between cell wall and cytoplasm, are frequently observed in material prepared for electron microscopy (Esau, 1977). The frequent presence of tubules and vesicles in such pockets has led researchers to believe that the pockets are functional organelles concerned with cell wall growth and other relations between the cell wall and cytoplasm (Esau, 1977). Invagination pockets observed in G. lepidota also contain vesicles as described by Esau.

Figures 8, 9, and 10 deal primarily with xylem cells and the xylem parenchyma cells which surround them. Figures 8 and 9 depict mature xylem tracheary elements and differentiating tracheary elements. The differentiating tracheary elements contain mitochondria, dictyosomes, and endoplasmic reticulum within their cytoplasm. This stage of development in the tracheary element is characterized by secondary wall deposition. Pickett-Heaps (1968) indicated that endoplasmic reticulum and dictyosomes are associated with incorporation of material into the cell wall, possibly by means of vesicles that move toward the cell periphery, unite with the plasmalemma, and release their contents toward the developing cell wall. After completion of secondary wall deposition, the cell enters a lysis stage in which the protoplast and parts of the primary cell wall are digested via hydrolytic enzyme activity (Esau, 1977). Hydrolases are released into the cytoplasm from ruptured tonoplasts and eventually digest primary wall parts not covered by lignified secondary wall layers (Esau, 1977). Figure 10 serves as an example of two mature xylem tracheary elements in which the hydrolytic stage has been completed and the primary cell wall between them has been at least partially digested.

A cross section of a transfer cell is displayed in Figure 11. Gunning, et al. (1968) were among the first to

describe in detail transfer cells located in minor veins of leaves. Gunning and Pate (1969) described transfer cells as those plant cells possessing ingrowths of wall materials which increases the surface area of plasma membrane available for transmembrane flux of solutes. The ingrowths are a specialized form of secondary wall deposition (Gunning, 1977). Formation of the secondary wall apparatus appears to be inducible and most plants may have the genetic competence to produce transfer cells as the requirement for short distance solute transport changes (Gunning, 1977). The implication is that familiar cell types such as xylem parenchyma, phloem parenchyma, and companion cells can have a common specialization related to short-distance transport processes (Gunning and Pate, 1974).

As minor veins in more species were examined the taxonomic distribution of transfer cells became clearer (Pate and Gunning, 1969). In their survey of minor veins of mature leaves of 975 species and 242 families of angiosperms, Pate and Gunning (1969) indicated that transfer cells are fairly common in herbaceous dicotyledons, rare in woody dicotyledons, and virtually absent in monocotyledons. In the Fabaceae, 90 of 482 genera representing 34 of 50 tribes were examined. More

than half of the genera contained transfer cells of which most were herbs (Pate and Gunning, 1969).

Transfer cells of four types were recognized in minor leaf veins of plants (Pate and Gunning, 1969). A-type transfer cells contain ingrowths distributed all around their periphery, while B-type cells have ingrowths polarized on one side or another (Gunning and Pate, 1969). These two types occur mainly in the phloem. Type C and D transfer cells occur in Xylem parenchyma and bundle sheath respectively, and have ingrowths on walls close to vessels or tracheids (Gunning and Pate, 1969).

Figure 11 is a cross section of an A-type transfer cell located within the vascular system of G. lepidota. Wall protruberances on one side of the cell are attached out of the plane of sectioning, but part of the ingrowths are visible in this plane. Cytoplasm within the cell is dense, with numerous mitochondria and ribosomes throughout. There are also four chloroplasts near the center of the cell. Gunning (1977) speculated that organelles such as mitochondria and chloroplasts provided energy for active transport of solutes across the enlarged plasma membrane.

Cellular ultrastructure of Glycyrrhiza provided new information concerning its anatomy and potential forage value.

Anatomically, leaf mesophyll cells and vascular cells observed were very similar to those found in other dicotyledonous plants (Esau, 1965; Esau, 1977). However, the chloroplasts exhibited short stacked grana and limited starch and lipid accumulation. Two cell types previously unknown in Glycyrrhiza were transfer and tannin cells. Both cell types are considered to be parenchyma cells with functions which are inducible or somewhat plastic in nature. Transfer cells have the genetic competence to produce cell wall ingrowths which increases plasmalemma area and facilitates rapid short distance solute transport. Tannin cells package and store large amounts of tannin within cellular vacuoles. These tannin cells may account for inconsistencies in reported forage values for Glycyrrhiza depending on genotype and maturity of the plant, and climate and soil type on which it was grown.

Future research on Glycyrrhiza should involve chemical isolation of tannin and evaluation of diverse ecotypes for genotypic differences in tannin production. A possible selection scheme could involve both high and low tannin producing ecotypes. The low tannin producing ecotypes could be utilized as a high protein grazing type Glycyrrhiza. The high tannin producing ecotypes could be utilized as a high protein roughage to reduce proteolysis and the incidence of bloat.

Ultrastructure of *Uromyces glycyrrhizae*

The vegetative or developmental stage of *U. glycyrrhizae* is depicted by the intercellular hyphae in Figures 12, 13, 14, and 15A. Intercellular hyphae, as the name suggests, grow between host cells. Fungal cell walls are more electron-opaque than plant cell walls and thus appear darker in cross section (Figs. 12 and 13). An amorphous outer layer of the fungal wall (Fig. 14) has been suggested as having a role in adhesion of hypha to each other or to host cells (Hardwick, et al., 1971). Presumably, this layer is secreted by the fungus, but is questionable if it is considered to be an actual cell wall constituent or not (Littlefield and Heath, 1979). Intercellular hyphae of basidiomycete rusts are typically septate, as indicated by Figures 14 and 15A. Septa are divided into types based on development characteristics and presence or absence of septal pores (Littlefield and Heath, 1979). Septa observed in *U. glycyrrhizae* contained no visible pore apparatus. This septum, termed a complete or nonperforated type, resembles the perforate type in all respects except it contains no pore (Littlefield and Heath, 1979).

Intercellular hyphae contain mitochondria, ribosomes, vacuoles, nuclei, and nucleoli. The cellular

plasmalemma, which is extended and folded in certain areas of the hypha, form structures called invaginated fungal plasmalemmas (Figs. 14 and 15A). This term is more descriptive than several others which describe the same cellular phenomenon (Shrief, 1984). The occurrence of invaginated plasmalemma in fungal species is well documented, but their function within the organism is still unknown (Shrief, 1984). Mitochondria are common in intercellular hyphae (Figs. 12, 13, 14) and appear as round, oval, and elongated bodies containing internal membranous structures. The nuclear condition of the hyphae is dikaryotic (Fig. 12) with an occasional view of a fungal nucleolus (Fig. 15A).

Rusts characteristically produce "branches" of intercellular hyphae, called haustorial mother cells, which are adjacent to host cell walls (Littlefield and Heath, 1979). Haustorial mother cells subsequently penetrate host cells and form intracellular structures known as haustoria (Bushnell, 1972). Bushnell (1972) defined the fungal haustorium as a specialized organ formed inside a living host cell as a branch of an intercellular hypha, which terminates within that host cell, and which has a role in substance interchange between host and fungus. Figures 16, 17, 18, and 19

depict haustorial mother cell formation, host cell penetration, and early haustorium formation within the host cell.

Actual penetration of the host cell by the pathogen is preceded by the delimitation of a haustorial mother cell from the intercellular hypha (Ehrlich and Ehrlich, 1971). Figures 16 and 17 illustrate a haustorial mother cell which was formed when a septum separated it from the intercellular hypha. A large invaginated fungal plasmalemma is also evident in the cytoplasm of the haustorial mother cell (Fig. 17).

Penetration of the host cell is considered to be an enzymatic degradation rather than a mechanical process (Ehrlich and Ehrlich, 1971). After host penetration, elongation of the haustorial neck and expansion of the haustorium within the host cell cytoplasm occurs. Figures 18 and 19 depict a successful penetration and early haustorium formation within the host cell. Figure 18 shows a nucleus, containing a nucleolus, which moved from the haustorial mother cell up the haustorial neck and into part of the new haustorium. The haustorium also contains a number of mitochondria, ribosomes, and a single invaginated fungal plasmalemma. An enlargement of the penetration area is provided by Figure 19. Formation of a

"collar" of host cell wall material around the immediate haustorial neck in response to the fungal penetration is a common occurrence (Littlefield and Heath, 1979). The boundary between collar and cell wall is not discernible in this particular penetration. The host plasma membrane lies along the surface of the collar, haustorial neck, and around the body of the haustorium. This host membrane is described as the extrahaustorial sheath or membrane (Figs. 15B, 18, 19, 20, and 21) when it surrounds and delimits the haustorial body from the host cytoplasm (Coffey, 1972; Littlefield and Heath, 1979). The extrahaustorial sheath is separated from the haustorial body by the extrahaustorial matrix (Fig. 15B). The composition and even the existence of the extrahaustorial matrix is a matter of controversy (Littlefield and Heath, 1979). Littlefield and Heath (1979) have speculated that the matrix may arise through loss of turgor during fixation, allowing fungal walls to shrink from the extrahaustorial sheath creating an artifact of preparation.

Haustoria can be filamentous, vesicular, or club-shaped, and may branch and coil in a variety of ways (Bushnell, 1972). Figures 15B, 20, 21, and 22 depict a variety of haustorial shapes and sizes. Host cells containing multiple haustoria (Figs. 15B and 21) may have

multiple penetrations or may involve folding of a single haustorium which produces a multiple haustorial appearance in the plane of sectioning. Cellular contents of haustoria are very similar to the organelles observed in intercellular hyphae. Most of the haustoria have a number of mitochondria and contain many ribosomes (Figs. 15B, 18, 19, and 21). Figure 22 contains a single haustorium in the normal dikaryotic nuclear condition.

In most respects the infection of G. lepidota by U. glycyrrhizae is very similar ultrastructurally to what is commonly observed in other host-pathogen interactions of this type (Littlefield and Heath, 1979; Hardwick, et al, 1971). Organelles observed are similar in shape, composition, and location to those reported in other rust pathogens. Intercellular hyphae and haustoria, with respect to nuclear condition, are both typically dikaryotic. U. glycyrrhizae contained very few microbodies within its cytoplasm and quite a few invaginated fungal plasmalemmas in intercellular hyphae, haustorial mother cells, and haustoria. Septa observed are of the complete or nonperforated type, which is not typical of the majority of rust pathogens (Littlefield and Heath, 1979). Thus, it is possible that septal pores do exist within the fungal septa, but were not sectioned in the correct plane to be revealed.

Further microscopic studies could reveal whether septa are perforate or nonperforate and if the paucity of microbodies is a typical situation for this fungus. Further ultrastructure work could be focused on host infection by various Uromyces spore types. Co-ordination of this ultrastructure work with infectivity of various spore types in controlled greenhouse experiments could reveal a more complete picture of the fungal life cycle.

The lack of pathogenic studies on native legumes provides opportunities to study unique host-pathogen systems. The discovery of as yet unreported pathogens on native legumes is also a real "possibility" for future research.

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ABBREVIATIONS USED IN FIGURES

C	Collar
Ch	Chloroplast
D	Dictyosome
EHM	Extrahaustorial Matrix
EHS	Estrahaustorial Sheath
FM	Fungal Mitochondrion
FN	Fungal Nucleus
FPL	Fungal Plasmalemma
FV	Fungal Vacuole
FW	Fungal Wall
G	Granum
H	Haustorium
HMC	Haustorial Mother Cell
HNK	Haustorial Neck
HMB	Host Microbody
HM	Host Mitochondrion
HN	Host Nucleus
HPL	Host Plasmalemma
HV	Host Vacuole
ICH	Intercellular Hypha
IFP	Invaginated Fungal Plasmalemma
Nu	Nucleolus
R	Ribosomes
S	Septum
SW	Secondary Wall
T	Tannin Inclusion
TC	Tannin Cell
W	Wall of Host Cell
X	Xylem

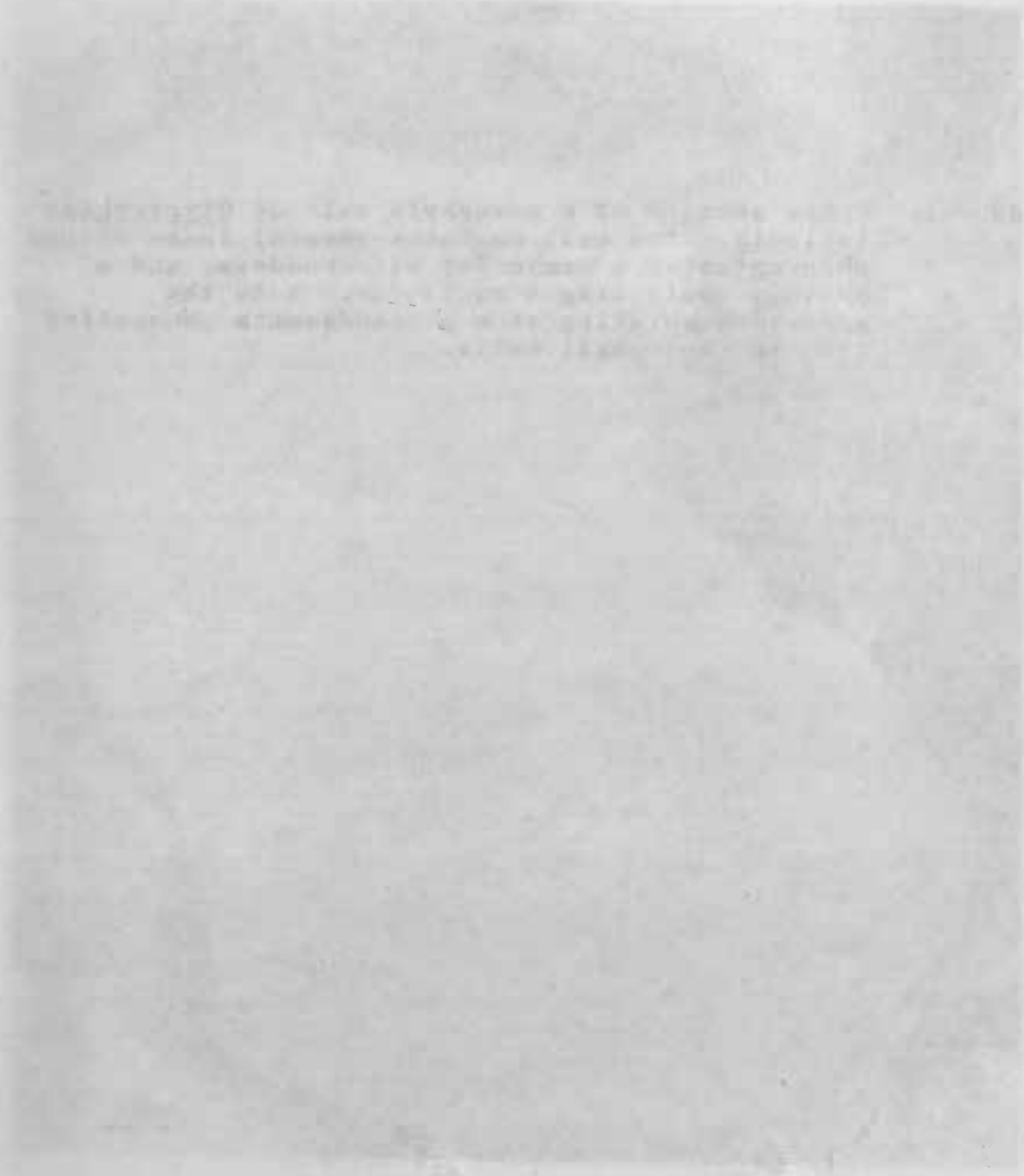


Figure 1. Cross section of a mesophyll cell of *Glycyrrhiza lepidota*. The cell contains several lense-shaped chloroplasts, a number of mitochondria, and a nucleus containing a nucleolus. Note the arrowhead pointing at a plasmodesmata connecting two leaf mesophyll cells.

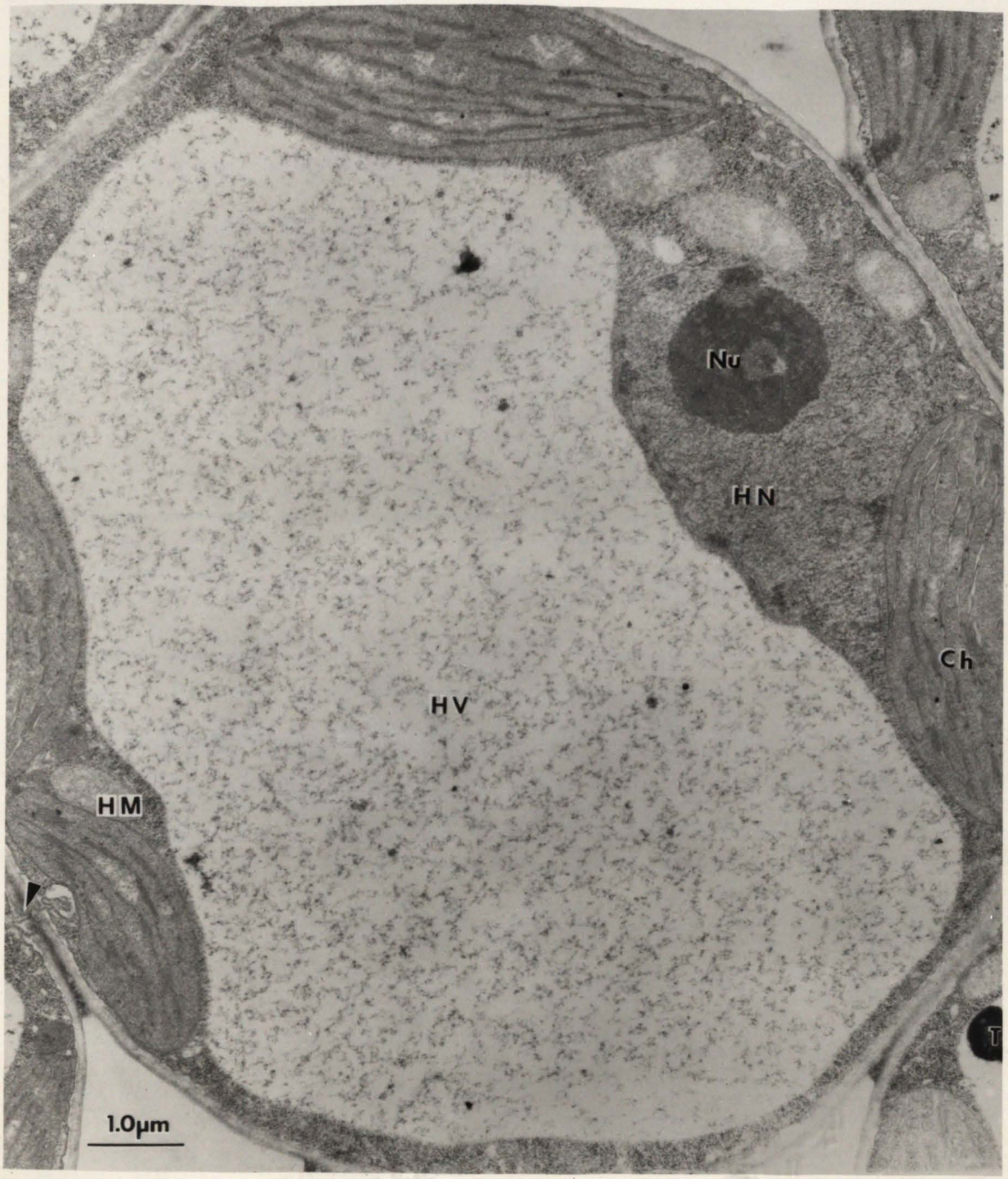

Nu
HN
Ch
HV
HM
1.0μm

Figure 2. Cross section of leaf mesophyll cells of *Glycyrrhiza* and a portion of an intercellular hypha (ICH) of *Uromyces*. The large central vacuole of the leaf cell contains a dark tannin inclusion and the open arrow indicates a smaller vacuole which also contains tannin. Note the closed arrow pointing to an amorphous substance between the mesophyll cell and the ICH.

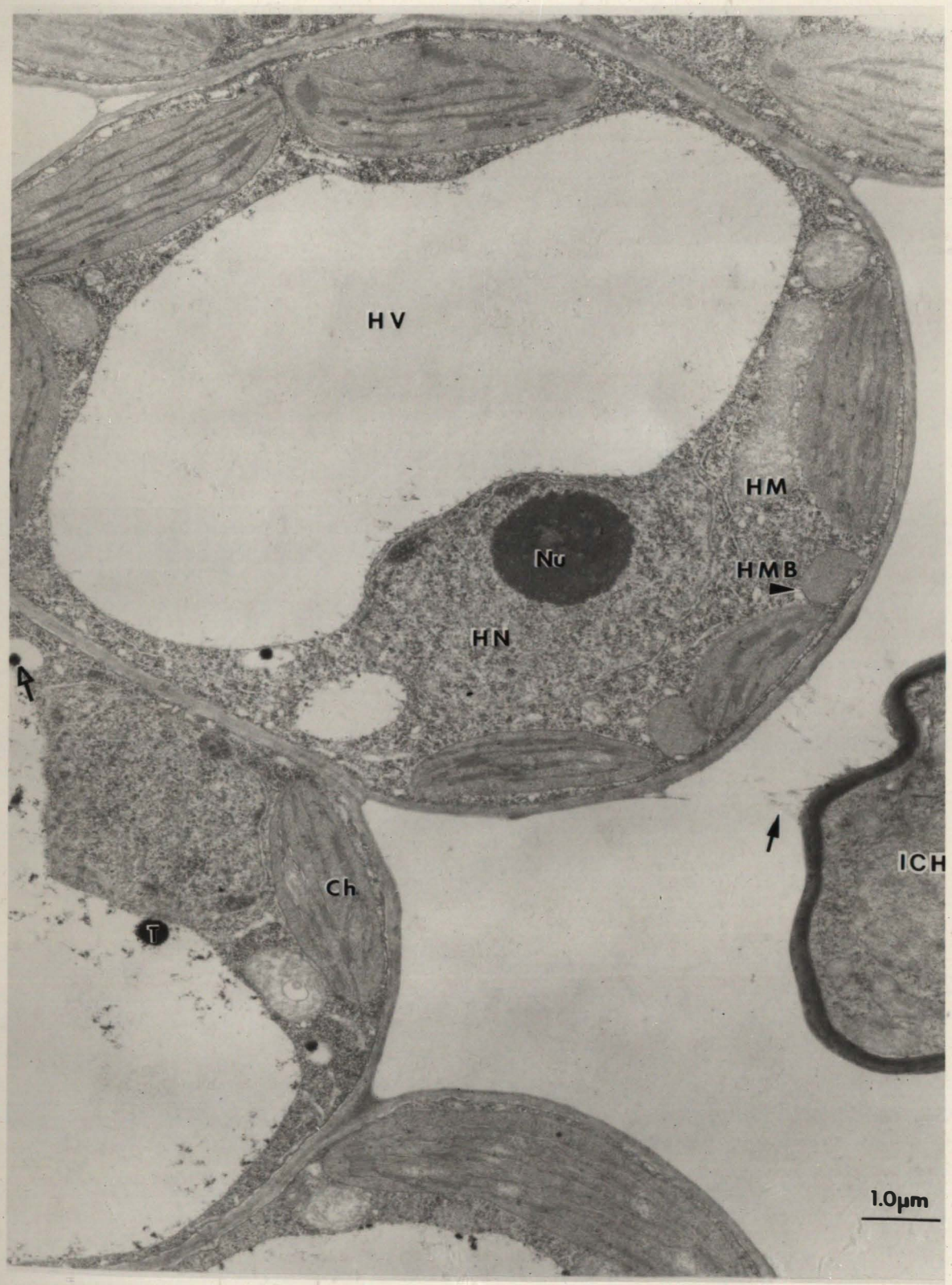

HV
HM
Nu
HMB
HN
Ch
T
ICH
1.0μm

Figure 3. An enlargement of a section of leaf mesophyll cells containing chloroplasts with short stacked grana and numerous ribosomes surrounding mitochondria.


R
HM
Nu
W
HN
G
Ch
0.5μm

Figure 4. An enlargement of a section of a leaf mesophyll cell showing a chloroplast, with numerous grana, surrounded by a bilayer membrane. The electron lucent areas between grana stacks may be the location of chloroplast DNA. The nucleus, delimited by the nuclear envelope (arrowhead) contains a large nucleolus.

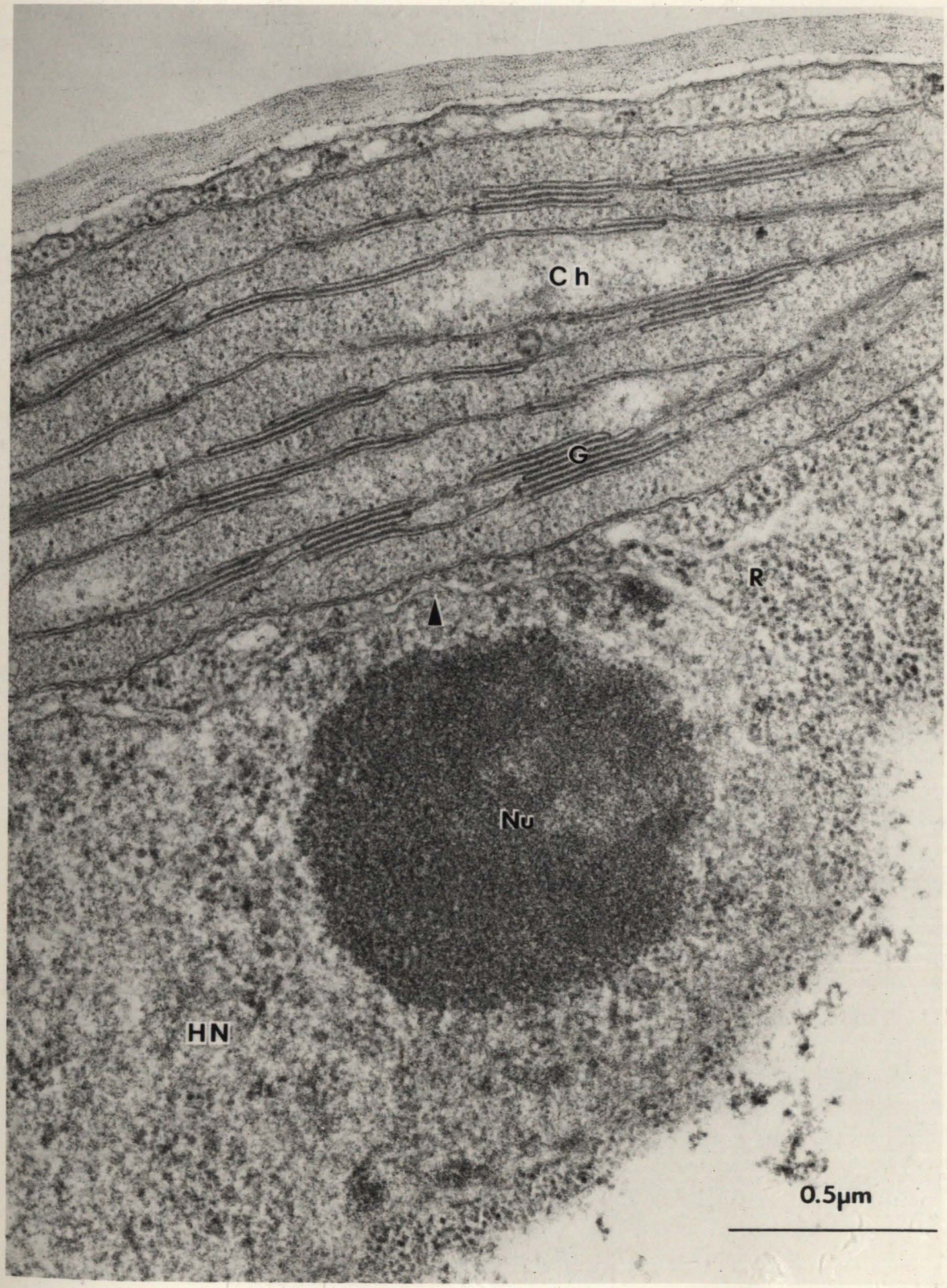

Ch
G
R
Nu
HN
0.5µm

Figure 5. Cross section of a tannin cell surrounded by leaf mesophyll cells. Note that the vacuole in the cell in the lower right is beginning to accumulate tannin.

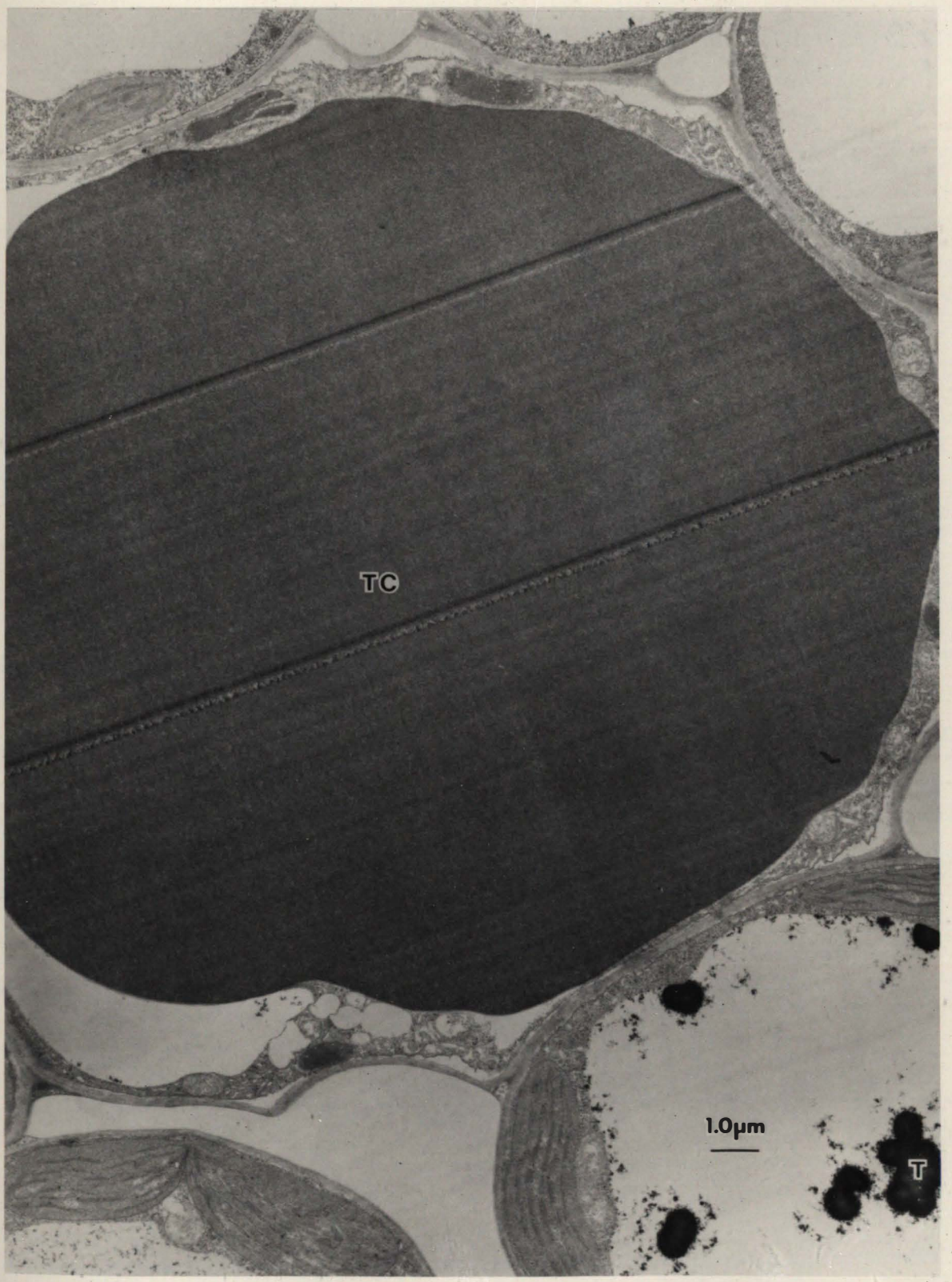

TC
1.0µm
T

Figure 6. Enlargement of a Glycyrrhiza tannin cell with its vacuole filled almost entirely by tannin. Note the host mitochondrion closely appressed to the cell wall with very little cytoplasm remaining in the cell.


TC
W
HM
0.5µm

Figure 7. Ultrastructure of *Glycyrrhiza* vascular parenchyma cells. The central cell contains many smaller vacuoles, very few plastids and three examples of invaginated host plasmalemma.

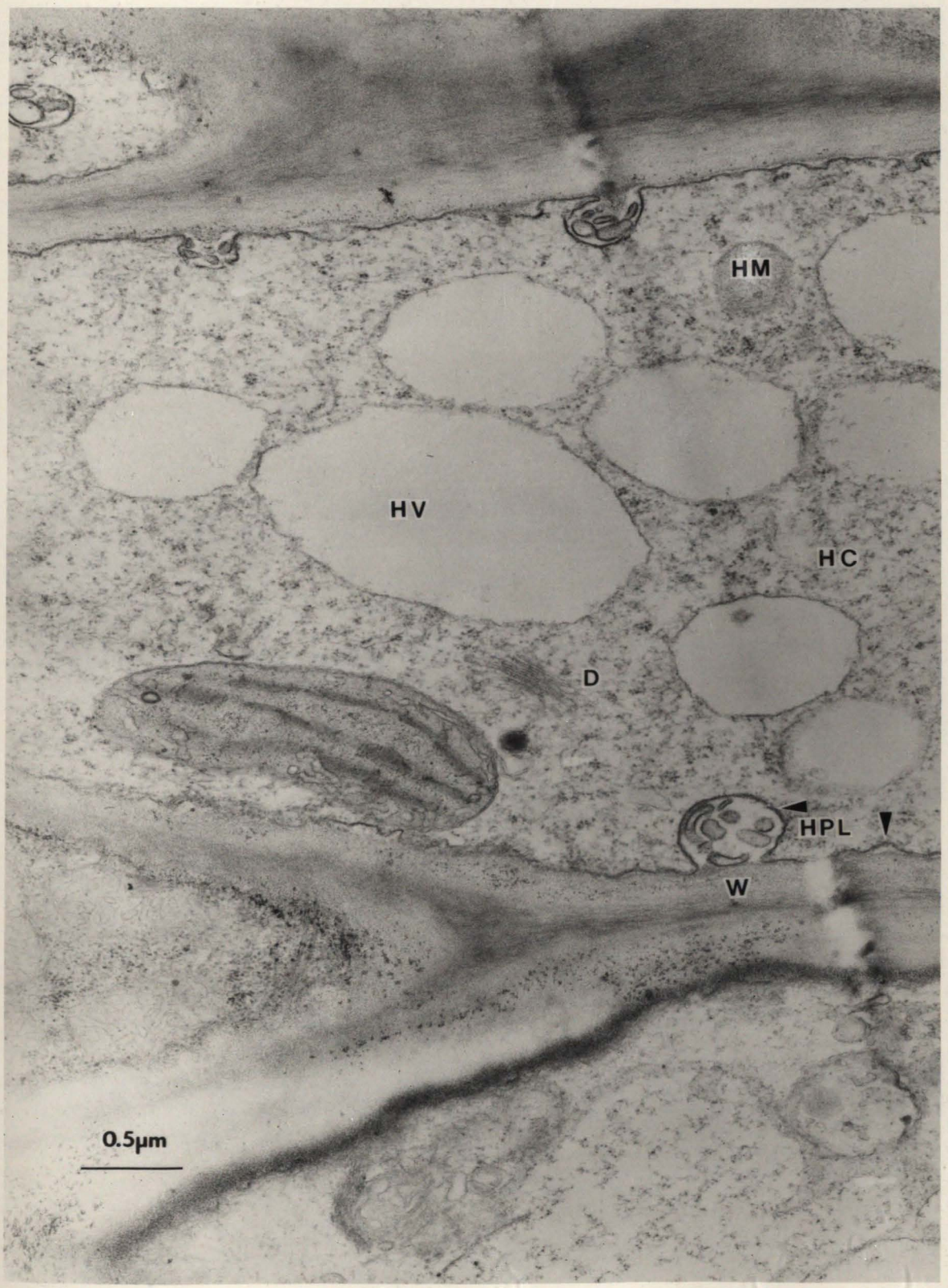

HM
HV
HC
D
HPL
W
0.5µm

Figure 8. Ultrastructure of Glycyrrhiza vascular system containing two mature xylem trachied cells, an immature xylem trachied cell (star), and xylem parenchyma cells. The micrograph also contains examples of rust intercellular hypha and a rust haustorium located within a vascular parenchyma cell.

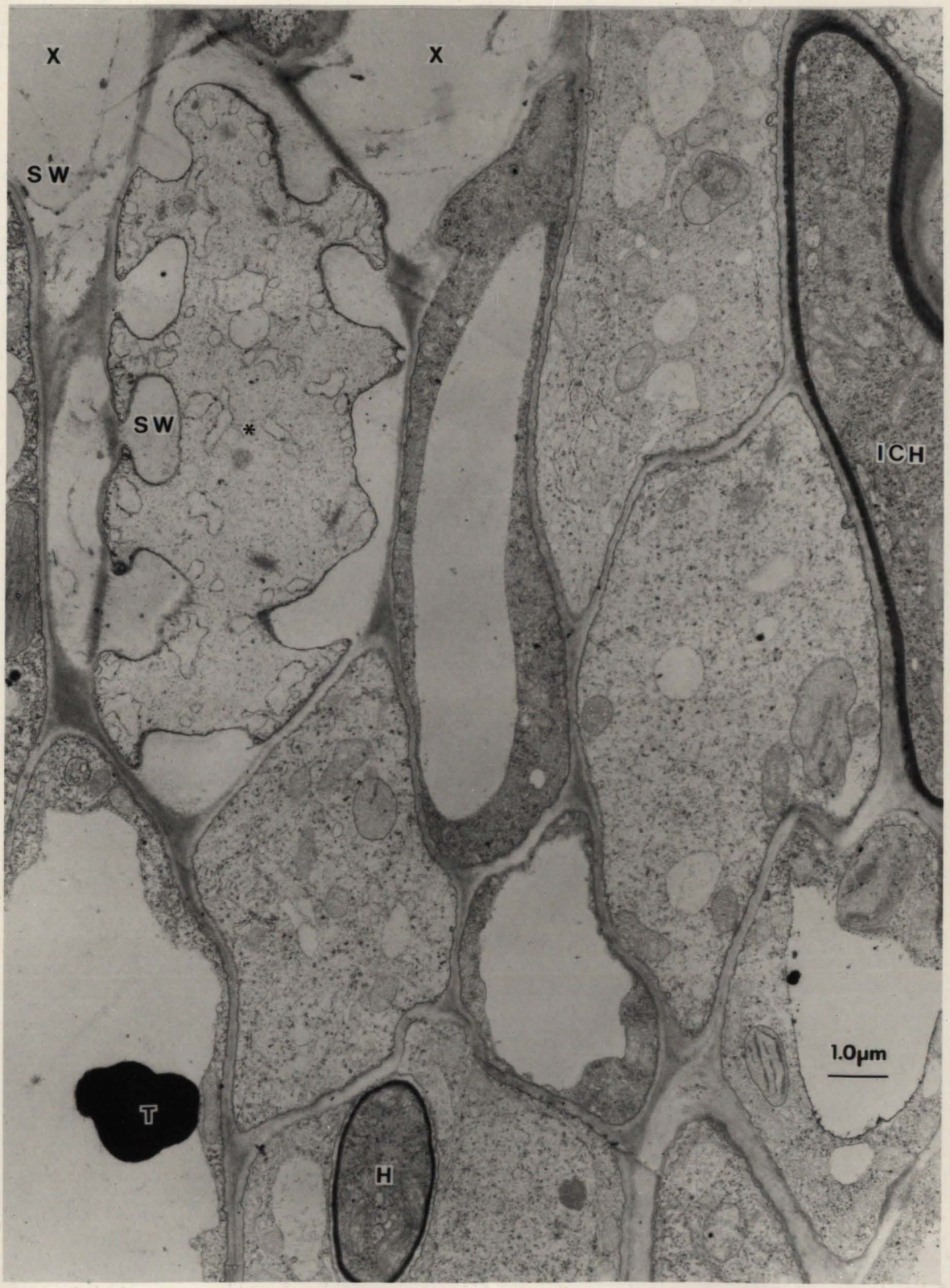

X
X
SW
SW
*
ICH
1.0µm
T
H

Figure 9. Vascular tissue of *Glycyrrhiza* with a mature xylem trachied cell adjacent to an immature xylem trachied cell (star). Note the similar secondary wall thickening in both cells and the dense cytoplasma, containing numerous mitochondria, in the immature xylem cell.

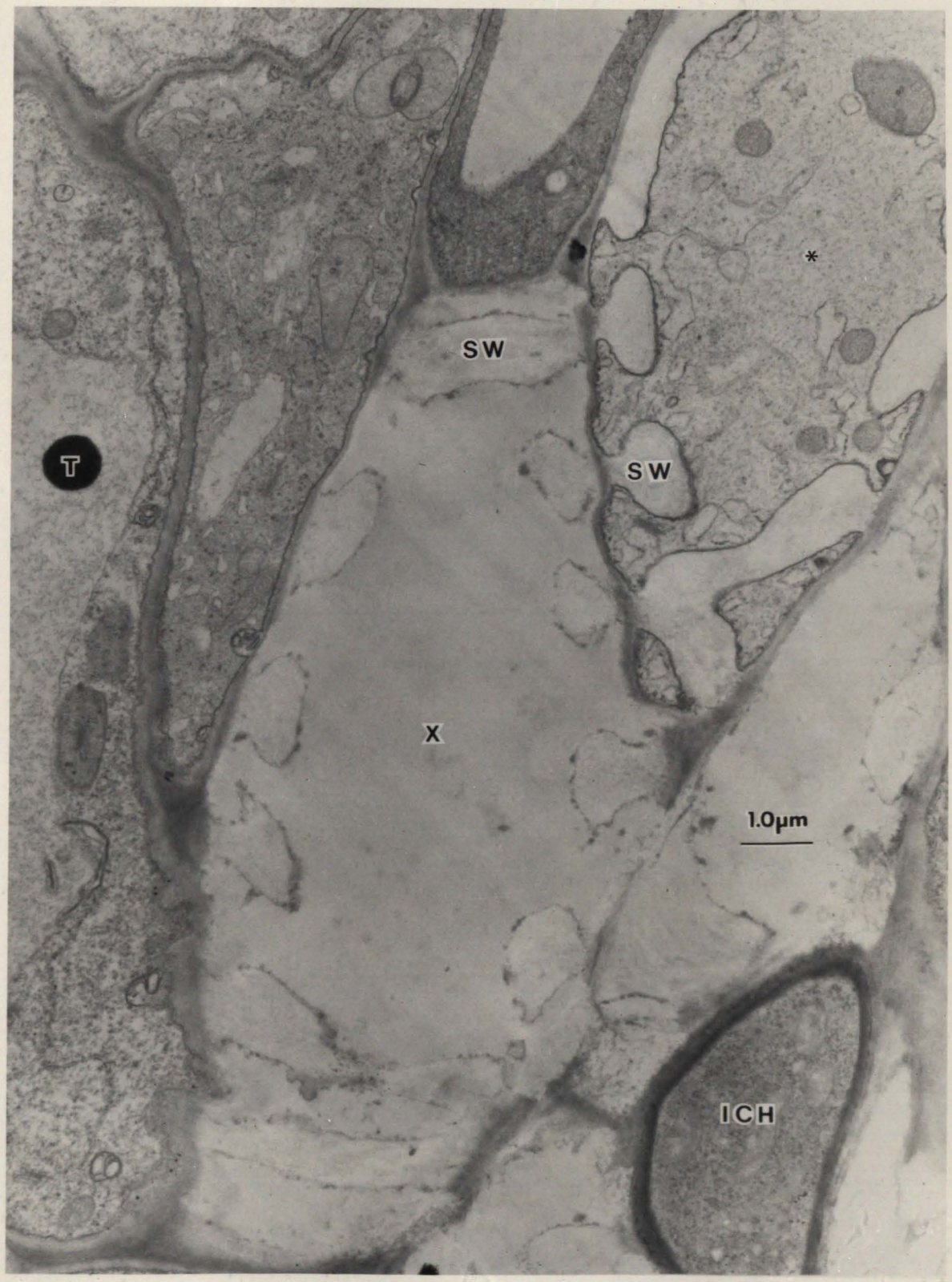

SW
SW
T
X
*
1.0µm
ICH

Figure 10. Ultrastructure of Glycyrrhiza vascular tissue showing two mature xylem trachied cells and xylem parenchyma cells containing vacuoles with tannin inclusions. Note the microbodies and mitochondria in the cell adjacent to one xylem parenchyma cell (lower right).

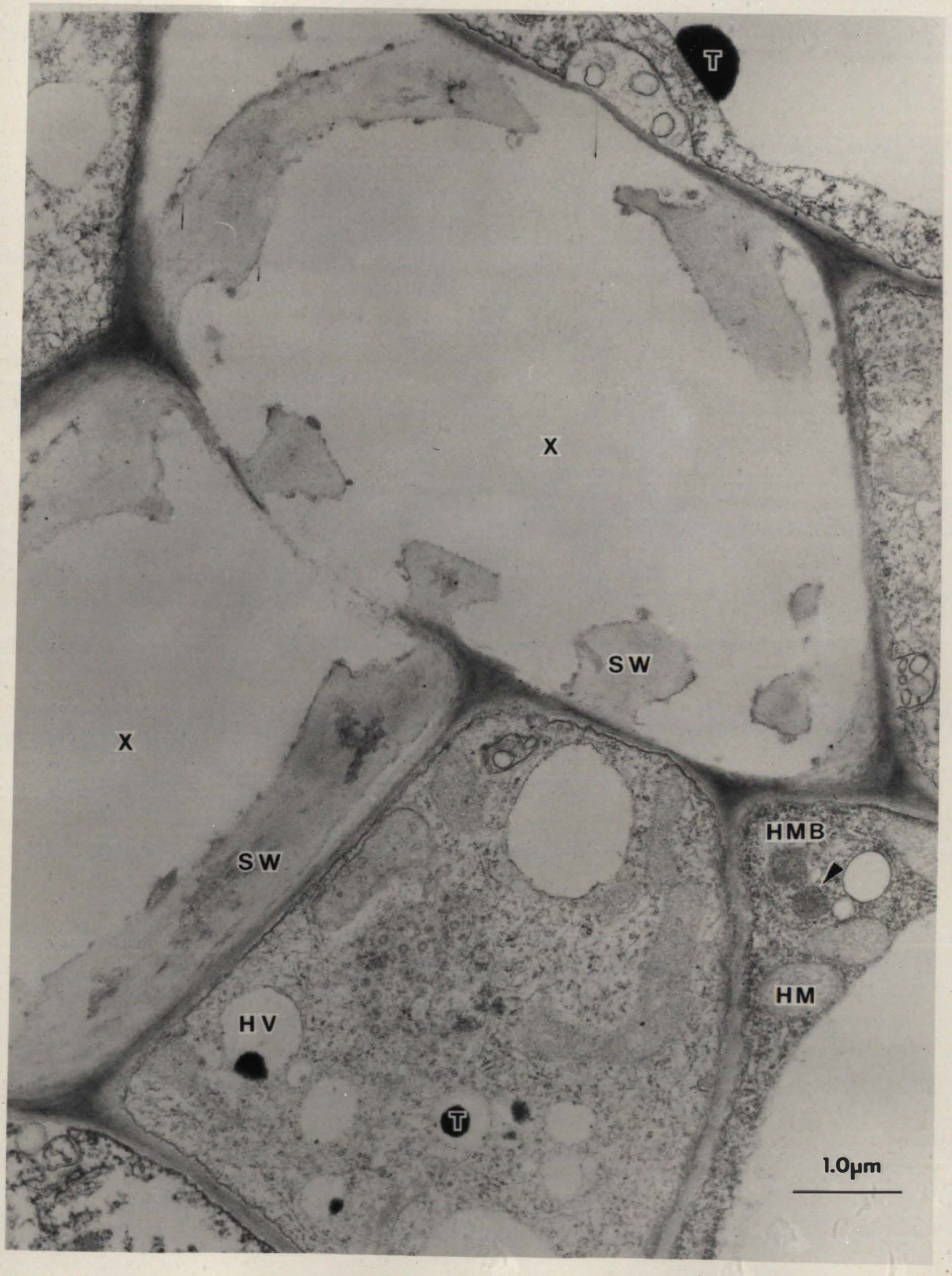

T
X
SW
X
SW
HMB
HM
HV
T
1.0μm

Figure 11. Cross section of an A-type transfer cell located in the vascular tissue of a minor leaf vein. Note the dense cellular cytoplasm containing closely packed clusters of ribosomes and numerous mitochondria surrounding the cell wall protruberances (stars).

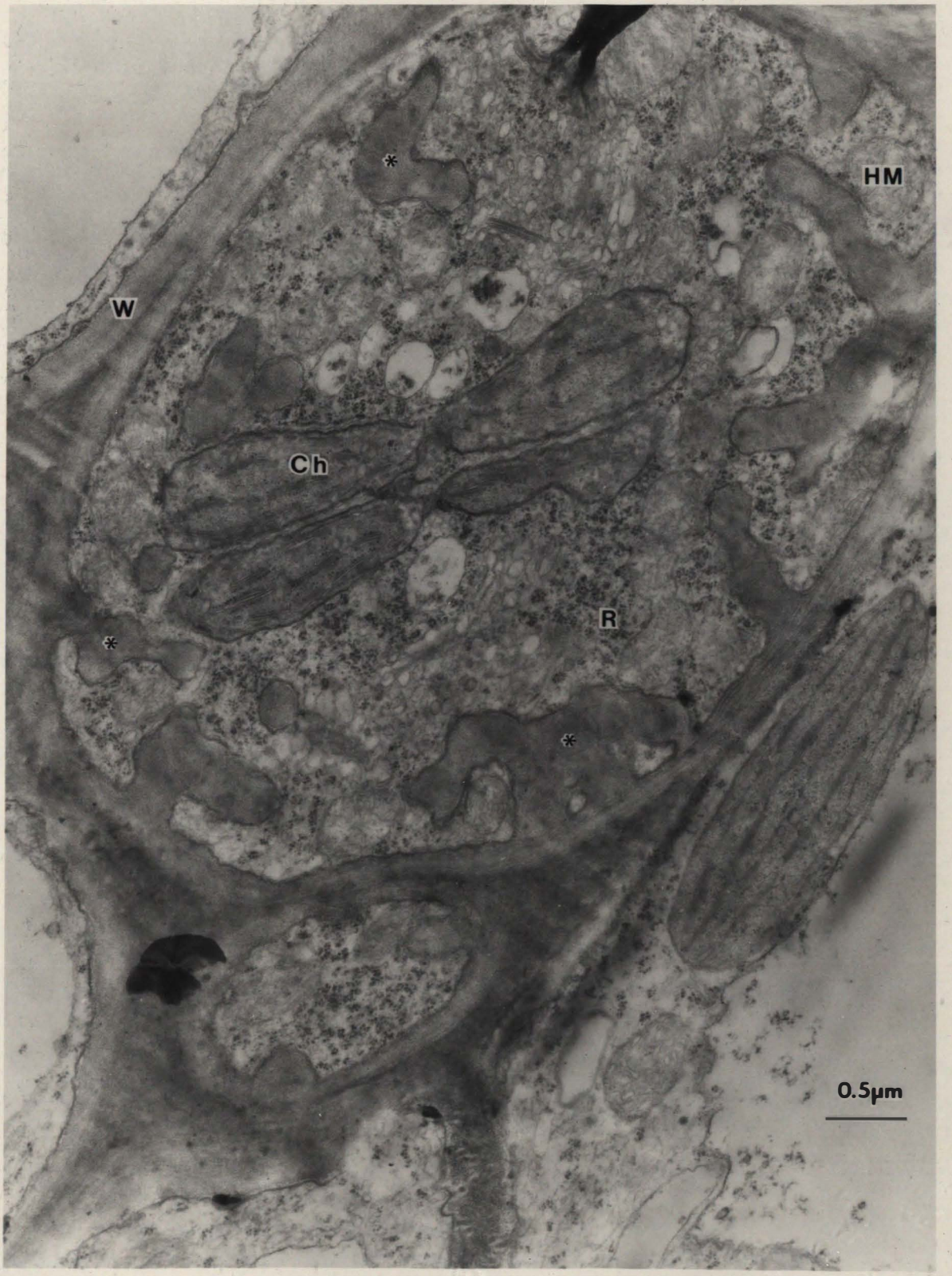

HM
W
Ch
R
0.5μm

Figure 12. Cross section of a rust hypha between two Glycyrrhiza mesophyll cells. Observe the dikaryotic nature of the hypha and compare fungal and host mitochondria, vacuoles, walls, and plasmalemmas. Tannin inclusions are located in the host's vacuole. The host ribosomes appear scattered, but many polyribosomes are evident in the fungal cytoplasm.

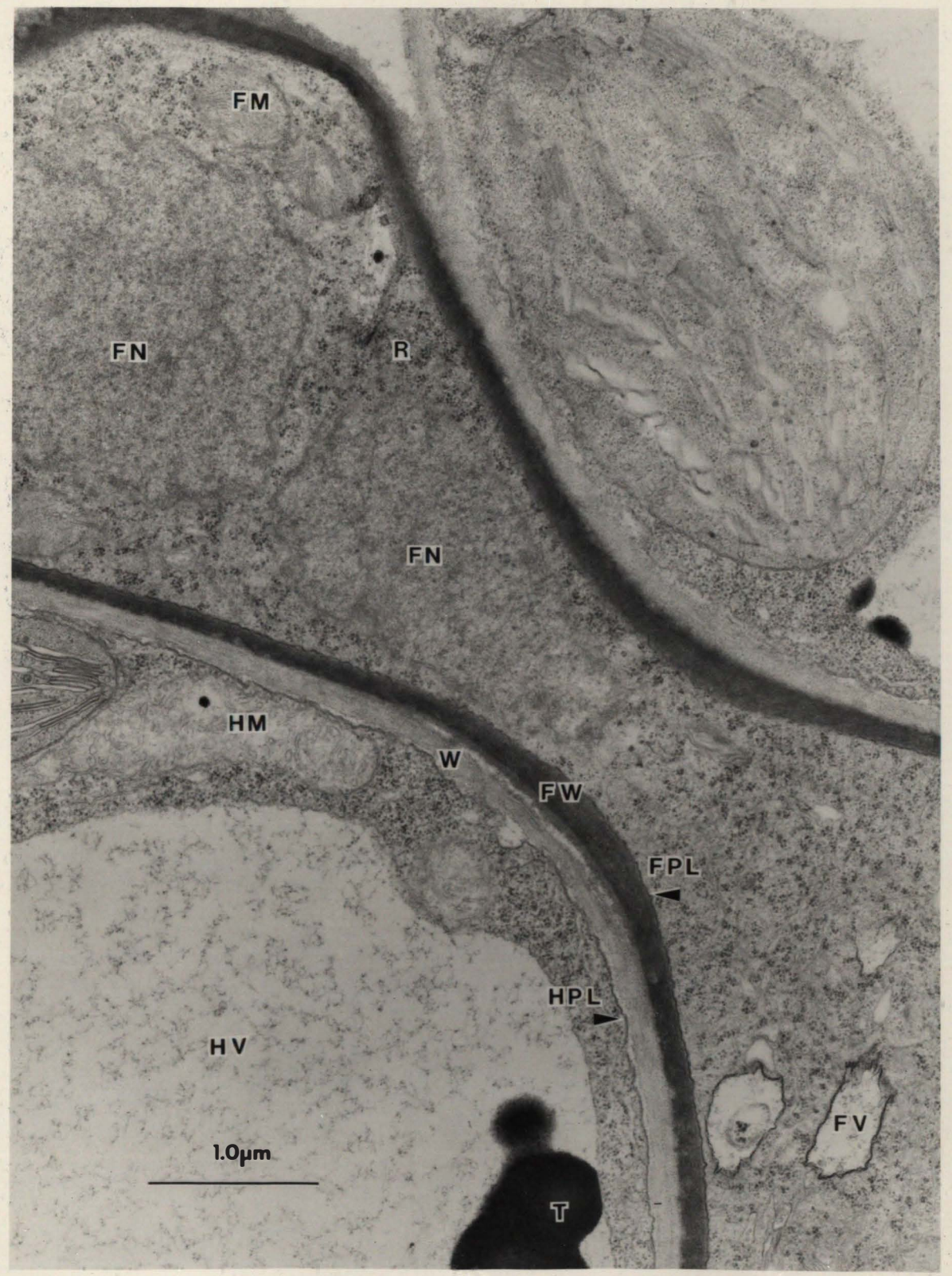

FM
FN
R
FN
HM
W
FW
FPL
HPL
HV
FV
1.0µm
T

Figure 13. Ultrastructure of an intercellular hypha containing a multitude of ribosomes and several mitochondria.

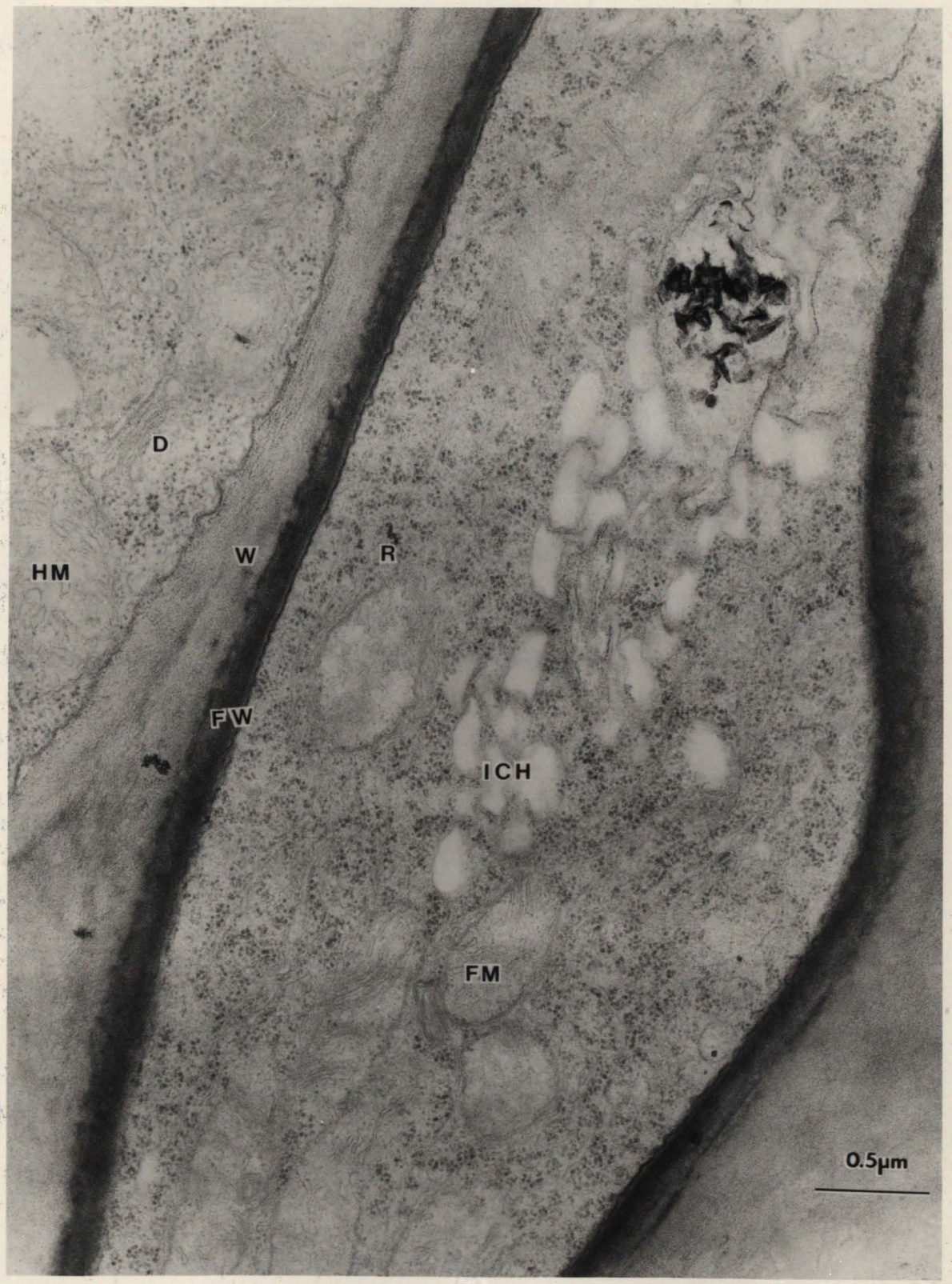

D
HM
W
R
FW
ICH
FM
0.5µm

Figure 14. Ultrastructure of two intercellular hyphae between Glycyrrhiza leaf mesophyll cells. The upper hypha contains a septum and two examples of invaginated fungal plasmalemma (IFP). The arrowhead points to the connection between the IFP and the plasmalemma. The arrow indicates an amorphous substance observed between host cells and fungal hyphae which may act as an adhesive.

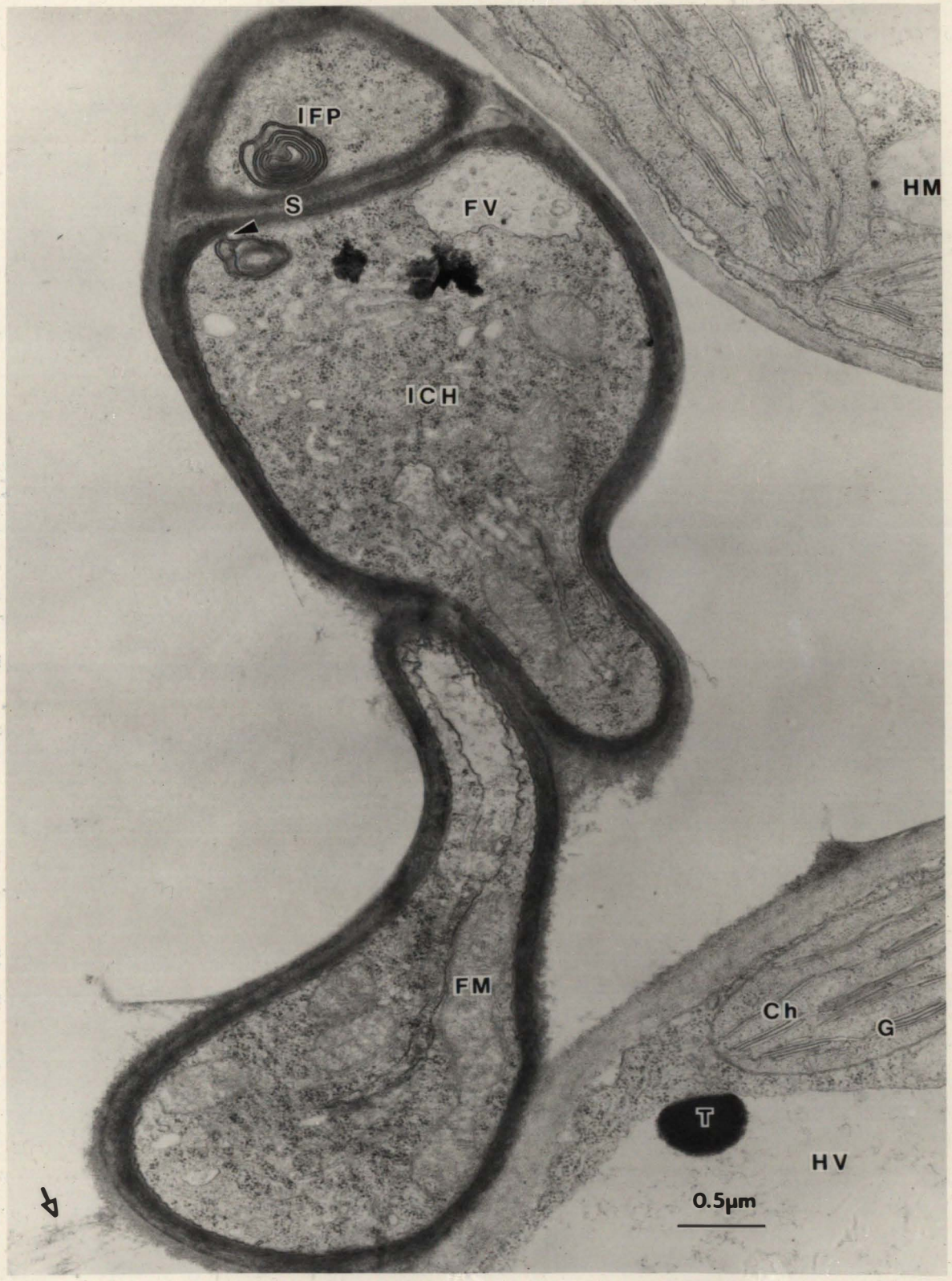

IFP
S
FV
HM
ICH
FM
Ch
G
T
HV
0.5μm
A

Figure 15A. Cross section of two segments of intercellular hyphae. One segment contains a septum and the other contains a nucleus and nucleolus. The star is located on a secondary wall thickening.

Figure 15B. Ultrastructure of multiple haustoria within a single host cell. Haustoria are surrounded by an extrahaustorial matrix which is bound by the extrahaustorial sheath. The open arrow is pointing to a plasmodesmata and the star is situated on a secondary wall thickening that may indicate a host transfer cell.

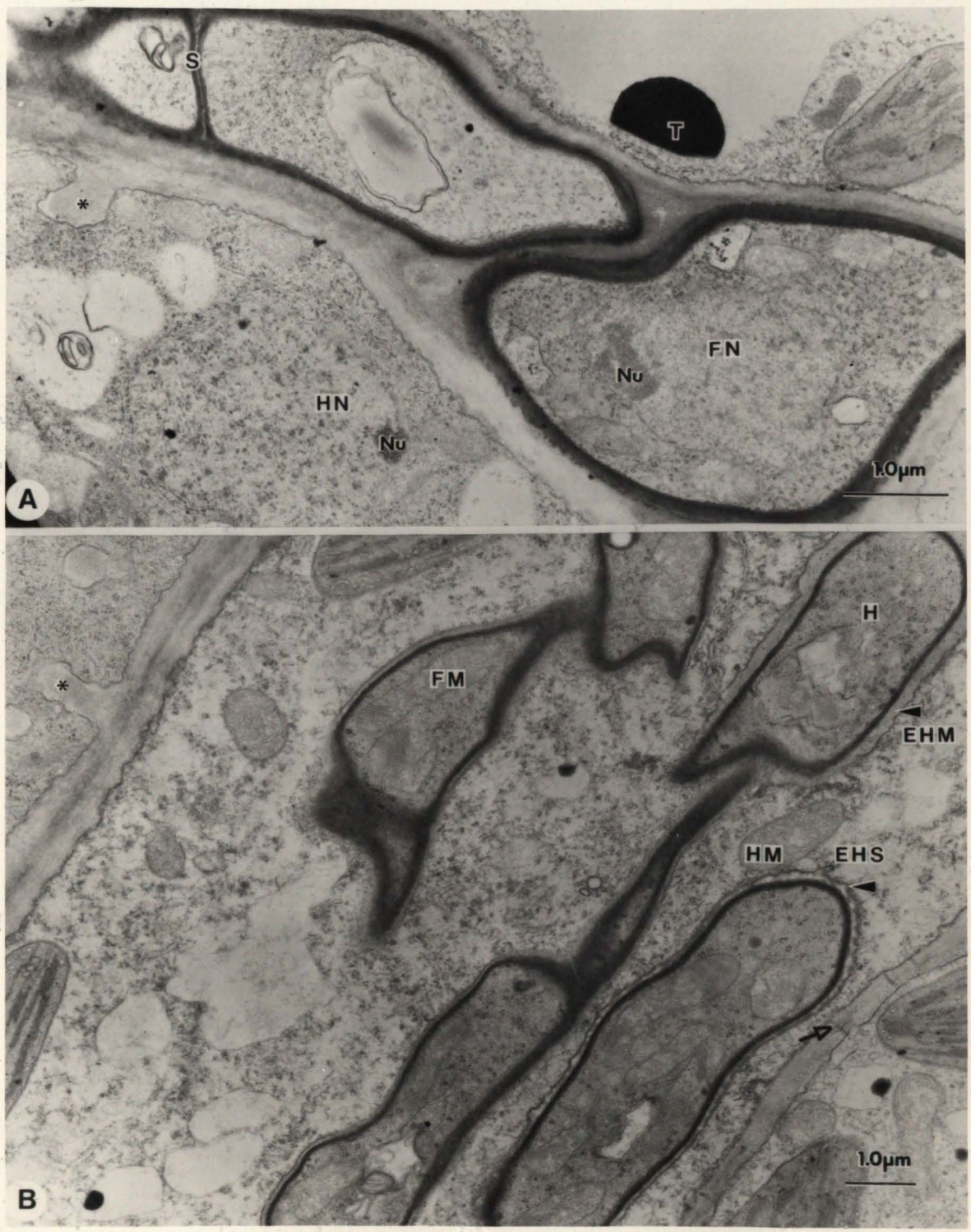

S
T
*
FN
Nu
HN
Nu
1.0μm
A
H
FM
*
EHM
HM
EHS
1.0μm
B

Figure 16. Early stages of host cell penetration by the haustorial mother cell which has been separated from the intercellular hypha by a septum. The star is located on a starch grain within a host chloroplast.

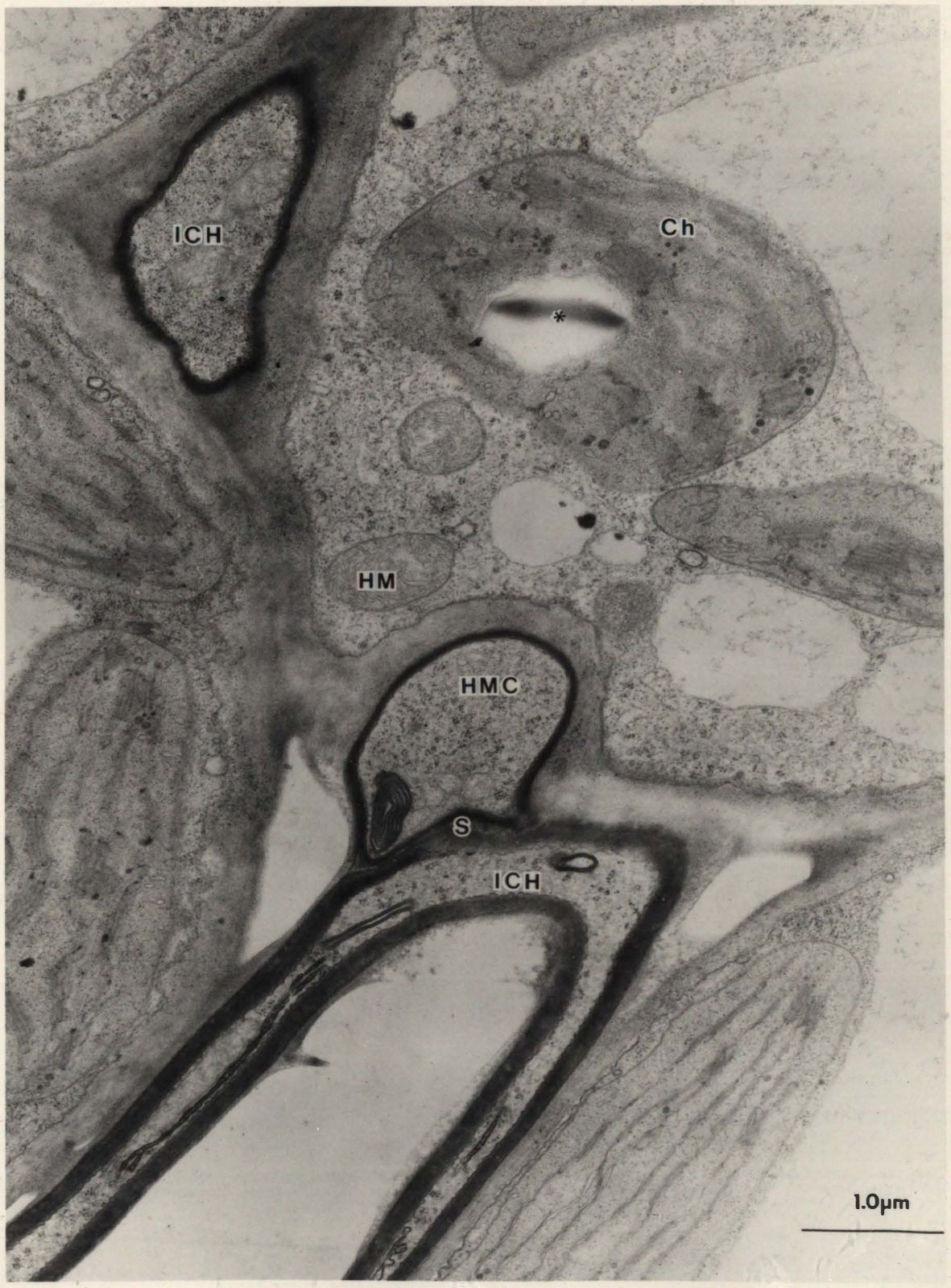

ICH
Ch
*
HM
HMC
S
ICH
1.0μm

Figure 17. Enlargement of haustorial mother cell (HMC) distending the host cell wall to form the collar during the penetration process. Note the invaginated fungal plasmalemma within the HMC and intercellular hypha.

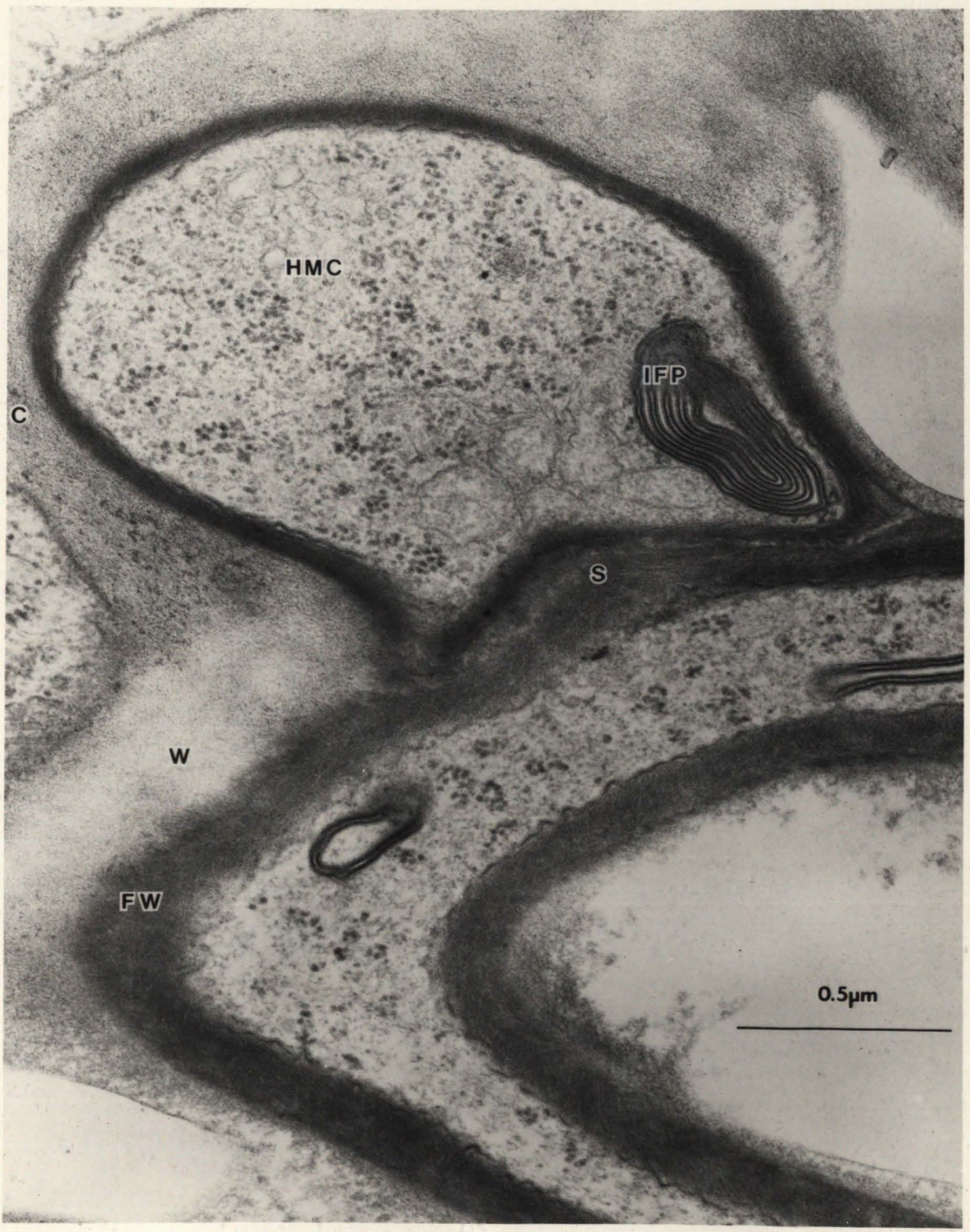

HMC
IFP
C
S
W
FW
0.5µm

Figure 18. Successful penetration of a host cell and formation of a haustorium by the haustorial mother cell (HMC). The fungal nucleus passed through the penetration pore from the HMC and migrated into the haustorial neck and haustorium.

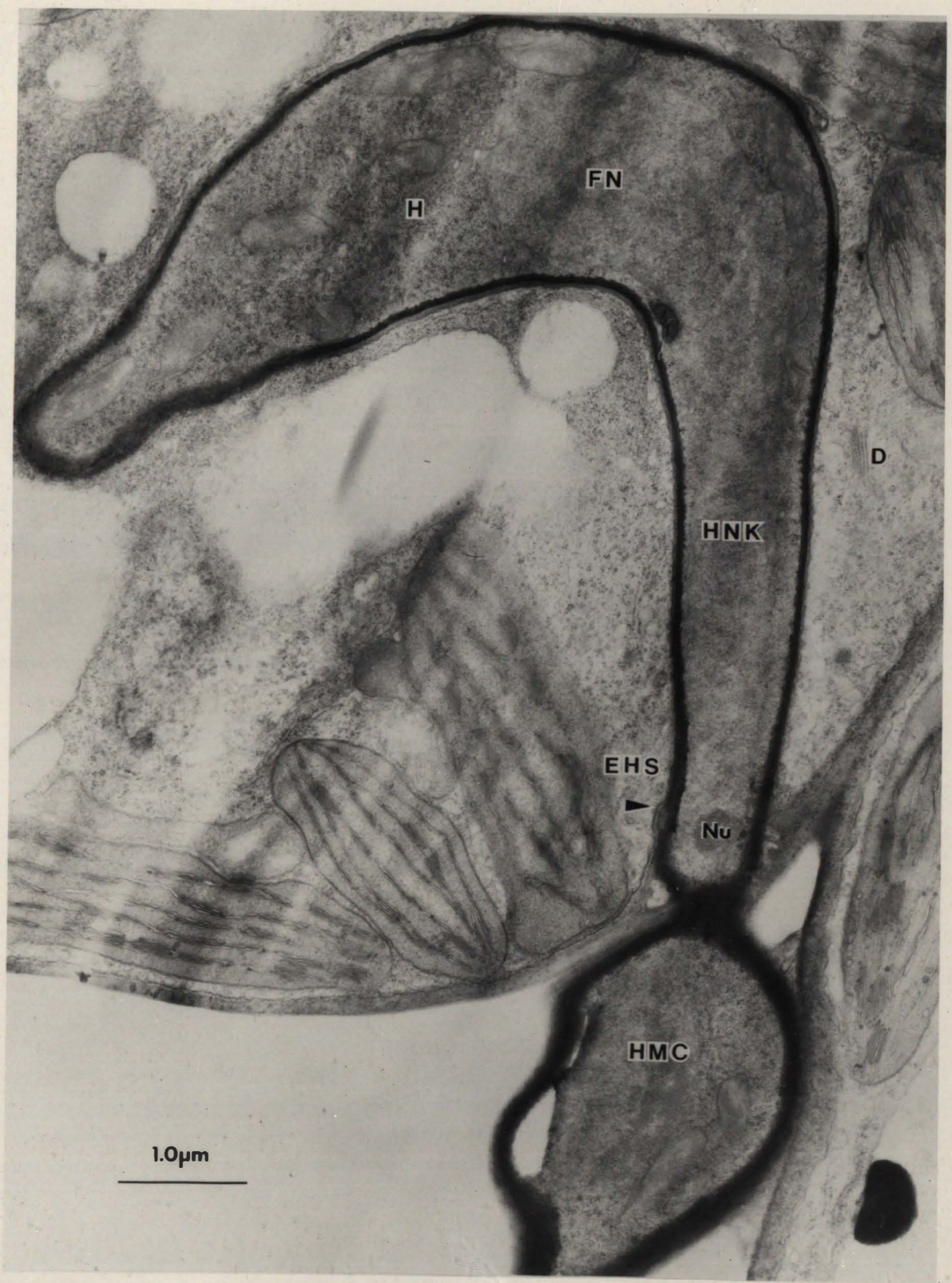

FN
H
D
HNK
EHS
Nu
HMC
1.0μm

Figure 19. Enlargement of a cell penetration site showing details of a haustorial mother cell and haustorial neck. Note the collar region near the penetration and the extrahaustorial sheath which surrounds and delimits haustorial contact with host cytoplasm.

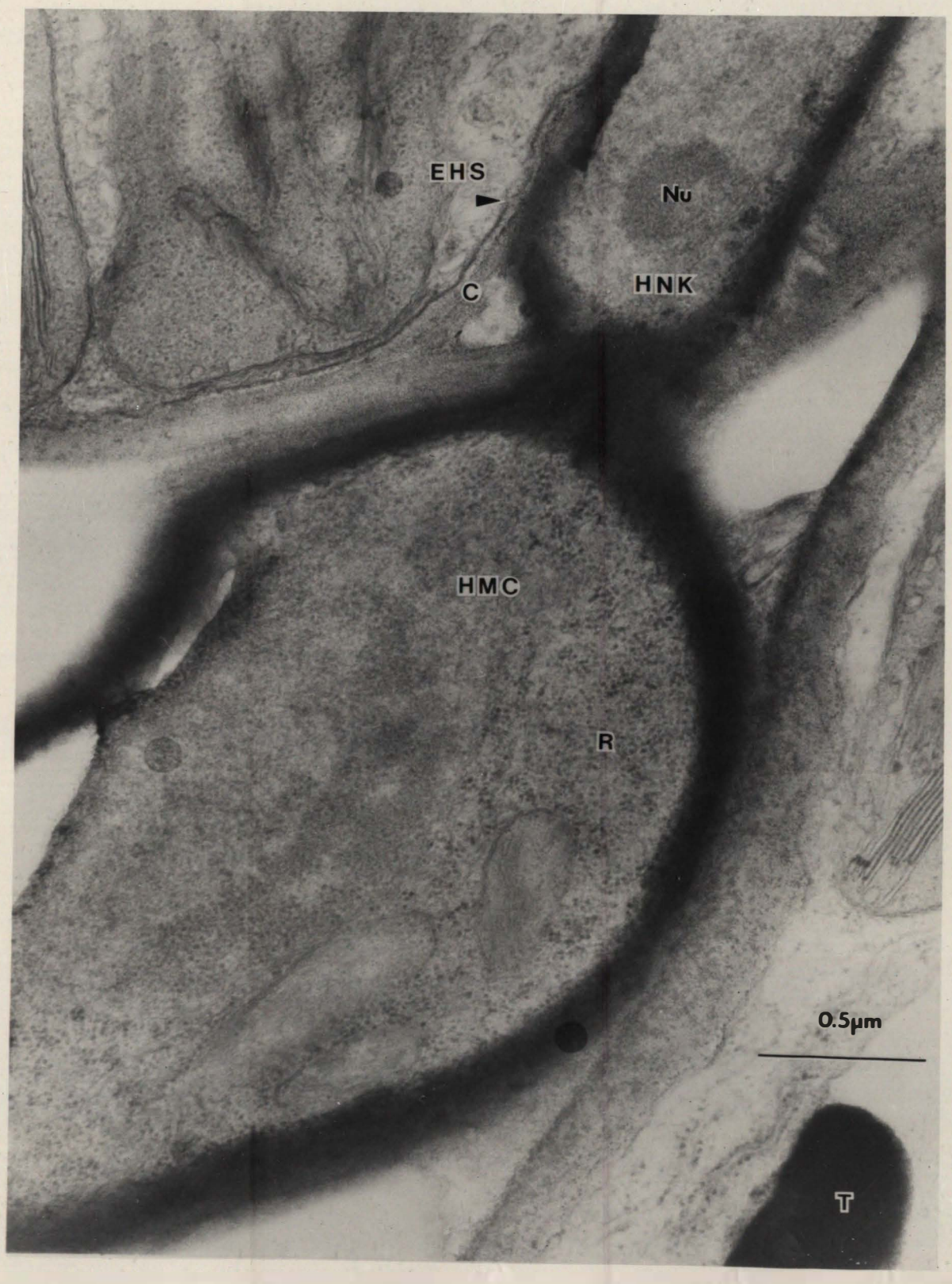

EHS
Nu
C
HNK
HMC
R
0.5µm
T

Figure 20. Ultrastructure of a host cell containing a haustorium and part of a haustorial neck. The actual penetration site and haustorial mother cell are out of the plane of sectioning and all than can be observed here is the haustorial neck and the collar which surrounds it.

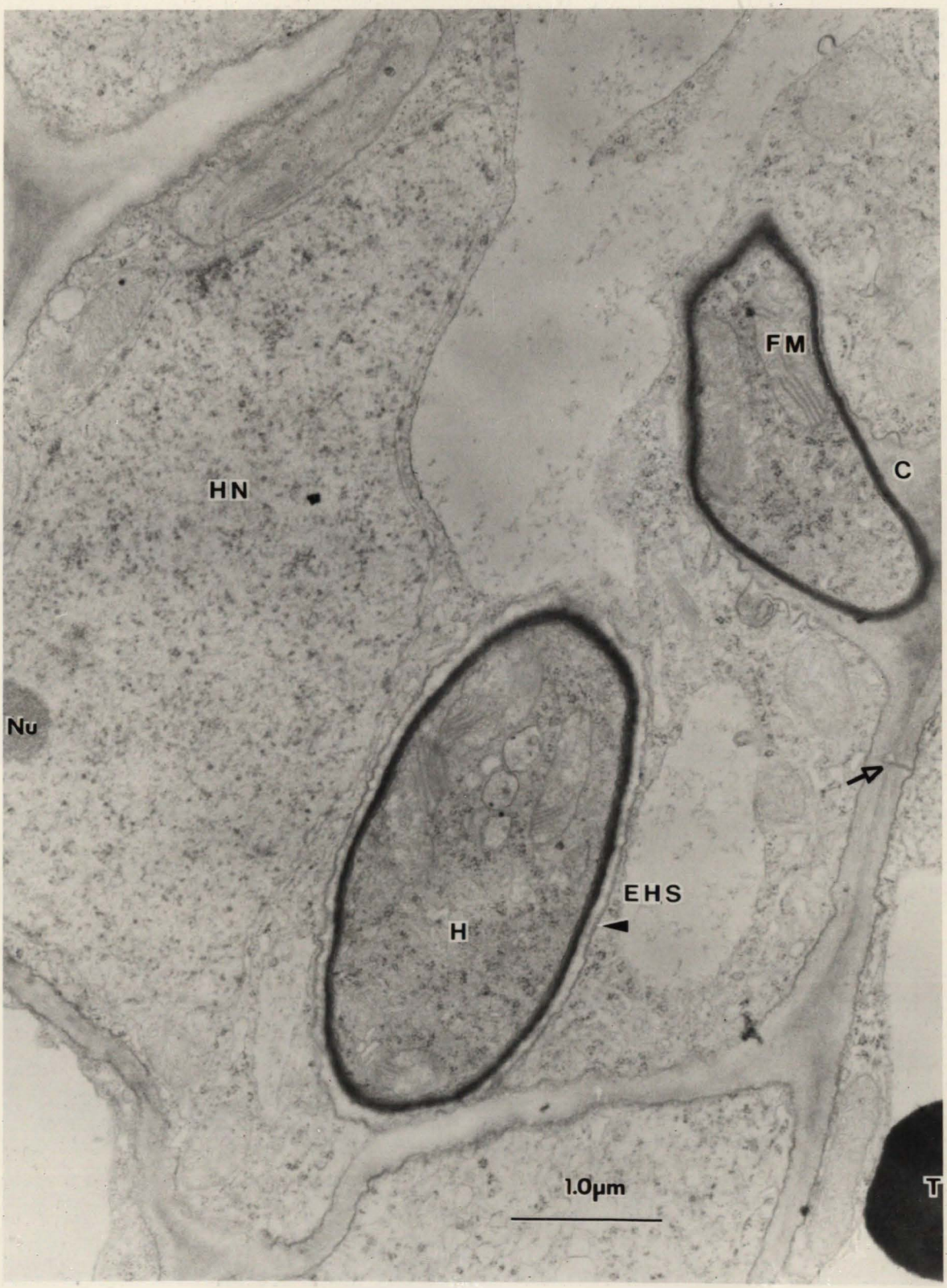

FM
C
HN
Nu
EHS
H
1.0μm
T

Figure 21. Multiple haustoria within a host cell. The haustoria vary with respect to shape, but all are surrounded by an extrahaustorial sheath and most have numerous ribosomes and mitochondria visible. Note the presence of lipid globules (star) within a host mitochondrion.

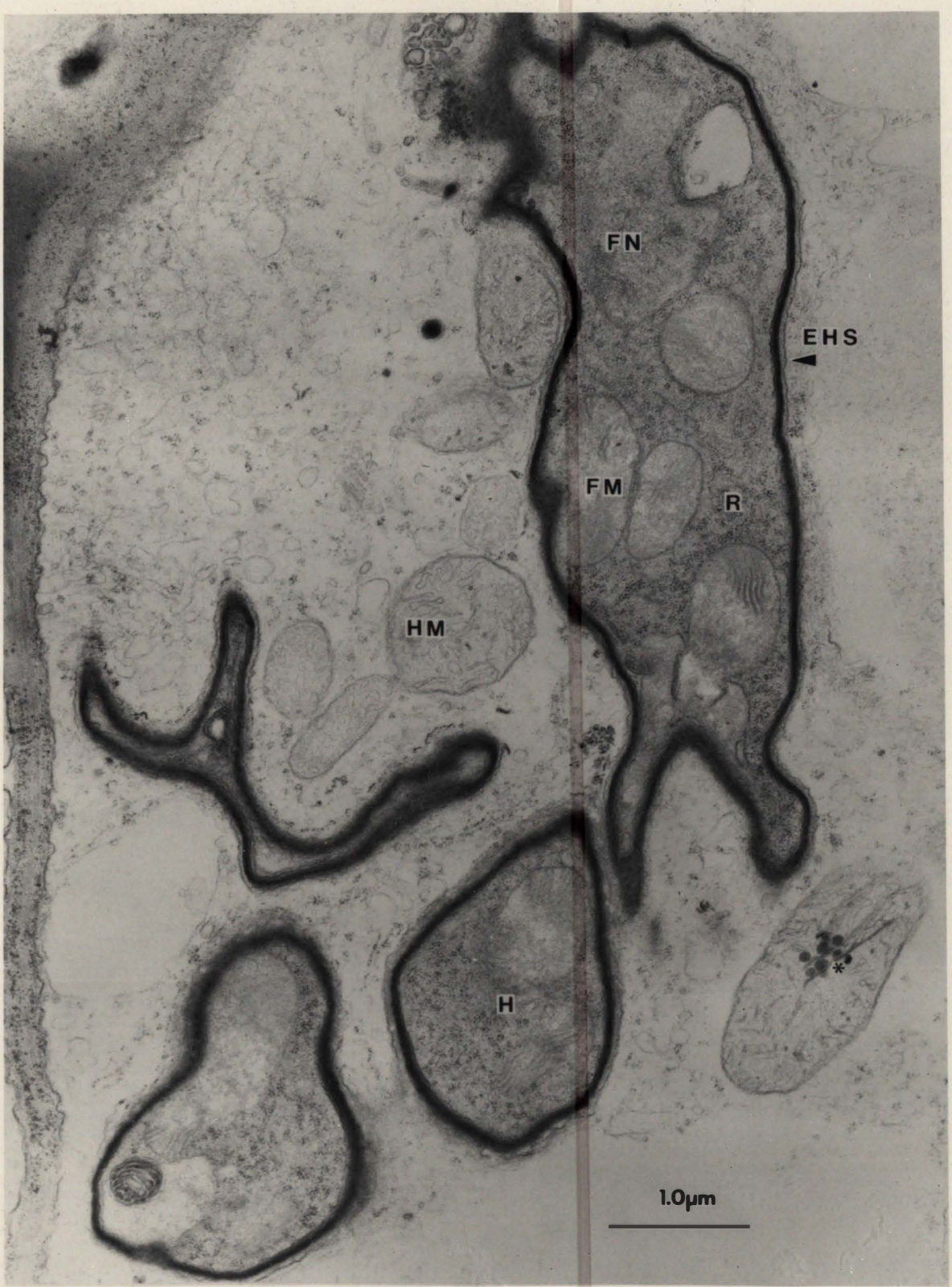
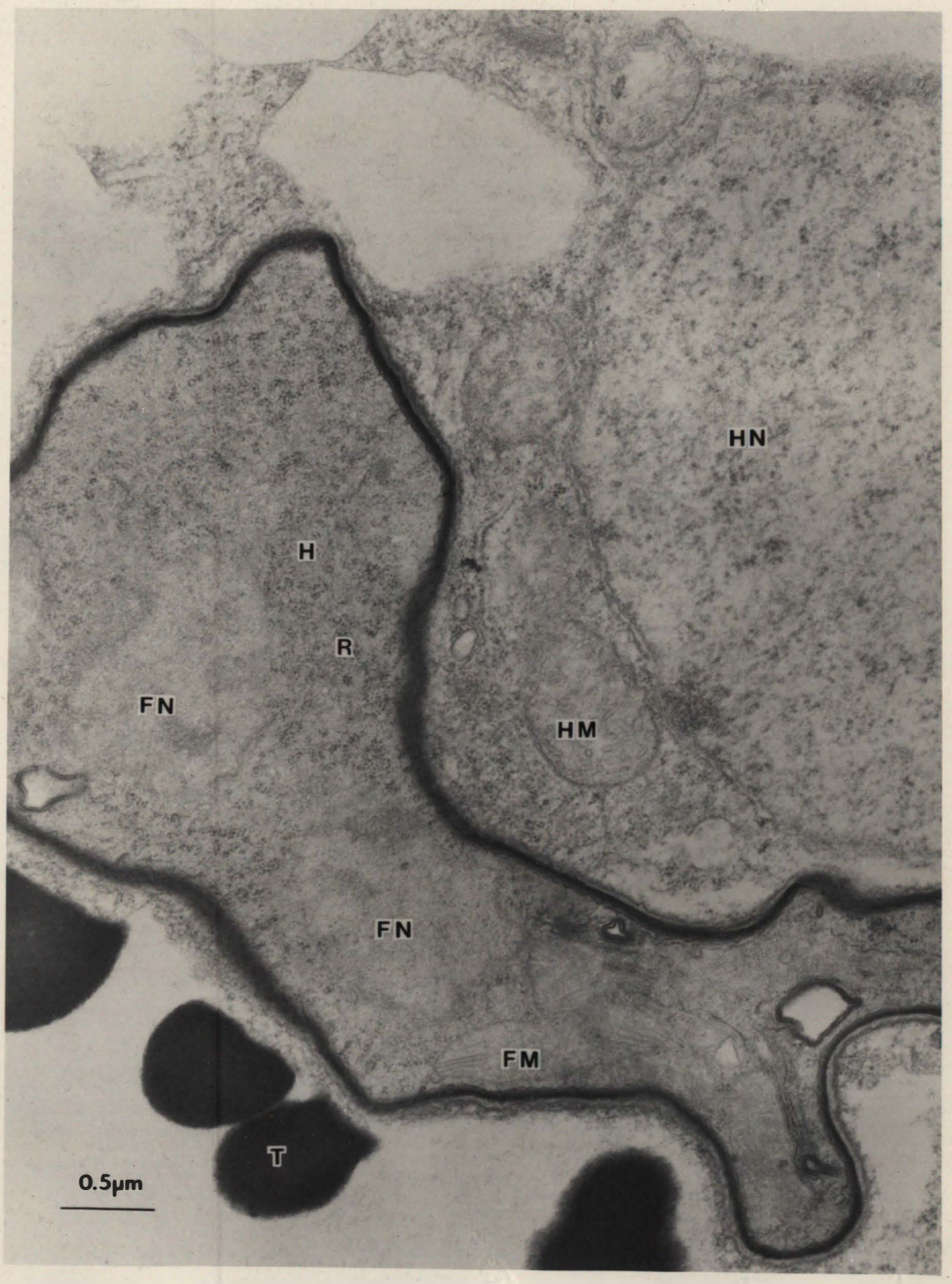

FN
EHS
FM
R
HM
H
1.0μm

Figure 22. Dikaryotic fungal huastorium containing numerous ribosomes and mitochondria is located within the host cell near a vacuole containing tannin inclusions.


HN
H
R
FN
HM
FN
FM
T
0.5μm